Veterinary Microbiology 148 (2011) 341–347

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Research article

Host-dependent differential expression of factor H binding proteins, their affinity to factor H and complement evasion by Lyme and relapsing fever borreliae

Lucia Kišová-Vargová [a], Rastislav Mucha [b], Dana Čerňanská [c], Mangesh Bhide [a,b,*]

[a] Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and pharmacy, Košice, Slovakia
[b] Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
[c] Parasitological Institute, Slovak Academy of Sciences, Košice, Slovakia

ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form 11 September 2010
Accepted 24 September 2010

Keywords:
Borrelia
Factor H
Complement
CRASP
Coiled-coil domain

ABSTRACT

Binding of complement factor H is crucial for the resistance of *Borrelia* to complement-mediated lysis. This study was aimed to assess the correlation between the expression of fH binding proteins (FHBPs) during the early phase of infection (48 h after the entry of *Borrelia* into the blood circulation) and complement resistance of the *Borrelia* genus. As expected, *B. afzelii*, *B. burgdorferi* sensu stricto and *B. garinii* (Serotype 4, PBi) showed resistance to complement mediated lysis when incubated with human and dog complement, which coincided with the significantly higher expression ($P < 0.05$) of the FHBPs. Similarly, *B. coriaceae* showed resistance to cattle complement. In non-reservoir hosts borreliae failed to induce expression of FHBPs within 48 h of complement challenge, and did not survive. It is important to note that not only the expression of FHBP but also their binding to fH is required for borrelial resistance to the complement. fH binding may depend on the coiled-coil (CC) motifs observed in the FHBPs, especially at the C terminus. A loss of the C-terminal CC motif in BgCRASP-1 of SKT-1 strain was found in in-silico CC prediction, and may be coupled with SKT-1's inability to bind factor H and evade complement-mediated attack. In contrast, the C-terminal CC motif was observed (P – 1.0) in BgCRASP-1 of PBi that may contributed to its factor H binding and human complement resistance.

1. Introduction

Borrelia employ various immune evasion strategies during the early phase of infection, such as binding of complement regulatory proteins like factor H (fH) and differential expression of cell surface antigens (Alitalo et al., 2001; Behera et al., 2005; Kraiczy et al., 2001b; McDowell et al., 2003). Binding of fH is crucial to avoid the formation of the membrane attack complex and thus borrelial cell lysis (Kraiczy et al., 2001c). Complement fH serves as a cofactor for the factor I mediated cleavage of C3b (Pangburn et al., 1977). Both Lyme and relapsing fever related borreliae express a variety of fH binding proteins (FHBPs) (Alitalo et al., 2002; Hellwage et al., 2001; Kraiczy et al., 2004a; Bhide et al., 2009).

Members of the *B. burgdorferi* sensu lato complex can infect a wide range of host species, however, certain host specificity exists for each *Borrelia* species. The host specificity matches with complement resistance of the given *Borrelia* species, while complement resistance depends on the binding affinity of FHBPs to the fH (Bhide et al., 2005; Kurtenbach et al., 1998). The binding ability of given FHBP to the fH of various animals is not equal

* Corresponding author at: Laboratory of Biomedical Microbiology and Immunology, Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Komenského 73, 04181, Košice, Slovakia., Tel.: +421 91 5984604; fax: +421 55 6323173.
E-mail address: mangeshbhide@hotmail.com (M. Bhide).

0378-1135/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2010.09.026

(Bhide et al., 2009). Among the repertoire of FHBPs, BbCRASP-1 (B.b. sensu stricto CRASP-1), BaCRASP-1 (*B. afzelii* CRASP-1) and FhbA (*B. hermsii* fH binding protein A) play a significant role in complement evasion (Alitalo et al., 2002; Hellwage et al., 2001; Hovis et al., 2004, 2006b; Kraiczy et al., 2001b; Metts et al., 2003). A ~19 kDa FHBP protein of *B. garinii* (PBi), an ortholog of OspE, also possesses significant human-fH binding affinity (Bhide et al., 2009).

Although in the recent years a plethora of authors have studied and characterized FHBPs, numerous unanswered questions remain: (1) what is the expression pattern of these major FHBPs during the very early phase of infection in humans and animals (first 48 h after their entry into the blood); (2) whether there is a correlation between FHBPs expression during the early phase of infection and the complement resistance in various animals; and (3) whether the fH binding ability of FHBPs depends on the CC motifs. The present study attempts to unravel these questions.

2. Materials and methods

2.1. Borrelia isolates and sera

The *Borrelia* strains, *Borrelia burgdorferi* sensu stricto (SKT-2, B.b.s.s), *B. afzelii* (SKT-4), *B. garinii* serotype 4 (PBi), *B. garinii* serotype 6 (SKT-1), *B. hermsii* (HS1) and *B. coriaceae* (Co53) were cultured in BSK-II medium at 33 °C until they reached the log phase (cell density ~10^5 cells/ml).

Human and animal (rabbit, hamster, goat, dog, mouse, rat, guinea pig, cattle, horse, sheep) serum samples were purchased from Sigma–Aldrich. During shipment all sera were transported on dry ice to avoid thermal inactivation of complement proteins. All sera were tested with ELISA to confirm the absence of anti-*Borrelia* antibodies. Sera were filtered through 0.22 μm syringe filters (Minisoft), pooled, aliquoted and stored at −80 °C until use.

2.2. In vitro quantification of expression of FHBPs

To 280 μl of fresh *Borrelia* culture 120 μl of serum was added and samples were incubated at 33 °C for 24 and 48 h, respectively. After incubation, the motility of spirochetes was examined by dark-field microscopy, which enabled us to determine the complement sensitivity of *Borrelia*. Total RNA was isolated using PureZol RNA isolation reagent (Bio-Rad, USA). Possible DNA contamination of total RNA was removed by DNase I (Ambion, USA) treatment. RNA was then reverse transcribed (iScript cDNA Synthesis Kit, Bio-Rad; 25 °C 5 min; 42 °C 30 min; 85 °C 5 min; 4 °C) and cDNA was stored at −80 °C. As a negative control, *Borrelia* cultures were incubated with inactivated sera (56 °C for 30 min).

Levels of mRNA expression of FHBPs (BbCRASP-1, BgCRASP-1, BaCRASP-1, 19 kDa protein, FhbA and integral Omp of *B. coriaceae*) were measured by quantitative real-time PCR (iQ5, Bio-Rad). The reaction mix contained 33 μM of target specific primers (Table 1), iQ SYBR Green Supermix (Bio-Rad), milliQ water and template cDNA. Amplification conditions are depicted in Table 2. To exclude the possibility of non-specific amplification, PCR was followed by melting curve analysis. The flaB gene (Table 1) served as housekeeping gene in all real time experiments. For negative control no cDNA template was added.

Table 1
Primers used in the study.

Borrelia strain	Gene target/Primer	Sequence	Annealing temperature (°C)	Product length (bp)
SKT-4	*BaCRASP-1 F*	*5'GCCGAAGAGCTACTAATG*	45.8	137
	BaCRASP-1 R	*5'TGTTTTGCTAATTCTTCATC*		
	L-BaCRASP-1 F	**5'ACAAAAACCAACCTGAATAC**	46.6	705
	L-BaCRASP-1 R	**5'AGAATCAAGGTCTTTGTAAT**		
SKT-2	*BbCRASP-1 F*	*5'TTCCGAACATTACAATATAATTGG*	48.0	249
	BbCRASP-1 R	*5'TGTTCTGCCAGTATTTTCTCATTA*		
	L-BbCRASP-1 F	**5'AAAGCCAAACTAAATATAATCAAGA**	48.4	745
	L-BbCRASP-1 R	**5'AAAAGGCAGGTTTTAAGTATC**		
SKT-1 and PBi	*BgCRASP-1 F*	*5'CAAAAAGCAAAACCAACACT*	47.3	245
	BgCRASP-1 R	*5'AATAGTATCATGGGGCTCAGA*		
	L-BgCRASP-1 F	**5'AAGCTTAATATTCTTACAACGATA**	47.3	706
	L-BgCRASP-1 R	**5'TTCATCCATATGTTTTGCTAAT**		
SKT-1 and PBi	*19 kDa PBi F*	*5'AAGCCCTGAGAAATCGAATGAAA*	51.7	191
	19 kDa PBi R	*5'GGCCGCCAAGCAGCATAG*		
	L-19 kDa PBi F	**5'ACTGCCCAATCAAAAAGCAAATCT**	54.0	496
	L-19 kDa PBi R	**5'GGCCGCCAAGCAGCATAG**		
HS-1	*FhbA F*	*5'ATCCTGATTTGCAAGATAGACTTA*	48.7	228
	FhbA R	*5'TATATTGCAATGCCCGTTCT*		
	L- FhbA F	**5'ATGCAATTAACAAAAAAATATAT**	46.7	533
	L- FhbA R	**5'TTTAAATATTCCATTATAGTTTCA**		
Co53	*Omp F*	*5'GCTTGGAACACAGATGAAGGAG*	53.7	211
	Omp R	*5'TGATTTTGCTGCTGCGATTTGCTG*		
	L Omp F	**5'GATTTTTCTATACTTGGACAC**	49.0	696
	L Omp R	**5'TGTCAATTTAATTAAGTTGTC**		
	flaB F	*5'CACATATTCAGATGCAGACAGAGG*	55.0	331
	flaB R	*5'CCGGTGCAGCCTGAGCAGTTTGAG*		

Primers depicted in italics were used for the FHBP expression study, while primers in bold letters were used to amplify the protein coding region of the FHBPs encompassing the CC domains.

Table 2
Amplification conditions for factor H binding proteins in the expression study.

Gene target	Amplification conditions
BbCRASP-1 and BgCRASP-1	95 °C for 5 min → 35 cycles [95 °C-30 s, 50 °C-50 s (real time acquisition)] 72 °C-5 min → melting curve
BaCRASP-1	95 °C for 3 min → 35 cycles [95 °C-40 s, 45.7 °C-40 s (real time acquisition)] 72 °C-2 min → melting curve
19 kDa protein	95 °C for 3 min → 35 cycles [95 °C-40 s, 51.7 °C-40 s (Real time acquisition)] 72 °C-2 min → melting curve
FhbA	95 °C for 5 min → 35 cycles [95 °C-40 s, 48.7 °C-50 s (Real time acquisition)] 72 °C-5 min → melting curve
Omp-P66	95 °C for 5 min → 35 cycles [95 °C-40 s, 53.7 °C-50 s (Real time acquisition)] 72 °C-5 min → melting curve
flaB	95 °C for 3 min → 35 cycles [95 °C-40 s, 55 °C-50 s (Real time acquisition)] 82 °C-5 min → melting curve

2.3. In silico analysis of CC motif structures in FHBPs

As CC domains were demonstrated to be involved in the presentation of the fH binding sites (McDowell et al., 2004, 2005), putative CC formation analysis was performed for reference sequences as well as the sequences of our strains used in this experiment. The regions encompassing all possible CC domains in the FHBPs were amplified, sequenced, subjected to in silico CC prediction and compared with the CCs of the reference strains: BaCRASP-1 (AJ786368.1, MMS strain), BbCRASP-1 (AE000790.1, B31 strain), BgCRASP-1 (AJ786369.1, ZQ1 strain), 19 kDa (AAU07257, PBi strain), FhbA (AAY42861.1, YOR strain) and Omp-P66 (AF016651, *B. coriaceae*). The primers were constructed with the help of DNASTAR-Lasergene, USA. The primers used are shown in Table 1 and the amplification conditions are presented in Table 3. The amplicons derived from PCRs were sequenced by using a BigDye terminator kit 3.1 on Avant 3100 (Applied biosystem, USA). FHBP sequences of our strains were aligned with the reference sequences (SeqScape V2.1, Applied biosystem).

The impact of the amino acid changes on CC formation was determined using COILS and PepCoil softwares (Lupas et al., 1991) (http://bioweb.pasteur.fr/seqanal/interfaces/pepcoil.html) and http://www.ch.embnet.org/software/COILS_form.html). In short, a pair-wise residue correlation that distinguishes two-stranded from three-stranded CCs as well as modeling of the coordinates of the core residues in CCs were used. To achieve closer correlation between in silico prediction and actual structure, both weighted and unweighted parameters were assessed while predicting CCs.

Nucleotide sequences of the FHBPs of the strains used in this experiment were submitted to the GenBank™ (USA) under the accession numbers – GQ344483, GQ344484, GQ344485, GQ344486, GQ344487 and GQ344488.

2.4. Statistical analysis

For the comparison of gene expression the ΔCt method (relative quantity gene expression) was applied using iQ5 software (Bio-Rad). Relative quantity gene expression (ΔCt) was determined as follows:

$$[\text{Relative quantity}_{\text{sample}} = E_{\text{gene} \times (Ct(\text{control}) - Ct(\text{sample}))}]$$

where E = efficiency of primer set (% efficiency × 0.01 + 1), Ct (control) = average Ct for control, and Ct (sample) = average Ct for the sample.

The paired t-test (STATGRAPHICS plus 5.1) was used to compare the differences between FHBP gene expression in various *Borrelia* when challenged with serum complement of animal and human.

3. Results

3.1. Expression of FHBPs mRNA

Expression of BaCRASP-1 mRNA in SKT-4 was induced significantly ($P < 0.05$) within the first 24 h when incubated with human, dog, hamster and mouse sera. This expression was found to be consistent even after 48 h of incubation. BaCRASP-1 expression was lower or absent in SKT-4 when challenged with sera from other animals (Fig. 1). An evident correlation was found between BaCRASP-1 expression and serum complement resistance of SKT-4. SKT-4 was resistant to human, dog, hamster and mouse serum complement (Table 4). Similarly, BbCRASP-1 mRNA expression in SKT-2 was significantly ($P < 0.05$) upregulated in dog, human and mouse sera; however, in the presence of hamster serum no notable upregulation of BbCRASP-1 was observed (Fig. 1). BbCRASP-1 mRNA was significantly upregulated in SKT-2 when challenged with horse serum complement, however only within the first 24 h. SKT-2 showed resistance to dog and human sera; however, it was sensitive to mouse complement despite evidently higher BbCRASP-1 expression (Fig. 1, Table 4).

Expression of FHBPs and complement sensitivity were assessed in two *B. garinii* strains namely SKT-1 (serotype 6, nonpathogenic strain) and PBi (serotype 4, pathogenic neuroinvasive strain). As expected, the expression of BgCRASP-1 and 19 kDa protein was upregulated and persisted even after 48 h of the incubation with human, dog and mouse serum in PBi. On the other hand, in case of

Table 3
PCR conditions for the amplification of the fragment encompassing CC domains.

Gene target	Amplification conditions
BbCRASP-1, BaCRASP-1, BgCRASP-1 and Omp P66	95 °C for 3 min → 35 cycles [95 °C-35 s, 49 °C-50 s, 72 °C-35 s] 72 °C-5 min 30 s → 4 °C
19 kDa protein	95 °C for 3 min → 35 cycles [95 °C-40 s, 55 °C-50 s 72 °C-40 s] 72 °C-2 min → 4 °C
FhbA	95 °C for 3 min → 35 cycles [95 °C-35 s, 46,7 °C-50 s 72 °C-35 s] 72 °C-5 min 30 s → 4 °C

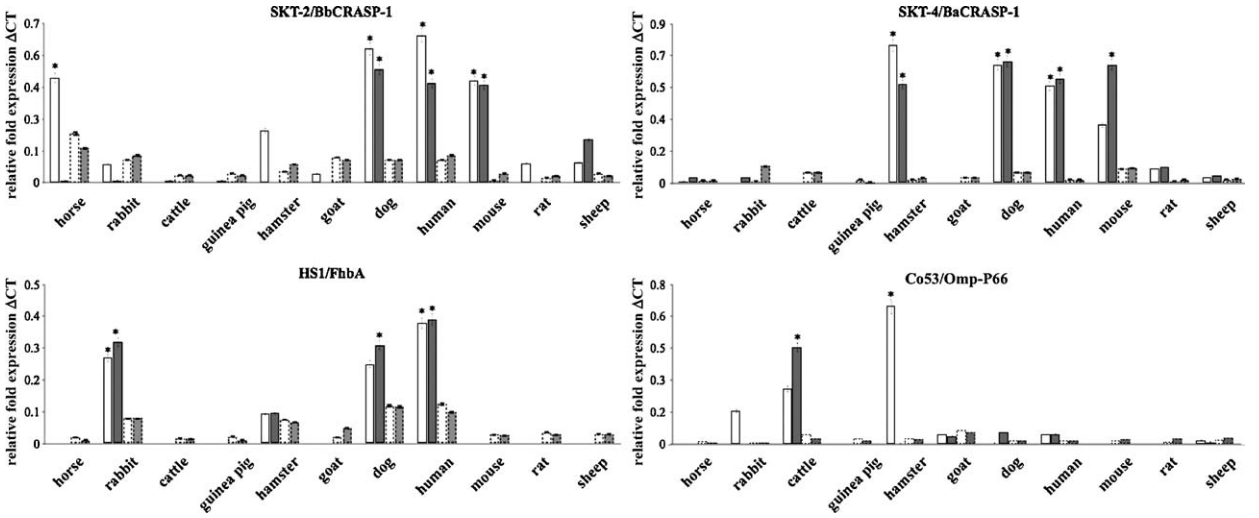

Fig. 1. Expression of FHBPs by B.b.s.s., *B. afzelii*, *B. hermsii* and *B. coriaceae*. White columns indicate expression of the given FHBP after 24 h of incubation; gray columns indicate expression of the given FHBP after 48 h of incubation. Columns with interrupted lines indicate expression of FHBP in the presence of inactivated sera. Columns bordered with continuous lines depict expression of FHBP in the presence of active sera. An asterisk indicates statistically significant ($P < 0.05$) expression of FHBP.

Table 4
Sensitivity of *Borrelia* strains to the complement from various hosts.

	SKT-2		SKT-4		Co53		PBi		SKT-1		HS1	
	AS	IS	AS	IS	AS	IS	AS	IS	AS	IS	AS	IS
Horse	M/N	M/M	N/N	M/M^B	M^B/N	M/M^B	M/S	M/M	S/N	M/M^B	N/N	M/M^B
Rabbit	S/N	M/M^B	N/N	M/M^B	N/N	M/M	M/S	M/M	S/N	M/M	M/M	M/M^B
Cattle	N/N	M/M	N/N	M/M^B	M/M^B	M/M^B	S/N	M/M^B	N/N	M/M^B	N/N	M/M^B
Guinea pig	N/N	M/M	N/N	M/M	N/N	M/M	S/N	M/M^B	S/N	M/M	N/N	M/M
Hamster	S/N	M/M	M/M	M/M	M^B/N	M/M	M/S	M/M	S/N	M/M	S/S	M/M
Goat	S/N	M/M^B	N/N	M/M^B	N/N	M/M^B	M^B/M^B	M/M^B	S/N	M/M^B	N/N	M/M
Dog	M/M	M/M	M/M	M/M	N/N	M/M	M/M	M/M	S/N	M/M	M/S	M/M
Human	M/M	M/M	M/M	M/M	N/N	M/M	M/M	M/M	S/N	M/M	M/M	M/M
Mouse	M/S	M/M^B	M/M	M/M^B	N/N	M/M	M/M	M/M^B	N/N	M/M^B	N/N	M/M
Rat	S/N	M/M^B	M^B/S	M/M^B	N/N	M/M	M^B/S	M/M^B	S/N	M/M^B	N/N	M/M
Sheep	S/S	M/M^B	M^B/S	M/M	N/N	M/M	S/S	M/M	N/N	M/M	N/N	M/M^B

AS – active serum; IS – serum with inactivated complement; N – 100% non-motile; M – actively motile; M^B – motile, however with sporadic occurrence of blebs; S – sluggish motility.

SKT-1 the expression of these proteins was drastically reduced after 48 h of incubation. This indicates that the PBi strain is relatively more resistant than SKT-1 to the serum complement of humans and various animals like dogs and some rodents (Fig. 2, Table 4).

Expression of FhbA was significantly ($P < 0.05$) induced in *B. hermsii* – HS1 – when challenged with human complement. Moreover, we found significantly higher expression of this protein in the presence of rabbit and dog complement. The complement resistance pattern of the HS1 strain was in concordance with the FhbA expression (Fig. 1, Table 4). Binding of bovine factor H by a causative agent of bovine enzootic abortion, *B. coriaceae,* was observed previously (Bhide et al., 2009). Within the first 24 h, the FHBP of *B. coriaceae* was upregulated and after 48 h the level of mRNA expression was even higher (Fig. 1). Although the induction of FHBP of *B. coriaceae* was highest in the early phase (first 24 h) of the challenge with hamster serum, an abrupt drop in the expression was noticed after 48 h of incubation.

3.2. In silico analysis of CC motif formation in FHBPs

The amino-acid sequence alignment of BaCRASP-1 [SKT-4 and MMS strain (GenBank^{TM} AJ786368.1)] revealed no variation except for two amino acids (H85Y and A143P). Both these variations were outside the predicted CC domains, thus might not alter the interaction between BaCRASP-1 and fH. The putative CC domains in both BaCRASP-1 spanned between amino acid residues: 53–73 (Probability, *P* – 0.4), 116–136 (*P* – 1.0) and 199–219 (*P* – 0.4) (Fig. 3).

We found only a 75.6% homology between the amino acid sequences of the BbCRASP-1 of SKT-2 and B31 (AE000790.1). The sequence variation altered the predicted CC structure of BbCRASP-1 in SKT-2. The B31 BbCRASP-1 had four predicted CC structures formed by amino acid residues 76–96 (*P* – 0.9), 136–156 (*P* – 1.0), 178–198 (*P* – 0.8) and 200–220 (*P* – 0.8). In BbCRASP-1 of SKT-2 all four predicted CC structures had higher probabilities (*P* – 0.9–1.0) of motif formation (Fig. 3).

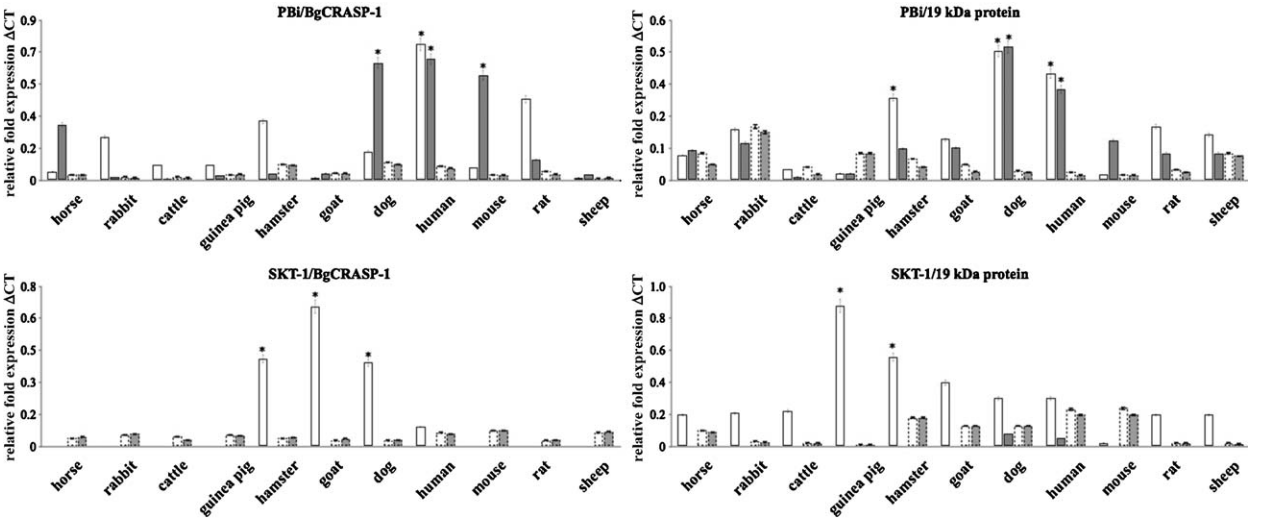

Fig. 2. Expression of FHBPs by *B. garinii*. White columns indicate expression of the given FHBP after 24 h of incubation; gray columns indicate expression of the given FHBP after 48 h of incubation. Columns with interrupted lines indicate expression of FHBP in the presence of inactivated sera. Columns bordered with continuous lines depict expression of FHBP in the presence of active sera. An asterisk indicates statistically significant ($P < 0.05$) expression of FHBP.

Significant variations were found between the probabilities of CC structure formation in BgCRASP-1 of the neuroinvasive strain PBi, the nonpathogenic SKT-1 and the previously described ZQ1 strain (AJ786369.1) (Fig. 4). The BgCRASP-1 sequence homology between PBi and ZQ1 was 78.6%, whereas it was at 72.4% between SKT-1 and ZQ1. The sequence variation in SKT-1 caused a significant decrease in the probability of predicted CC structure formation compared to what was observed in PBi (Fig. 4).

	*		#		
CC1	53	QKTKASKLEIIGKNLEDQNKQ	0.4	MMS	
	53	QKTKASKLEIIGKNLEDQNKQ	0.4	SKT-4	
CC2	116	LNYEKQKIETLKEILEKLNAN	1.0	MMS	BaCRASP-1
	116	LNYEKQKIETLKEILEKLNAN	1.0	SKT-4	
CC3	199	AETLNATLEAYNQNSQNIQYN	0.4	MMS	
	199	AETLNATLEAYNQNSQNIQYN	0.4	SKT-4	
CC1	76	LKAIGKELEDQKKEENIQIAK	0.9	B31	
	87	KELEDRKNQYDIQIAKITNEE	1.0	SKT-2	
CC2	136	KENIEKLKEILEILKKNSKHY	1.0	B31	
	141	KENIETLKEILEKLINNYEND	1.0	SKT-2	BbCRASP-1
CC3	178	LELIQNGVENLSQEESKSMLM	0.8	B31	
	178	LKLEKHLKSINEKLDTLSKEN	1.0	SKT-2	
CC4	200	IKSNLEIKQRLKKTLNETLKV	0.8	B31	
	203	LEALLEQVKSALQLQEKFKKT	0.9	SKT-2	
CC1	75	LEKMIKDLEDQKDQEDTEIAK	1.0	ZQ1	
	75	LEKIGKKLEAQKEKDSAEIAT	0.7	PBi	
	75	LKEIGKKLEAQKEKDSAKIAT	0.4	SKT-1	
CC2	131	LNYEKQKIDTLKEILEKLLAK	0.7	PBi	BgCRASP-1
	131	LNYEKQKINTLKEILEKLLTK	0.4	SKT-1	
CC2	173	IENALELMKEEIEDASEILNQ	1.0	ZQ1	
CC3	217	LNETIKAYNQDLDNIKSNEDQ	1.0	ZQ1	
	217	LNKTIEAYNQDLDNIKSNEDQ	1.0	PBi	
	211	LNKTIESYKQNLEIIKPIRNQ	0.1	SKT-1	
CC1	125	NEKLEEEEENEAQQVNSLQNR	1.0	ZQ1	
	125	NEKLEEEEENEAQQVNSLQNR	1.0	PBi	19 kDa protein
	125	NEKLEEEEENEAQQVNSLQNR	1.0	SKT-1	
CC1	31	ADLLKTLDNLLKTLDNNQKQA	0.8	YOR	
	31	ADLLKTLDNLLKTLDNNQKQA	0.8	HS1	
CC2	65	LNDLMEQQKSFLDNLQKKKED	0.9	YOR	FhbA
	65	LNDLMEQQKSFLDNLQKKKED	0.9	HS1	
CC3	147	LQNLEQKKERALQYINGKLYV	0.6	YOR	
	147	LQNLEQKKERALQYINGKLYV	0.6	HS1	
CC1	106	AAKSFKTLNAEISTYEDNKKG	0.8	Co53	Omp-P66

Fig. 3. Coiled-coil motif structures in various FHBPs. CC1 – putative first CC motif, CC2 is the second, CC3 is the third and CC4 is the fourth putative motif. *Indicates the number of the amino acid residue. #The probabilities of CC motif formation are mentioned as numeric units for each putative CC motif.

In the case of 19 kDa protein, variations in the amino acid sequences of SKT-1 and PBi were found, however all variations were located outside CC motifs. The predicted CC motif in the 19 kDa protein of both *B. garinii* strains was located between amino acid residues 125–145 (P – 1.0). *In silico* analysis of FhbA YOR (AAY42861.1) and HS1 (CAL64012.1) strains of *B. hermsii* revealed three putative CC structures between amino acid residues 31–51 (P – 0.8), 65–85 (P – 0.9) and 147–167 (P – 0.6) with no sequence variations (Fig. 3).

4. Discussion

In the early phase of infection, the host–Borrelia association is reliant on the interactions between the host's innate immune system (especially the complement system) and borrelial surface proteins (Alitalo et al., 2002; Hellwage et al., 2001; Kraiczy et al., 2001a, 2001b, 2003, 2004a, 2004b). The complement-regulating fH binding ability of *Borrelia* is often correlated with their complement resistance and ability to evade host's innate immunity (Alitalo et al., 2001; Hellwage et al., 2001; Kraiczy et al., 2001a, 2001b; Kurtenbach et al., 2002). Furthermore, the complement resistance of *Borrelia* is frequently linked to the ecology of the Lyme disease and host specificity (Bhide et al., 2005, 2009; Kurtenbach et al., 1998; van Dam et al., 1997).

In order to establish infection, *Borrelia* must regulate the expression of numerous proteins (Miller and Stevenson, 2006). In addition to CRASP-1 and OspE, many OspE related proteins (Erps) like ErpA (CRASP-5), ErpC (CRASP-4), ErpP (CRASP-3) serve as receptors for fH of various vertebrate hosts, which enables *Borrelia* to block the

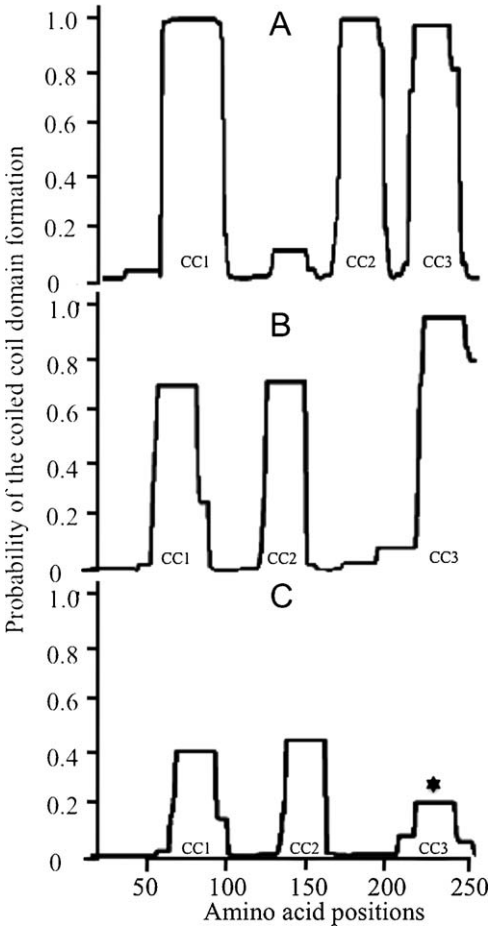

Fig. 4. Variations in the CC motif formation in BgCRASP-1 in the ZQ1, PBi and SKT-1 strains. Probabilities of the formation of CC structures in ZQ1 (panel A), PBi (panel B) and SKT-1 (panel C). *Indicates a loss in the formation of CC domain that may affect the affinity to fH. CC1 – putative first CC motif formed in the given FHBP at the N-terminus. CC2 is the second, CC3 is the third putative motif.

activation of the alternative complement pathway in different animals. The temporal analysis of FHBPs expression throughout the mammal–tick infection cycle indicates that these surface proteins have an important role in the establishment of the infection (Miller et al., 2003, 2005; Miller and Stevenson, 2006). Hitherto, numerous FHBPs were described from different *Borrelia* species, for example: *B.b.s.s* – BbCRASP-1 to BbCRASP-5, *B. afzelii* – BaCRASP-1 to BaCRASP-5, *B. garinii* – BgCRASP-1, *B. hermsii* – FhbA, etc. We (Bhide et al., 2009) and others (Hartmann et al., 2006; Kraiczy et al., 2001a, 2003, 2004b; Wallich et al., 2005) have found apparent fH binding affinity of BbCRASP-1, BaCRASP-1, BgCRASP-1, FhbA and novel Bg19 kDa proteins than other FHBPs. However, the binding affinities of FHBPs of various *Borrelia* strains to human and animal fH are not equal (Bhide et al., 2009). It is important to note that the expression of FHBPs differs from host to host depending on the chemical signaling (Miller and Stevenson, 2004). This host-dependent FHBP expression in *Borrelia* and binding affinity to fH may contribute to the differential resistance of *Borrelia* species to animal and human complement. In the present study the FHBP expression was correlated with the complement resistance of *Borrelia*. Significantly higher expression ($P < 0.05$) of FHBPs in SKT-2, SKT-4, HS1 and PBi strains were in correlation with their resistance to human and dog complement (Figs. 1 and 2; Table 4). A similar correlation was also observed between upregulation of FHBP in Co53 and its resistance against bovine complement (Fig. 1 and Table 4). No induction in the expression of FHBPs was observed in all borrelial strains, except *B. coriaceae*, when incubated with cattle serum. This indicates that these strains lack effective complement evasion mechanism against cattle complement and thus they are sensitive to cattle serum (Figs. 1 and 2, Table 4). Interestingly, BbCRASP-1 in SKT-2 was induced rapidly within 24 h when challenged with horse complement, however, the expression decreased within next 24 h of incubation (Fig. 1). Similar rapid induction within first 24 h and rapid decline in the next 24 h was also observed for BgCRASP-1 and 19 kDa protein in SKT-1 (Fig. 2), which may be the cause of insufficient resistance of *Borrelia* to the given host's complement (Table 4). These results may also suggest that not only the expression of FHBPs, but also their binding to fH is pivotal to resist complement attack.

Several researchers have studied the molecular basis of interactions between fH and FHBPs (Alitalo et al., 2004; Kraiczy et al., 2003; Metts et al., 2003). It was confirmed that the fH binding domain is conformational or discontinuous and dependent on the CC structures (Hovis et al., 2006a; McDowell et al., 2004, 2005; Metts et al., 2003). CC domains, as heptad repeats $(abcdefg)_n$, consist of two or more right handed α helices. Amino acid residues "*a*" and "*d*" are usually hydrophobic, and residues "*e*" and "*g*" are generally charged. Nonconservative amino acid substitution of the hydrophobic or charged residues may abolish the structure and/or fH binding ability (McDowell et al., 2004). In the present study, computer based CC prediction of the BgCRASP-1 from ZQ1 strain revealed three putative CC domains (Fig. 4 Panel A), whereas substitutions of conservative residues in the predicted CC structures of BgCRASP-1 in SKT-1 substantially reduced the probability of domain formation (Figs. 3 and 4 Panels A and C). The C-terminal CC domain is the most critical motif that may determine the fH binding ability (McDowell et al., 2004). Amino acid substitutions in this CC domain may have abolished the fH affinity of BgCRASP-1 in SKT-1 and thus this strain has lost its ability to evade complement-mediated attack. Interestingly, the C-terminal domain in pathogenic PBi was unchanged (Figs. 3 and 4). Sequentially, the failure of SKT-1 and the ability of PBi to induce and maintain the expression of FHBPs in an in vitro complement challenge can be justified (Fig. 2). No variations in the predicted CC forming amino acid residues in the FHBPs of SKT-4, HS1 and PBi were observed (Fig. 3).

5. Conclusion

The study revealed the facts: (1) the expression pattern of FHBPs in *Borrelia* varies from host to host during the very early stage of infection; (2) a correlation exists between FHBPs expression during the early phase of infection and

the complement resistance of *Borrelia* in various animals; and (3) the mutations/irregularities in CC domains of FHBPs may affect the fH binding ability.

Acknowledgements

We thank Bc. Martina Cepková for her excellent assistance with sequencing. The research activities and authors of this study are supported by the research grants VEGA-1/0621/09, 1/0608/09 and EU structural funds for centers of excellence-26220120002 (INFEKTZOON). We thank Dr. Paul Nyitray, DVM, PhD and Dr. Peter Novak, Ph.D for English language editing.

References

Alitalo, A., Meri, T., Chen, T., Lankinen, H., Cheng, Z.Z., Jokiranta, T.S., Seppala, I.J., Lahdenne, P., Hefty, P.S., Akins, D.R., Meri, S., 2004. Lysine-dependent multipoint binding of the *Borrelia burgdorferi* virulence factor outer surface protein E to the C terminus of factor H. J. Immunol. 172, 6195–6201.

Alitalo, A., Meri, T., Lankinen, H., Seppala, I., Lahdenne, P., Hefty, P.S., Akins, D., Meri, S., 2002. Complement inhibitor factor H binding to Lyme disease spirochetes is mediated by inducible expression of multiple plasmid-encoded outer surface protein E paralogs. J. Immunol. 169, 3847–3853.

Alitalo, A., Meri, T., Ramo, L., Jokiranta, T.S., Heikkila, T., Seppala, I.J., Oksi, J., Viljanen, M., Meri, S., 2001. Complement evasion by *Borrelia burgdorferi*: serum-resistant strains promote C3b inactivation. Infect. Immun. 69, 3685–3691.

Behera, A.K., Hildebrand, E., Scagliotti, J., Steere, A.C., Hu, L.T., 2005. Induction of host matrix metalloproteinases by *Borrelia burgdorferi* differs in human and murine lyme arthritis. Infect. Immun. 73, 126–134.

Bhide, M.R., Escudero, R., Camafeita, E., Gil, H., Jado, I., Anda, P., 2009. Complement factor H binding by different Lyme disease and relapsing fever Borrelia in animals and human. BMC Res. Notes 2, 134.

Bhide, M.R., Travnicek, M., Levkutova, M., Curlik, J., Revajova, V., Levkut, M., 2005. Sensitivity of *Borrelia* genospecies to serum complement from different animals and human: a host–pathogen relationship. FEMS Immunol. Med. Microbiol. 43, 165–172.

Hartmann, K., Corvey, C., Skerka, C., Kirschfink, M., Karas, M., Brade, V., Miller, J.C., Stevenson, B., Wallich, R., Zipfel, P.F., Kraiczy, P., 2006. Functional characterization of BbCRASP-2, a distinct outer membrane protein of *Borrelia burgdorferi* that binds host complement regulators factor H and FHL-1. Mol. Microbiol. 61, 1220–1236.

Hellwage, J., Meri, T., Heikkila, T., Alitalo, A., Panelius, J., Lahdenne, P., Seppala, I.J., Meri, S., 2001. The complement regulator factor H binds to the surface protein OspE of *Borrelia burgdorferi*. J. Biol. Chem. 276, 8427–8435.

Hovis, K.M., Jones, J.P., Sadlon, T., Raval, G., Gordon, D.L., Marconi, R.T., 2006a. Molecular analyses of the interaction of *Borrelia hermsii* FhbA with the complement regulatory proteins factor H and factor H-like protein 1. Infect. Immun. 74, 2007–2014.

Hovis, K.M., McDowell, J.V., Griffin, L., Marconi, R.T., 2004. Identification and characterization of a linear-plasmid-encoded factor H-binding protein (FhbA) of the relapsing fever spirochete *Borrelia hermsii*. J. Bacteriol. 186, 2612–2618.

Hovis, K.M., Tran, E., Sundy, C.M., Buckles, E., McDowell, J.V., Marconi, R.T., 2006b. Selective binding of *Borrelia burgdorferi* OspE paralogs to factor H and serum proteins from diverse animals: possible expansion of the role of OspE in Lyme disease pathogenesis. Infect. Immun. 74, 1967–1972.

Kraiczy, P., Hartmann, K., Hellwage, J., Skerka, C., Kirschfink, M., Brade, V., Zipfel, P.F., Wallich, R., Stevenson, B., 2004a. Immunological characterization of the complement regulator factor H-binding CRASP and Erp proteins of *Borrelia burgdorferi*. Int. J. Med. Microbiol. 293 (Suppl 37), 152–157.

Kraiczy, P., Hellwage, J., Skerka, C., Becker, H., Kirschfink, M., Simon, M.M., Brade, V., Zipfel, P.F., Wallich, R., 2004b. Complement resistance of *Borrelia burgdorferi* correlates with the expression of BbCRASP-1, a novel linear plasmid-encoded surface protein that interacts with human factor H and FHL-1 and is unrelated to Erp proteins. J. Biol. Chem. 279, 2421–2429.

Kraiczy, P., Hellwage, J., Skerka, C., Kirschfink, M., Brade, V., Zipfel, P.F., Wallich, R., 2003. Immune evasion of *Borrelia burgdorferi*: mapping of a complement-inhibitor factor H-binding site of BbCRASP-3, a novel member of the Erp protein family. Eur. J. Immunol. 33, 697–707.

Kraiczy, P., Skerka, C., Brade, V., Zipfel, P.F., 2001a. Further characterization of complement regulator-acquiring surface proteins of *Borrelia burgdorferi*. Infect. Immun. 69, 7800–7809.

Kraiczy, P., Skerka, C., Kirschfink, M., Brade, V., Zipfel, P.F., 2001b. Immune evasion of *Borrelia burgdorferi* by acquisition of human complement regulators FHL-1/reconectin and Factor H. Eur. J. Immunol. 31, 1674–1684.

Kraiczy, P., Skerka, C., Kirschfink, M., Zipfel, P.F., Brade, V., 2001c. Mechanism of complement resistance of pathogenic *Borrelia burgdorferi* isolates. Int. Immunopharmacol. 1, 393–401.

Kurtenbach, K., De Michelis, S., Etti, S., Schafer, S.M., Sewell, H.S., Brade, V., Kraiczy, P., 2002. Host association of *Borrelia burgdorferi* sensu lato—the key role of host complement. Trends Microbiol. 10, 74–79.

Kurtenbach, K., Sewell, H.S., Ogden, N.H., Randolph, S.E., Nuttall, P.A., 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. 66, 1248–1251.

Lupas, A., Van Dyke, M., Stock, J., 1991. Predicting coiled coils from protein sequences. Science 252, 1162–1164.

McDowell, J.V., Harlin, M.E., Rogers, E.A., Marconi, R.T., 2005. Putative coiled-coil structural elements of the BBA68 protein of Lyme disease spirochetes are required for formation of its factor H binding site. J. Bacteriol. 187, 1317–1323.

McDowell, J.V., Tran, E., Hamilton, D., Wolfgang, J., Miller, K., Marconi, R.T., 2003. Analysis of the ability of spirochete species associated with relapsing fever, avian borreliosis, and epizootic bovine abortion to bind factor H and cleave c3b. J. Clin. Microbiol. 41, 3905–3910.

McDowell, J.V., Wolfgang, J., Senty, L., Sundy, C.M., Noto, M.J., Marconi, R.T., 2004. Demonstration of the involvement of outer surface protein E coiled coil structural domains and higher order structural elements in the binding of infection-induced antibody and the complement-regulatory protein, factor H. J. Immunol. 173, 7471–7480.

Metts, M.S., McDowell, J.V., Theisen, M., Hansen, P.R., Marconi, R.T., 2003. Analysis of the OspE determinants involved in binding of factor H and OspE-targeting antibodies elicited during *Borrelia burgdorferi* infection in mice. Infect. Immun. 71, 3587–3596.

Miller, J.C., Narayan, K., Stevenson, B., Pachner, A.R., 2005. Expression of *Borrelia burgdorferi* erp genes during infection of non-human primates. Microb. Pathog. 39, 27–33.

Miller, J.C., Stevenson, B., 2004. Increased expression of *Borrelia burgdorferi* factor H-binding surface proteins during transmission from ticks to mice. Int. J. Med. Microbiol. 293 (Suppl 37), 120–125.

Miller, J.C., Stevenson, B., 2006. *Borrelia burgdorferi* erp genes are expressed at different levels within tissues of chronically infected mammalian hosts. Int. J. Med. Microbiol. 296 (Suppl 40), 185–194.

Miller, J.C., von Lackum, K., Babb, K., McAlister, J.D., Stevenson, B., 2003. Temporal analysis of *Borrelia burgdorferi* Erp protein expression throughout the mammal–tick infectious cycle. Infect. Immun. 71, 6943–6952.

Pangburn, M.K., Schreiber, R.D., Muller-Eberhard, H.J., 1977. Human complement C3b inactivator: isolation, characterization, and demonstration of an absolute requirement for the serum protein beta1H for cleavage of C3b and C4b in solution. J. Exp. Med. 146, 257–270.

van Dam, A.P., Oei, A., Jaspars, R., Fijen, C., Wilske, B., Spanjaard, L., Dankert, J., 1997. Complement-mediated serum sensitivity among spirochetes that cause Lyme disease. Infect. Immun. 65, 1228–1236.

Wallich, R., Pattathu, J., Kitiratschky, V., Brenner, C., Zipfel, P.F., Brade, V., Simon, M.M., Kraiczy, P., 2005. Identification and functional characterization of complement regulator-acquiring surface protein 1 of the Lyme disease spirochetes *Borrelia afzelii* and *Borrelia garinii*. Infect. Immun. 73, 2351–2359.