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Borrelia burgdorferi complement regulator-acquiring surface proteins (BbCRASPs): expression patterns during the mammal-tick infection cycle

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Abstract

Host complement is widely distributed throughout mammalian body fluids and can be activated immediately as part of the first line of defense against invading pathogens. The agent of Lyme disease, *Borrelia burgdorferi* sensu lato (s.l.), is naturally resistant to that innate immune defense system of its hosts. One resistance mechanism appears to involve binding fluid-phase regulators of complement to distinct borrelial outer surface molecules known as CRASPs (complement regulator acquiring surface proteins). Using sensitive molecular biology techniques, expression patterns of all three classes of genes encoding the CRASPs of *B. burgdorferi* sensu stricto (BbCRASPs) have been analyzed throughout the natural tick-mammal infection cycle. Each class shows a different expression profile in vivo and the results are summarized herein. Studies on the expression of *B. burgdorferi* genes using animal models of infection have advanced our knowledge on the ability of the causative agent to circumvent innate immune defenses, the contributions of CRASPs to spirochete infectivity, and the pathogenesis of Lyme disease.

Keywords

Borrelia burgdorferi; Gene regulation; CRASP; Factor H; Tick; Infection cycle

Introduction

Borrelia burgdorferi sensu lato (s.l.) has a complicated enzootic life cycle. To perpetuate, spirochetes depend on a vertebrate host, often a small mammal or a bird, and a vector tick of the genus *Ixodes*. Those ticks have three postembryonic stages: larva, nymph, and adult, each of which takes only one blood meal. Bacteria from an infected reservoir host may be acquired by feeding larvae, which then colonize the tick midgut and are retained through the molt into

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the nymph stage. As an infected nymph feeds, spirochetes are transmitted into the blood and skin of the host and subsequently spread into distant host tissues to establish disseminated and persistent infection (Stanek and Strle, 2003; Wormser, 2006). Occasionally, infected ticks feed on humans, which may lead to the development of Lyme disease (Lyme borreliosis). Dissecting molecular mechanisms underlying the ability of *B. burgdorferi* s.l. to persistently infect immunocompetent mammalian hosts is crucial to understanding Lyme disease pathogenesis and the development of improved therapies to prevent and treat these infections. Components of the host complement system are widely distributed throughout body fluids and can be activated spontaneously to mediate potent responses to infections (Janeway et al., 1999). Complement was originally discovered as a powerful enhancer of antibody-mediated killing, but it also constitutes an important part of innate immunity. Complement can be activated on surfaces of invading organisms through the alternative pathway, before the specific adaptive response develops. By this pathway, spontaneous activation of C3 on cell surfaces triggers a cascade of enzymatic events leading to formation of the membrane-attack complex as well as opsonization and inflammatory response. Yet, like many other blood-borne pathogens, most infectious isolates of *B. burgdorferi* s.l. are naturally resistant to this arm of the host innate immune defenses (Brade et al., 1992; Breitner-Ruddock et al., 1997; van Dam et al., 1997). *B. burgdorferi* s.l. produces several different outer surface proteins collectively termed CRASPs (complement regulator-acquiring surface proteins). These lipoproteins share affinities for the host fluid phase negative regulators of complement factor H and/or FHL-1 (factor H-like protein 1) (Hellwage et al., 2001; Kraiczy et al., 2001, 2003, 2004a, 2004b; Alitalo et al., 2002, 2005; Stevenson et al., 2002; McDowell et al., 2003; Hartmann et al., 2006; Kraiczy and Würzner, 2006; Herzberger et al., 2007). Those two host proteins promote breakdown of C3b and inactivation of the alternative pathway C3 convertase (Janeway et al., 1999; Kraiczy and Würzner, 2006). Mice and many other mammals do not produce FHL-1, while humans do, so the ability of CRASPs to bind FHL-1 may play a role in human disease, although not in infection of natural reservoir hosts. Some CRASPs also bind other, similar serum proteins such as FHR-1 (factor H-related protein 1) (Park and Wright, 1996; Hellwage et al., 1999, 2006; Zipfel et al., 2002, 2007; McRae et al., 2005; Haupt et al., 2007).

Genetic analyses of Lyme disease spirochetes led to division of *B. burgdorferi* s.l. into several genospecies, with names including *B. burgdorferi* sensu stricto (s.s.), *B. garinii*, *B. spielmanii*, *B. afzelii*, and others (Baranton et al., 1992; LeFleche et al., 1997). Since this review primarily discusses the CRASPs of *B. burgdorferi* s.s., for clarity in reading we will refer to that organism as *B. burgdorferi* and to Lyme disease spirochetes in general as *B. burgdorferi* s.l. The CRASPs produced by each borrelial genospecies are indicated by the genus and species initials, e.g. *B. burgdorferi* CRASPs are designated BbCRASP, those of *B. afzelii* are BaCRASP, etc. *B. burgdorferi* produces up to five different BbCRASPs, which are reviewed herein. At least two of the BbCRASPs contribute to complement resistance in vitro (Brooks et al., 2005; Hartmann et al., 2006) but the question of why *B. burgdorferi* produces multiple distinct factor H-binding proteins remains unsolved. Confounding matters further, both wild-type and factor H-deficient mice can be infected by Lyme disease spirochetes to equal degrees (Woodman et al., 2007), suggesting that factor H-binding is not essential for efficient mammalian infection.

Those data suggest five, non-exclusive possibilities:

1. Each class of CRASP is expressed at different times during the spirochete infectious cycle.
2. Co-expressed CRASPs cooperate with each other in their functions.
3. Binding of factor H by *B. burgdorferi* is not the only mechanism used by the Lyme disease spirochete to avoid being killed by complement.

4. Binding of factor H is not be the only function of BbCRASPs, and therefore not the only mechanism by which BbCRASPs contribute to complement resistance and/or mammalian infection.

5. Binding of factor H and other, similar host proteins by BbCRASPs serves other, unrelated functions, such as adherence to host tissues.

Studies of *B. burgdorferi* transmission from infected ticks into mammalian hosts using animal models have greatly advanced our knowledge on the ability of spirochetes to circumvent innate immune defenses. Shedding more light on the regulation of BbCRASP expression during the tick-mammal infection cycle can assist in determination of their additional functions and may help in developing efficient vaccines that will directly target these proteins at the time they are produced. Alternative therapeutic strategies could involve specific disruption of signaling pathways triggering synthesis or coordinating action of BbCRASPs.

BbCRASP-encoding genes

BbCRASP-1 is encoded by *cspA*, a gene located on the linear DNA replicon lp54 (Fig. 1). Although the genome of every Lyme disease spirochete carries multiple genes that share homology to *cspA*, only *cspA* is capable of binding factor H (Fraser et al., 1997;Casjens et al., 2000;Kraiczy et al., 2006). The *B. afzelii* BaCRASP-1 is also encoded by an orthologous gene, likewise located on its lp54 homolog (Wallich et al., 2005). The *cspZ* gene, encoding BbCRASP-2, is located on another linear DNA element, lp28-3, but is not closely related to any other gene within the *B. burgdorferi* genome (Fraser et al., 1997;Casjens et al., 2000;Hartmann et al., 2006). Orthologous genes have been identified in all other Lyme disease spirochete genospecies, although it is not yet clear whether or not the encoded proteins bind factor H and FHL-1 (Rogers and Marconi, 2007, and our unpublished results). BbCRASP-3, -4, and -5 lipoproteins all belong to the Erp paralog family, and their respective genes are named *erpP*, *erpC*, and *erpA*, or, collectively, *ospE* (Stevenson et al., 1996,2002;Casjens et al., 2000;Hellwage et al., 2001;Alitalo et al., 2002,2004;Kraiczy et al., 2003,2004a;Metts et al., 2003). *erp* loci are all located on borrelial prophages which replicate as circular episomes known as cp32s (Stevenson et al., 2001,2006).

Tick nymphs and the process of transmission

Bacteria colonizing the midguts of unfed nymphs generally do not produce detectable levels of BbCRASPs (Miller et al., 2003; von Lackum et al., 2005; Bykowski et al., 2007) (Fig. 2). When infected ticks begin to feed, *B. burgdorferi* begins production of Erp proteins (Miller et al., 2003), but BbCRASP-1 or -2 remain largely undetectable during this time (von Lackum et al., 2005; Bykowski et al., 2007, and our unpublished results). Ingested host complement is ineffective inside the tick midgut, presumably due to components of tick saliva that block complement activation (Ribeiro, 1987; Lawrie et al., 1999; Wikel, 1999; Valenzuela et al., 2000; Rathinavelu et al., 2003; Schroeder et al., 2007).

As ticks continue feeding, bacteria cross the gut epithelium, migrate through the hemolymph, target and penetrate the salivary glands, and are deposited into the bite wound with tick saliva (Benach et al., 1987; Ribeiro et al., 1987; Zung et al., 1989). In host dermis at the bite site, almost all bacteria produce detectable levels of BbCRASP-1 and all examined Erp proteins (Miller et al., 2003; von Lackum et al., 2005). However, only a small percentage of bacteria produce detectable BbCRASP-2 levels in skin at the tick bite site (Bykowski et al., 2007).

Disseminated mammalian infection

At the time of disseminated mammalian infection, *B. burgdorferi* resides extracellularly at low densities in a variety of tissues (Schwan et al., 1999), making it very difficult to directly examine

bacterial protein production. However, at this stage transcript levels can be assessed by the highly sensitive method of quantitative RT-PCR (Miller, 2005). Analyses of antibody production can also serve to assess borrelial protein expression during mammalian infection. Transcripts from *erp* genes have been detected in various tissues of infected laboratory animals, including non-human primates, throughout the course of disseminated and persistent infection (Miller et al., 2003, 2005; Miller and Stevenson, 2006). *B. burgdorferi*-infected mice mount rapid antibody responses to Erp proteins (Lam et al., 1994; Akins et al., 1995; Suk et al., 1995; Wallich et al., 1995; Stevenson et al., 1998; Miller et al., 2000b; Hefty et al., 2001, 2002; McDowell et al., 2001). Erp-directed antibodies persist at high levels and periodically increase during prolonged infection, suggesting sustained exposure (and re-exposure) to Erp proteins throughout chronic infection (Miller et al., 2003).

In contrast, *cspA* transcripts become undetectable within two weeks of establishing mammalian infection (Wallich et al., 2003; Lederer et al., 2005; McDowell et al., 2006; Bykowski et al., 2007). Humans and laboratory mice produce limited antibody responses to BbCRASP-1, consistent with brief exposure of that protein to host immune systems (McDowell et al., 2006; Rossmann et al., 2006).

By two weeks of mammalian infection, transcription of *cspZ* increases dramatically and is significantly higher than in bacteria residing in ticks or during laboratory cultivation (Bykowski et al., 2007). Humans and laboratory animals infected with *B. burgdorferi* produce robust antibody responses to BbCRASP-2, also indicating substantial production of that protein during vertebrate infection (Hartmann et al., 2006, and our unpublished results).

Acquisition of bacteria by larval ticks

Almost all of the bacteria acquired by feeding larvae produce BbCRASP-1, indicating that *cspA* expression is re-stimulated during the mammal-tick transmission stage (von Lackum et al., 2005). BbCRASP-2 protein is expressed in low abundance at this time, as *cspZ* undergoes transcriptional repression (Bykowski et al., 2007). Erp proteins are produced by essentially all recently-acquired borreliae in the feeding larval midgut. Following completion of larval feeding and detachment from the host, *B. burgdorferi* reduces levels of all BbCRASPs (Miller et al., 2003; von Lackum et al., 2005; Bykowski et al., 2007)

Molecular mechanisms underlying expression of BbCRASPs

Transcription start sites for *cspA* and *cspZ* have not yet been mapped, and the sequences upstream from open reading frames show no obvious similarities to each other or to *erp* loci. All the *erp* coding sequences, including *erpA*, *erpC*, and *erpP*, are preceded by almost identical 5′-non-coding sequences, including binding sites for at least 3 distinct DNA-binding proteins (Babb et al., 2004, 2006). Co-regulation of *erp* genes on different cp32 plasmids suggests the existence of similar molecular mechanisms that could coordinate expression of genes encoding BbCRASP-3, -4, and -5 at the transcriptional level (Stevenson et al., 1998; Babb et al., 2001; El-Hage and Stevenson, 2002; Babb et al., 2004, 2006).

Potential for direct or indirect effects on the synthesis of BbCRASPs by the two *B. burgdorferi* alternative RNA polymerase sigma factors RpoS (σ^S) and RpoN (NtrA, σ^{54}), has recently been ruled out (Bykowski et al., 2007). All data indicate that each of the BbCRASP-encoding genes are transcribed using the housekeeping sigma, RpoD (σ^{70}) and that the alternative sigma factors do not directly influence expression of these genes.

Conclusions

Three types of genetically distinct but functionally related *B. burgdorferi* factor H-binding lipoproteins have been described to date. Each class has a distinct expression pattern during the mammal-tick infection cycle. BbCRASP-1 is produced exclusively during stages of bacterial transmission from mammal to tick and vice versa, while BbCRASP-2 is produced during established mammalian infection. Erp proteins are up-regulated when nymphal ticks begin to feed on mammals, are produced at all stages of mammalian infection, and are then repressed following acquisition by feeding ticks. All other genospecies of Lyme disease spirochetes contain cp32 prophage elements and *erp* genes, with at least some encoded proteins sharing the ability to bind factor H (our unpublished results). These *erp* operons contain the conserved 5′-non-coding regions as do the *B. burgdorferi erp* loci, suggesting that they also share the same expression patterns. Likewise, it is likely that the BaCRASP-1-encoding gene of *B. afzelii* has an expression profile similar to that of its *B. burgdorferi* ortholog, due to similarities between those genes' promoter elements (Wallich et al., 2005). As noted above, other genospecies possess orthologs of *cspZ*, but the 5′-non-coding regions of *cspZ* genes identified in strains of *B. garinii* are significantly different from those of *B. burgdorferi*, so it is very possible that these genes are controlled through different mechanisms (our unpublished results). Those differences indicate that continued studies of CRASPs produced by the many different types of Lyme disease spirochetes are warranted. Since factor H-deficient and wild type mice can be infected with *B. burgdorferi* to essentially identical levels, the possible binding of factor H to the surface of bacteria may not be the only mechanism employed to avoid complement-mediated killing (Woodman et al., 2007). Progress in dissecting other mechanisms of *B. burgdorferi* complement resistance, defining novel ligands or roles for each BbCRASP, and studies on interactions between factor H-binding proteins may bring novel explanations for the diverse BbCRASP regulation patterns. Completing these analyses will have a significant impact on our understanding of the infectious properties of Lyme disease spirochetes.

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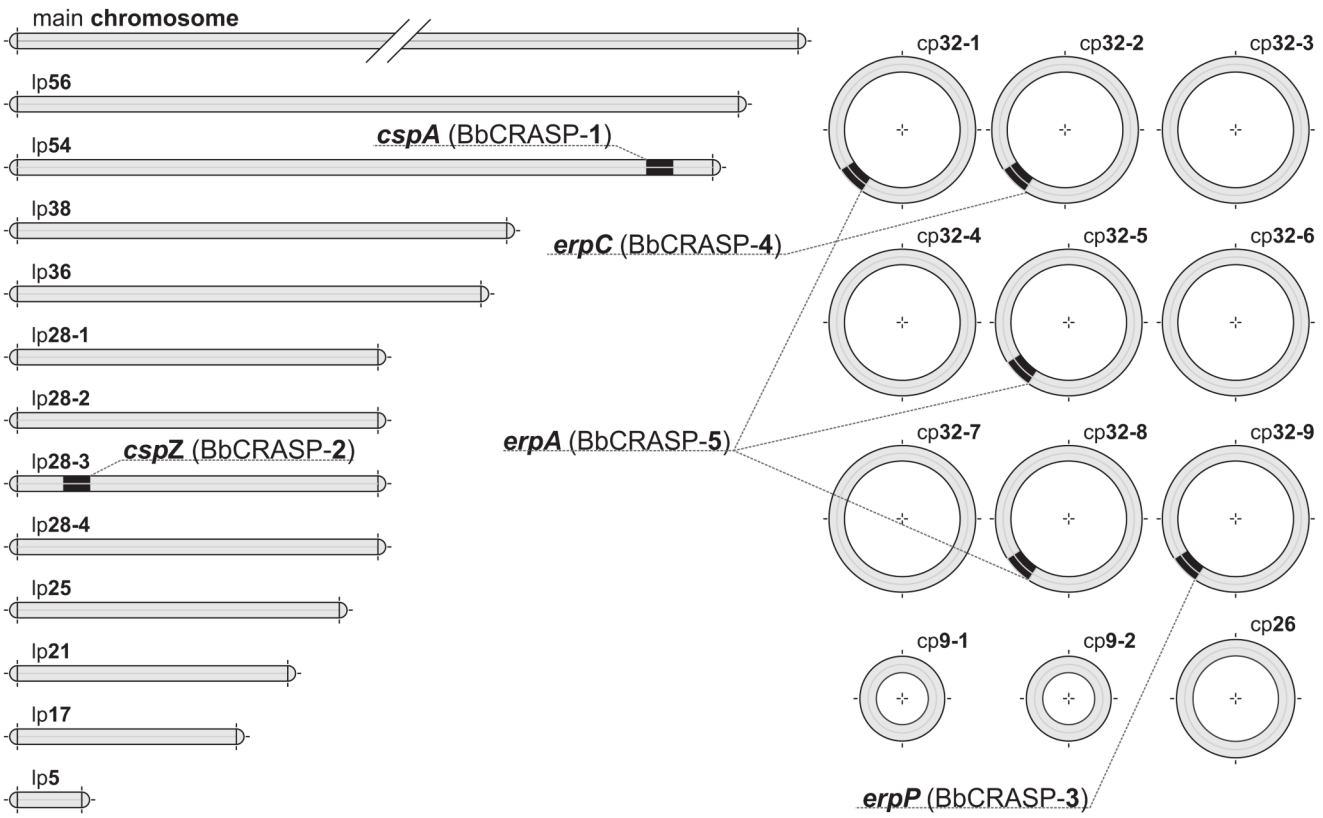

Fig. 1.

BbCRASP-encoding genes in the genome of *B. burgdorferi* strain B31. The segmented genome of *B. burgdorferi* B31 is composed of a linear main chromosome and at least 24 other linear and circular replicons (Fraser et al., 1997; Casjens et al., 2000; Miller et al., 2000a). Several publications have referred to BbCRASP-encoding genes of the strain B31 by the open reading frame (ORF) numbers assigned following sequencing and annotation of the genome (Casjens et al., 2000). The sequenced B31 subculture had lost cp32-2, cp32-5, and other DNAs, so not all genes known to exist in strain B31 were given ORF numbers. BbCRASP-1 is encoded by *cspA* (ORF BBA68) gene located on the linear DNA element lp54. BbCRASP-2 is encoded by *cspZ* (ORF BBH06) carried by another linear replicon, lp28-3. Genes *erpP* (ORF BBN38, encoding BbCRASP-3) and *erpC* (encoding BbCRASP-4) are carried by plasmids cp32-9 and cp32-2, respectively. Strain B31 can contain three identical copies of *erpA*, encoding BbCRASP-5, on prophages cp32-1 (ORF BBP38), cp32-5, and cp32-8 (ORF BBL39).

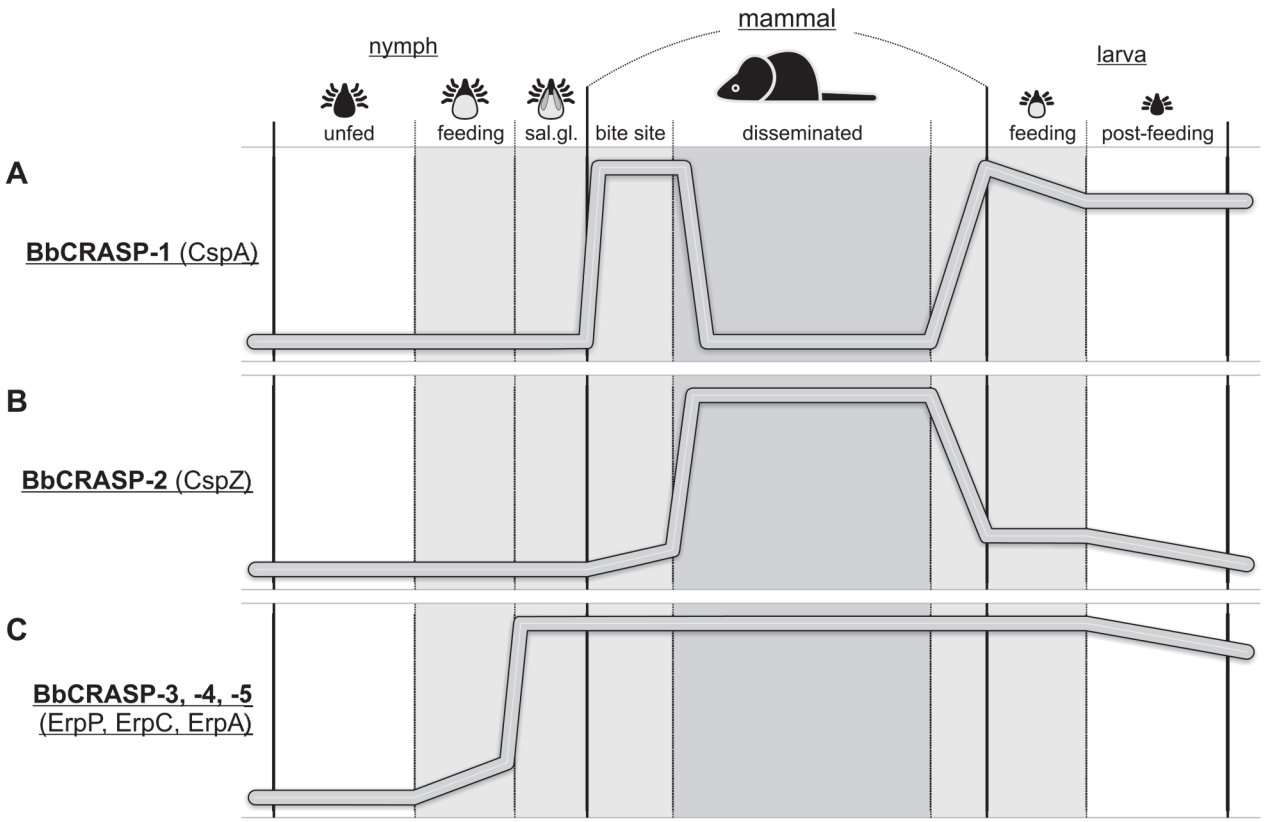

Fig. 2.

Comparison of BbCRASP in vivo expression profiles (relative levels of each BbCRASP or gene transcript) during the mammal-tick infection cycle. (A) BbCRASP-1 likely functions only during stages when bacteria are moving from the infected nymphs to the mammalian host and again to naïve larval ticks (von Lackum et al., 2005; Bykowski et al., 2007). Limited antibody responses to this lipoprotein have been observed (Wallich et al., 2005; McDowell et al., 2006; Rossmann et al., 2006). (B) BbCRASP-2 is produced predominantly during established mammalian infection and induces strong antibody production by the host (Hartmann et al., 2006; Bykowski et al., 2007, and our unpublished results). (C) Erp proteins are produced during all stages between transmission from infected nymphs to acquisition by subsequently feeding larvae (Das et al., 1997; Gilmore et al., 2001; McDowell et al., 2001; Hefty et al., 2002; Liang et al., 2002; Miller et al., 2003, 2005, 2006; Miller and Stevenson, 2006). Infected mammals mount rapid antibody responses to Erp proteins which persist at high levels over several months of infection (Lam et al., 1994; Akins et al., 1995; Suk et al., 1995; Wallich et al., 1995; Stevenson et al., 1998; Miller et al., 2000b; Hefty et al., 2001, 2002; McDowell et al., 2001). Please see text for additional details.