Tick-host-pathogen interactions in Lyme borreliosis
Hovius, J.W.R.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Summary and general discussion
Lyme borreliosis is an emerging zoonosis caused by the spirochetal agent *Borrelia burgdorferi* sensu lato, which is primarily transmitted by *Ixodes* ticks [1–3]. Recently we have began to realize that the pathogenesis of Lyme borreliosis is dependent on a plethora of complex interactions between tick, host and pathogen [4], of which a selection is described in this thesis. We have divided this thesis in multiple sections and herein describe in molecular detail newly identified tick-host-pathogen interactions and how these could influence the course and outcome of *Borrelia* infection.

As an introduction to the thesis, in chapter 1, we review the literature on tick-host-pathogen interactions currently known to play a role in the pathogenesis of Lyme borreliosis [4]. We elaborate on how tick salivary proteins modulate host immune responses and how this could affect both the process of tick feeding and pathogen transmission from the tick to the host. In addition, we review *B. burgdorferi* genes that are preferentially expressed while *Borrelia* resides in the tick and the vertebrate host and describe how direct interaction of the gene products with either tick or host proteins facilitates spirochete survival throughout the enzootic life cycle.

**PART I: Tick-host interactions**

Ticks acquire a bloodmeal over a period of days, giving the host sufficient time to generate anti-tick immune responses [5]. However, the tick, in turn, has developed mechanisms to protect itself against host inflammation and immune responses. Tick saliva that is introduced in the host skin during tick feeding has multiple effects, including inhibition of the complement cascade [6], impairment of NK [7] and dendritic cell function [8], reduction of antibody titers [9], repression of production of cytokines, such as interleukin (IL)-2 and IFN-γ [10–14], IL-4 [15] and IL-10 [16], blocking of chemokine activity [17], and inhibition of T lymphocyte proliferation [13,18]. Importantly, immunosuppression by tick saliva may result in more efficient transmission of several tick-borne pathogens [19,20]. By screening a cDNA *I. scapularis* salivary gland expression library Das and colleagues identified several salivary gland proteins that were recognized by antibodies from so-called ‘tick-immune’ animals; rabbits that were repeatedly exposed to ticks and that developed antibodies against tick salivary gland components resulting in an acquired resistance to tick infestation [21,22]. One of these proteins was Salp15, a secreted 15 kDa salivary protein that was expressed in fed, but not in unfed, *I. scapularis* ticks. Other salivary proteins that were identified by this method include Salp14 and Salp9pac, which were later shown to possess anticoagulant activity [23–25]; Salp20, that inhibited the alternative complement cascade [26,27]; Salp16, that facilitated uptake from another tick-borne pathogen, *Anaplasma phagocytophilum*, by the tick [28]; and Salp25D, that inhibited the oxidative burst from neutrophils enabling acquisition of *B. burgdorferi* from the host by the tick [29], a prerequisite for the continuation of the enzootic life cycle of the pathogen. Salp15 showed weak homology to Inhibin A, a protein that is part of the transforming growth factor (TGF)-β superfamily, a family of proteins with immunosuppressive activity. Therefore, in this section of the thesis, we investigated the immunosuppressive activity of Salp15.
In chapter 2 we describe how the feeding induced and secreted *I. scapularis* salivary protein, Salp15, directly inhibits T cell activation and proliferation. Salp15 interferes with early T cell receptor (TCR)-triggered interleukin (IL)-2 production by CD4+ T cells *in vitro*, and also inhibits T cell-mediated immune responses *in vivo* [30]. Recently the research group of Anguita showed that the C-terminus of Salp15 binds to the CD4 co-receptor on T cells [31], interfering with early TCR-signaling events [32]. These data provide a molecular basis for understanding the immunosuppressive activity of *I. scapularis* saliva and vector-host interactions.

In chapter 3 we further investigated the immunosuppressive effects of *I. scapularis* Salp15 and demonstrate that Salp15 is capable of inhibiting human dendritic cell (DC) function [33]. DCs are sentinel cells abundantly present in the dermis (the site of the tick-bite), and are of crucial importance for the initiation of an adequate host immune response [34]. We show that Salp15 inhibits production of the pro-inflammatory cytokines IL-6, TNF-α and IL-12p70 by DCs, as well as DC-induced T cell activation. Salp15 binds to the C-type lectin receptor DC-SIGN on the surface of DCs, which results in activation of the serine/threonine kinase Raf-1. Strikingly, Raf-1 activation by Salp15 leads to mitogen-activated protein kinase kinase (MEK)-dependent decrease of IL-6 and TNF-α mRNA stability and impaired nucleosome remodeling at the IL-12p35 promoter.

Immunosuppression induced by a tick salivary protein is likely to be instrumental to both tick feeding and *B. burgdorferi* infection by making the tick-host-pathogen interface a less hostile environment for both the tick as well as the spirochete. This section of the thesis provides new insights into the molecular mechanism of immunosuppression by a specific tick salivary gland protein and might lead to the development of novel anti-inflammatory or immunosuppressive agents. Noteworthy, studies to investigate the potential application of tick salivary proteins as immunosuppressive agents are currently being performed at the Academic Medical Center and are part of future research projects.

**PART II: Tick-pathogen interactions**

The main vector for Lyme borreliosis in Europe is the tick *I. ricinus*, whereas in the United States *B. burgdorferi* sensu stricto is mainly transmitted by *I. scapularis*. In chapter 4 we set out to identify whether *I. ricinus* also contained Salp15-like proteins. Using reverse transcriptase polymerase chain reaction (RT-PCR) techniques we show that indeed *I. ricinus* saliva contains at least three Salp15-like proteins, with one of the proteins, Salp15 Iric-1, sharing 80% similarity evenly distributed over the entire amino acid sequence [35]. Our findings were corroborated by others that identified principally similar Salp15-like proteins in fed *I. ricinus* salivary glands [36]. Comparison of our protein sequences with those deposited in several databases indicated that these proteins are part of a Salp15 family of which members are conserved among different *Ixodes* tick species, which are
all capable of transmitting \textit{B. burgdorferi} sensu lato. This suggests that these proteins might have similar activities as \textit{I. scapularis} Salp15.

Recently, it was shown that the interaction between tick, host and \textit{B. burgdorferi} was even more complex than previously assumed. Ramamoorthi et al. showed that \textit{I. scapularis} Salp15 binds to \textit{B. burgdorferi} outer surface protein (Osp)C. This was shown to coat the outer surface of the spirochete with Salp15, protecting \textit{B. burgdorferi} from antibody-mediated killing in mice immune to \textit{B. burgdorferi} \cite{37}. In Europe, apart from \textit{B. burgdorferi}, \textit{B. garinii} and \textit{B. afzelii} are transmitted by \textit{I. ricinus}. These \textit{Borrelia} species can also cause Lyme borreliosis \cite{38,39}. Therefore, we investigated the interaction of a Salp15 homologue in \textit{I. ricinus}, Salp15 Iric-1, with \textit{Borrelia} isolates representative of the three major European pathogenic \textit{Borrelia} species. In chapter 5 we demonstrate that Salp15 Iric-1 binds to OspC from all European \textit{Borrelia} species in vitro. However, only \textit{B. burgdorferi} was protected from antibody-mediated killing by Salp15 Iric-1 in vivo, putatively giving this \textit{Borrelia} species a survival advantage in nature \cite{40}.

In the aforementioned study by Ramamoorthi and colleagues \cite{37} it was shown that Salp15 also provided \textit{B. burgdorferi} with a survival advantage in naive mice, i.e. mice not previously exposed to \textit{B. burgdorferi}. Obviously, naive mice do not have specific antibodies against \textit{B. burgdorferi}. In chapter 6 we show that binding of \textit{I. scapularis} Salp15, but also Salp15 Iric-1, to isolates from European \textit{Borrelia} species that are sensitive to killing by the complement system provides protection against complement-mediated borreliacidal activity in vitro \cite{41}. Others have shown that specific \textit{B. burgdorferi} strains can also produce complement regulating-acquiring surface proteins (CRASPs) \cite{42} and Osp E/F related proteins (Erps) \cite{43–45} to bind factor H or factor H-like (FHL)-protein and consequently inhibit complement-mediated borreliacidal activity by itself.

In nature, the mechanisms described in PART I and II might contribute in parallel to the modulation of host immune responses by tick saliva and \textit{B. burgdorferi} and collectively assist the spirochete in establishing an infection. Our research has delineated new molecular interactions that occur at the tick-host-pathogen interface and provide insight into the (early) pathogenesis of Lyme borreliosis and other tick-borne illnesses. Besides, research such as presented in PART I and II may lead to the identification of novel tick salivary protein candidates for anti-tick vaccines, since interfering with tick-host-pathogen interactions that are crucial for pathogen transmission, e.g. by an anti-tick vaccine based on specific tick salivary proteins, could prevent the transmission of \textit{B. burgdorferi} from the tick to the host.

**PART III: Host-pathogen interactions**

A variety of interactions between host molecules and \textit{B. burgdorferi} proteins determine the outcome of \textit{B. burgdorferi} infection. During the initial stages of mammalian infection, when adhesion to extracellular host components may assist in survival of spirochetes that are transmitted
through a tick bite, *Borrelia* upregulates several proteins on its outer surface, for instance OspC [46–51], Decorin binding protein A and B [52] and the fibronectin binding protein BBK32 [53]. By contrast, most likely due to host immune pressure, during later stages of infection many exposed lipoproteins are downregulated [51,54]. Some *B. burgdorferi* genes are even either differentially up- or downregulated dependent on the tissue that is colonized [55,56]. In addition, as previously discussed, the spirochete attempts to evade the host immune system by inhibiting the complement cascade [42–45]. Importantly, during persistent infection, *B. burgdorferi* also tries to evade host immune responses by recombination at the variable major protein like sequence (*vls*) locus [57,58]. The *B. burgdorferi* *vls* locus consists of a *vls* expression site (*vlsE*) and 15 unexpressed upstream silent cassettes [59]. Segments of the silent *vls* cassettes randomly recombine into the *vlsE* expression site, resulting in multiple variations of the VlsE protein during the course of infection [59–61]. The examples detailed above underscore how well the spirochete is adapted to survive in the mammalian host.

The host in turn, upon infection with *B. burgdorferi*, mounts an immune response in an attempt to eradicate the spirochete. A great deal of our current knowledge on the host immune response against *B. burgdorferi* comes from the murine model for Lyme borreliosis [62]. As for all infections, the initial defense against *Borrelia* is formed by innate immune responses. A crucial line of defense in the innate immune response against *Borrelia* is the complement cascade, consisting of the classical, the lectin, and the alternative pathways. These three pathways merge at the common intersection of the complement factor C3 and can lead to formation of the membrane attack complex (MAC), which causes lysis of complement sensitive *Borrelia* isolates [63]. In addition, activation of the complement cascade can lead to opsonization followed by phagocytosis of *Borrelia* by, for instance, polymorphonuclear leukocytes, monocytes and macrophages [64–66]. These cells can also produce pro- or anti-inflammatory cytokines and chemokines upon recognition of Borrelial lipoproteins through a variety of invariant cell-surface receptors, among which Toll-like receptors (TLRs). *B. burgdorferi* is predominantly recognized by TLR2 [67]. Indeed, mice deficient for TLR2 have spectacularly increased systemic *Borrelia* numbers upon experimental infection [68,69]. Strikingly, patients with a polymorphism in the TLR2 gene were less susceptible for developing symptoms of late Lyme borreliosis [70], indicating that ongoing inflammation could be responsible for disease manifestations.

The outcome of the clash between *B. burgdorferi* and the host immune system, i.e. clearance or persistence of the spirochete, is dependent on virulence factors of the spirochete and the efficacy of the immune response raised by the host. In most animal models, in which 100% infectivity is pursued, *B. burgdorferi* is able to cause a persistent infection despite the development of vigorous innate and adaptive immune responses against the pathogen. It is here to mention that, although *B. burgdorferi* has been isolated from patients many years after the initial infection, in general *B. burgdorferi* is incapable of causing a persisting infection in most individuals, even without
antibiotic treatment. In this section of the thesis we investigated novel host and pathogen factors that could influence the outcome of *Borrelia* infection.

Lyme borreliosis can also occur in naturally infected dogs, manifesting as malaise and lameness [71]. Hovius and Hovius investigated the course of infection and immune responses in a cohort of pet dogs, naturally infected through tick-bite [72]. In this study we show that all naturally exposed dogs have moderate to high antibody titers, but symptomatic dogs produce a much wider spectrum of antibodies, including immobilizing antibodies, compared to asymptomatic infected dogs (Chapter 7). This indicates that the magnitude of the immune response directed against the bacterium is associated with disease activity in dogs. In human clinical practice we do not yet have access to reliable (serological) diagnostic tests distinguishing between active infection, subclinical infection and *B. burgdorferi* infection in the past. Importantly, to some extent, these tests are available for syphilis, another spirochetal disease [73–75]. Possibly, the data as presented in chapter 7 could contribute to the development of such tests for *B. burgdorferi*, and research on this complicated topic should have high priority on the research agenda [76].

In Europe, a considerable percentage of ticks is coinfected with multiple *Borrelia* species [77]. A tick can therefore transmit more than one *Borrelia* species to the host through a single tick-bite [78–80]. In chapter 8 we assessed the effect of simultaneous infection with the non-arthritogenic *B. garinii* strain PBi and the arthritogenic *B. burgdorferi* strain B31 on the course of experimental murine Lyme borreliosis [81]. We demonstrate that mice co-infected with PBi and B31 show significantly more paw swelling and arthritis, long-standing spirochetemia, and significantly higher systemic numbers of B31 spirochetes than mice infected with B31 alone. Mice infected with *B. garinii* strain PBi alone did not develop any symptoms. These data indicate that coinfection of *B. garinii* and *B. burgdorferi*, which is not unlikely in nature, results in an aggravated course of Lyme borreliosis and might even play an important role in the pathogenesis of human Lyme borreliosis in Europe. Interestingly, the evolutionary origin of *B. burgdorferi* has been a topic of debate. Recently, it was argued that *B. burgdorferi* originates from Europe [82], where as earlier studies suggested that *B. burgdorferi* ancestors must have been located primarily in the USA and were introduced into Europe in the post-Columbian era [83]. Our findings in chapter 8, but also in chapter 5, suggest a preferential survival advantage for *B. burgdorferi* over the other European *Borrelia* species. Therefore, regardless of the origin of *B. burgdorferi*, our findings provide experimental evidence of how this preferential protection could have influenced, or will influence, the introduction and persistence of *B. burgdorferi* in tick populations.

In *in vitro* studies *B. burgdorferi* has been shown to upregulate expression of the urokinase Plasminogen Activator Receptor (uPAR; CD87; PLAUR) [84,85], a receptor that can bind urokinase Plasminogen Activator (uPA) and is expressed on a variety of immune cells [86]. The urokinase receptor also affects cellular migration, adhesion, but also phagocytosis by facilitating intracellular signaling in conjunction with other surface receptors, including many integrins. This
can occur independently of the proteolytic activity of uPA [87]. We here show that uPAR is not only upregulated on murine and human leukocytes upon *B. burgdorferi* exposure, but that uPAR is involved in phagocytosis of the spirochete both in vitro as well as in vivo in the mouse model of Lyme borreliosis (Chapter 9). Better understanding of how the immune system copes with acute and persistent *Borrelia* infection might lead to novel or additional treatment strategies in conjunction to antibiotic treatment. This could be specifically true for understanding the role of uPAR, since several antagonists or agonists of the uPA/uPAR system have already been tested in oncological clinical trials [88,89], paving the way for research on the effects of these molecules in other disease entities.

**PART IV: Spin-off of research on tick-host-pathogen interactions**

Research on tick-host-pathogen interactions assists in understanding the complex processes that occur at the site of the tick-bite and how these could affect the (early) pathogenesis of Lyme borreliosis. However, there is more to be learned from the data presented in this thesis. Tick saliva contains a wide range of physiologically active molecules that are crucial for attachment to the host and/or for the transmission of pathogens [37] and that interact with host processes, including coagulation and fibrinolysis, and immunity and inflammation [90,91]. In this section we speculate how immunologically targeting specific tick salivary proteins could prevent the transmission of tick-borne pathogens from the tick to the host [92]. As an example we discuss an anti-tick vaccine based on Salp15 (Chapter 10). Importantly, we also discuss molecules in tick saliva that have been extensively studied in vitro or in animal models for human diseases, and that, due to their specificity, are potential future anticoagulant or immunosuppressive agents (Chapter 10). The *I. scapularis* tick genome is currently being sequenced, an effort known as the *Ixodes* Genome Project [93,94]. Once the genome is known this might give an enormous additional impulse to research in this field.

**Concluding remarks**

Since its discovery approximately 30 years ago, Lyme borreliosis has become the most important vector-borne disease in the Western world. The pathogenesis of this tick-borne disease is influenced by a plethora of tick-host-pathogen interactions and is still not entirely understood. This thesis describes in molecular detail novel tick-host, tick-pathogen and host-pathogen interactions in Lyme borreliosis, contributing to the understanding of the pathogenesis of this emerging zoonotic disease.

We have focused on the interaction of the *Ixodes* tick salivary gland protein, Salp15, with both *B. burgdorferi* as well as the mammalian immune system. The inhibition of host immune responses on the one hand, and the protection of *B. burgdorferi* on the other hand, by this pleiotropic tick salivary protein are exemplary of the complexity of tick-host-pathogen interactions that collectively
determine the outcome of an infection with *B. burgdorferi*. Unquestionably, in the future we will be able to use tick salivary proteins, or compounds based thereon, to our own benefit, and develop new tools to combat or prevent tick-borne diseases. Noteworthy, we have some catching up to do, since the majority of the interactions described in this thesis are likely to be the result of millions of years of co-evolution.

In addition, in both the experimental murine model for Lyme borreliosis as well as in naturally *B. burgdorferi* infected dogs, we show a delicate role for the host immune response in the genesis of Lyme borreliosis symptoms. The mammalian immune system should not generate a weak immune response, since this may fail to eradicate the spirochete, however an excessive immune response will lead to (irreversible) tissue damage and clinical symptoms; this is not an enviable task with both the arthropod vector as well as the bacterium trying to tip the balance of this fragile equilibrium. Importantly, better understanding of the host immune response to *Borrelia* brings us closer to the development of clear-cut diagnostic tests and therapeutic compounds that can specifically and favorably target the immune response against the bacterium.
Reference List


