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Role of the VlsE Lipoprotein in Immune Avoidance by the Lyme Disease Spirochete *Borrelia burgdorferi*

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Abstract

Borrelia burgdorferi is the causative bacterial agent of Lyme disease, the most prevalent tick-borne infection in North America. The ability of *B. burgdorferi* to cause disease is highly dependent on its capacity to evade the immune response during infection of the mammalian host. One of the ways in which *B. burgdorferi* is known to evade the immune response is antigenic variation of the variable major protein (VMP)-like sequence (Vls) E lipoprotein. Past research involving the *B. burgdorferi* antigenic variation system has implicated a gene-conversion mechanism for *vlsE* recombination, analyzed the long-term dynamic changes occurring within VlsE, and established the critical importance of antigenic variation for persistent infection of the mammalian host. However, a role for the VlsE protein other than providing an antigenic disguise is currently unknown, but it has been proposed that the protein may function in other forms of immune evasion. Although a substantial number of additional proteins reside on the bacterial surface, VlsE is the only known antigen that exhibits ongoing variation of its surface epitopes. This suggests that *B. burgdorferi* may use a VlsE-mediated system for immune avoidance of its surface antigens. Several recent experimental studies involving host reinfection, superinfection, and the importance of VlsE antigenic variation during the pathogen's enzootic cycle have been used to address this question. Here, the cumulative results from these studies are reviewed, and the knowledge gaps that remain regarding the role of VlsE for immune avoidance are discussed.

Keywords

Lyme disease; *Borrelia*; immune evasion; antigenic variation; VlsE

I. INTRODUCTION

The multisystem disease known as Lyme disease is the most prevalent vector-borne infection affecting humans in both North America and Europe.[1,2] *Borrelia burgdorferi*, the causative bacterial agent of the disease in the United States, is transmitted by hard-bodied ticks of the genus *Ixodes*. When feeding, infected ticks transmit *B. burgdorferi* to humans, which can result in a localized infection (erythema migrans) at the site of the tick bite. After transmission, disseminated and chronic stages of infection occur that are characterized by neurological, cardiological, and arthritic manifestations of disease. Infection with *B. burgdorferi* can last from months to years due to avoidance of the host immune response,

and key to its successful evasion tactics is recombination within the *vls* locus located on the 28-kb linear plasmid (lp28-1).[3–5]

The *vls* locus consists of an expression site that encodes the 35-kDa variable major protein (VMP)-like sequence (Vls) E lipoprotein and a tandem array of 15 silent cassettes (*vls2–16*; each ~500 bp in length) that are oriented in the opposite direction from the *vlsE* gene (Fig. 1).[3,5,6] A short intergenic region (~160 bp) separates the *vlsE* locus and the silent cassettes, and this intergenic space contains a near-perfect 51-bp inverted repeat sequence capable of forming a highly stable DNA stem loop.[7] In addition, a portion of the promoter required for *vlsE* expression is located within this inverted repeat.[7] The *vlsE* expression region is comprised of a central variable cassette (Fig. 1) that is flanked by constant regions. At the junction of the variable and constant regions are 17-bp direct repeats that are also found at either end of most of the silent cassettes. The *vlsE* cassette region exhibits a roughly 90% sequence identity with each of the silent cassettes,[5] and the majority of sequence differences reside in six variable regions (Fig. 1) that are flanked by six highly conserved, or invariant, sequences.

It has been shown that recombination events can be detected as early as 4 d after infection of mice and continue to occur throughout infection.[9] Previous work also demonstrated that antibodies specific for the variable regions of VlsE were produced during experimental infection of mice.[10] Clones lacking lp28-1 have been shown to exhibit an intermediate infectivity phenotype, whereby these spirochetes are able to disseminate to tissue sites but unable to persist in the murine host.[11,12] However, these same clones are capable of long-term survival in severe-combined immunodeficient (SCID) mice that lack an effective antibody response.[13,14] lp28-1–Deficient isolates also grow normally in a dialysis membrane chamber implanted in the peritoneal cavity of rats, where exposure to either antibodies or immune cells is restricted.[14] Finally, immunocompetent mice infected with an lp28-1^{-} strain complemented with only the *vlsE* gene (lacking the *vls* silent cassette region) are able to clear infection, demonstrating that it is not the mere presence of VlsE that provides the capacity for persistent infection, but rather the ability to undergo *vls* gene conversion to produce VlsE variants.[15]

Infection experiments involving a *vls* deletion mutant demonstrated that this *B. burgdorferi* clone is completely cleared from immunocompetent C3H mice by d 21 after infection,[16] matching the phenotype observed with clones that lacked lp28-1. Consistent with the findings that lp28-1 is not required for persistent infection in the absence of an adaptive immune response,[13–15] the *vls* deletion mutant exhibits long-term survival in SCID mice. Targeted deletion was also used to obtain lp28-1 mutants containing mainly the *vls* locus and the genes necessary for autonomous replication of the plasmid. The only other potential genes retained on the mutant lp28-1 were eight very small open reading frames (ORFs; *bbf19–22* and *bbf27–30*) predicted to encode for proteins consisting of only 82 amino acids or less. Infectivity experiments showed that these *B. burgdorferi* clones are fully infectious and persistent in immunocompetent mice, providing further evidence that the silent *vls* cassettes and *vlsE* are the primary lp28-1–resident genes involved in spirochete persistence.[16] Moreover, these mutant clones carried out *vlsE* recombination in immunocompetent C3H

mice, indicating that protein factors required for antigenic switching are likely not carried on lp28-1 but encoded elsewhere.

Although gene conversion has been implicated in *vlsE* antigenic variation, much is still unknown regarding the mechanistic details and proteins involved in recombination. The proposed model for *vlsE* antigenic switching involves a nonreciprocal gene-conversion mechanism, whereby segments within the *vlsE* central cassette region are replaced with sections of varied length and location from the silent cassettes (Fig. 2). Only the cassette region of *vlsE* displays sequence variation resulting from gene conversion; the sequence and organization of the silent cassettes remain unaltered.[9] A detailed analysis of the *vlsE* sequence changes found that the *vls* antigenic variation system promotes both short and long recombination events within each cassette region. Along with gene-conversion events, template-independent changes that lead to additional sequence variation of *vlsE* also occur. The end result of these accumulated changes is a new *vlsE* sequence with a mosaic structure. Although disruption of a large number of genes involved in DNA recombination, repair, and replication ruled out the involvement of their respective encoded proteins in *vlsE* switching, it was discovered that the RuvAB complex of *B. burgdorferi* is required for *vls* antigenic variation, presumably by promoting branch migration of Holliday junctions during *vlsE* recombination.[17,18]

An interesting study by Embers and coworkers reported that expression of the immunodominant outer-surface protein (Osp) C by an lp28-1–deficient *B. burgdorferi* clone was abnormally high *in vivo*, suggesting that down-regulation of this protein is impaired.[19] It has been well documented that OspC is down-regulated in *B. burgdorferi* shortly after establishing infection in the animal host, and it has been suggested that this provides the spirochete with a mechanism to avoid clearance mediated by anti-OspC antibodies.[20–22] The overall conclusion from the Embers et al. study was that failure of OspC repression by lp28-1–deficient spirochetes renders them susceptible to immune-mediated clearance, and this could potentially be responsible, in part, for the intermediate infectivity phenotype associated with these *B. burgdorferi* clones. Thus, the possibility was raised that one or more genes involved in *ospC* repression may be present on lp28-1.

As mentioned above, previous mutational analysis of lp28-1 did not address the potential role of the ORF regions *bbf19–22* and *bbf27–30* for *B. burgdorferi* persistence.[16] However, it was recently reported that *B. burgdorferi* mutants lacking either region *bbf19–22* or *bbf27–30* were found to be capable of persistent infection of immunocompetent C3H mice for up to 91 d.[23] Results from this study also showed that deletion of these gene regions had no effect on *ospC* expression during infection of mice. Together with previously published results involving additional lp28-1–deletion mutants,[16] these findings increase the likelihood that the *vls* locus is the only lp28-1–resident genetic system responsible for persistence during infection of the mammalian host.

The overall strengths of past investigations include numerous independent analyses of VlsE sequence changes implicating a gene-conversion mechanism, the long-term dynamics of those changes, and the critical importance of VlsE antigenic variation for persistent infection by *B. burgdorferi*. However, a role for the VlsE protein other than providing an antigenic

disguise is currently unknown. Additionally, no studies on the importance of VlsE antigenic variation for the pathogen's enzootic cycle had previously been conducted until recently. This review focuses on recent investigations that have attempted to determine whether a VlsE-mediated immune avoidance system is at work in the Lyme disease pathogen and assess the importance of VlsE antigenic variation for the enzootic cycle of *B. burgdorferi*.

II. THE ROLE OF VlsE IN HOST REINFECTION AND SUPERINFECTION

Despite the multitude of expressed surface proteins that are immunogenic, VlsE is the only known *B. burgdorferi* antigen that exhibits variation of its surface epitopes. A long-standing question regarding *B. burgdorferi* immune escape has been how such a feat is accomplished through sequence variation of this single lipoprotein, despite the presence of a substantial number of additional antigens residing on the bacterial surface. One possibility is that VlsE acts as a shield to obscure the epitopes of other surface antigens. In fact, crystallography data suggest that the binding of VlsE to other proteins on the membrane surface may block antibody binding to the lateral surface of VlsE.[24] This in turn may protect other surface antigens that are tightly juxtaposed to VlsE from antibody recognition (Fig. 3A). A precedent for this type of interaction has been demonstrated in studies with the *Borrelia* protein P66, in which the protein is protected from proteolytic cleavage in Lyme *Borrelia* expressing high levels of OspA.[25] It has also been shown that OspA potentially serves an antibody-shielding role in the tick vector during a blood meal uptake from an immune host.[26]

A. Host Reinfection

With respect to VlsE-mediated shielding, experiments using either *in vitro*–grown or host-adapted wild-type *B. burgdorferi* were conducted to determine whether VlsE expression could provide Lyme disease spirochetes with the capacity to reinfect mice.[28] The levels of VlsE expression were shown to be up-regulated by 32-fold during host infection relative to those measured *in vitro*.[29] Unlike the highly susceptible *in vitro*–grown spirochetes, *B. burgdorferi* that have adapted within the animal host were demonstrated to be relatively invulnerable to the protective effects of immune sera.[30,31] The reinfection study showed that cultured (low VlsE expressing) wild-type *B. burgdorferi* are unable to reinfect mice, whereas host-adapted (high VlsE expressing) wild-type spirochetes are fully competent for host reinfection (summarized in Table 1).[28] To determine whether variable or static VlsE could provide a capacity for reinfection, researchers used a VlsE-deficient clone and a mutant clone expressing nonvariable or "static" VlsE.[28] The results from these experiments involving wild-type and VlsE mutant clones found that only the wild type could reinfect mice initially infected and cleared of spirochetes devoid of VlsE. In other words, the immune response of these mice was sufficient to prevent reinfection by a VlsE-deficient clone but could not block reinfection by spirochetes capable of expressing variable VlsE (Fig. 3B). It was also shown that SCID mice treated with immune sera generated against a VlsE-deficient mutant were resistant to infection by this same clone but could be successfully challenged by host-adapted wild-type spirochetes expressing VlsE.[28] The finding that passively transferred antibodies developed to non-VlsE surface antigens can provide immunity against the VlsE-deficient clone, but are not borreliacidal to wild-type

spirochetes, may hint at a possible VlsE-mediated shielding mechanism. Moreover, the data from this study indicate that the adaptation state of infecting spirochetes, likely due to its respective effects on VlsE expression, can greatly influence *B. burgdorferi* evasion from the host antibody-mediated response (Fig. 3C).

It has also been proposed that VlsE may be a T-cell–independent antigen that could directly stimulate certain B-cell subsets.[16,32] The resulting humoral response generated by VlsE may serve to override antibody production against other potential surface antigens. It has been observed that *vls* mutant *B. burgdorferi* clones complemented with a nonswitchable form of *vlsE* cleared more quickly in immunocompetent mice relative to non-VlsE–complemented mutants.[16] It is conceivable that this outcome could be the result of direct stimulation of B cells by VlsE, and this modulating ability could result in more effective clearance of these spirochetes due to the absence of VlsE antigenic variation in those clones. In support of this, experiments from the reinfection study found that sera derived from nude mice infected with wild-type *B. burgdorferi* contained anti-VlsE T-cell independent antibodies at sufficient levels to prevent infection by *in vitro*–grown wild-type *B. burgdorferi* but were at inadequate quantities to prevent infection by the VlsE-deficient mutant clone.[28] This finding suggests that the T-cell–independent antibody response is directed primarily to VlsE, with subdominant titers of these antibodies against non-VlsE surface antigens present, as demonstrated by their inability to prevent infection by the VlsE-deficient mutant clone.

B. Superinfection

Mixed infections with various *B. burgdorferi* genotypes have been reported in questing ticks,[33–35] reservoir animals,[36] and humans.[37] In *Peromyscus leucopus* mice, infections by heterologous *B. burgdorferi* populations are fairly common and are potentially acquired by either coinfection or superinfection.[36] Although host superinfection by heterologous strains has been experimentally established,[36,38] the ability of homologous clones to superinfect a host has not been studied in the past. Additionally, a role for VlsE in host superinfection has not been previously investigated.

To address the above knowledge gaps regarding host superinfection, experiments were conducted using the homologous B31 wild-type and VlsE-deficient strains and the heterologous A297 wild-type strain.[39] Results from experiments addressing the ability of homologous B31 wild-type and VlsE-mutant *B. burgdorferi* clones to superinfect various mouse models demonstrated an inability of intrastrain clones to superinfect immunocompetent mice (see Table 1). In contrast, heterologous *B. burgdorferi* strains exhibited the capacity to establish superinfection in immunocompetent mice, supporting findings from previous studies. Experiments also showed that homologous *B. burgdorferi* clones were unable to superinfect different types of antibody-deficient mice,[39] suggesting that the host innate system is a factor in preventing *B. burgdorferi* superinfection. Importantly, data from additional immunodeficient mice indicate that murine complement is likely a major barrier to intrastrain superinfection. Finally, experiments involving VlsE-deficient mutant *B. burgdorferi* demonstrated that the presence of *vlsE* is not required for either intrastrain or interstrain superinfection.[39] The data from this study also suggested that VlsE is likely a major specific target of the host antibody response during superinfection.

Unlike the wild-type B31, the VlsE-deficient clone exhibited the capacity to establish spirochetemia in immunocompetent C3H mice persistently infected with the heterologous A297 wild-type clone. Thus, the ability of the VlsE-deficient clone to establish spirochetemia in an A297-infected host may indicate that the antibody response is directed primarily to VlsE, with antibodies against non-VlsE antigens potentially generated at subdominant levels. Despite the absence of spirochetes in blood, superinfecting wild-type B31 *B. burgdorferi* was detectable from other tissue sites, indicating an ability of heterologous wild-type *B. burgdorferi* to disseminate to and colonize occupied niches of the infected murine host.[39] Given that spirochetes are presumably required to enter the bloodstream to travel to distal tissue sites, this result may suggest that superinfecting spirochetes are not completely prevented from transiently colonizing blood; instead, the titer of blood-residing spirochetes might simply be maintained at undetectable levels. Thus, during interstrain superinfection, host anti–*B. burgdorferi* antibodies seem to be responsible for primarily targeting *B. burgdorferi* that specifically express variable VlsE.

In nature, *B. burgdorferi* is propagated in a life cycle that involves an arthropod vector and mammalian reservoir host.[40–42] The capacity to superinfect may provide *B. burgdorferi* with the advantage of being maintained in the enzootic cycle, especially in ecological situations when naive *Peromyscus* mice populations are of limited availability. The findings reported from the superinfection study suggested that murine complement is a barrier to superinfection by homologous, but not heterologous, *B. burgdorferi*. Host specificity by *B. burgdorferi* can be mediated by the alternative pathway of the complement system.[44–46] Consequently, the repertoire of genes that encode high-affinity ligands for complement inhibitors can dictate the host range of *B. burgdorferi*.[45,47] Thus, it is plausible that during superinfection, innate immunity in a reservoir host could act as a selective driving force for *B. burgdorferi* heterogeneity.

A proposed simplistic model based on the findings from this work illustrates this selection process (Fig. 4).[39] In this model, the innate immune response of a persistently infected mouse impedes secondary infection by spirochetes that are homologous to primary-infecting *B. burgdorferi*. In contrast, heterogeneous *B. burgdorferi* have the capacity to overcome this innate barrier and thus eventually establish superinfection. Although both B31 wild-type and VlsE-deficient spirochetes have the capacity to superinfect A297-infected C3H mice, only the wild-type clone was able to persist. The ability of the wild-type B31 strain to establish persistence in the face of antibody response induced by heterologous *B. burgdorferi* may indicate that the VlsE-variant repertoire of B31 is not identical to that of A297 *B. burgdorferi*. Thus, once superinfection has been initially established, a distinct *vls* repertoire may be required for superinfecting *B. burgdorferi* to maintain persistence. Ultimately, only genetically diversified *B. burgdorferi* that have the capacity to overcome both innate and acquired immune responses will be the most likely candidates for continuation of the *B. burgdorferi* life cycle.

III. IMPORTANCE OF VlsE ANTIGENIC VARIATION FOR THE ENZOOTIC CYCLE OF *B. BURGDORFERI*

Survival of the Lyme pathogen in nature is completely dependent on its enzootic cycle. Thus, it is important to assess whether the variant-generating capacity of the *vls* system is a must for the Lyme pathogen to be efficiently and successfully perpetuated throughout its life cycle. Although previously untested at the time, the expected outcome would be that antigenic variation of VlsE is required for persistence in the natural reservoir host. Also unknown, but potentially more interesting, were the effects of *vls* mutation on the ability of *B. burgdorferi* to be acquired, persist, and be transmitted to naïve mice by *Ixodes* ticks.

The findings reported in a recent study (summarized in Fig. 5) show that devoid of the *vls* locus, both *in vitro*–grown and tick-transmitted *B. burgdorferi* lost the capacity to persist in *Peromyscus* mice,[48] consistent with previous studies that showed an inability of VlsE-deficient *B. burgdorferi* to establish a persistent infection in laboratory strains of mice.[16,28] Additionally, the data from this work demonstrated that antibodies generated in the natural reservoir were also borreliacidal to *B. burgdorferi* in the absence of the *vls* locus.[48] Infection of SCID mice with the host-adapted VlsE-deficient clone was prevented by passive immunization with antibodies raised against this mutant clone in *Peromyscus* mice, whereas host-adapted wild-type *B. burgdorferi* was able to establish infection in the immunized animals. In contrast, an intact *vls* system was insufficient to allow tick-transmitted wild-type *B. burgdorferi* to resist these same antibodies, indicating that VlsE is unlikely to be functionally involved at the time of tick-mediated *B. burgdorferi* transmission. Indeed, *vlsE* recombination does not occur during infection of the tick vector,[49] and very few spirochetes (<1%) express VlsE.[50] Moreover, the level of VlsE expression is low in ixodid nymphs, compared to that found during murine infection, which further supports the insignificant role of the *vls* locus during the initial tick and murine host interaction.[29,49–51] Overall, the combined findings provided further evidence of a VlsE-mediated immune avoidance system that functions to prevent *B. burgdorferi* surface antigens from being recognized by host antibodies once host infection has been established.

The *B. burgdorferi* enzootic cycle depends on efficient infection of not only the vertebrate host but also the arthropod vector to ensure continual maintenance of Lyme disease spirochetes in nature. Tick experiments involving VlsE-deficient and static VlsE clones demonstrated that *Ixodes scapularis* larvae were able to acquire both mutant clones.[48] However, the acquisition rate for the static VlsE mutant was found to be significantly lower when larvae were allowed to feed on either C3H or *Peromyscus* mice, demonstrating that the presence of nonswitchable *vlsE* impaired the ability of these mutant spirochetes to infect larval ticks. In contrast, the VlsE-deficient mutant could be acquired by tick larvae from infected mice at levels comparable to those of the wild type. This latter finding correlates well with previously published data that demonstrated unimpaired tick acquisition of lp28-1–deficient *B. burgdorferi* clones.[52,53] The higher tick acquisition rate of the VlsE-deficient clone compared to that of the static VlsE mutant from immunocompetent mice potentially indicates that a static VlsE variant constitutes a specific target of host antibodies present in the murine blood meal. This is supported by results showing that the static VlsE

clone exhibits an acquisition rate comparable to that of wild-type *B. burgdorferi* when ticks were fed on SCID mice lacking an effective antibody response.[48] The transstadial survival rates of both wild-type and VlsE-mutant *B. burgdorferi* clones in flat nymphs correlated well overall with the corresponding acquisition rates. This not only served to validate the acquisition rates but also suggested that the *vls* locus was not required for ticks to remain infected during the molting period.

The significant reduction in tick acquisition exhibited by the static VlsE mutant suggests that, in the long run, the presence of a fully functional *vls* system is an obligate requirement for Lyme disease spirochetes to be successfully propagated through continuous *B. burgdorferi* enzootic cycles. This study provided the first direct evidence for the significance of VlsE during the *B. burgdorferi* enzootic cycle and suggests that the variant-generating capacity of the *vls* system is crucial for the Lyme disease pathogen to be efficiently and successfully perpetuated throughout the *B. burgdorferi* life cycle.

IV. REMAINING KNOWLEDGE GAPS

Although the studies described above have provided support for the presence of VlsE-mediated immune avoidance in *B. burgdorferi*, experiments thus far have only offered indirect evidence for such a system. Future studies must focus on determining the actual identity of *B. burgdorferi* surface antigens that are potentially protected by the presence of VlsE. In turn, this would allow for more direct experimental approaches required to detect epitope shielding. Yet another remaining question is whether host molecules act in complex with VlsE to protect the epitopes of adjacent surface antigens from antibody recognition. As an example, the T-cell–independent immune response that is generated by VlsE may result in an IgM-bound VlsE complex that is large enough to effectively shield the epitopes of other surface antigens, thereby allowing escape from host IgG antibody recognition. A precedent for IgM masking of IgG epitopes was previously demonstrated for the malarial pathogen *Plasmodium falciparum*.[54] These authors found that binding of IgM to the antigenically variable Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1) protein Variant surface antigen 2-CSA (VAR2CSA), which is displayed on the surface of infected erythrocytes, allowed evasion from acquired immunity in the infected host. Interestingly, binding of IgM to this PfEMP1 protein did not compromise its function nor increase susceptibility to complement-mediated cell lysis.

As mentioned previously, the biological function of the VlsE protein is currently unknown, but an elegant study by Tilly et al. demonstrated that VlsE and OspC may share similar but distinct roles during host infection.[55] Unfortunately, the exact function(s) of the OspC protein also remains undefined, so exactly what common role these two lipoproteins may share is still a mystery. Based on previous studies, it was speculated that they might serve to protect the pathogen from host defenses. Alternatively, they may aid in stabilizing the bacterial structure during host infection, similar to that recently demonstrated for OspA and B in lipid rafts on the spirochete membrane.[56,57] Although OspC is not associated with these lipid rafts,[57] high levels of either OspC or VlsE may still function in some fashion as an essential component for overall stability of the bacterial outer membrane.

Additional functions of VlsE independent of its antigenic variation properties have also been proposed in the past. Previous indications found that VlsE may be function as an adhesin protein that could serve a role in tissue tropism.[58] It has been shown that in addition to a role in immune evasion, variable large protein (Vlp) / variable small protein (Vsp) variants can determine tissue tropism in the related relapsing fever agent *Borrelia turicatae*.[59,60] Indeed, higher VlsE expression levels have been observed in spirochetes recovered from joint and skin tissues than from heart tissue.[51] However, a previous study found no obvious differences in the amino acid sequence of VlsE variants recovered from different tissue sites, suggesting the absence of any VlsE role in tissue tropism.[61] Despite this failure, a study by Baum et al. involving sera obtained from field-caught *Peromyscus* mice infected with either high- or low-prevalent *B. burgdorferi* strains demonstrated that the anti-VlsE antibody responses displayed limited overall reactivity.[62] This is in sharp contrast to the more broadly cross-reactive responses observed in humans and laboratory strains of mice. Thus, it is possible that the highly segmental nature of VlsE antigenic variation that results in a vast number of variants during infection of laboratory mice has complicated efforts to associate specific protein sequences to a given tissue site.

In total, continued pursuit toward identifying the mechanism responsible for *B. burgdorferi* immune escape has important implications for the development of vaccines against the pathogen and other *Borrelia* species. If the protective effects of VlsE can be minimized using therapeutics, an effective vaccine can be developed and used in conjunction to prevent infection or reinfection by the Lyme disease spirochete. Additionally, such knowledge could lead to the development of novel strategies for targeting Lyme disease *Borrelia* in the tick vector and/or reservoir host. Overall, such future studies will significantly advance our knowledge of immune evasion by *B. burgdorferi* and in turn could have broad implications for other animal and human pathogens.

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ABBREVIATIONS

vls	vmp-like sequence

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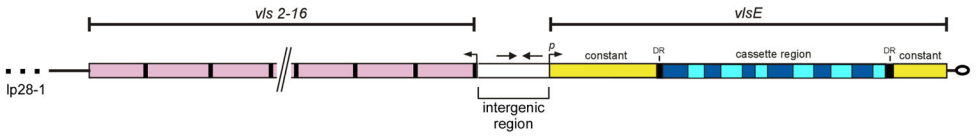

FIG. 1.

The *vls* locus of *B. burgdorferi* B31. Arrangement of the *vls* expression site, *vlsE*, and the contiguous array of 15 silent cassettes comprising the *vls* locus on the right telomere end of lp28-1. The six variable regions of the central vlsE cassette and the six invariant regions. The bars flanking the vlsE cassette region and silent cassettes represent the 17-bp direct repeats. The silent cassettes (*vls2–16*) are not drawn to scale. Arrows positioned at the beginning of *vlsE* and silent cassettes indicate the respective orientations. Arrows located within the intergenic region denote the inverted DNA repeat. DR, 17-bp Direct repeat; *p*, *vlsE* promoter (reprinted with permission from Springer, Copyright 2012).[8]

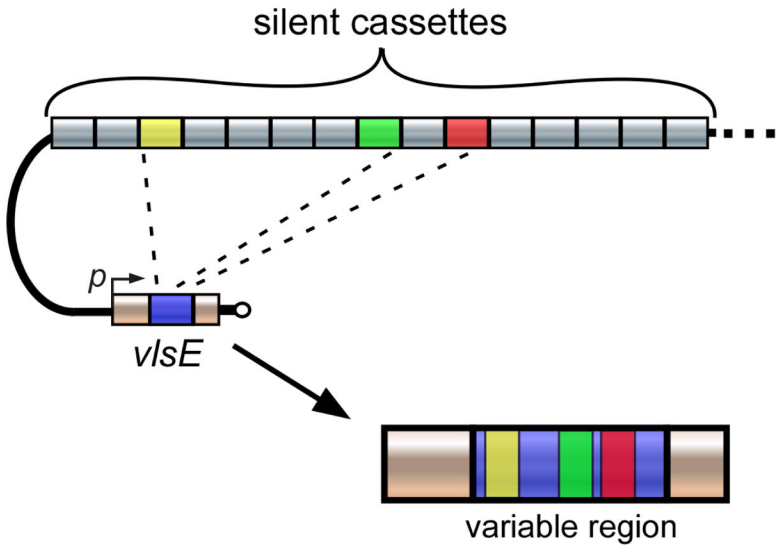

FIG. 2.

Overview of *vlsE* antigenic switching in *B. burgdorferi*. Variant-specific segments act as a source of DNA for nonreciprocal recombination events with the *vlsE* expression locus. Through this process, segments of the variable region are replaced by sections of varied length and location from the donor sequences. In the example shown, three sequential gene-conversion events (dashed lines) occur within each expression site through recombination with the colored donor sections to generate a new expression site sequence with a mosaic structure. *p*, *vlsE* promoter (reprinted with permission from John Wiley & Sons, Inc., Copyright 2009).[4]

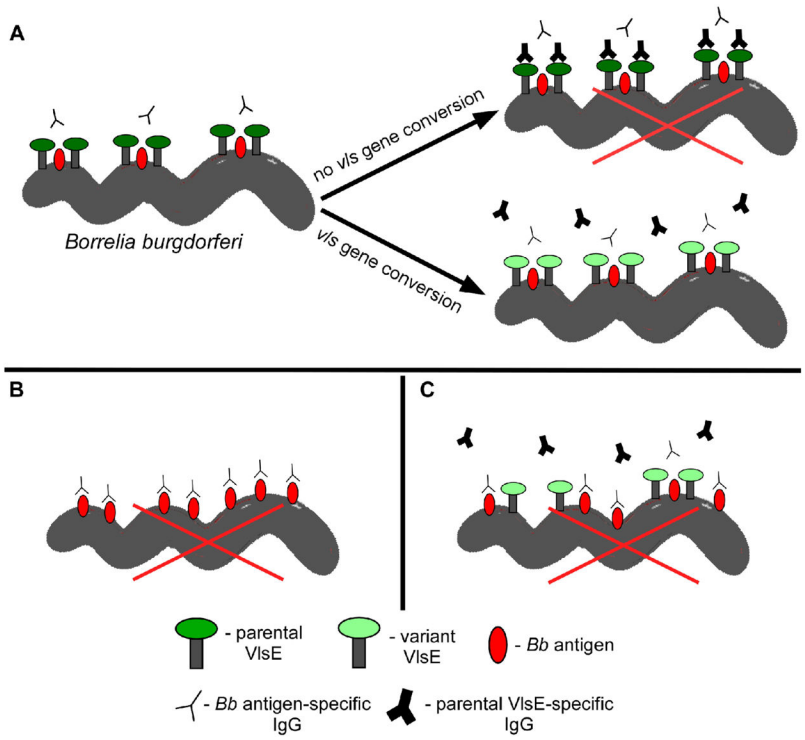

FIG. 3.

Model for VlsE-mediated protection of *B. burgdorferi* surface antigens. (A) Shortly after host infection, up-regulation of *vlsE* expression leads to surface localization of the encoded lipoprotein. Interaction of VlsE with other proteins results in a complex that works to shield epitopes of these surface antigens. Continued *vls* gene conversion leading to production of VlsE variants is necessary to avoid killing by antibodies raised against the parental and subsequent VlsE variants, allowing for sustained epitope masking. (B) Absence or (C) low expression of VlsE allows binding of antibodies to *B. burgdorferi* surface antigens that ultimately leads to spirochete death (red Xs). A legend indicating the identity of the various molecular depictions is provided at the bottom of the figure. IgG, immunoglobulin G (reprinted with permission from the American Society for Microbiology, Copyright 2016).[27]

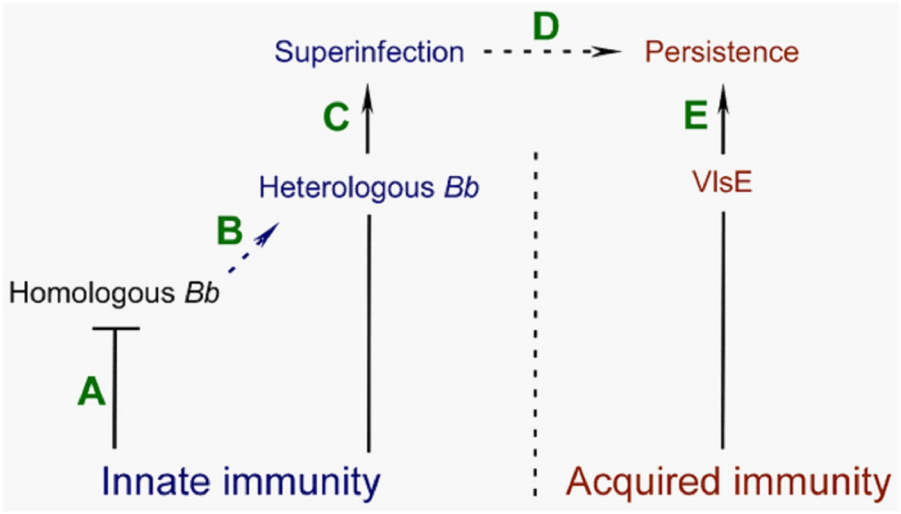

FIG. 4.

Innate immunity as a driving force for *B. burgdorferi* heterogeneity during superinfection. (A) Innate immunity of a persistently infected mouse host blocks ("T" [horizontal] end of the line) secondary-infecting spirochetes that are homologous to initial-infecting *B. burgdorferi*. (B) Immune pressure mediated by the host innate response acts as a driving force for selection of heterologous *B. burgdorferi*. (C) In turn, only heterologous *B. burgdorferi* have the capacity to overcome this innate barrier (arrow end of the line) and establish superinfection. (D) and (E) Initially, VlsE is uninvolved in this selection process. However, variable VlsE is required for evasion of the host-acquired immune response that leads to persistent *B. burgdorferi* superinfection. Established persistence of secondary-infecting *B. burgdorferi* in the reservoir host increases the likelihood of transmitting a selected *B. burgdorferi* heterogeneity to seasonally available questing ticks (reprinted with permission form the American Society for Microbiology, Copyright 2014).[39]

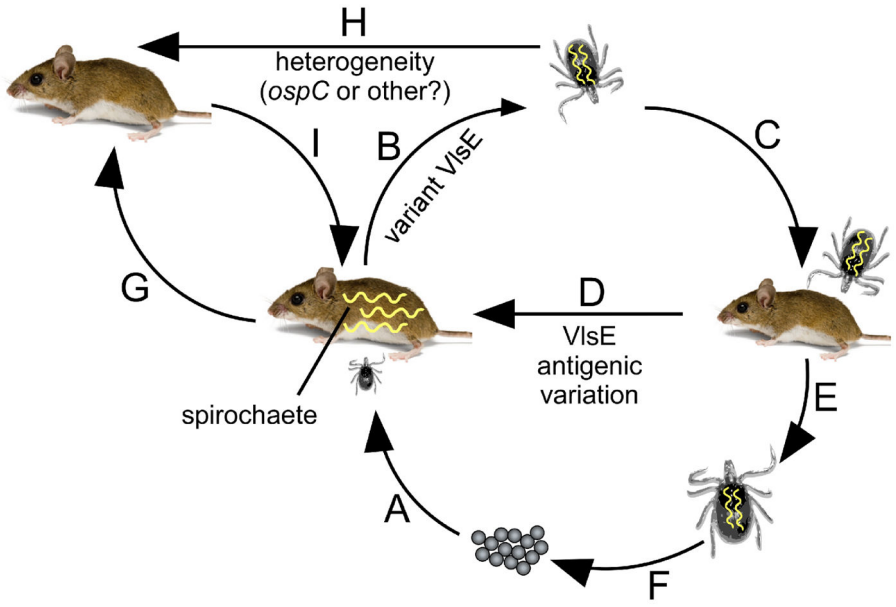

FIG. 5.

Summary of the importance of VlsE antigenic variation during the enzootic cycle of *Borrelia burgdorferi*. (A)–(I) Stages of the life cycle of *B. burgdorferi* involving the tick vector and reservoir host. (A) Acquisition of spirochetes occurs when hatched tick larvae feed on infected *Peromyscus* mice during the summer months. (B) The production of VlsE variants by spirochetes is necessary to escape anti-*Borrelia* antibodies present in the blood meal, allowing for efficient acquisition by larval ticks that are transstadially retained when the larvae molt into nymphs. (C) Infected nymphal ticks transmit spirochetes when feeding on young, uninfected mice during the spring. (D) Antigenic variation of VlsE by infecting *B. burgdorferi* in these young mice allows spirochetes to persist at least until the summer months to be acquired by tick larvae, thereby perpetuating the life cycle of the pathogen. (E) and (F) Infected nymphs molt into adult ticks, which are not considered to be important for maintaining *B. burgdorferi* in nature. Adults typically feed and mate on large mammals such as deer, resulting in the next generation of tick vectors. (G)–(I) Although not well studied, it is possible that immune clearance of spirochete infection occurs in certain numbers of mice. These mice may become reinfected by feeding nymphs that carry a strain of *B. burgdorferi* that is heterogeneous in some way to the original infecting strain. This capacity for reinfection might be highly advantageous for maintenance of the pathogen during ecological situations when immunologically naïve mammalian reservoir populations are of limited availability (reprinted with permission from PLoS, Copyright 2015).[48]

TABLE 1

Summary of findings regarding host reinfection and superinfection by *B. burgdorferi* wild-type and VlsE-mutant clones

***B. burgdorferi* clone**	**VlsE status**	**Persistence?**	**Reinfection?**	**Superinfection?**
In vitro–grown wild type	Parental (switchable)	Yes	No	Intrastrain, no Interstrain, yes
Host-adapted wild type	Variable	Yes	Yes	Intrastrain, no Interstrain, yes
VlsE deficient	Absent	No	No	Intrastrain, no Interstrain, yes
Static VlsE	Parental (nonswitchable)	No	No	Intrastrain, no Interstrain, yes