Chapter 10

Summary and general discussion

Tick-host-\textit{Borrelia} interaction: Implications for host immunity and vaccination strategies
Part I of this thesis focuses on novel candidates and vaccination strategies to prevent Lyme borreliosis. There are various possibilities to prevent Lyme borreliosis in individual humans such as personal protection, antibiotic prophylaxis after a tick bite, or vaccination. While protective measures such as the use of adequate clothing during outside activities, the use of insect repellent and to check for ticks after visiting tick-infested areas are advised, they obviously are not sufficiently effective: over one million people are yearly bitten by ticks in The Netherlands, and the incidence of Lyme borreliosis is rising despite increased awareness. The potential effects of antibiotic prophylaxis after a tick bite are promising, yet prophylaxis has a high number-needed-to-treat, potential side-effects and does not fit well with the restrictive antibiotic policy we have in the Netherlands. Moreover, many Lyme borreliosis patients do not recall a tick bite and disease could not have been prevented by antibiotic prophylaxis in those cases. Vaccination against Lyme borreliosis therefore could be an important preventive measure in high-risk populations. A recombinant OspA-based vaccine was available for human use for four years, however it was taken off the market for various reasons, one of which being a need for boosters. Other vaccination candidates and techniques should therefore be explored.

OspC antibody responses are highly important for B. burgdorferi s.l. clearance. Chapter 2 focuses on a novel vaccination technique that uses a tattoo needle to apply an OspC-based DNA vaccine in a rapid prime-boost schedule. Previously, this technique was demonstrated to induce a superior cellular immune response compared to intramuscular DNA vaccination, accomplishing effective protective immunity against Influenza infection and HPV-mediated skin tumors, but it was not yet tested against bacteria. We found this technique to work very efficiently against B. afzelii PKo, a European B. burgdorferi s.l. isolate. Interestingly, this technique elicited strong antibody responses with preferable IgG subtype distribution compared to recombinant protein vaccination, and full protection against B. afzelii needle inoculation. This could extend the application of this technique beyond viral or oncological targets, and future studies should elucidate its potential against other extracellular bacteria and in settings where rapid induction of immunity is required.

Another vaccination strategy to prevent Lyme borreliosis (as well as other tick-borne diseases) is to target the vector. In Western Europe, I. ricinus ticks transmit B. burgdorferi s.l. to humans via saliva that is inserted into the skin by a bite that lasts several days. Although still under debate, it is believed that B. burgdorferi s.l. is usually transmitted after the tick has bitten at least 24-48 hours, because the spirochetes need to migrate from the tick midgut to its salivary glands before
entering the saliva\textsuperscript{4,114}. Indeed, human infection was found to occur more often after 72 hours of tick attachment\textsuperscript{115}. Via several different mechanisms, vaccines directed against tick salivary proteins are thought to inhibit transmission of \textit{B. burgdorferi} s.l. as well as other tick-borne pathogens: transmission could be inhibited by impairment of tick feeding and early tick detachment, while a hostile environment for pathogens can be created at the bite site by antibody-mediated inhibition of immunosuppressive or anticoagulant tick saliva proteins and by induction of a local hypersensitivity response. Indeed, both immunization with tick saliva extract as well as immunization using repeated tick infestations in guinea pigs and rabbits can induce early tick detachment and diminished \textit{Borrelia burgdorferi} transmission\textsuperscript{55,116}. Another approach is to target vaccines against the tick midgut, thus disrupting the tick from the inside when it feeds on an immune host. This approach has been very successful in preventing bovine babesiosis in cows, and commercial vaccines based on the \textit{Rhipicephalus microplus} midgut protein Bm86 have successfully entered the market. These vaccines impaired tick feeding and reproduction, leading to a dramatic decrease in cattle mortality by bovine babesiosis and also in a reduction in the use of acaricides by farmers\textsuperscript{117,118}. In \textit{chapter 3} we describes a vaccine against an \textit{I. ricinus} Bm86 homologue, entitled Ir-86. Despite the efficiency observed against \textit{R. microplus}, feeding and oviproduction of \textit{I. ricinus} adults were not affected by Ir-86 vaccination in rabbits. A plausible explanation for this observation is that \textit{R. microplus} are one-host ticks, feeding on the same vaccinated cow several times during their life-cycle; \textit{I. ricinus} ticks only bite the same host one time during their life cycle, and are thus only exposed to host antibodies during one bloodmeal. However, we did not investigate the effect of these vaccines on \textit{Borrelia} transmission, which should be a subject for further investigation.

To understand how \textit{Ixodes} ticks can remain attached for days and how they help pathogens to evade the host immune response at the bite site, one should identify individual immunosuppressive tick salivary proteins. Previously, a tick salivary lectin pathway inhibitor (TSLPI) in \textit{I. scapularis} was found to inhibit complement-mediated killing of \textit{B. burgdorferi}, to play a role in the transmission of \textit{B. burgdorferi} s.s. from \textit{I. scapularis} ticks to mice and to be important in the acquisition of \textit{B. burgdorferi} s.s by larval \textit{I. scapularis} ticks. Moreover, silencing of TSLPI and passive immunization with anti-TSLPI antibodies reduced \textit{B. burgdorferi} s.s. loads in mice after infection by \textit{I. scapularis} tick\textsuperscript{63}. In \textit{chapter 4}, the discovery and function of an \textit{I. ricinus} TSLPI homologous protein is described. We demonstrate that \textit{I. ricinus} TSLPI clustered within a larger family of \textit{Ixodes} salivary proteins, and that this protein reduced MBL-dependent C4 deposition and inhibits human complement-mediated killing
of (intermediate) complement-sensitive *B. burgdorferi* s.l. isolates. *I. ricinus* TSLPI is upregulated in the tick salivary glands during feeding, and seems to be implicated in the transmission of *B. burgdorferi* s.l. to humans at the bite site.

In **part II** of this thesis, a “new” *Borrelia* species is introduced, designated *Borrelia miyamotoi*. Although the presence of *B. miyamotoi* in *Ixodes* ticks has been acknowledged for decades, its pathogenicity in humans was only discovered in 2011, when *B. miyamotoi*-infected patients in Russia were discovered to experience viral-like illness accompanied by high fevers, sometimes with a relapsing disease pattern. Interestingly, the discovery of this novel infectious disease was made in a “reverse order”: instead of searching for a pathogen to match a clinical syndrome, in this case the presence of the pathogen in its vector was established first, followed by a search for human disease correlates. **Chapter 5** of this thesis is a literature review on *Borrelia miyamotoi*. It describes that *B. miyamotoi* is a relapsing fever spirochete that is present in the same tick species and –populations as *Borrelia burgdorferi* s.l., but exerts completely different pathogenesis and clinical manifestations. While *B. burgdorferi* mainly infects host tissues, relapsing fever spirochetes replicate in the blood stream, leading to a febrile viral-like illness designated hard tick-borne relapsing fever (HTBRF). Due to very limited research on *B. miyamotoi* and a lack of awareness among clinicians, *B. miyamotoi* is currently rarely diagnosed, and the incidence and implications of infection are still unclear. We found that *B. miyamotoi* is present in around 2% of *Ixodes* ticks across the northern hemisphere, with higher infection prevalences in regions with a higher prevalence of *B. burgdorferi* s.l. A recent study showed a transmission efficiency to humans of approximately 8% (by infected *I. persulcatus* adults), suggesting a substantial exposure of humans at a population level: In The Netherlands alone, over one million people are annually bitten by *I. ricinus* ticks. Theoretically therefore, well over a thousand humans could be yearly exposed to *B. miyamotoi* in the Netherlands, although this would not necessarily lead to clinical manifestations in all cases. Interestingly, *B. miyamotoi* is vertically transmitted from adult ticks to their larval offspring (unlike *B. burgdorferi* s.l.), and indeed we found that Dutch *I. ricinus* larvae can infect mice with *B. miyamotoi*. If *B. miyamotoi* is transmitted to humans by larvae, these bites are likely to be missed, and these patients might frequently present with a summertime fever without any history of tick bite. This, combined with a lack of widely available (clinically validated) diagnostic tests make HTBRF an infection that is hard to diagnose at the moment, and because antibiotics prescribed for presumed Lyme borreliosis will also clear HTBRF, HTBRF is easily mistaken for Lyme borreliosis. This was also the case for the Dutch patient that we diagnosed with HTBRF, as is described in **Chapter 6**.
This immunocompromised patient presented with a chronic meningoencephalitis, and was initially diagnosed to have a possible Lyme neuroborreliosis. However, we retrospectively diagnosed the patient with *B. miyamotoi* infection by a PCR on spinal fluid. This discovery instigated further research efforts, which are described in chapters 7 to 9.

**Chapter 7** describes the detection of *B. miyamotoi* in Dutch ticks, rodents and birds. Furthermore, the absence of *B. miyamotoi* is described retrospectively in 31 skin biopsies from patients suspected of cutaneous Lyme borreliosis. While 9 biopsies were positive for *B. burgdorferi* s.l., none were positive for *B. miyamotoi*. These data support the notion that *B. miyamotoi* infection does not cause skin infections. Since *B. miyamotoi* incidence is estimated to be lower than for Lyme borreliosis, future studies should confirm this finding in larger patient cohorts. Hopefully, prospective studies can be conducted to assess HTBRF by PCR on blood in patients presenting with fever after a tick bite, in order to acquire an estimate of disease incidence and burden in The Netherlands.

Our final goal was to gain insight into *B. miyamotoi* pathogenesis and to discover novel serologic markers for infection. Because *B. miyamotoi* was considered uncultivable, we first needed to optimize a culture medium and method to propagate *B. miyamotoi* spirochetes in vitro. After developing a new culture medium (designated MKP-F), we demonstrated that *B. miyamotoi* is resistant against human complement, which could facilitate human infection by the spirochete (**chapter 8**). In contrast, the relapsing fever spirochete *Borrelia anserina* was sensitive to human complement, suggesting that its lack of host adaptation to humans plays a role in the absence of human infections. Further collaborative studies are currently being performed to identify the protein(s) responsible for complement resistance in *B. miyamotoi*. Also, using our newly developed culture medium, we were able to isolate *B. miyamotoi* from human patients (unpublished), which should provide more insight into pathogenesis and further tools to improve serologic diagnosis.

**Chapter 9** reveals the discovery of several variable major proteins (Vmps) that can be expressed by *Borrelia miyamotoi*. These immunodominant surface proteins comprise of variable small proteins (Vsps, which are homologues of *B. burgdorferi* s.l. OspC) or variable large proteins (Vlps, which are homologues of *B. burgdorferi* s.l. VlsE). At each time, relapsing fever spirochetes can only express one Vsp or Vlp, which can be switched by gene conversion, leading to a clinical relapsing fever pattern. For *B. miyamotoi*, we show that antibodies against Vsp1 eliminate Vsp1-expressing
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spirochetes from the murine bloodstream. However, a small subpopulation expressing a Vlp is not eliminated by anti-Vsp1, leading to outgrowth of this particular serotype. This suggests a similar mechanism of antibody evasion by Vmp expression switching as was shown in other TBRF species. This mechanism is responsible for the clinical picture of relapsing febrile episodes, which was described in several human patients with HTBRF. Next, we showed that B. miyamotoi Vmps are highly immunogenic in humans, and we detected antibodies against Vsp1 or other Vmps in 6/9 acute HTBRF patients (compared to 5/9 when detecting GlpQ antibodies) tested 7 to 17 days after disease onset. Because of the strong, rapid and specific antibody responses elicited by Vmps (which are not expressed by B. burgdorferi s.l.), detecting antibodies against Vmps might aid in the early diagnosis of B. miyamotoi infection. Further studies should be performed to assess which Vmps (or peptides thereof) should be combined to optimize sensitivity. Due to cross-reactivity it might be possible to detect antibodies against most serotypes by combining representative Vmps from different subfamilies. These Vmp subfamilies could be determined from their homology to B. hermsii Vmp subfamilies α to δ.²

To conclude, B. miyamotoi is a relapsing fever spirochete that is transmitted by the same ticks and in the same regions as B. burgdorferi s.l., and patients might have previously been under-diagnosed or misdiagnosed as having Lyme borreliosis. The use of specific serologic tests and the routine employment of B. miyamotoi PCR on blood in patients with risk of tick bites and unexplained summertime fever should help to identify more patients across the Northern hemisphere. It is important to acknowledge that a high fever with viral-like illness, elevated liver enzymes and thrombopenia within weeks after a tick bite should raise suspicion of HTBRF, or another tick-borne disease, rather than Lyme borreliosis.