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ORIGINAL INVESTIGATION

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Molecular and immunological characterization of the p83/100 protein of various *Borrelia burgdorferi* sensu lato strains

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Abstract The complete coding regions of the chromosomally encoded p83/100 protein of four *Borrelia garinii* strains and one *Borrelia burgdorferi* sensu stricto strain have been amplified by the polymerase chain reaction (PCR), cloned and sequenced. From alignment studies with the deduced amino acid sequences presented here, and five other published p83/100 sequences, the most heterologous region of the p83/100 molecule was identified to be located between amino acid position 390–540. To study the structure of this heterogeneous region, and internal fragment of the *p83/100* genes from 11 additional *B. burgdorferi* sensu lato strains was amplified by PCR. The PCR products were analyzed by DNA sequencing and restriction enzyme analysis. These internal *p83/100* fragments varied in size and sequence. Cluster analysis of internal *p83/100* fragments, as well as restriction enzyme analysis, revealed three major groups in accordance with grouping into the three species causing Lyme disease. Strains within the same species (six *B. burgdorferi* sensu stricto and six *B. afzelii* strains) showed similar p83/100 partial structures. Nevertheless, nine *B. garinii* strains showed more sequence variations and could be further divided into two major subgroups. One group is represented by OspA serotype 4 strains, the other more heterogeneous group is represented by OspA serotypes 3, 5, 6 and 7 strains. Phenotypic analysis with four p83/100-specific monoclonal antibodies revealed four distinct reactivity patterns. Antibody L100 1B4 recognized a common epitope of *B. burgdorferi* sensu stricto and *B. afzelii*. Antibodies L100 17D3 and L100 18B4 were reactive with an epitope shared by strains of all three species. The broadest reactivity was shown by L100 18B4 which, in constrast to L100 17D3, additionally recognized the relapsing fever borreliae *B. turicatae* and *B. hermsii*. L100 8B8 detected a subgroup of the *B. burgdorferi* sensu stricto strains. Since comparison of the p83/100 molecule with sequences from protein databases showed similarities with characteristics of eukaryotic cell structures, the p83/100 might mimic these structures and may, therefore, be involved in the immune escape mechanism of the pathogenic agent of Lyme disease.

Key words Lyme borreliosis · *Borrelia burgdorferi* sensu lato · p83/100 Antigen · Molecular analysis · Phenotypic analysis

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Introduction

A protein with a molecular mass of approximately 83-100 kDa is an immunodominant protein of the immune response in patients with Lyme borreliosis [17, 32]. This chromosomally encoded protein [24], designated p83/100 in this study, is associated either with the protoplasmic cylinder [18] or with the flagella [9]. Although p83/100 homologues [14, 33] were designated p100, p83 [24], p93 [31] and p97 [9], the molecular masses deduced from their amino acid (aa) [14, 32] sequences do not differ very much. Since p83/100 is usually expressed in low amounts, the cloning of this protein was an important step for its use as diagnostic antigen.

The respective genes of *B. burgdorferi* sensu stricto [24, 31] and *B. afzelii* [14] have been cloned in *E. coli*. The p83/100 homologues belonging to different species of *B. burgdorferi* sensu lato share a high degree of identity (>88%) but are more heterogeneous than the homologues of flagellin, which is another immunodominant chromosomally encoded protein of *B. burgdorferi* sensu lato [15, 19, 25]. In contrast to the flagellin, the p83/100 protein is a highly specific protein of *B. burgdorferi* sensu lato, show-

ing no immunological cross reactions outside the genus *Borrelia* [4]. Recombinantly expressed p83/100 is a highly sensitive antigen for detection of IgG antibodies in late Lyme borreliosis [26, 36]. However, a study using p83/100 proteins from *B. burgdorferi* sensu stricto and *B. afzelii* suggested that antigenic heterogeneity of this protein might play a role in the sensitivity of antibody detection [38].

One aim of this work was to gain new insights into the molecular structure of the p83/100 molecules. Therefore, the complete *p83/100* gene of *B. burgdorferi* sensu lato strains PBr (*B. garinii*, OspA serotype 3), PBi (*B. garinii*, OspA serotype 4), TN (*B. garinii*, OspA serotype 6), Gö2 (*B. garinii*, OspA serotype 6) and PBre (*Borrelia burgdorferi* sensu stricto, OspA serotype 1) were amplified and cloned. Based on alignments of these sequences and other previously published sequences, [8, 14, 24], the region of the *p83/100* gene between aa position 390–540, (p83 numbering [24], turned out to be the most heterogeneous part of the molecule. A section of this *p83/100* heterogeneous region was sequenced and subsequently analyzed from 11 additional *B. burgdorferi* sensu lato strains.

Another aim of this study was the immunological analysis of p83/100. Therefore, we established a panel of monoclonal antibodies (mAbs) using recombinantly produced p83/100 derived from either *B. afzelii* or *B. burgdorferi* sensu stricto. Molecular as well as immunological analysis with mAbs revealed differences between the p83/100 proteins among the three species of *B. burgdorferi* sensu lato. In addition, a high degree of heterogeneity among *B. garinii* strains was observed by cluster analysis of p83/100 sequences and one mAb even identified a subgroup among *B. burgdorferi* sensu stricto strains.

Material and methods

Borrelia spp. strains and cultivation

Designation and origin of *B. burgdorferi* sensu lato strains used in this study are given in Table 1. All strains have been described and classified previously [35, 37]. Strains were cultured in modified Kelly medium (MKP) as described [22].

Polymerase chain reaction, restriction analysis, cloning and sequencing

Total genomic DNA was extracted as described [20]. The coding region of *p83/100* and the internal fragment were polymerase chain reaction (PCR)-amplified from genomic DNA by the PCR, in a Perkin-Elmer 9600 thermal cycler under standard conditions with a total of 30 cycles (96 °C 1 min, 37 °C annealing 1 min, 72 °C 2 min), [28]. PCR was carried out in an 100-µl assay (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM dNTP each, 1.0 Unit of AmpliTaq DNA polymerase, Perkin-Elmer, Weiterstadt, Germany), and 20 pmol each of the 5′- and 3′-primers.

The 5′-forward and 3′-reverse primers for cloning of the complete *p83/100* sequences were 5′-GAGGGATCCATCATGAAAGAACTTGATAAGGAAAAATTAA-3′ (position 76–91, p83 numbering [24]) and 5′-GAGCTGCAGTTATTTAACTTTCTTTAAAG-3′ (position 2078–2103), respectively. Primers for amplification and DNA sequencing of a section of the heterogeneous region (from now on referred to as "internal fragment") were 5′-foreward primer "R" (position 1140–1158, 5′-GAAAAGCAAATTGAAATC-3′) and 3′-reverse primer "W" (position 1411–1427, 5′-TCAACCTCAGAAACAGG-3′) determined from *p83/100* sequence alignments.

For cloning of the complete *p83/100* sequences, the amplified products were cut with restriction enzymes *Bam*HI and *Pst*I, purified by agarose gel-electrophoresis, eluted (QIAquick, Gel extraction kit, Qiagen, Hilden, Germany) and approximately 100 ng of PCR product was ligated into pUC18 following standard procedures [29]. Subsequent transformation of competent *E. coli* XL1 blue cells (Stratagene, Heidelberg, Germany) and DNA preparation (Qiagen pack 100) was carried out following the manufacturer's instructions.

Internal PCR fragments were analyzed for size on 3% agarose gels (Metaphor agarose; FMC, Biozym, Hess. Oldendorf, Germany) stained with ethidium bromide. Prior to DNA sequencing and restriction analysis (*Bgl*II, Boehringer, Mannheim, Germany) PCR mixtures were purified using using the Qiaquick PCR purification Kit (Qiagen). DNA sequencing of the complete *p83/100* genes and the internal fragments was carried out by the dideoxynucleotide chain-termination method on an ABI 373 DNA-sequencer (Taq cycle sequencing reactions) following the manufacturer's instructions. For sequencing of cloned *p83/100* genes, M13 primers were used followed by 12 *p83/100*-specific primers derived from previously obtained sequences. Internal *p83/100*-fragment sequences were obtained with primers "R" and "W" (see above).

Sequence analysis and accession numbers

For comparison of protein sequences the GCG programm package vers. 7.1 [7] and Clustal V (German Cancer Research Center, Heidelberg, Germany) were used. Database searches were performed with the Fasta A (MIPS, Martinsried) and BlastP (German Cancer Research Center) programms (PIR/swissprot protein database, release 42.0). The sequences were assigned the following EMBL/GenBank/DDBJ Nucleotide Sequence Data Libraries under the following accession numbers: complete p83/100 sequences: PBr:X83557, TN:X75212, PBi:X75213, PBre:X813557, Gö2:X77749. Partial sequences of p83/100 internal fragment: PLe:X81529, PGau:X81527, PLj7:X81530, PWudI:X81535, PKa2:X81528, 297:X81514, T255:X81531, B. pac.:X81520, TIsI:X81533, T25:X81532, WABSou:X81534.

Monoclonal antibodies

Four different mAbs against p83/100 were used in this study: L100 18B4, L100 17D3, L100 1B4 and L100 8B8. They were raised in two BALB/c mice which had been immunized three times intraperitoneally with a mixture of recombinantly expressed p83/100 protein from *B. burgdorferi* sensu stricto strain B31 and *B. afzelii* strain PKo (60 µg/dose on days 0, 21, 43) and had received two booster injections (120 µg on days 48 and 49). In both mice, the first immunization was performed with complete Freund's adjuvant. Immunization on day 21 was done with incomplete Freund's adjuvant and on day 43 without supplement. The booster injections on days 48 and 49 were given without adjuvant. Hybridomas were produced by standard procedures [10].

Identification of antibody-producing hybridomas was accomplished by ELISA using recombinantly expressed p83/100 and immunoblots with whole cell lysates of strains PKo and B31 as antigens [39]. Cloning and determination of the immunoglobulin class and isotype was performed as described [34].

SDS-PAGE and Western immunoblots

Spirochetes were harvested, washed and prepared for SDS-PAGE and Western immunoblots as described [36]. Protein concentration was determined by the method of Bradford [3] and electrophoresis was carried out on an Mini-Protean II Electrophoresis apparatus (Bio-Rad, München, Germany). Samples (5 µg) were subjected to discontinuous SDS-PAGE (12% polyacrylamide) at 100 V. Westernblot was

Table 1 *Borrelia burgdorferi* sensu lato strains analyzed for molecular heterogeneity of p83/100

Strain[a]	Biological origin	Geographic origin	Species	OspA serotype[b]	p83/100 sequence [reference]
B31	*I. dammini*	United States	*B. burgdorferi*	1	[24]
PBre	Human skin	Germany	sensu stricto	1	This study
PKa2	Human CSF	Germany		1	This study
T255	*I. ricinus*	Germany		1	This study
B. pac.	*I. pacificus*	United States		1	This study
297	Human CSF	Untied States		1	This study
PKo	Human skin	Germany	*B. afzelii*	2	[14]
PBo	Human CSF	Germany		2	[8]
PLe	Human skin	Germany		2	This study
PLj7	Human skin	Slovenija		2	This study
PGau	Human skin	Germany		2	This study
PWudI	Huamn skin	Germany		2	This study
PBr	Human CSF	Germany	*B. garinii*	3	This study
PBi	Human CSF	Germany		4	This study
PTrob	Human skin	Germany		4	[8]
WABSou	Human skin	Austria		5	This study
TN	*I. ricinus*	Germany		6	This study
TIsI	*I. ricinus*	Germany		6	This study
Gö2	*I. ricinus*	Germany		6	This study
T25	*I. ricinus*	Germany		7	This study
K48	*I. ricinus*	Czech Republik		6	[8]

[a] Strains are described in [35, 37]
[b] OspA serotype was determined according to [35]

performed by the semi-dry blotting technique [16] on nitrocellulose membranes (BA 45; Schleicher und Schüll, Dassel, Germany). Transfer of proteins was verified by staining with Ponceau S, and immunoblotting with mAbs following standard procedures. Antibody binding was detected using anti-mouse immunoglobulin-specific horseradish peroxidase-conjugate (Dakopatts, Copenhagen, Denmark) as described [36].

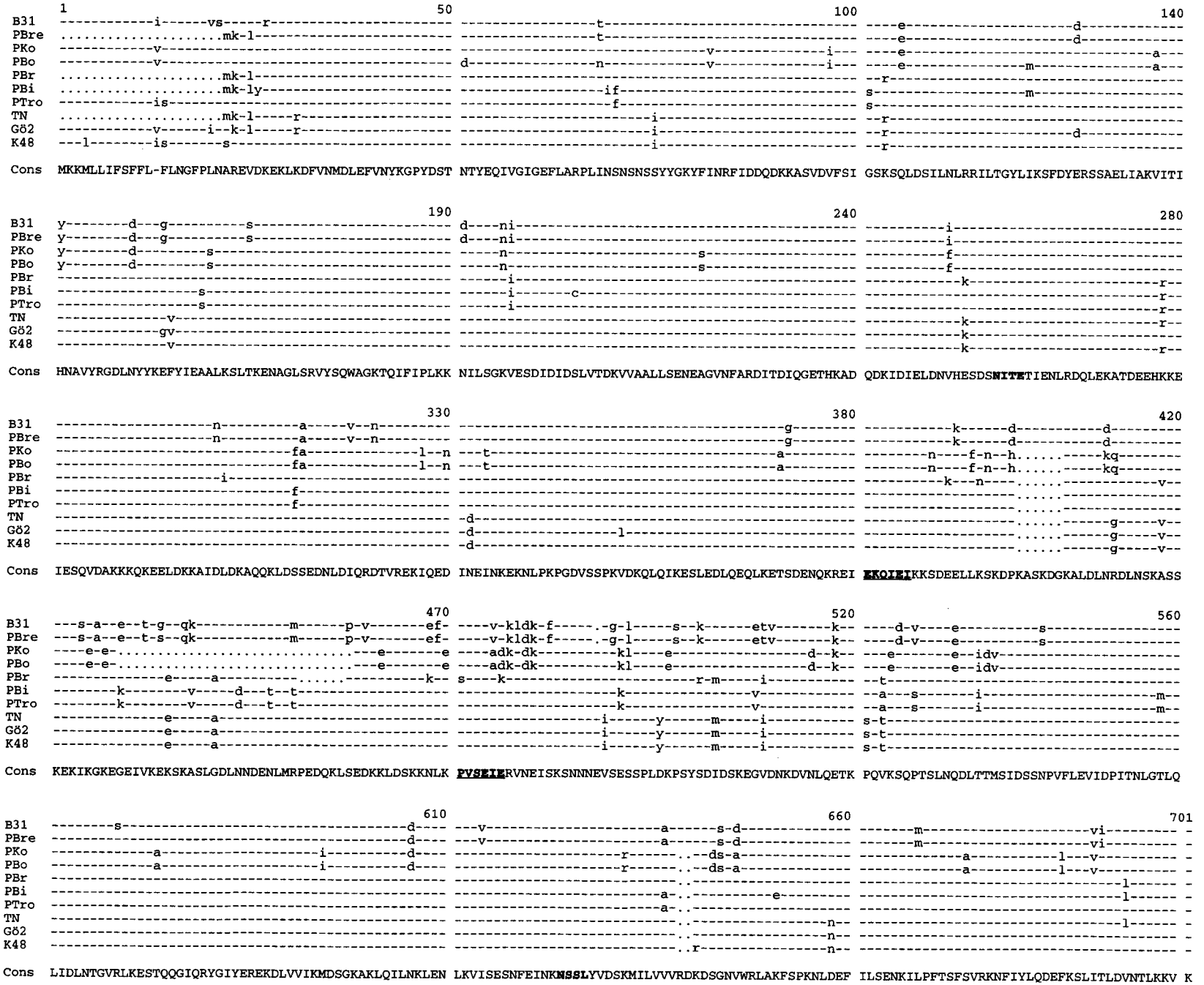
Results

Analysis of overall p83/100 sequences

The coding regions of *p83/100* sequences of five *B. burgdorferi* sensu lato strains PBr, PBre, TN, PBi and Gö2 were PCR-amplified, cloned and sequenced. Comparison studies with their deduced aa sequences and five previously published p83/100 sequences of other strains (*p83/100* sequences: p83/B31 [24]; p93/K48, p93/BPo, p93/PTrob [8]; p100/PKo, [14]) showed high degrees of identity (88.2–99.1%). In alignment studies (Fig. 1) and cluster analysis (Fig. 2) the strains were divided into four main clusters: group 1, *B. burgdorferi* sensu stricto (B31, BPre); group 2, *B. afzelii* (PKo, PBo); group 3, *B. garinii* (PTrob, Pbi); and group 4, *B. garinii* (PBr, K48, TN, Gö2). In two *B. burgdorferi* sensu stricto strains (B31 and PBre), we found a 6-aa insertion at position 400–405 (p83 numbering, Fig. 1) and a 2-aa insertion at position 639–640, whereas a striking 30-aa deletion (position 429–459) was sole present in p83/100 of *B. afzelii* strains PKo and PBo. In this region, *B. garinii* strain PBr showed a small 6-aa deletion between position 452–457. In addition to the observed insertions, deletions and aa exchanges, most variations of the p83/100 molecule are located in the internal region (aa 390–540), Fig. 1.

Analysis of PCR amplified internal p83/100 fragments

The internal region of *p83/100* between nucleotide position 1141–1427 (aa, position 381–474) was PCR-amplified from additional 11 *B. burgdorferi* sensu lato strains. The PCR-amplification products were analyzed by restriction analysis with *Bgl*II and their sequences were determiend. The amplified DNA fragment contains a 18-pb insertion typical for *B. burgdorferi* sensu stricto strains and a 90-bp deletion typical for *B. afzelii* strains. Internal PCR fragments of the investigated species were of the expected 287 bp in size for *B. burgdorferi* sensu stricto strains B31, PBre, PKa2, 297 and T255; 197 bp for *B. afzelii* strains PKo, BPo, PLe, PLj7, PGau and PWudI; and 269 bp for *B. garinii* strains PBi, WABSou, TN, T25 and TIsI, respectively (Fig. 3a). All PCR-amplified fragments showed the expected size with the exception of the internal fragment of strain *B. pacificus*. Surprisingly this fragment had a size of 269 bp normally typical for *B. garinii* strains (Fig. 3a). No PCR product was obtained with strain PBr (Fig. 3a), apparently due to several mismatches in the binding region of reverse primer "W". Restriction enzyme analysis of the amplified PCR fragments with *Bgl*II showed distinct fragment patterns which were typical for each of the three spe-


1
50
100
140
B31
PBre
PKo
PBo
PBr
PBi
PTro
TN
Gö2
K48
Cons MKKMLLIFSFFL-FLNGFPLNAREVDKEKLKDFVNMDLEFVNYKGPYDST NTYEQIVGIGEFLARPLINSNSNSSYYGKYFINRFIDDQDKKASVDVFSI GSKSQLDSILNLRRILTGYLIKSFDYERSSAELIAKVITI
190
240
280
330
380
420
Cons HNAVYRGDLNYYKEFYIEAALKSLTKENAGLSRVYSQWAGKTQIFIPLKK NILSGKVESDIDIDSLVTDKVVAALLSENEAGVNFARDITDIQGETHKAD QDKIDIELDNVHESDSNITETIENLRDQLEKATDEEHKKE
470
520
560
Cons IESQVDAKKKQKEELDKKAIDLDKAQQKLDSSEDNLDIQRDTVREKIQED INEINKEKNLPKPGDVSSPKVDKQLQIKESLEDLQEQLKETSDENQKREI EKQIEIKKSDEELLKSKDPKASKDGKALDLNRDLNSKASS
610
660
701
Cons LIDLNTGVRLKESTQQGIQRYGIYEREKDLVVIKMDSGKAKLQILNKLEN LKVISESNFEINKNSSLYVDSKMILVVVRDKDSGNVWRLAKFSPKNLDEF ILSENKILPFTSFSVRKNFIYLQDEFKSLITLDVNTLKKV K

◀ **Fig. 1** Comparison of deduced, overall p83/100 protein sequences of *Borrelia burgdorferi* sensu lato strains. Potential glycosylation sites are in *bold type*; corresponding flanking regions of the internal part investigated in this study via PCR are *underlined* and *bold*. (References for p83/100 gene sequences are given in Table 1)

cies (Fig. 3b). We were not able to amplify *p83/100* homologues from whole DNA preparations of *B. hermsii*, *B. turicatae* and *T. phagedenis* using primers for internal- or overall-DNA sequences as described above.

Alignment studies with internal fragments

The amplified internal *p83/100* fragments were sequenced and the deduced aa sequences were compared (Fig. 4). Internal *p83/100* fragments of strains B31, PKo, and PBo were amplified as positive controls and their sequences were checked for identity with already published sequences.

Alignment studies obtained from partial sequences confirmed the four clusters (Fig. 5) derived from alignments with overall p83/100 sequences. Within the *B. burgdorferi* sensu stricto group only the internal p83/100 sequence of strain *B. pacificus* showed a 6-aa deletion typical for *B. afzelii* and *B. garinii* strains (Fig. 4). However, the *B. pacificus* sequence showed high identity with the sequences of *B. burgdorferi* sensu stricto strains. All *B. afzelii* strains investigated had the 30-aa deletion as a typical marker for this group.

L100 mAb isolation and characterization

Four different mAbs were obtained after screening with recombinantly produced p83/100 proteins as antigens. Their reactivity was analyzed by immunoblotting with whole cell lysates of 18 different *B. burgdorferi* sensu lato strains, the relapsing fever borreliae *B. turicatae* and *B. hermsii* as well as *Treponema phagedenis* (Fig. 6).

Broadest reactivity was shown by mAb L100 18B4 which recognized all *B. burgdorferi* sensu lato strains and both relapsing fever borreliae. In contrast, mAb L100 17D3 did not recognize the relapsing fever borreliae but was also reactive with all *B. burgdorferi* sensu lato strains. mAb L100 1B4 recognized an epitope common to *B. burgdorferi* sensu stricto- and *B. afzelii* strains. mAb L100 8B8 bound only to p83/100 of strains B31, PBre and PKa2 within the *B. burgdorferi* sensu stricto cluster.

Computer analysis of deduced amino acid sequences and database retrieval results

Compared to the overall identities of the p83/100 sequences (88–99%), the internal region between aa position 387–470 (or 387–540, respectively) shares a lower degree of identity. Between the three genospecies investigated within this study, identities ranged from 71% to 93%. Search for homologous structures and similar proteins in databases revealed no significant identities (17%) to other proteins, if overall p83/100 sequences were used as query.

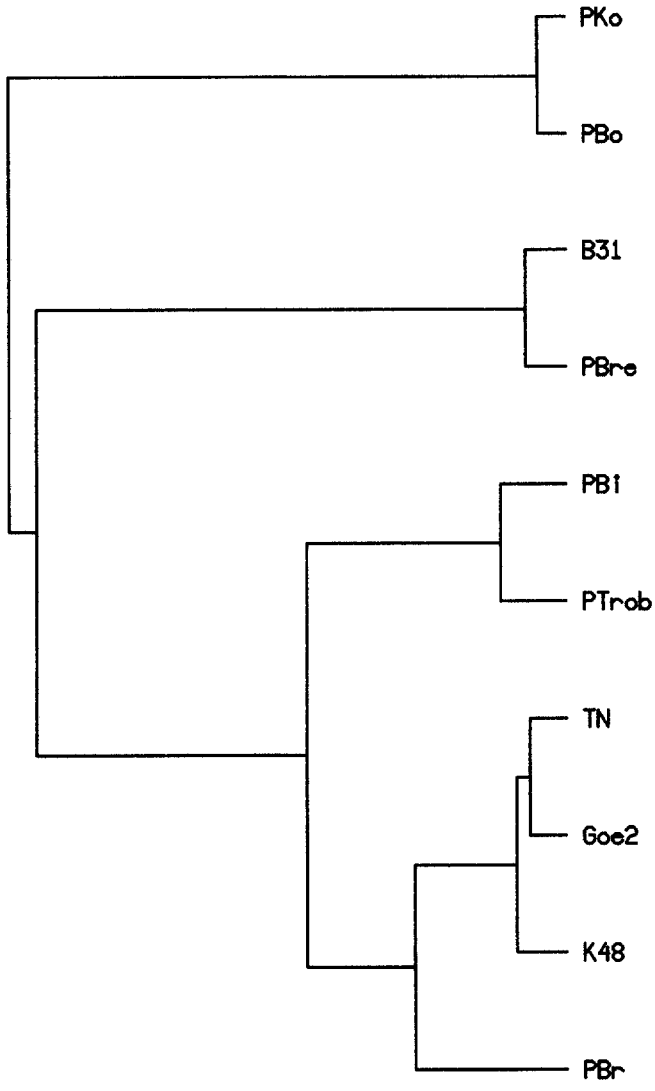


Fig. 2 Cluster analysis of overall 83/100 protein sequences of *B. burgdorferi* sensu lato strains

Discussion

In this study complete sequences of p83/100 were obtained from 5 of the 16 investigated *B. burgdorferi* sensu lato strains. A section of the most variable region of the p83/100 gene was identified from 11 different *B. burgdorferi* strains to characterize the molecular heterogeneity of this immundominant antigen.

Cluster analysis of the p83/100 sequences derived either from total or partial sequences resulted in three major clusters corresponding to the species definition of Baran-

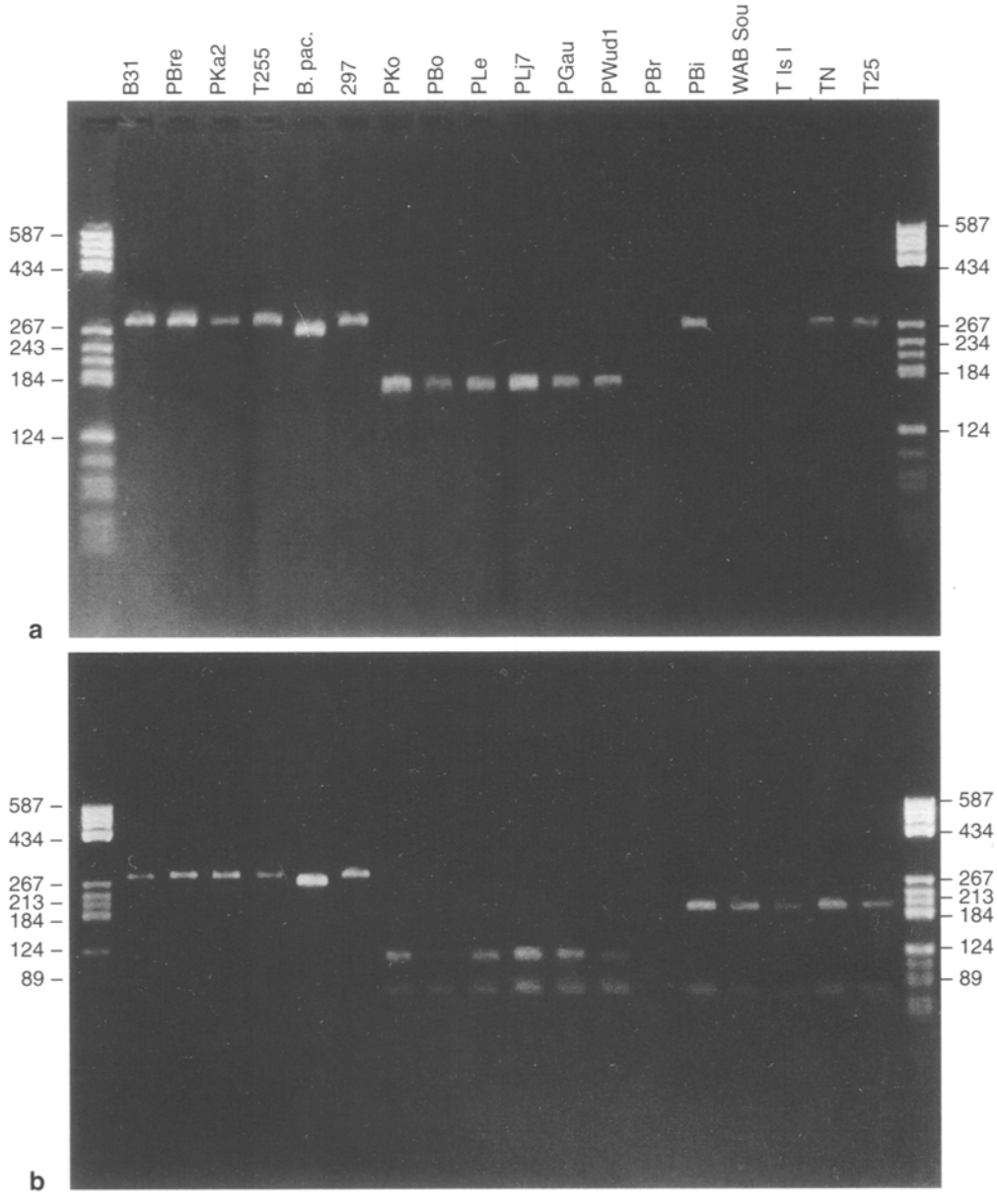


Fig. 3 **a** Agarose gels (3%) stained with ethidium bromide after electrophoresis of PCR amplified p83/100 internal fragments of *B. burgdorferi* sensu lato strains. DNA length standards (in base pairs) are indicated. **b** Restriction analysis (*Bgl*II) of PCR amplified *p83/100* internal fragments from **a**

ton et al. [2]. Within the *B. afzelii* and *B. burgdorferi* sensu stricto group only minor sequence differences were detectable. In contrast, the *B. garinii* strains are considerably more heterogeneous. Surprisingly, the two OspA serotype 4 strains, PBi and PTrob, formed as separate cluster within the *B. garinii* group. Since OspA serotype 4 strains are more often isolated from human cerebrospinal fluid (CSF) than from skin or ticks [35], the finding that a specific type of p83/100 is associated with OspA serotype 4 strains appears of special interest in respect to possible virulence factors involved in Lyme disease.

Restriction fragment analysis of the *p83/100* internal fragment PCR-amplification product appears to be a method for determination of the species. Restriction enzyme analysis of whole *Borrelia* DNA followed by hybridization with the *p83* gene was used by Zingg et al. [40]. These authors found three groups (I, II and VII) among US isolates and three groups (III, IV and VI) among 12 isolates from Europe. In their study, *B. garinii* strains formed a homogeneous group, and thus, their results are in accordance with our results using restriction fragment analysis of PCR-amplified *p83/100* genes. Our method appears to be easier to perform since no hybridiztion step is necessary. However, both methods failed to distinguish between the two *B. garinii* clusters obtained by sequence analysis.

Chou-Fasman prediction of the five overall sequences presented here, revealed a hydrophilic central region of approximately 300 aa (position 250–550) of the molecule and hydrophobic parts at the N and C terminus which is in accordance to results from Perng et al. [24]. The central

Borrelia (species)	strain	amino acid sequence of p83/100 internal fragment											ospA serotype
		387	400									470	
B. burgdorferi sensu stricto	B31[24]	---- -k-------- ----g----- d--------- ---s-a--e- -t-g--qk-- ---------- m------p-v -------ef-											1
	PBre	---- -k-------- ----g----- d--------- ---s-a--e- -t-s--qk-- ---------- m------p-v -------ef-											1
	PKa2	---- -k-------- ----g----- d--------- ---s-a--e- -t-g--qk-- ---------- m------p-v -------efn											1
	T255	---- -k-------- ----g----- d--------- ---s-a--e- -t-g--qk-- ------k-f- m------p-v -------ef-											1
	297	---- -k-------- ----g----- d--------- ---s-a--e- -t-g--qk-- ---------- m------p-v -------ef-											1
	B.pac	---- -k---..... .---g----- d--------- ---s-a--e- -t-g--qk-- ---------- m------p-v -------ef-											1
B. afzelii	PKo[14]	--n- -----..... .n--h----- kq-------- ----e-e-.. ....-- -e-------e											2
	PBo[8]	--n- -----..... .n--h----- kq-------- ----e-e-.. ....-- -e-------e											2
	PLe	--n- -----..... .n--h----- kq-------- ----e-e-.. ....-- -e-----k-e											2
	PLj7	--n- -----..... .n--h----- kq-------- ----e-e-.. ....-- -e-----k-e											2
	PGau	--n- -----..... .n--h----- kq-------- ----e-e-.. ....-- -e-----k-e											2
	PWudI	--n- -----..... .n--h----- kq-------- ----e-e-.. ....-- -e-------e											2
B. garinii	PBr	---- k---n..... .---------- --d----v-- --------g- ----e--a-- a--------- r.....--- -------k--											3
	PBi	---- -----..... .---------- --d------- --------k- -------v-- ---d---t-- t--------- ----------											4
	PTro[8]	---- -----..... .---------- --d------- --------k- -------v-- ---d---t-- t--------- ----------											4
	WAbSou	---- -v---..... .---------- -gd----v-- --------g- ----e--a-- a--------- r--------- ----rn----											5
	TIsI	---- ----t..... .---------- -gd----v-- --------g- ----e--a-- a--------- r--------- -------k--											6
	TN	---- -----..... .---------- -gd----v-- --------g- ----e--a-- a--------- r--------- ----------											6
	G82	---- -----..... .---------- -gd----v-- --------g- ----e--a-- a--------- r--------- ----------											6
	K48[8]	---- -----..... .---------- -gd----v-- --------g- ----e--a-- a--------- r--------- ----------											6
	T25	---- -----..... .---------- --d----v-- --------g- ----e--v-- a--------- r---k----- ----------											7
	Cons.	KKSD EELLKSKDDK ASKDPKALDL NRELNSKASS KEKIKGKE-E IVKEKSK-SL GDLNNDENLM -PEDQKLSED KKLDSKKNLK											

Fig. 4 Comparison of deduced amino acid sequences from p83/100 internal fragments of various *B. burgdorferi* sensu lato strains. References for sequences are given in brackets

region is not only more hydrophilic but shows a considerably higher degree of sequence variability (71–93%) than the flanking regions.

Since this variable region comprises approximately 40% of the molecule, one might expect the production of antibodies with varying paratopes during immunization of mice with the two purified recombinant proteins of highest diversity (B31 and PKo p83/100). These immunoglobulins may be capable of differentiating within the *B. burgdorferi* sensu lato species. Immunization of mice with recombinant antigens induced synthesis of the antibody L100 1B4, which showed reactivity only with *B. burgdorferi* sensu stricto and *B. afzelii* a strains but not with *B. garinii* strains. No species-specific mAb was found to react with the p83/100 of all investigated strains of anyone of the three species. However, one antibody L100 8B8 was reactive with certain *B. burgdorferi* sensu stricto strains (B31, PBre and PKa2) but not with others. The capability of mAb L100 8B8 to distinguish between *B. burgdorferi* sensu stricto strains is not due to differences within the aa 387–470 partial sequence, since this part of p83/100 is completely identical in all *B. burgdorferi* sensu stricto strains. For epitope mapping, determination of overall sequences of *B. burgdorferi* sensu stricto strains PKa2, T255, *B. pacificus* and 297 are necessary to find antigenic target structures of L100 8B8.

mAb L100 18B4 detected an epitope conserved in the p83/100 homologues among *B. burgdorferi* sensu lato strains and the relapsing fever *B. turicatae* and *B. hermsii*. Perng et al. [24] were not able to detect a copy of the *p83* gene in *B. hermsii* by hybridization with a *p83* probe. We also failed to amplify either an homologue of the internal part, or the whole of *p83/100* gene from the relapsing fever borrelia *B. turicatae* using PCR (data not shown). For

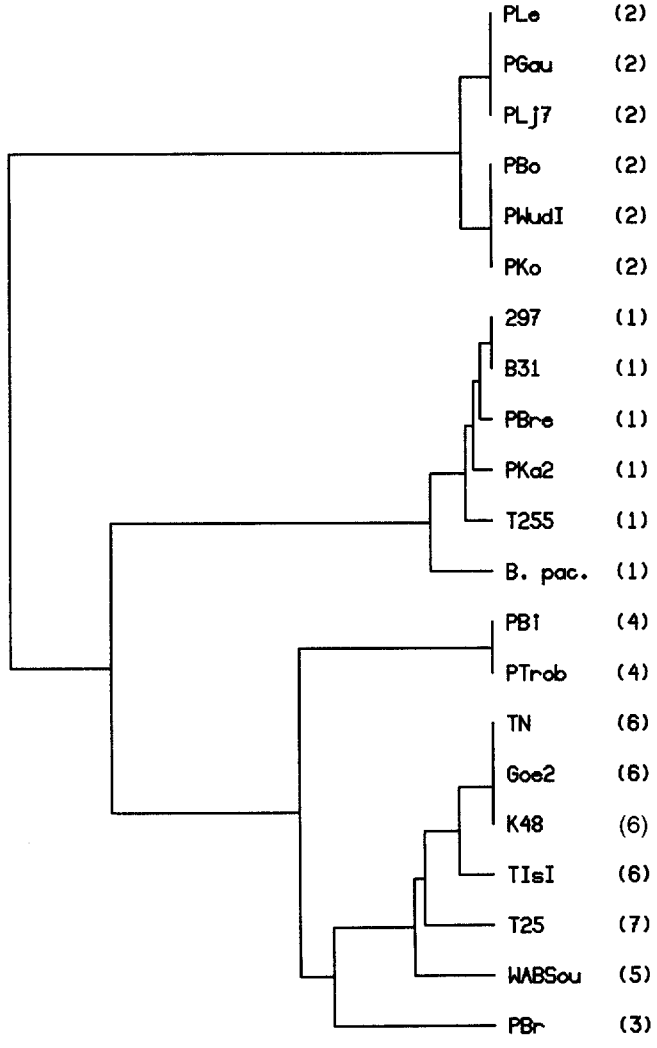


Fig. 5 Cluster analysis of p83/100 internal fragment protein sequences of various *B. burgdorferi* sensu lato strains. Corresponding OspA serotypes are shown in parentheses

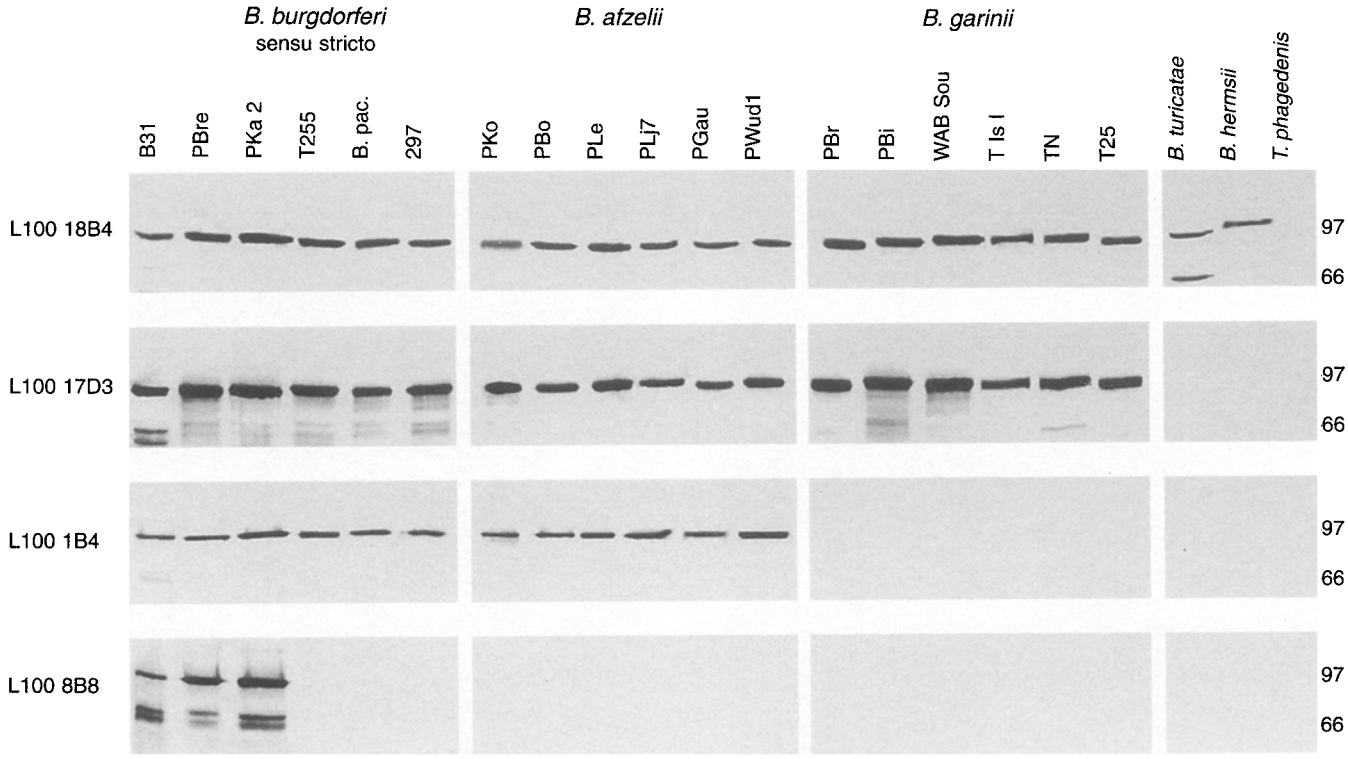


Fig. 6 Immunological characterization (immunoprint) of *B. burgdorferi* sensu lato strains, relapsing fever borreliae and *Treponema phagedenis* with p83/100-specific L100 monoclonal antibodies. Molecular mass standards (kDa) are indicated on the *right*

that reason, the target structure of mAb L100 18B4 in relapsing fever borreliae is still elusive. In contrast, mAb L100 17D3 reacted specifically with p83/100 of Lyme disease spirochetes of all three species of *B. burgdorferi* sensu lato but not with relapsing fever borreliae.

This set of mAbs enables future analysis of epitopes specifically recognized by antibodies from patients with late stage Lyme borreliosis [32, 36]. Since we found not only molecular, but also immunological diversity between the different p83/100 homologues, it appears necessary to evaluate whether the combination of certain p83/100 homologues could increase the sensitivity of antibody detection. This antigen shows a higher degree of diversity than the flagellin (p41), which is also chromosomally encoded. Thus, p83/100 might be a better marker for typing strains.

Little is known about the role of the p83/100 molecule for pathogenesis of Lyme borreliosis. Recently Anda et al. [1] reported that p93 shares epitopes with DnaK and glyceraldehyde-3-phosphate dehydrogenase of vertebrates. Database research with overall sequences performed by us showed minor identities (approx. 17%) to some muscle- or cytoskeletal proteins (e.g., myosin, troponin). Thus, this molecule might mimic host cell surfaces or even subcellular structures of the cytoskeleton and, therefore, might enable Lyme disease spirochetes to escape the immune response of the host as suggested by Szczepanski and Benach [30].

The three-aa portion Arg-Gly-Asp (RGD) is a common, versatile cell recognition signal of, for example, integrins. This sequence was first discovered in fibronectin (for review see [27] or [12]), and plays a role in the adhesion process of *B. burgdorferi* sensu lato to host cell surfaces like platelets [5]. Recently Coburn et al. [6] showed that infectious strains of *B. burgdorferi* sensu lato bind to human platelets via the integrin $\alpha_{IIb}\beta_3$ and that binding to platelets was not caused by OspA, OspB or OspC. Since p83/100 has the RGD region at position 146–148 (Fig. 1), it may be a candidate for binding to the RGD receptors.

Other mechanisms for the attachment process of *B. burgdorferi* to the surface of host cells have also been discussed, such as the binding of a 39-kDa borrelial polypeptide to eukaryotic protoglycans [13]. For other bacterial species, glycoproteins have been reported to act as surface aggregation substances [11, 23].

Computer analysis of the overall p83/100 sequences showed two hypothetical Asn-glycosylation sites [21] in conserved regions of the p83/100 protein (aa position 257–260 – NITE – and position 624–627 – NSSL –, bold in Fig. 1). Glycosylation of the molecule was confirmed in SDS-PAGE band-shift experiments by glycan staining and immunoblot of native p83/100, which had been cleaved with *N*-glycosidase and *O*-glycosidase (data not shown). Additionally, p83/100 could be purified from the ultrasonicate of whole cells by lectin-affinity chromatography, supporting these findings (manuscript in preparation). These hints point to a possible binding mechanisms of the borrelial cell to host cell surfaces via the p83/100 RGD sequence receptor mechanism or via adhesion, facilitated by carbohydrate side chains. An alternating glycosylation pattern of p83/100 might be one additional reason (besides the

length of the aa chain) for differences in molecular mass, observed by several authors [9, 14, 18, 31].

This study with its 5 new total and 11 new partial sequences and the reactivity pattern of four different mAbs recognizing type-specific, species-specific and genus-specific epitopes of p83/100 homologues may help to enlarge our knowledge about the molecular diversity of the p83/100 antigen and its immunological target structures.

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