Tick-host-Borrelia interaction
Implications for host immunity and vaccination strategies
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Chapter 1

General Introduction
**GENERAL INTRODUCTION**

*Lyme borreliosis*

Lyme borreliosis is a tick-borne disease caused by *Borrelia burgdorferi* sensu lato spirochetes, which was discovered during an outbreak of arthritis in 1975 in the Connecticut village of Old Lyme, and is currently the most common vector-borne disease in the Northern hemisphere. The causative spirochetes reside in the midgut of *Ixodes* ticks (*I. scapularis* in the northeastern USA, *I. pacificus* in western USA, *I. ricinus* in Europe and *I. persulcatus* in Russia/Asia), from which they migrate into the tick salivary glands upon ingestion of the host’s blood. During this process, *B. burgdorferi* s.l. spirochetes downregulate Outer surface protein A (OspA) and upregulate Outer surface protein C (OspC); expression of OspC is essential to infect the host. When inside the host, *B. burgdorferi* s.l. needs to evade the immune system in order to cause persistent infection. The complement system for example, is an innate immune mechanism where the classical and lectin pathways are amplified by the alternative pathway, resulting in spirochete lysis by the formation of membrane attack complexes (MACs), among other detrimental effects. The complement resistance of these strains can be specific for the complement of several host species, allowing them to infect these specific hosts. This mechanism plays an important role in *B. burgdorferi* ecology, which involves feeding of the tick on three different hosts during its lifetime; as a larvae, nymph and adult. Unfed larvae are not infected with *B. burgdorferi* s.l., and humans are bitten more frequently by nymphs than adult ticks. Therefore, the acquisition of *B. burgdorferi* by *Ixodes* larvae from infected mammals and birds is crucial for nymph-mediated transmission to incidental hosts, such as humans (Figure 1). When humans are bitten by infected *Ixodes* ticks, *B. burgdorferi* spirochetes can be transmitted from the moment they enter the tick saliva. However, not all humans will become infected, and not all infected individuals will become symptomatic: While 0.3% to 5.2% of tick bites in endemic areas in Europe lead to erythema migrans (EM, a “bulls eye rash”), seroconversion occurs in 3.5–8.2% after tick bites that do not lead to EM, suggesting asymptomatic exposure and adequate clearance by the host immune response. Indeed, in a Dutch cohort military personnel followed for one year, only one out of 14 seroconverters had noticed an EM. *B. burgdorferi* s.l. species differ in their geographic distribution: *B. burgdorferi* sensu stricto (s.s.) and the
recently discovered \textit{B. mayonii} infect humans in the USA\textsuperscript{17}, while \textit{B. afzelii}, \textit{B. garinii} and \textit{B. burgdorferi} s.s. are the main pathogens causing Lyme borreliosis in Europe\textsuperscript{18}. When \textit{B. burgdorferi} s.l. causes Lyme borreliosis, it can manifest as (multiple) EM, lymphocytoma, oligoarthritis, carditis, acrodermatitis chronica atrophicans (ACA), carditis or neuroborreliosis\textsuperscript{19,20}. Treatment with doxycycline or ceftriaxone usually clears the infection\textsuperscript{2}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Ecology of \textit{B. burgdorferi} s.l. and ticks}
\textit{Ixodes} ticks feed three times in their life cycle: once as a larva (left) on small rodents and birds, after which they molt into a nymph (top). Nymphs molt into adults after they take a blood meal (right). These adults mate during feeding on large hosts such as deer, after which around 1000 eggs are laid (bottom). When \textit{B. burgdorferi} s.l. is acquired by larvae or nymphs during feeding on infected hosts, their subsequent stage is able to transmit the pathogen, and infection in the tick is lost after the female lays eggs, which are not infected. Humans are often bitten by nymphs, and are a dead-end host for \textit{B. burgdorferi} s.l. which is maintained in nature in ticks and reservoir hosts. Source: Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. Nature Reviews Microbiology 10, 87-99 (February 2012). With the publisher’s permission.
Other tick-borne diseases

Examples of other pathogens transmitted by *Ixodes* ticks are *Anaplasma phagocytophilum* (bacteria causing human granulocytic anaplasmosis), *Rickettsia* species (bacteria causing various clinical entities), *Francisella tularensis* (bacteria causing tularemia), tick-borne encephalitis virus (TBEV, causing tick-borne encephalitis), *Babesia microti/divergens* (parasites causing babesiosis), and *Borrelia miyamotoi* (bacteria causing tick-borne relapsing fever).

*Borrelia miyamotoi* was known to infect ticks for two decades, although it was only identified as a human pathogen in 2011, when Alexander Platonov described the first infected patients\(^{21,22}\). Interestingly, *B. miyamotoi* is the only relapsing fever *Borrelia* species that is present in *Ixodes* ticks. Because *Borrelia miyamotoi* infects *Ixodes* ticks, it occurs in the same areas where Lyme borreliosis is endemic. Unlike *Borrelia burgdorferi* s.l. however, it is regularly transovarially transmitted by female *Ixodes* ticks to their larval offspring, and bites by larvae can transmit *B. miyamotoi*\(^{23-27}\).

Moreover, the clinical symptoms of *Borrelia miyamotoi* infection seem to differ from those caused by Lyme borreliosis, as will be presented in a literature review that is included in this thesis. Furthermore, several studies are presented in this thesis that explore this emerging pathogen that is present across the Northern hemisphere.

All other currently known relapsing fever species reside in lice (*Borrelia recurrentis*, causing louse-borne relapsing fever in the horn of Africa, LBRF) or soft ticks (various species around the world, causing tick-borne relapsing fever, TBRF). LBRF caused large epidemics in Europe, North Africa and Russia in various 20\(^{th}\) century world- and civil wars, causing millions of deaths, and has recently been described in refugees traveling from the horn of Africa to The Netherlands and other European countries\(^{28,29}\). The most well-studied TBRF species is *B. hermsii*, which is found in the Western USA, but is not a common pathogen in humans with only 450 described cases between 1987 and 2000\(^{30}\). In contrast, *Borrelia crocidurae* in Senegal was found responsible for a community incidence of 14 per 100 person years, the highest of any bacterial infection reported in Africa, but has been scarcely studied\(^{31}\). African tick-borne relapsing fever was already described by David Livingstone in 1857 during his travels across the continent, and the relationship between relapsing fever and tick bites has been long known in some African communities\(^{32}\). In Nyasaland for example, people knew that newcomers were more susceptible to TBRF, and that immunity to tick bites in locals waned after after living in a different place without exposure to ticks. They have even been said to bring ticks to feed on them when they would temporarily live somewhere else, in order to preserve immunity. Joseph Everett Dutton, the British
parasitologist who discovered *Trypanosoma brucei* (the agent of sleeping sickness), apparently was not immune: he died in The Congo Free State from infection with the TBRF species later named *Borrelia duttonii* \(^{33, 34}\).

**Borrelia burgdorferi s.l. vaccination**

OspA is the most commonly targeted antigen in *B. burgdorferi* vaccination studies, and has been described already in 1990 \(^{35}\). Antibodies against the highly immunogenic OspA not only target infecting spirochetes in the host, but have also been described to kill *B. burgdorferi* inside ticks feeding on vaccinated animals \(^{36}\). While an effective human vaccine based on OspA (LYMErix®) was introduced in 1998 \(^{37}\), it was voluntarily withdrawn from the market in 2002 due to commercial reasons, the need for boosters and speculations of an epitope in OspA potentially causing auto-immune arthritis \(^{38}\), although vaccine safety studies did not support this hypothesis \(^{39, 40}\). Because of the vaccine potential of OspA and because the incidence of Lyme borreliosis has since then dramatically increased, vaccine trials are again targeting OspA in humans \(^{41}\). A second promising vaccine candidate against *B. burgdorferi* s.l. is OspC. In contrast to OspA, OspC is upregulated when *B. burgdorferi* infects the host, and OspC vaccines are very effective in preventing transmission by ticks as well as needle-inoculation with the spirochete, while spirochete migration from the tick midgut to its saliva is also inhibited \(^{42-45}\). A problem with OspC as a vaccine candidate however is that there are many OspC serotypes with low cross-protectivity, and therefore multivalent OspC vaccines are currently being investigated to protect against *B. burgdorferi* isolates with various OspC serotypes \(^{46, 47}\). Furthermore, recombinant OspC vaccination might induce local skin reactions, which might limit its use in humans \(^{48}\).

**Tick proteins and their vaccine potential**

William trager described in 1929 that repeated infestations with dog ticks (*Dermacentor variabilis*) induce “tick immunity” in guinea pigs \(^{49}\). Tick immune hosts have acquired immunity to tick proteins by repeated exposure, and subsequently feeding ticks will feed shorter and ingest smaller amounts of blood. The mechanisms behind “tick immunity” are poorly understood, however it is believed basophils play an important role in antibody-mediated acquired immunity to *Haemaphysalis longicornis* ticks, causing a delayed-type hypersensitivity response at the bite site \(^{50}\). For Rhipicephalus (*Boophilus*) microplus, a tick that feeds on the same cow in all of its stages (a “one-host tick”), vaccination of cattle with a *R. (B.) microplus* midgut protein (Bm86) causes tick mortality after feeding on antibody-containing blood. Bm86 vaccination is also successful in diminishing tick reproduction on cattle, and combied these effects have led to a strong reduction in the incidence of bovine
babesiosis (“cattle fever”) after vaccination. Two Bm86-based commercial vaccines are currently widely used in cows in endemic areas, preventing cattle disease, decreasing the use of acaricides and saving farmers millions of dollars in Australia and Latin America.

The next obvious step is to develop a vaccine against *Ixodes* ticks, which are most relevant for human disease. For *Ixodes* ticks, “tick immunity” has been described in guinea pigs by immunizing with 24-hour fed *Ixodes scapularis* salivary gland extracts, resulting in decreased tick feeding time, fed tick weights and transmission of *B. burgdorferi*. “Tick immune” rabbit serum transferred to mice also reduced *B. burgdorferi* transmission by infected ticks. Interestingly, some humans also seem to be able to acquire immunity to *Ixodes* ticks: in an American study, people who reported more than 3 itching tick bites (a sign of delayed type hypersensitivity) had the lowest incidence of Lyme borreliosis, despite a high exposure to tick bites.

During a tick bite, various anticoagulant and immunosuppressive tick saliva proteins are inserted into the host skin, enabling tick feeding as well as infection by tick-borne pathogens. An example of such proteins is Salp15, which suppresses CD4 T-cells and dendritic cells, and also protects *B. burgdorferi* at the bite site from host antibody-mediated killing by binding and shielding OspC. Interestingly, although Salp15 increases infectivity by *B. burgdorferi* and enables prolonged tick attachment, it was also proven effective as an anti-tick vaccine.

Another *Ixodes* salivary protein that impairs host immunity is tick salivary lectin pathway inhibitor (TSLPI). This protein was identified in *I. scapularis* based on its binding to anti-tick antibodies in repeatedly infested “tick immune” rabbits. It inhibits the lectin complement pathway, thus protecting complement-sensitive *B. burgdorferi* s.l. from complement-mediated killing at the bite site. Interestingly, both the transfer of TSLPI antibodies to mice as well as silencing of TSLPI by RNA interference in ticks reduced the transmission of *B. burgdorferi* s.l. from ticks to mice. Finally, vaccination against a tick cement protein was shown to protect mice against transmission of TBEV, proving that vaccines against *Ixodes* ticks could not only prevent Lyme borreliosis, but also other tick-borne diseases.
Figure 2. Inhibition of skin innate immune responses by tick saliva.

Tick saliva that is introduced into host skin contains various proteins with immunosuppressive functions, thus aiding prolonged tick attachment and feeding as well as pathogen transmission. AMP, antimicrobial peptide; IFN-γ, interferon γ; IL, interleukin; NO, nitric oxide; TNF-α; tumor necrosis factor-α.

Outline of the dissertation

**Part I: Novel vaccine candidates and methods to prevent Lyme borreliosis**

In part I, novel vaccination approaches are investigated against Lyme borreliosis in Europe, targeting either *B. afzelii* or its European vector, *I. ricinus*. In chapter two, a novel technique to apply DNA vaccines using a tattoo machine was tested against *B. afzelii* OspC in mice. This method resulted in full protection from needle-inoculation with *B. afzelii* and an improved antibody subclass profile compared to recombinant OspC vaccination. Chapter three describes vaccination with *I. ricinus* homologues of Bm86, in order to inhibit adult *I. ricinus* tick feeding on rabbits. Unfortunately, the strong phenotype in diminishing *Rhipicephalus* tick infestations on Bm86-vaccinated cattle was not observed in rabbits infested with *Ixodes* ticks. Chapter four describes the identification of a TSLPI homologue in *I. ricinus*. This tick salivary protein can aid *B. burgdorferi* transmission by inhibiting complement activity at the tick bite site, and is a homologue of a previously described potential anti-tick vaccine candidate.

**Part II: Host immunity against Borrelia miyamotoi**

*Borrelia burgdorferi* s.l. species are not the only *Borrelia* spirochetes transmitted by *Ixodes* ticks, and part II of this thesis aims to expand knowledge on the emerging tick-borne relapsing fever spirochete *B. miyamotoi*. Chapter five gives a broad literature-based overview on *B. miyamotoi* and its ecologic and clinical aspects, highlighting the widespread occurrence of this pathogen. Chapter six reports on the first *B. miyamotoi* infected patient described in Europe, and the second case of meningoencephalitis linked to this pathogen. It was the start point of our focus on this emerging pathogen, and the excitement from observing live *B. miyamotoi* in CSF from this patient inspired the pursual of the research projects included in the following chapters. Chapter seven describes the occurrence of *B. miyamotoi* in wild animals in The Netherlands, and reports on PCRs we have performed on biopsies of patients with Lyme borreliosis (LB)-suspected skin lesions that had been previously tested for *B. burgdorferi* in our hospital. While we found ticks and wild animals to be infected, *B. miyamotoi* was not found in patient skin biopsies. This suggests that *B. miyamotoi* is not associated with LB-suspected skin lesions, although this requires further investigation and more patients should be tested.
Chapter eight describes the development of a culture medium for *B. miyamotoi*. Using this medium, we were able to demonstrate that *B. miyamotoi* is resistant to human complement, which provides insight into its survival mechanism in the human bloodstream. Finally, Chapter nine describes a novel mouse model for infection with *B. miyamotoi*. In C3H/HeN mice, a minority experienced a relapse of spirochtemia, which had previously been described to occur in a minority of human patients. For the first time, the expression of Variable Major Proteins (Vmps) by *B. miyamotoi* is described, as well as the role of Vmp antibodies in spirochete clearance and Vmp serotype switching, a mechanism crucial for immune evasion by relapsing fever spirochetes. Moreover, *B. miyamotoi*-infected patients were revealed to rapidly produce Vmp antibodies during infection, which might serve as a basis for more sensitive serologic tests to diagnose *B. miyamotoi* infection. Finally, in Chapter ten, our findings are summarized and discussed in broader context.