Lyme disease, or Lyme borreliosis, the most prevalent arthropod-borne disease in the Western world, is caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato group and is predominantly transmitted through *Ixodes* ticks. There is currently no vaccine available to prevent Lyme borreliosis in humans. *Borrelia* outer membrane proteins are reviewed which have been investigated as vaccine candidates. In addition, several tick proteins are discussed, on which anti-tick vaccines have been based, or are interesting future candidates, to prevent transmission of the spirochete from the tick vector to the mammalian host. Finally, novel vaccination strategies to prevent Lyme borreliosis are proposed, based on multiple *Borrelia* antigens, tick antigens or a combination of both *Borrelia* as well as tick antigens.
Ixodes ticks and Borrelia burgdorferi

Lyme borreliosis, caused by spirochetes belonging to the Borrelia burgdorferi sensu lato group, and transmitted through Ixodes ticks, is a prevalent zoonosis in the Western world with yearly incidences peaking over 100 cases per 100 000 residents in many European countries and in endemic areas in the United States [1]. B. burgdorferi mainly survives in a tick-mouse cycle. In the US, B. burgdorferi sensu stricto is primarily transmitted by Ixodes scapularis ticks, while the three predominant pathogenic Borrelia species in Europe, B. burgdorferi sensu stricto, Borrelia garinii and Borrelia afzelii, are transmitted through Ixodes ricinus ticks.

Ixodes ticks normally take several days to feed, allowing the host to mount an immune response against exposed tick antigens. Therefore, tick saliva, introduced into host skin during the feeding process, contains a wide range of proteins with immunosuppressive [2], anticomplement [3] and antihaemostatic activity [4]. Tick saliva-induced suppression of local host immune responses is instrumental for both tick feeding as well as transmission of spirochetes from the arthropod vector to the mammalian host [5].

Tick control using acaricides (e.g. cyfluthrin, carbaryl or deltamethrin) represent one strategy to prevent Lyme borreliosis [6]. A downside of using acaricides is that this method is not useful in all ecologic settings [7]. In addition, acaricide resistance in ticks, toxicity to humans and other animals and environmental pollution are severe drawbacks [8].

Because vaccination approaches using Borrelia antigens have proven to be cumbersome (see below), interfering with local tick-host-pathogen interactions, using tick saliva proteins as vaccine candidates might be the way forward. Novel vaccination strategies to prevent Lyme borreliosis and potentially other tick-borne diseases will be discussed.

Variable Borrelia gene expression during the enzootic life cycle

To identify novel Borrelia and tick proteins that could be used as vaccine candidates, a proper understanding of the variable expression of both Ixodes and Borrelia genes encoding proteins that are pivotal in the transmission process of the spirochete is mandatory. In order to survive in the tick-host cycle Borrelia up- and/or downregulates several genes, including genes that code for lipoproteins and non-lipidated proteins on the membrane of Borrelia, so-called outer membrane proteins (OMPs). For example, Borrelia upregulates outer surface protein A (OspA), together with OspB, while entering the tick [9], binding to the tick receptor for OspA (TROSPA) [10]. This enables Borrelia to persist inside ticks in between blood meals. Once Borrelia-infected ticks start taking a subsequent blood meal, Borrelia downregulates OspA and upregulates other genes, including OspC, while migrating towards the tick salivary glands, from where spirochetes are transmitted to the host. In the salivary glands, the feeding-induced tick saliva protein Salp15 binds to Borrelia OspC, protecting the spirochete against both innate and
adaptive immune responses [11,12]. BB0323, also a membrane associated protein, is essential for both borrelial persistence and successful transmission of *Borrelia* in the tick-mouse cycle, and its expression is induced during tick feeding [13]; *Borrelia* was shown to be well-adapted to the hostile environment of the mammalian host, partially due to upregulation of outer membrane proteins like decorin-binding proteins A and B (DbpA and B) and BBK32 and RevA/B that bind to host decorin and fibronectin, respectively [14,15,16]. In the mammalian host, *Borrelia* also upregulates expression of ErpP, ErpA and ErpC, which enables binding to human plasminogen [17]. *Borrelia* downregulates immunogenic lipoproteins, such as OspC, to evade the host immune response [18], and upregulates proteins like the surface exposed lipoprotein VlsE, a protein essential for *Borrelia* survival in mammals. The *vls* gene consists of the *vls* expression site (*vls*E) that encodes the surface-exposed lipoprotein VlsE and 15 silent cassettes located upstream of *vls*E [19]. Throughout the course of infection in the mammalian host these cassettes randomly recombine into the *vls*E expression locus, resulting in expression of different VlsE variants [19] and altered antigenic properties ensuring protection of the spirochetes from killing by anti-VlsE antibodies. Finally, *Borrelia* expresses several proteins to protect itself against the host complement system (Box 1) such as complement regulating-acquiring surface proteins (CspA, CspZ), Osp E/F related proteins (Erps) and CD59-like proteins [20,21].

**Box 1. Complement resistance of Borrelia spirochetes**

Serum resistant *Borrelia* spirochetes express proteins on their outer membrane, named Erps and CRASPs or Csp’s, which can bind host complement regulators such as factor H (FH) and factor H-like proteins (FHL-1). Factor H regulates the alternative pathway of complement activation on the surface of host’s cells, where it enhances breakdown of C3b and C3bBb convertase, protecting host cells from complement activity. To date, five CRASP proteins have been identified. CRASP-1 and CRASP-2 both bind FH and FHL-1, while CRASP-3, -4 and -5 bind only FH. By binding these factors and by expressing CD59-like proteins [20], *Borrelia* inhibits formation of C5b-9, also named the membrane attack complex (MAC) [21], inferring (partial) resistance to complement-dependent killing by the host.

**The former vaccine against Lyme borreliosis: the OspA vaccine**

Previous studies indicated that immunization with OspA induced a long term protective immune response in mice [22]. Spirochetes were killed inside the midgut of engorging ticks in the presence of anti-OspA antibodies [23]. The OspA vaccine successfully completed Phase I, II and III trials and the OspA vaccine was approved by the Federal Drug Administration in the US in 1998. Vaccinated individuals showed approximately 80% protection against *B. burgdorferi* infection after receiving three vaccine doses with OspA using aluminum as
an adjuvant [24]. One drawback of the OspA vaccine was that protective immunity correlated with high titres of OspA antibodies after immunization, and it was shown that ~5% of the vaccine recipients developed insufficient antibody responses against OspA. This was associated with decreased cell surface expression of Toll-like receptor (TLR)-1 [25]. Thus, high antibody titres did not persist long after vaccination and additional boosters would be necessary to maintain protective titers [24] causing the vaccine to be withdrawn from the market four years after its release. Different adjuvants and/or carriers could be used to enhance presentation of a possible second-generation OspA vaccine [26,27]. Nevertheless, since a Lyme vaccine is still undoubtedly compulsory, other vaccine candidates and novel vaccination approaches should be explored. In contrast to the human OspA vaccine, several canine OspA vaccines are still commercially available (Box 2).

**Box 2. Canine lyme borreliosis vaccination**

Not only humans, but also dogs can suffer from Lyme borreliosis and other tick borne diseases [77]. Dogs frequently contract infection with *Borrelia*, however, the majority of dogs do not become ill after infection. Several commercial canine Lyme vaccines are available in the US and commonly used to protect dogs against Lyme borreliosis, especially in endemic areas. Several commercial canine OspA based vaccines were compared showing that a three-dose vaccination schedule significantly raised higher antibody responses compared to two doses of antigen [78]. However, in the setting of pet dogs living in an endemic region (Lyme, Connecticut, USA), still 25% of the dogs vaccinated with two doses of the OspA vaccine was infected with *B. burgdorferi* (compared to 63% in placebo vaccinated dogs) [79]. Recently, high level of protection was found in dogs immunized with a bivalent bacterin, consisting of a conventional *B. burgdorferi* strain and an *ospA*- and *ospB*-negative strain expressing high amounts of OspC. This approach resulted in high titres of borreliacidal antibodies directed against OspA and OspC [27]. One year after immunization OspC antibody titres vanished completely in these dogs and also the OspA titres waned significantly. Still, after *Borrelia*-infected tick challenge, 40% of the vaccinated dogs were infected with *Borrelia*, but infection was cleared after 2 months and dissemination to distinct organs did not occur, underscoring that vaccination strategies using multiple OMPs is also effective in dogs [80].

**Alternative Borrelia OMP vaccine candidates**

An alternative vaccine could be an OspC-based vaccine based on its protective ability, expression profile and antigenicity (Table 1). OspC vaccination has been shown to provide protective immune responses [28]. A disadvantage of the OspC vaccine is the heterogeneity of OspC proteins among *Borrelia* strains. Therefore, a multivalent OspC-based chimeric
vaccinogen has been developed [29]. Another drawback of the OspC vaccine was that approximately half of individuals in a Phase I trial developed erythema and swelling at the injection site [30]. Several other OMPs could be used as vaccine candidates (Table 1), such as DbpA. Immunization with DbpA elicited a strong protective antibody response against *Borrelia* in mice [31]. Nonetheless, this protective response was only described in mice that were syringe-inoculated with *Borrelia*. When spirochetes were transmitted through tick-bite this protective response was no longer apparent [32]. OspB plays an important role in colonizing the tick gut[33], and several studies have shown that vaccination with OspB elicits a protective response against *Borrelia* [34,35]. Recently it was found that the borreliacidal effect of an antibody specific for OspB was complement-independent [36].

Table 1. *Borrelia* antigens that can be used for future vaccine development

<table>
<thead>
<tr>
<th>Vaccine candidate</th>
<th>Function</th>
<th>Mechanism of action</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OspA</td>
<td>Binds tick midgut</td>
<td>Borrelicidal effect inside tick</td>
<td>[10,23]</td>
</tr>
<tr>
<td>OspC</td>
<td>Tick-host transmission</td>
<td>Borrelicidal effect outside tick</td>
<td>[28]</td>
</tr>
<tr>
<td>DbpA</td>
<td>Bind host decorin</td>
<td>Protects only after needle inoculation, not after tick transmission</td>
<td>[31,32]</td>
</tr>
<tr>
<td>OspB</td>
<td>Binds tick midgut</td>
<td>Borrelicidal effect inside tick</td>
<td>[33,32,36]</td>
</tr>
<tr>
<td>BBK32</td>
<td>Binds fibronectin</td>
<td>Borrelicidal effect in- and outside tick</td>
<td>[16,38]</td>
</tr>
<tr>
<td>BB0323</td>
<td>Tick-host transmission</td>
<td>Not determined</td>
<td>[13]</td>
</tr>
<tr>
<td>RevA</td>
<td>Binds fibronectin</td>
<td>Not determined</td>
<td>[14]</td>
</tr>
<tr>
<td>ACGal</td>
<td>Unknown</td>
<td>Not determined</td>
<td>[42,43]</td>
</tr>
</tbody>
</table>

This antibody was able to disrupt the outer membrane of *Borrelia* resulting in osmotic lysis of the spirochetes. *Borrelia* antigens that can elicit complement-independent bactericidal antibody responses are interesting vaccine candidates since *Ixodes* ticks have several proteins with complement inhibiting properties [3,11,37]. Complement-independent bactericidal antibodies could kill *Borrelia* inside the tick vector, even in the absence of functional complement. Another potential vaccine candidate could be BBK32. In the presence of BBK32, anti-serum mice were partially protected against *Borrelia* infection. In addition, BBK32 anti-serum also reduced spirochetal loads in the tick vector during feeding and after molting [38]. BB0323 and RevA are also upregulated during tick feeding and are therefore possible interesting vaccinogens, but until now no vaccination studies have been reported with these proteins.
A mixture of multiple *Borrelia* antigens could be used to enhance the protective effect of the vaccine (Fig.1A). A synergistic protective effect was found when mice were vaccinated with OspA and DpbA together, compared to a single antigen [39]. Likewise, a combination vaccine of OspC, BBK32 and DpbA was shown to be more effective than a single or double antigen vaccine in mice [40]. It might be possible to generate long-term immunity by an appropriate immunization with a cocktail of *Borrelia* proteins in humans too, since re-infection in successfully treated Lyme borreliosis patients with late manifestations, such as arthritis, appears to be extremely rare. In contrast, re-infection in successfully treated patients with early symptoms like erythema migrans is common [41]. This difference might be due to an immune response directed against multiple spirochetal proteins generated throughout the course of infection.

Besides *Borrelia* proteins, glycolipids could also be promising vaccine candidates. Over 80% of patients in late stages of Lyme borreliosis developed antibody titers against the glycolipid ACGal, the most abundant glycolipid comprising 45% of *Borrelia*’s glycolipids [42]. Moreover, ACGal was abundant among *B. burgdorferi*, *B. afzelii* and *B. garinii*, and antibodies reacted with ACGal from all three strains. A combination of chemical synthesis and enzymatic transformation steps were used to make synthetic ACGal, which was reactive with patient sera [43]. Immunization studies with ACGal will have to be performed to investigate whether ACGal can elicit a protective response.

**Prevention of tick engorgement and/or *Borrelia* transmission**

‘Tick immunity’ refers to the phenomenon in which ticks are unable to feed successfully after several tick infestations and was first described by William Trager in 1939 [44]. He observed that *Dermacentor variabilis* ticks were killed while feeding on guinea pigs after two to four tick infestations. Other parameters to measure tick immunity are reduction in tick weight, the inability to molt after feeding, the number of ticks feeding on a host, and the time of attachment and egg mass. Interestingly, salivary proteins produced during the first 24 hours of tick feeding are able to induce tick immunity in the vertebrate host [45]. Tick immunity not only affects tick feeding but can also interfere with transmission of pathogens, such as *Babesia*, *Francisella* and *Borrelia* [46,47,48]. In addition, humans were found to develop immediate and delayed cutaneous hypersensitivity reactions after repeated tick-bites, and were less likely to develop Lyme borreliosis [49]. Strikingly, BALB/C mice do not become tick immune after tick infestations, but the host immune reaction directed against tick proteins is still able to decrease transmission of *Borrelia* from infected ticks to these mice [50]. Similar results were found when serum from tick immune rabbits was transferred to mice [45].
Figure 1. Novel vaccination strategies, which could lead towards the development of a future Lyme borreliosis vaccine.

When *Ixodes* ticks take a blood meal, *Borrelia* up- and down regulate several OMPs in order to successfully migrate from the tick midgut to the mammalian host by travelling through the tick hemolymph and salivary glands. During the feeding process, ticks introduce saliva into the host skin including soluble factors with immunosuppressive and antithromostatic activity. These factors play an important role in successful tick feeding and *Borrelia* transmission and therefore can be used to prevent Lyme borreliosis, as is depicted in the figure. A) Immunization with a cocktail of several *Borrelia* OMPs or other borrelial membrane components such as glycolipids, that are expressed when *Borrelia* is transmitted from *Ixodes* ticks to the host. Antigens that elicit a bactericidal effect in a complement-independent way are preferred, since this enables spirochete killing as soon as blood enters the tick midgut. B) Immunization with *Borrelia*-binding proteins, together with *Borrelia* antigens could have a synergistically protecting effect. *Borrelia* is more exposed to borreliacidal antibodies when *Borrelia*-binding proteins are neutralized. In addition, an antibody response against *Borrelia*-binding proteins can result in opsonisation and subsequently increased uptake of *Borrelia* by phagocytic cells.
C) Immunization with tick proteins inducing an immune response leading to impaired feeding and tick rejection. As an example an antibody response directed against digest cells of the tick midgut, resulting in lysis of tick gut cells and disruption of the gut is illustrated. D) Immunization with a tick salivary protein that leads to modulation of a local host immune response unfavourable for Borrelia, e.g. induction of Th-1 responses, facilitating clearance of the spirochetes. E) Immunization with tick proteins indirectly important for survival of Borrelia could result in a more efficient immune response against Borrelia. As an example, neutralization of tick proteins with anti-complement properties, resulting in complement opsonisation of Borrelia and the formation of the terminal membrane attack complex (MAC), subsequently leading to increased uptake by phagocytic cells and lysis of Borrelia spirochetes, respectively. F) Immunization with tick proteins, which interfere with other host defense responses e.g. the host coagulation system, resulting in increased coagulation and inflammation. For more details, see the text. Abbreviations: OMP, outer membrane protein; APC, antigen presenting cell; IFN-γ, interferon-gamma; IL-12, interleukin 12.

These observations further underscore that tick proteins could be used as vaccinogens to prevent Borrelia transmission (Fig.1E, Table 2). Different approaches, as discussed below, could lead to impaired Borrelia transmission (Fig.1).

**Impairment of tick feeding**

Several proteins have been shown to impair tick feeding when used as a vaccinogen (Fig.1C). One of the studied proteins is 64P, a secreted tick cement protein of Rhipicephalus appendiculatus. Immunization with recombinant truncated 64TRP protein resulted in a humoral, a cellular and a delayed type hypersensitivity (DTH) response at the tick bite site of guinea pigs, rabbits, hamsters and cattle [51]. Anti-64TRP antibodies reacted with epitopes in the tick midgut, followed by rupture of the midgut [51]. 64TRP-immunized mice were protected against tick-borne encephalitis (TBE) virus transmitted by Ixodes ricinus [52]. Vaccination with Bm86 and Bm95, both glycoproteins located on Boophilus microplus midgut epithelium, has been studied extensively for the control of these ticks in cattle and is commercially sold as TickGARD™ and Gavac™. Immunized cattle were partially protected against tick bites, with an efficacy ranging from 85.2% to 99.6% depending on the Boophilus species [53]. Moreover, vaccination lowered the incidence (from 53 to 1.9 per 1000 animals) and mortality (from 6 to 0.18 per 1000 animals) caused by Babesia infection [48]. Until now, cross-protection with Ixodes ticks has not been studied using Bm86 and Bm95 as a vaccine.

Furthermore, it has been shown that immunization with either the tick or mosquito protein subolesin, a regulatory protein important in several cellular pathways, provides similar protection against I. scapularis infestation [54]. Subolesin could be a potent vaccine candidate since it could give cross-protection against several vectors, among which are mosquitoes, sandflies and ticks, such as Anopheles atroparvus, Aedes caspius, Culex pipiens, Phlebotomus perniciosus and I. scapularis [54].
Table 2. Vector proteins that can be used for future vaccine development

<table>
<thead>
<tr>
<th>Vaccine candidate</th>
<th>Function</th>
<th>Mechanism of action</th>
<th>Interferes with pathogen transmission</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>64P/64TRP</td>
<td>Cement protein</td>
<td>DTH response at tick bite site and ruptures tick midgut</td>
<td>Tick-borne encephalitis virus transmission</td>
<td>[51,52]</td>
</tr>
<tr>
<td>Subolesin</td>
<td>Regulatory protein</td>
<td>Increased inflammation</td>
<td>Anaplasma acquisition</td>
<td>[54,55]</td>
</tr>
<tr>
<td>FER2</td>
<td>Iron transport</td>
<td>Not determined</td>
<td>Not determined</td>
<td>[56,57]</td>
</tr>
<tr>
<td>Sialostatin L2</td>
<td>Anti-inflammatory</td>
<td>Increased inflammation</td>
<td>Not determined</td>
<td>[2,58]</td>
</tr>
<tr>
<td>Iris</td>
<td>Anti-haemostatic and anti-inflammatory</td>
<td>Most likely increased inflammation</td>
<td>Not determined</td>
<td>[59,60,61]</td>
</tr>
<tr>
<td>Salp15</td>
<td>Anti-inflammatory</td>
<td>Enhanced phagocytosis of Salp15 bound Borrelia, enhanced protective effect against Borrelia antigens</td>
<td>Borrelia transmission</td>
<td>[11,12,69,70,72]</td>
</tr>
<tr>
<td>Salp25D</td>
<td>Antioxidant</td>
<td>Detoxified reactive oxygen species at tick bite site</td>
<td>Borrelia acquisition, not transmission</td>
<td>[73]</td>
</tr>
<tr>
<td>IGBP</td>
<td>Binds and excretes IgG back into the host</td>
<td></td>
<td>Not determined</td>
<td>[75,76]</td>
</tr>
<tr>
<td>BM86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Unknown, midgut protein</td>
<td>Lysis of digestive cells of the midgut</td>
<td>Babesia, Anaplasma</td>
<td>[48,53]</td>
</tr>
<tr>
<td>SP-15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Unknown, salivary protein</td>
<td>A strong Th-1 cellular response induced an anti-pathogen response at the bite site</td>
<td>Leishmania</td>
<td>[62]</td>
</tr>
</tbody>
</table>

<sup>a</sup>Vaccinogens derived from other species. Cross-protection with *Ixodes* ticks has not been studied.
Interestingly, *I. scapularis* ticks feeding on mice immunized with subolesin displayed impaired acquisition of *Anaplasma phagocytophylum*, an obligate intracellular bacterium causing human granulocytic anaplasmosis [55]. To date, impaired transmission of tick-borne pathogens to subolesin-immunized hosts has not been reported.

The recently described FER2, an *I. ricinus* protein secreted in the tick hemolymph, was shown to be important in supplying peripheral tissues, such as tick ovaries and tick salivary glands with iron, by transporting blood meal-derived iron. After silencing of FER2 in *I. ricinus* nymphs, tick feeding was dramatically impaired [56]. When immunization studies were performed with recombinant *I. ricinus* and *Rhipicephalus microplus* FER2, tick feeding was not only impaired when adult *I. ricinus* ticks fed on rabbits immunized with *I. ricinus* FER2 but also when adult *R. microplus* and *Rhipicephalus annulatus* ticks fed on *R. microplus* FER2 vaccinated cattle [57].

Proteins with anti-inflammatory characteristics represent a group of interesting vaccine candidates. Immunization with sialostatin L2, a protein with anti-inflammatory characteristics [2], was shown to impair *I. scapularis* feeding on guinea pigs [58]. Likewise, when vaccination studies were performed with Iris, a salivary protein identified in female nymphal and adult stages of *I. ricinus* and that interferes with both the haemostasis and immune response of the host [59,60], nymphal tick feeding on vaccinated rabbits was impaired [61]. Since Iris inhibits production of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, by host leukocytes [59], it could also be that vaccination with Iris results in more effective immune responses against *Borrelia* by neutralizing the effect of Iris (Fig.1D).

**Modulation of protective immune responses**

As indicated above, a different approach to prevent *Borrelia* transmission would be to induce an immune response unfavourable for *Borrelia*, an approach that has already been shown effective in the case of *Leishmania* transmission by sand flies [62]. Mice vaccinated with the sandfly salivary protein SP-15 were protected from *Leishmania* parasites transmitted through sandflies due to strong Th-1 responses directed to SP-15 at the bite site [62]. Comparable approaches might be effective for *Borrelia*, since systemic administration of interferon (IFN)-γ (a Th1 cytokine), alone or in combination with interleukin (IL)-2 and TNF-α, in BALB/C mice during feeding of *Borrelia*-infected ticks resulted in a reduction of *Borrelia* infection rates in these mice [63]. Thus, a tick salivary protein capable of inducing strong immune responses, e.g. Th1 responses, during tick feeding when used as a vaccinogen, could potentially enhance anti-*Borrelia* immune responses facilitating clearance of the spirochete.
Neutralization of proteins that inhibit host coagulation

Another possible approach would be to target a group of salivary proteins that inhibit another host defense mechanism, e.g. the coagulation cascade (Fig.1F). Since blood coagulation is a complicated system organized in different pathways, ticks interfere with several steps of the coagulation system by producing a variety of proteins with various anti-haemostatic activities. Activation of coagulation also results in induced inflammation since there is extensive cross-talk between these two systems [64]. Until now several *Ixodes* proteins with anticoagulant properties have been described [4,60,65,66,67,68], though neutralizing antibodies against these proteins have not yet been developed.

Targeting proteins that (in)directly interact with *Borrelia*

Tick proteins that interact with *Borrelia* are potential targets. The *Ixodes* salivary protein Salp15 specifically binds to *Borrelia*’s OspC and thereby protects the spirochetes against borreliacidal antibodies [12]. As we postulated before, by using Salp15 as a vaccine candidate specific antibodies could not only neutralize the immunosuppressive effects of Salp15 [11,69,70], but could also bind *Borrelia* surface coated Salp15, enhancing clearance by host phagocytic immune cells [5,71]. Indeed, very recently it was shown that the presence of Salp15 antibodies in mice provided partial protection against tick-transmitted *Borrelia* infection [72]. Interestingly, the presence of anti-Salp15 antibodies also enhanced the protective capacity of both OspA and OspC antibodies, indicating that immunization with a tick antigen can be used to complement the protective effect of vaccination with a *Borrelia* antigen (Fig.1B).

Salp25D, an antioxidant *I. scapularis* salivary protein, was shown to be important for *Borrelia* survival in the tick-mouse cycle by protecting *Borrelia* from reactive oxygen species produced by neutrophils [73]. Unlike Salp15, this protein does not play a role in transmission from the tick to the host, but is important in the migration from the murine host to the tick; both Salp25D-silenced ticks and ticks feeding on Salp25D-immunized mice were less able to acquire *Borrelia* while engorging on *Borrelia*-infected mice. Another example of a tick salivary protein indirectly interacting with a tick-borne pathogen to ensure acquisition from the host to the tick is Salp16 [74]. *A. phagocytophilum*, but not *B. burgdorferi*, failed to enter and survive in the tick vector in the absence of Salp16. Although Salp25D and Salp16 were originally described as salivary proteins, they are also expressed in the tick midgut. TROSPA, also expressed in *I. scapularis* midguts, is required for successful midgut colonization of *Borrelia* by directly interacting with OspA [10]. Blocking TROSPA within *I. scapularis* with TROSPA antiserum showed impaired colonization of *Borrelia*, and subsequently less spirochetes transmission to the host during a subsequent blood meal. For obvious reasons these are not useful as vaccine candidates for humans. By contrast, wildlife vaccination, resulting in impaired function of these
proteins and thereby impaired pathogen acquisition, could lead to decreased infection rates in the tick population lowering the risk of pathogen transmission to humans.

Human subjects have yet to be immunized with tick proteins, and cross-reactive immune responses against human proteins remain an issue that needs to be addressed. Another potential concern regarding vaccination against tick-borne diseases is the fact that several tick species including *Ixodes* ticks have mechanisms to eliminate host antibodies ingested by the blood meal [75]. IgG binding proteins (IGBPs) are able to bind, transport and excrete immunoglobulin back into the host. *I. scapularis* has been shown to eradicate ingested host IgG [76]. Both *Borrelia* antigens and tick proteins used as vaccine candidates acting inside the tick vector could be more effective if we would be able to target and disable IGBPs, especially for antibodies that exert their function inside the tick, such as the OspA vaccine.

**Concluding remarks**

Developing a vaccine to prevent Lyme borreliosis remains a challenge. It is important to keep in mind that *B. burgdorferi* is transmitted through ticks and that spirochetes exploit tick proteins to establish an infection. Several novel approaches were discussed that could be pursued to develop an effective *Borrelia* vaccine: (i) immunization with a cocktail of several *Borrelia* OMPs (including those that elicit a bactericidal effect in a complement-independent way); (ii) immunization with tick proteins inducing an immune response at the site of the tick bite and/or inside the tick, resulting in impaired feeding and tick rejection; (iii) immunization with tick proteins, which interfere with other host defense responses (e.g. the host coagulation system); (iv) immunization with tick proteins that (in)directly interact with *Borrelia*; (v) immunization with a tick salivary protein that leads to modulation of local host immune responses, e.g. induction of Th-1 responses; or (vi) a combination of a tick salivary protein and a *Borrelia* antigen, as has been shown for the *Ixodes* tick salivary protein Salp15. Thus, the way forward in the development of an effective *Borrelia* vaccine could be combining different vaccinogens, whether these are multiple *Borrelia* antigens, tick antigens or a combination, eliciting synergistic anti-*Borrelia* and anti-tick immune responses. These approaches might not only be applicable for the prevention of transmission of *B. burgdorferi* from the tick to the host, but could also prove to be instrumental for the prevention of transmission of arthropod-borne pathogens in general.
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