Interactions between *Borrelia burgdorferi* and ticks

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Abstract | *Borrelia burgdorferi* is the causative agent of Lyme disease and is transmitted to vertebrate hosts by *Ixodes* spp. ticks. The spirochaete relies heavily on its arthropod host for basic metabolic functions and has developed complex interactions with ticks to successfully colonize, persist and, at the optimal time, exit the tick. For example, proteins shield spirochaetes from immune factors in the bloodmeal and facilitate the transition between vertebrate and arthropod environments. On infection, *B. burgdorferi* induces selected tick proteins that modulate the vector gut microbiota towards an environment that favours colonization by the spirochaete. Additionally, the recent sequencing of the *Ixodes scapularis* genome and characterization of tick immune defence pathways, such as the JAK–STAT, immune deficiency and cross-species interferon-γ pathways, have advanced our understanding of factors that are important for *B. burgdorferi* persistence in the tick. In this Review, we summarize interactions between *B. burgdorferi* and *I. scapularis* during infection, as well as interactions with tick gut and salivary gland proteins important for establishing infection and transmission to the vertebrate host.

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Lyme disease is a tick-borne disease caused by the spirochaete *Borrelia burgdorferi*, which is transmitted enzootically between ticks and their hosts, resulting in approximately 300,000 cases annually in the United States[1,2]. Globally, several species within the *B. burgdorferi* sensu lato complex have been identified as human pathogens, however, in the United States, nearly all Lyme disease is caused by *B. burgdorferi* sensu stricto (referred to as *B. burgdorferi* in this Review). Erythema migrans, the characteristic expanding rash, is an indicator of early acute infection, although the disease can also present with a variety of non-specific clinical signs. Spirochaetes enter the human skin at the tick bite site and then use internal periplasmic flagella to migrate to distal tissues, including the heart and joints[3]. Untreated infections can progress to multisystemic manifestations including rheumatologic, neurologic and cardiac disease. Similar versions of Lyme disease occur throughout the Northern Hemisphere, where *Ixodes* tick species are present. In Europe, Lyme borreliosis is caused by *B. burgdorferi* sensu lato complex spirochaetes (BOX 1), which may infect as many as 85,000 persons annually, while in Asia fewer epidemiological studies have been reported, and it is likely that the true incidence is not well understood.

The genome of *B. burgdorferi* consists of an approximately 1-Mb linear chromosome and at least 17 circular and linear plasmids, many of which are highly stable and contain genes that are crucial for survival[4,5] (BOX 2). Gene expression is highly regulated to enable the spirochaete to adapt to the different environments as it cycles between an arthropod host and a vertebrate host[6]. External cues from the host, such as temperature, pH, CO_2 levels and other biotic factors, as well as host species are important factors that regulate gene expression in *B. burgdorferi*[7–10]. *B. burgdorferi* undergoes several changes during transmission from the tick to the host to adapt to the new conditions. At the bite site, the spirochaete must evade the immune defences of the mammalian host to extravasate and establish infection in other tissues. Although *B. burgdorferi* genome encodes several proteins to facilitate these functions, it also relies heavily on interactions with tick salivary proteins injected into the bite site during the initial stage of vertebrate infection. Understanding how the spirochaetes and the tick host interact is crucial to better understand infection, pathogen transmission and potential targeted therapies.

In the United States, most tick-borne infections are transmitted by the bite of the blacklegged tick, *Ixodes scapularis*, including infections with *B. burgdorferi*, *Borrelia miyamotoi*, *Borrelia mayonii*, *Babesia microti*, *Ehrlichia muris eauclairensis*, *Anaplasma phagocytophilum* and Powassan virus. This three-host tick species is the primary vector for Lyme disease-causing *B. burgdorferi* spirochaetes. The life cycle of *I. scapularis* spans 2–4 years and includes egg, larval, nymphal and adult stages.

Box 1 | **Lyme disease and Lyme borreliosis in the United States and Europe**

Tick vectors
In the United States, *Ixodes scapularis* is the primary tick species associated with human transmission except for the West Coast, where *Ixodes pacificus* is the most important vector. In Europe, *Ixodes ricinus* is the primary vector for human transmission, although *Ixodes persulcatus* is also a source of infections in certain regions[198]. In Asia, *I. persulcatus* as well as various other *Ixodes* species and *Haemaphysalis* species are vectors for *Borrelia burgdorferi*.

***Borrelia* genospecies**
In the United States, *B. burgdorferi* sensu stricto is the aetiological agent of Lyme disease. A more recently discovered species, *Borrelia mayonii*, is also present in the North Central region of the United States, where it can overlap in clinical presentation with Lyme disease caused by *B. burgdorferi* sensu stricto, yet accounts for a much smaller number of reported human infections[199]. In Europe, most cases of Lyme borreliosis are caused by *Borrelia afzelii*, *Borrelia garinii* and to a much lesser extent *B. burgdorferi* sensu stricto and *Borrelia bavariensis*[200,201].

Estimated annual infections
In the United States ~33,000 cases were reported in 2018, although the number of estimated annual infections is closer to 300,000 (REFS[1,2,202]). In Europe, Lyme borreliosis is not a mandatory reportable disease in many countries. However, ~85,000 cases are reported annually, which is likely to be a severe underestimate[203].

Geographic distribution
Lyme disease incidence is highest in the Northeast and North Central regions of the United States, although infections can be acquired in the West Coast and South as well[19,202]. In Europe, *I. ricinus* is widely distributed throughout the continent, and human cases are likely to be closely associated with the distribution of the vector.

Hosts
In both the United States and Europe, small mammals, especially rodents, are reservoirs for *Borrelia* spp. infections and are important hosts for immature *Ixodes* spp. stages. Deer have minimal importance as reservoirs for spirochaetes that cause Lyme disease, but as the primary hosts for adult stages, they are crucial for tick reproduction. Some birds, similarly to deer, are important sources for tick dispersal and seeding in new populations[204], as well as transmission of spirochaetes.

Clinical presentation
Lyme disease (in the United States) and Lyme borreliosis (in Europe) are highly similar in their primary clinical features and may include multisystemic disease of the skin, joints, heart and nervous system. However, in the United States, systemic disease, including a rapid advancement of erythema migrans, is more common (approximately 70% of infected individuals), and in the absence of antibiotic treatments, Lyme arthritis seems to be a more likely outcome than in Europe. In Europe, neuroborreliosis is more common, acrodermatitis chronica atrophicans and borrelial lymphocytoma are reported more frequently and erythema migrans expands more slowly with greater central clearing relative to the typical presentation in the United States[198,200]. The clinical features of Lyme borreliosis seem to be associated with distinct genotypes and tissue tropisms of specific species of *B. burgdorferi* sensu lato.

Most tick-borne pathogens, including *B. burgdorferi*, are acquired during the larval or nymphal feed and are transmitted by nymphs or adults (FIG. 1), with the exception of transovarially acquired pathogens. Immature *I. scapularis* ticks are generalist feeders, parasitizing small mammals, medium-sized mammals, birds and reptiles[11]. Adults also feed on medium-sized mammals, although the primary host for this stage is the white-tailed deer, *Odocoileus virginianus*[12].

I. scapularis activity patterns are highly seasonal and vary by geography[13,14]. Tick phenology is therefore an important factor in the epidemiology of tick-borne pathogens[14,15]. In the North Central region of the United States, larvae are most active during June and July, whereas larval emergence is bimodal in the Northeast, with peaks in the spring and late summer[16,17]. Nymphs are most active in June and July, and although adults can be active year-round under ideal conditions, they are encountered most often in spring and in autumn. The incidence of Lyme disease is greatest during the months when nymphs are most active[18,19] (BOX 1). Although *I. scapularis* is also present in regions of the United States other than the North Central and Northeast regions, several factors, including disparate host-seeking behaviour of immature stages, result in a lower prevalence of *B. burgdorferi* in ticks and a lower risk of Lyme disease in these other regions[20].

Whereas much of the ecology and epidemiology of *B. burgdorferi* and Lyme disease have been understood for decades, the sequencing of the *I. scapularis* genome in 2016 as well as studies examining the tick transcriptome and proteome have greatly advanced the current understanding of the tick immune defence pathways[21–26]. In this Review, we focus on these findings and how they have enabled researchers to better understand interactions between *B. burgdorferi* and *I. scapularis*[22,27–30]. We describe key interactions specific to the tick gut and highlight the influence of the tick gut microbiota, as well as mechanisms that the spirochaete uses to alter the microbiota[31–34]. Lastly, we highlight tick–*B. burgdorferi* interactions in the salivary glands that are important for transmission to mammalian hosts.

Interactions in the gut

B. burgdorferi spirochaetes are highly motile and use periplasmic flagella to propel themselves through host fluids and tissues[35]. When a tick feeds on an infected vertebrate host, spirochaetes are attracted to the tick feeding site by chemotactic signals where they are ingested during the feeding process[36]. The tick gut is the initial site of colonization of *B. burgdorferi*[37], and there the spirochaete must overcome several barriers to persist in the tick, such as evading tick immune defences[22,29,30] and avoiding endocytic digestion in tick gut epithelial cells[38]. Most spirochaetes remain in the lumen of the tick gut for the duration of the moulting process before migrating to the salivary glands during subsequent feedings[39]. To facilitate colonization and persistence in the gut, *B. burgdorferi* has evolved elaborate mechanisms to modulate the gut environment. Importantly, *B. burgdorferi* accomplishes these processes with minimal fitness cost to the tick[40]. Here we discuss interactions between *B. burgdorferi* and the tick in the gut environment, the tick immune response to infection with *B. burgdorferi* and the mechanisms that *B. burgdorferi* uses to establish infection in the tick.

Outer surface protein interactions in the gut. The presence of spirochaetes in tick salivary glands is essential for transmission to a new vertebrate host. However, the gut is the principal tissue of residence for *B. burgdorferi* during most of its arthropod phase and is therefore also a key site for its interactions with the tick. In an unfed infected tick, *B. burgdorferi* is found closely associated with the gut epithelial cells[37,41]. As blood flow and temperature shift during the tick bloodmeal, spirochaetes must adjust to a reduction in pH from 7.4 to 6.8 (REFS[42,43]). To rapidly adapt to changes encountered in hostile and physiologically dissimilar host

Enzootically
Describes a pathogen that is maintained through transmission among non-human animal reservoirs.

Three-host tick species
Ticks that leave the host after feeding during each stage of development. This is in contrast to single-host tick species, which remain attached to the same host from larva to adults.

Neuroborreliosis
Neurological manifestation of disease that can occur as part of systemic infection with *Borrelia* spirochaetes, including *Borrelia burgdorferi*.

Acrodermatitis chronica atrophicans
A late manifestation of chronic *Borrelia burgdorferi* infection characterized by blue-red skin lesions and swelling, typically on the extremities.

environments, for example in the tick gut, *B. burgdorferi* uses preferential gene expression.

A relatively well-studied example of tick–*B. burgdorferi* interactions involves several outer surface proteins. For successful acquisition of spirochaetes following the tick bloodmeal, the outer surface proteins OspA and OspB are important for adherence and persistent colonization of the tick gut. Binding of OspA to TROSPA, a tick gut protein upregulated during tick feeding and downregulated on repletion, contributes to this process[44–48] (FIG. 2). Whereas OspA expression facilitates establishment of *B. burgdorferi* in the gut of a previously uninfected tick, for transmission to a new vertebrate host during a subsequent bloodmeal, spirochaetes must exit the gut and pass through the salivary glands. There are contrasting reports about the mechanisms of spirochaete migration. Initial studies suggested that the incoming bloodmeal and alteration in temperature and pH result in downregulation of OspA and upregulation of OspC[10,42,49]. This led to the initial hypothesis that loss of OspA expression is associated with migration of the spirochaetes from the gut, and expression of OspC is linked with movement of *B. burgdorferi* out of the tick and the establishment of mammalian infection[44,49,50]. Later studies showed no reciprocal expression of OspA and OspC; instead, OspA expression was maintained throughout the feeding process[51–53]. Additionally, robust OspA expression was detected in nearly all spirochaetes throughout the course of the bloodmeal and decreased only in the mammalian host[52]. Live microscopy has shown a biphasic process of migration in which networks of replicating non-motile spirochaetes, by adhering to differentiating, hypertrophying and detaching epithelial cells, migrate towards the basolateral surface of the gut epithelium[41]. In the second phase, the spirochaetes transition into motile organisms that are able to traverse the basal membrane, enter the haemocoel and migrate to salivary glands[41].

OspA and OspC also have a role in protecting the spirochaetes from innate defence molecules in the incoming blood. Both OspC and OspA can bind plasminogen[54] and promote conversion to plasmin, a protease that negatively regulates the complement system, thus facilitating movement through tick and host tissues[55]. OspA has also been shown to shield spirochaetes from antibodies in the tick gut[56] and is regulated by several complex global gene expression pathways that facilitate the transition back and forth between tick and vertebrate hosts. One of these pathways is the RpoN–RpoS alternative sigma factor cascade, in which RpoS functions as a gatekeeper to repress tick-phase genes, including *ospA*, during the mammalian host phase[52]. Following spirochaete acquisition by ticks, RpoS is downregulated and a second pathway, the Hk1–Rrp1 two-component system, is activated. During the tick-borne phase of the *B. burgdorferi* life cycle, Hk1–Rrp1 controls a broad subset of genes that promote survival of spirochaetes in the gut through synthesis of the second messenger c-di-GMP, which in turn induces the expression of several outer surface proteins that inhibit complement-mediated lysis[57–62]. When either the sensor kinase (Hk1) or the diguanylate cyclase response regulator (Rrp1) is rendered non-functional, spirochaetes retain the ability to infect mice but are unable to survive acquisition by ticks[58,59,62]. It was reported that while Hk1–Rrp1-deficient spirochaetes were ingested and visible in the guts of ticks feeding on infected mice, the spirochaetes were later eliminated from the gut during the course of feeding. These same mutants were able to persist in the guts of ticks into which they had been injected via a needle; however, once the ticks were allowed to feed on mice, the spirochaete burden also declined, suggesting that the mutants were unable to survive mammalian host factors within the bloodmeal[58–60,62]. Later, during reciprocal tick-to-host transmission, synthesis of RpoS induces a set of genes that facilitate physiological adaptation to the bloodmeal, chemotactic migration out of the

Box 2 | **Biology of *Borrelia burgdorferi***

Borrelia burgdorferi belongs to the phylum Spirochaetes and the spirochaetes have a distinct spiral shape with a flat-wave morphology[205]. *B. burgdorferi* spirochaetes lack classic lipopolysaccharide in the outer membrane and are described as Gram-negative-like[206]. The spirochaetes contain both an outer lipid bilayer and an inner lipid bilayer, a compositionally distinct peptidoglycan layer with flagella in the periplasmic space between the two membranes, which protects from recognition by the host immune system[207]. Approximately 7 to 11 flagella are located at both ends of the spirochaete and form a ribbon that wraps around the spirochaete[207]. The flagella give *B. burgdorferi* its structural shape and enable motility in environments such as tick saliva and the highly viscous extracellular matrix network in the dermis of mammals[207]. In the skin, several immune signalling pathways, including those signalling through MyD88, have a role in controlling the initial colonization[208]; however, spirochaetes that can evade innate immune recognition disseminate to secondary infection sites, such as the heart, joint tissues, urinary bladder and nervous system. As *B. burgdorferi* lacks classic bacterial secretion apparatus and toxins, the carditis, arthritis and neuritis observed in persistently infected patients is likely caused by the inflammatory immune response at the site of infection, which can be induced by certain spirochaete antigens, including lipoproteins.

The genome of *B. burgdorferi* is composed of an approximately 1-Mb linear chromosome and at least 17 circular and linear plasmids[4]. Although the chromosome encodes many bacterial orthologues with known or housekeeping functions, the vast majority of plasmid-encoded genes are unique to *Borrelia* spp. and are unrelated to known proteins. The genome encodes relatively few genes involved in response to oxidative and nitrosative stress[4,136,137]. Additionally, *B. burgdorferi* encodes limited genes involved in metabolic pathways; therefore, it relies heavily on the host and uses transport systems to scavenge nutrients from the environment, such as the manganese transporter *bb0219* (REFS[80,81,209]), which maintains the metabolic flexibility needed to use the different nutrients available in arthropod and vertebrate environments. In mammals, glucose is the primary source of carbon in blood[82], whereas glycerol and, to a lesser extent, chitobiose are available to spirochaetes in the tick environment[62,83–85]. The second messenger c-di-GMP upregulates genes and induces an effector protein that enables spirochaetes to use alternative pathways of carbon metabolism[60]. Moreover, *B. burgdorferi* mutants lacking the ability to use glycerol could infect mice normally yet were present at much lower levels in experimentally infected nymphs than in wild-type spirochaetes[84]. The genome also does not encode components of the tricarboxylic acid cycle or enzymes required for nucleotide and fatty acid synthesis[4].

Much of the research on *B. burgdorferi* biology has focused on lipoproteins on the outer membrane, referred to as 'outer surface proteins', because of their important role in spirochaete survival, including adaptation to and navigation of physiologically and immunologically hostile host environments. Several studies have documented antigenic variation in outer surface protein expression as *B. burgdorferi* transitions between the tick host and the mammalian host[10,48–50,52]. In the mammalian host, certain outer surface proteins can activate neutrophils, B cells, T cells and dendritic cells, whereas others inhibit neutrophils, natural killer cells and complement activation[210]. Recent evidence demonstrated that *B. burgdorferi* can shield itself from immunogenic proteins with the highly variable VlsE surface protein[211]. The VlsE antigen is essential for persistence in the mammalian host and undergoes robust antigenic switching through recombination events in the *vlsE* locus and 15 silent *vls* cassettes[212]. Importantly, the high degree of heterogeneity, as well as the shielding and antigenic switching, complicates the development of an effective vaccine.

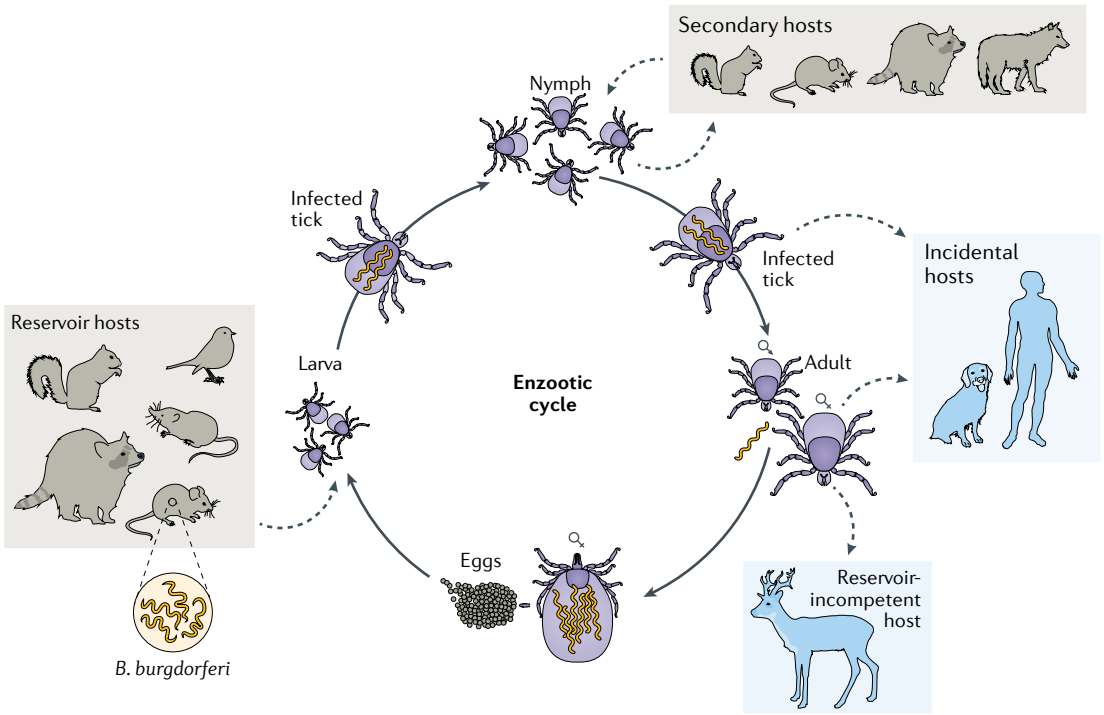

Fig. 1 | **The life cycles of *Ixodes scapularis* and *Borrelia burgdorferi*.** Uninfected larvae hatch and seek a host to feed on, which is typically a small mammal or bird, but may include larger animals. Because *Borrelia burgdorferi* is not transmitted transovarially, this life stage is the primary opportunity for spirochaetes to infect ticks that feed on an infected host. After feeding, the six-legged larvae moult and emerge as eight-legged nymphs, which may be infected with spirochaetes acquired during their initial bloodmeal. Nymphs seek a second host, typically a small or medium-sized mammal, and this bloodmeal may offer a second opportunity for spirochaetes to infect ticks. Importantly, nymphs infected during the larval bloodmeal can transmit spirochaetes to hosts, including humans and domestic animals. After fed nymphs have moulted to the adult stage, newly emerged adult *Ixodes scapularis* ticks search for a large animal host, typically white-tailed deer, for mating and a final bloodmeal. Although deer are the preferred hosts, adult female ticks will also feed on humans and domestic animals, which can acquire *B. burgdorferi*, but are relatively unimportant to further perpetuation of infections. Because ticks cannot acquire *B. burgdorferi* from deer, these hosts are not effective reservoirs for *B. burgdorferi*, although they are important for perpetuation of tick populations. After mating, engorged females release themselves from hosts and eventually oviposit an egg mass, which may contain hundreds to thousands of eggs. *I. scapularis* ticks produce only a single clutch of eggs and then die. Solid arrows denote progression steps in the tick life cycle and dashed arrows denote host preferences for specific tick life stages.

gut and transition to a form that is infectious for mammalian hosts[51,59,63–66]. In addition, BadR (*Borrelia* host adaptation regulator) represses the transcription of *rpoS*, whereas BosR activates *rpoS* transcription and represses *ospA* and *ospB*[67–70]. BadR-deficient spirochaetes cannot infect mice, presumably because they lack the ability to repress RpoS and lack the subsequent ability to transition from expression of tick-phase genes to mammalian phase genes[69,70].

BBE31 is another outer surface lipoprotein with a recognized role in *Borrelia*–vector interactions. Peak expression of BBE31 occurs in the gut of nymphs on days 2 and 3 after feeding, suggesting an effective role during the period when transmission of spirochaetes occurs[71]. Antibody fragments to BBE31 introduced during this period did not affect *B. burgdorferi* burden in the gut, yet reduced spirochaete presence in haemolymph and salivary glands, presumably though interference with migration out of the gut. TRE31 is a secreted tick protein and a specific binding partner of BBE31. *B. burgdorferi* infection induces TRE31 expression; it is expressed in the gut of fed and unfed *I. scapularis* but not in the salivary glands. Blocking expression of TRE31 in feeding ticks had no effect on the presence of spirochaetes in the gut; however, there was a clear reduction of the bacterial burdens in haemolymph and salivary glands, indicating that BBE31–TRE31 interaction is important for transmission to new hosts[71].

Another important factor in the vector stage of *B. burgdorferi* is the outer membrane surface protein BBA52, which is upregulated early during tick feeding[72]. A mutant lacking functional *bba52* was able to establish infection in needle-inoculated mice; however, tick-to-mouse transmission of spirochaetes was inhibited, and tick acquisition of mutants from hosts was also reduced[72]. Although the specific function of this gene is not well understood, it is clearly essential to the entry of spirochaetes to and their exit from the tick.

Recently, a yeast surface display library encoding tick gut proteins was used to identify interactions between whole *B. burgdorferi* spirochaetes and tick gut proteins. In this study, the authors identified two proteins, Ixofin3D and ISDLP, as potential *B. burgdorferi* interactants[73]. Ixofin3D is a fibronectin III domain-containing gut

Borrelial lymphocytoma
A skin lesion that presents as a blue-red skin nodule characterized by robust infiltration of B lymphocytes following *Borrelia* spp. infection.

Phenology
The study of how climate and seasons can impact the life cycle of a population.

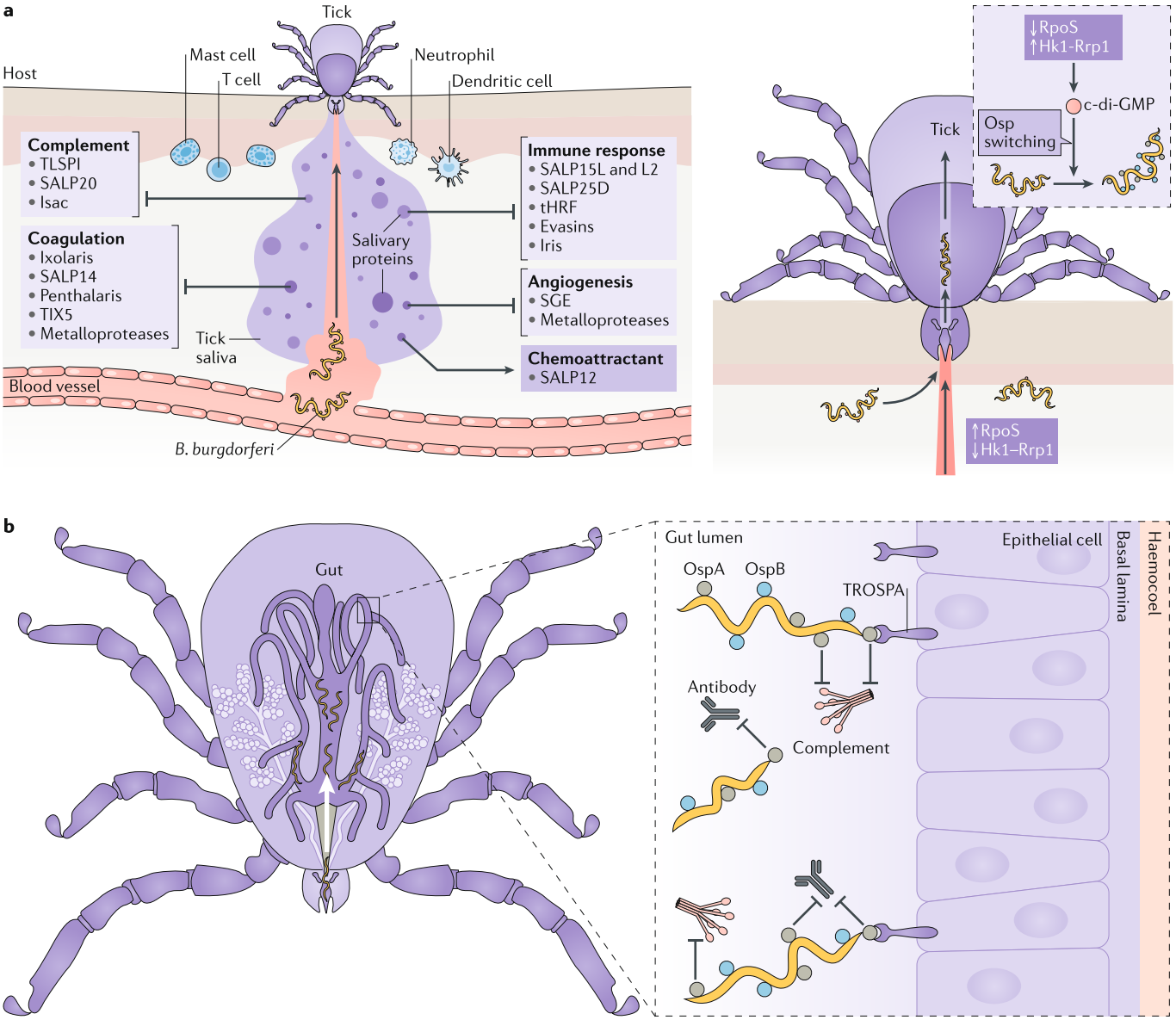

Fig. 2 | **Acquisition of *Borrelia burgdorferi*. a** | When feeding on an infected host, the *Ixodes scapularis* tick takes up *Borrelia burgdorferi* with the bloodmeal. The tick injects the salivary protein SALP25D into the host skin to dampen inflammation at the feeding site. SALP25D quenches reactive oxygen species generated by activated neutrophils at the bite site[186]. The activity of SALP25D enhances *B. burgdorferi* acquisition by the tick, possibly by increasing viability of the spirochaetes at the bite site. SALP12 functions as a chemoattractant for *B. burgdorferi* and increases the quantity of spirochetes entering the tick[36]. Entry of *B. burgdorferi* into the tick induces upregulation of Hk1–Rrp1 and downregulation of RpoS, resulting in production of c-di-GMP, an important molecule regulating expression of outer surface proteins. **b** | Spirochaetes ingested in the bloodmeal adhere to the tick gut and remain in this organ until a subsequent tick feeding. *B. burgdorferi* expresses the outer surface proteins OspA and OspB, which protect spirochaetes from harmful components in host blood[56], including antibodies and complement, and enable them to adhere to and persist in the gut[44,45,47]. OspA interacts specifically with the tick receptor TROSPA, which is located on the luminal surface of gut epithelial cells and is upregulated when spirochaetes are ingested[48]. tHRF, tick histamine release factor.

protein and binds *B. burgdorferi*, facilitating its congregation towards epithelial cells. Although the *B. burgdorferi* genome encodes at least two proteins that bind host fibronectin, BBK32 (REF.[74]) and RevA[75], they are associated with infection in mice[76,77] but they are not essential in the tick phase[78], and neither protein bound Ixofin3D. Therefore, the specific mechanism of interaction of this protein with *B. burgdorferi* remains unclear; however from a functional perspective, it was suggested that the aforementioned congregation would help direct the spirochaete away from the gut lumen and promote its exit from the gut[73]. ISDLP is a dystroglycan-like tick protein and is expressed abundantly on the surface of gut epithelial cells. It bound to *B. burgdorferi* and facilitated spirochaete migration from the gut by mechanisms that also remain to be determined[79].

Similarly to many zoonotic pathogens, *B. burgdorferi* lacks certain metabolic pathways and depends on its hosts for essential nutrients[4]. Because *I. scapularis* feeds only once per life stage, during much of the tick lifespan, available nutrients are limited, and *B. burgdorferi* must compete with the tick and its microbiota for nutrients. However, to assist with this, *B. burgdorferi* uses a transport system to scavenge nutrients from its hosts[80,81] and maintain metabolic flexibility to use the different nutrients available in arthropod and vertebrate environments. This includes acquisition of carbon, where during infection of mammals, glucose is the primary source of carbon in blood[82], whereas glycerol and, to a lesser extent, chitobiose are available to spirochaetes in the tick environment[62,83–85]. This was demonstrated by the observation that *B. burgdorferi* mutants lacking a gene necessary for glycerol metabolism could infect mice yet demonstrated a reduced capacity to replicate in ticks[84].

Influence of the tick microbiota. All multicellular eukaryotes coexist with a collection of commensal microorganisms primarily in the gut[86,87]. This mutualistic partnership provides important benefits for overall gut health, immune responses, pathogen sensing and metabolic functions[88]. Although most microbiota research has been performed in vertebrates, the importance of the microbiota in arthropods has become an exciting topic recently[89,90]. Similarly to the microbiota of other metazoans, the tick microbiota is likely composed of bacteria, archaea, fungi and viruses[91], but bacteria are the predominant members. Several recent studies and reviews have characterized microbiota diversity in ticks[34,91–97]. There are differing opinions on the extent of the diversity of the tick microbiota. Although several studies have suggested that hundreds of bacterial genera are associated with *Ixodes* ticks, it is becoming apparent that the abundance and diversity of the tick microbiota may be inflated by spurious contamination due potentially to the low biomass of tick samples[34]. There is growing evidence that the tick microbiota is likely less complex than initially thought[34,98], composed predominantly of the endosymbiont *Rickettsia buchneri*[99] and other bacterial genera, such as *Enterococcus*, *Pseudomonas*, *Staphylococcus*, *Lysinibacillus* and *Bacillus*, at much lower abundance[31,33,34,98]. The rickettsial endosymbiont of *Ixodes pacificus* has been suggested to provide folate, a key vitamin absent in the bloodmeal, to the tick[100]. Thus, it is likely that rickettsial endosymbionts are preferred and vital inhabitants of the tick. Although the tick microbiota might be unstable, with the exception of *R. buchneri*, the bacterial members that associate with the tick, even if transiently, may impact the tick and its interactions with the pathogens it harbours. Differences in findings are in part due to the development of technologies and approaches to study the microbiota, in addition to technical issues, such as geography, sex, temperature, stage of development and wild versus laboratory-grown ticks[31,32,91,93,101–107]. Here we focus on several studies that have associated microbiota composition in ticks with susceptibility to *B. burgdorferi* infection[31,33,108].

A study in 2014 demonstrated that the microbiota has an important role in facilitating *B. burgdorferi* colonization[32]. In this study, the authors produced dysbiosed *I. scapularis* larvae by placing surface-sterilized female progenitors in sterile containers, thus limiting the exposure of eggs and hatching larval ticks to the normal environmental microbiota. Larval ticks raised in sterile containers harboured decreased relative abundance of *Acinetobacter* spp., *Brevibacterium* spp., *Lysinibacillus* spp. and *Staphylococcus* spp. compared with ticks grown in normal laboratory conditions. Dysbiosis resulted in decreased *B. burgdorferi* colonization when the larvae were allowed to feed on infected mice, suggesting that the microbiota impedes effective *B. burgdorferi* colonization[32]. While the state of the microbiota can influence colonization efficiency, *B. burgdorferi* can also actively alter the microbiota during the course of infection to generate an environment that is conducive for colonization[33]. When *I. scapularis* ticks fed on *B. burgdorferi*-infected mice, spirochaete presence increased gene expression of several tick genes in the gut such as the gene encoding PIXR (protein of *I. scapularis* with a reeler domain). PIXR inhibits biofilm formation, and RNA interference-mediated knockdown of PIXR decreased *B. burgdorferi* colonization, suggesting that *B. burgdorferi* induces PIXR expression to enhance colonization in the tick[33]. Changes to biofilm formation could impact the ability of *B. burgdorferi* to adhere to the gut epithelium and traverse intercellular junctions in order to access the haemocoel and migrate to the salivary glands[41].

The microbiota could impact *B. burgdorferi* colonization through various mechanisms. The genome of *B. burgdorferi* lacks several genes important for different metabolic pathways, such as synthesis of nucleotides, fatty acids and thiamin[4,28,109]; therefore, spirochaetes rely on the arthropod host and the bloodmeal to acquire these essential nutrients and metabolic products. Endosymbionts have an important nutritional role for arthropods[110]; therefore, alterations to the microbiota could impact *B. burgdorferi* infection. A study queried the genomes of several *Borrelia* species, including *B. burgdorferi and Borrelia afzelii*, and showed that *Borrelia* spp. lack interbacterial effector and immunity genes that would be crucial for survival in a polymicrobial milieu[34]. Consistent with this rationale, the abundance of *Pesudomonas*, *Bacillus* or Enterobacteriaceae was negatively correlated with *B. burgdorferi* abundance[34,91]. Additional studies are required for a mechanistic understanding of the impact of the microbiota on *B. burgdorferi* survival in the tick.

Alterations to the tick microbiota also disrupt the structural integrity of the peritrophic matrix, which provides a barrier between the incoming bloodmeal and the gut epithelium[32,111]. Changes to the microbiota decreased expression of peritrophin, a key structural component, and disruption of the peritrophic membrane, which reduced *B. burgdorferi* colonization and adherence to the gut lumen[32]. These alterations to the microbiota were accompanied by decreased immune activation through the JAK–STAT pathway[32] (FIG. 3). Demonstrating a functional link between STAT and the integrity of the peritrophic membrane, the study showed that STAT was a transcriptional activator of several members of

Peritrophic matrix
A matrix of carbohydrates and proteins secreted by the tick gut during feeding, which acts as a barrier to protect the mucosal surface of the gut epithelium from abrasion by the incoming bloodmeal.

the peritrophin family of genes on the basis of the presence of canonical STAT-binding sites in their promoter regions[32]. Differential STAT activation could also potentially impact the tick immune responses towards the spirochaetes, as discussed in the next subsection[32].

The tick immune system. Ticks, like arthropods, have an immune system to detect and control potential pathogens. Therefore, *B. burgdorferi* has evolved strategies to evade tick immune defences. Most of our knowledge of the arthropod immune system comes from research in *Drosophila melanogaster*[112,113]. The arthropod immune system consists of the immune deficiency (IMD) pathway, the JAK–STAT pathway and Toll receptor signalling[113–117]. Activation of these pathways can occur through various mechanisms, including recognition of pathogen-associated molecular patterns and activation of the Toll receptor ligand Spaetzle, or induction of cytokine-like molecules[118]. In *Drosophila*, activation of these pathways triggers the production of several effector molecules, including antimicrobial peptides, which contributes to controlling infection by invading bacteria, viruses or fungi[118]. Unlike *Drosophila*, in which specific antimicrobial peptides are activated by specific immune pathways, relatively limited information is available in ticks. Several recent computational studies have characterized the immune system in ticks and identified key similarities and differences with *Drosophila*[25,26,119]. Initial studies to characterize the *I. scapularis* immune system identified 234 genes that were categorized into nine immune pathways: gut–microorganism homeostasis, agglutination, leucine-rich repeat proteins, proteases, coagulation, non-self recognition and transduction via Toll, IMD pathway and JAK–STAT pathways, free radical defence, phagocytosis and antimicrobial peptides[119]. The recent sequencing of the *I. scapularis* genome expanded on these findings and identified additional components encoded in the IMD, JAK–STAT and Toll signalling pathways[21]. Despite missing several components of the immune signalling pathways, ticks maintain a functional immune response against invading pathogens[22,26,30,120–122].

The IMD pathway in *Drosophila* has been well characterized and is primarily important for resistance to Gram-negative bacteria[118,123–125]. The IMD pathway is activated by diaminopimelic peptidoglycans binding

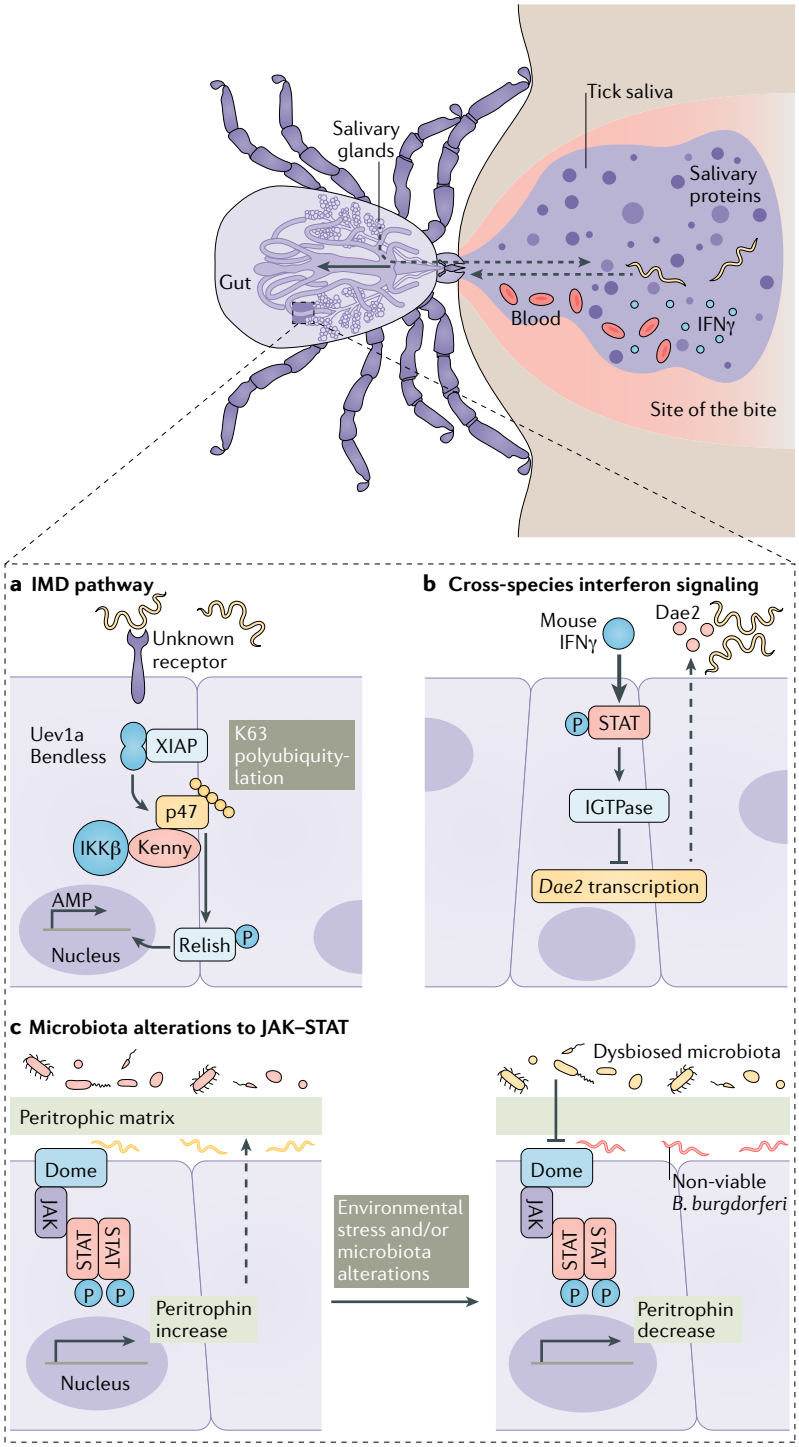

Fig. 3 | **Tick immune defences against *Borrelia burgdorferi* infection. a** | Infection with *Borrelia burgdorferi* activates the immune deficiency (IMD) pathway in *Ixodes scapularis*. On activation, p47 is polyubiquitylated by X-linked inhibitor of apoptosis (XIAP) in complex with the Bendless–Uev1a heterodimer. Downstream signalling through Kenny results in phosphorylation of Relish and transcription of antimicrobial peptides (AMPs). **b** | Uninfected *I. scapularis* can ingest mouse interferon-γ (IFNγ) along with the bloodmeal when feeding on *B. burgdorferi* infected mice. Mouse IFNγ signals in the tick gut through an unknown receptor, resulting in STAT-mediated activation of a Rho-like GTPase (IGTPase) and production of the AMP Dae2. **c** | Certain microbiota compositions enable activation of the JAK–STAT pathway by an unknown molecular signal and in turn induce the expression of peritrophin genes. Peritrophins are crucial for the formation of a structurally intact peritrophic matrix. *B. burgdorferi* uses the peritrophic matrix as a shield or barrier protecting it from toxic contents during colonization of the gut epithelium. STAT-induced immune effectors, as well as environmental changes, can alter the microbiota composition. Certain changes in the microbiota composition impair JAK–STAT signalling, and this results in decreased expression of peritrophins, leading to a thinner and compromised peritrophic matrix. A compromised peritrophic matrix no longer functions as a protective shield and thus impairs *B. burgdorferi* colonization of the gut epithelium.

to peptidoglycan recognition proteins, resulting in the production of antimicrobial peptides[112,116]. *I. scapularis* encodes several genes involved in IMD pathway activation, such as *caudal*, *relish*, *tak1*, *posh* and *caspar*; however, several signalling components are absent[21,25,26]. Although ticks are missing various proteins involved in the IMD pathway signalling cascade, several important proteins have been identified that are involved in the activation of the IMD pathway on infection with *B. burgdorferi*, as well as *A. phagocytophilum*[21,22,25,29]. On infection and pathogen detection, the tick E3 ubiquitin ligase X-linked inhibitor of apoptosis (XIAP) ubiquitylates p47, allowing p47 to activate the NF-κB regulator Kenny[29] (FIG. 3). This interaction between XIAP and p47 is critical for IMD pathway activation and nuclear translocation of the NF-κB homologue Relish, which is important for antimicrobial peptide expression[29,126]. *B. burgdorferi* does not contain diaminopimelic peptidoglycans in its envelope[127]. Instead, lipids that make up the bacterial membrane, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol and 1-palmitoyl-2-oleoyl diacylglycerol, activate the IMD pathway[22]. Additionally, RNAi knockdown of several tick genes involved in IMD signalling, such as *caspar*, *relish*, *uev1a* and *bendless*, resulted in increased *B. burgdorferi* burden in ticks allowed to feed on infected mice[22]. Overall, these results clearly demonstrate activation of the IMD pathway on infection with *B. burgdorferi*; however, additional studies to identify the effector antimicrobial peptides involved in killing and/or inhibiting *B. burgdorferi* are still needed.

Several recent studies have identified a role for the JAK–STAT pathway in controlling *B. burgdorferi* infection in ticks. The JAK–STAT pathway is conserved between *Drosophila* and ticks[128], and *I. scapularis* maintains several components of the JAK–STAT pathway, such as *stat*, *hop*, *dome*, *pias* and *socs*[21]. *I. scapularis* does not encode the canonical ligand Upd, a secreted protein in *Drosophila*, which binds and activates the JAK receptor Dome[21,129]. Nevertheless, despite lacking *upd*, ticks maintain a functional JAK–STAT pathway and use this pathway to control pathogen burden[30,121]. A recent study identified cross-species cytokine signalling between mice and ticks[30]. In this study, when *I. scapularis* ticks were allowed to feed on *B. burgdorferi*-infected mice, interferon-γ (IFNγ) was also ingested with the bloodmeal. Murine-derived IFNγ activated *I. scapularis* STAT in the gut through an unknown receptor[30]. Activated STAT induced activation of a tick-encoded Rho-like GTPase (IGTPase) and production of the antimicrobial peptide Dae2 (FIG. 3). Dae2 is suggested to be an important antimicrobial peptide for controlling *B. burgdorferi* during acquisition, although a direct effect of Dae2 on *B. burgdorferi* remains to be demonstrated[122]. Knockdown of Dae2 in *I. scapularis* resulted in increased *B. burgdorferi* burden after feeding on infected mice. Importantly, activation of the JAK–STAT pathway has also been observed on infection with *A. phagocytophilum*[121]. *A. phagocytophilum* infection activated the JAK–STAT pathway, resulting in expression of a 5.3-kDa antimicrobial peptide. RNAi knockdown of STAT increased *A. phagocytophilum* burden in salivary glands and increased transmission[121].

The Toll signalling pathway has been well studied in *Drosophila* and has an important role in defence against Gram-positive bacteria[118,130,131]. Activation of the Toll pathway occurs through the Toll receptor ligand Spaetzle, which is secreted in an inactive form and cleaved on extracellular detection of bacterial components, such as lysine-type peptidoglycan. Spaetzle binding activates the Toll receptor, resulting in binding to the adaptor protein MyD88 and downstream signalling[132]. *I. scapularis* encodes several components of the Toll signalling cascade, such as *toll*, *myd88*, *spaetzle*, *tube*, *pelle*, *cactus* and *dorsal*[21]. Additionally, expression of several *toll* genes and *myd88* is upregulated on infection with *B. burgdorferi*[30]. However, the role of Toll activation in controlling *B. burgdorferi* infection has not been determined and is an important area of future research.

The previously described studies demonstrate that the JAK–STAT and IMD pathways have important roles in controlling *B. burgdorferi* infection[22,29,30,122]. However, although *B. burgdorferi* infection activates the JAK–STAT and IMD pathways, these defence systems are not able to eliminate the spirochaete from the tick. Little is known regarding how *B. burgdorferi* can circumvent restriction by the IMD and JAK–STAT pathways in the tick gut.

The *B. burgdorferi* genome encodes several proteins to control immune activation in mammals during transmission, such as BBA57, which dampens interferon activation[133]. Whether the *B. burgdorferi* genome encodes proteins that impair tick immune activation pathways through direct protein–protein interactions is unknown and requires further investigation. A possible mechanism to dampen activation of tick immune defences could be through the formation of a molecular barrier that surrounds the gut epithelial layer, termed the 'dityrosine network' (DTN)[134]. In mosquitoes, formation of the DTN limits immune activation during a bloodmeal, which could potentially disrupt the beneficial gut microbiota[134]. Similarly to other arthropods, *I. scapularis* encodes a dual oxidase (*duox*) and a peroxidase (*ISCW017368*) involved in DTN formation[135]. Knockdown of *duox* or the peroxidase impaired DTN formation, resulting in activation of tick immunity and reduced *B. burgdorferi* colonization[135]. Silencing of *duox* also increased nitric oxide synthase activity. Production of reactive nitrogen species is an additional protective mechanisms that ticks use to control pathogens, such as *B. burgdorferi*, by targeting DNA[136,137]. However, *B. burgdorferi* can counter the nitrosative stress through expression of the nucleotide excision repair gene *uvrB*[137]. Alternatively, as *B. burgdorferi* infection persists through multiple life stages of the tick, *B. burgdorferi* has evolved strategies to minimize fitness costs to the tick. It is possible that *I. scapularis* becomes tolerant of *B. burgdorferi* infection because immune activation could be detrimental to the host.

Interactions in the salivary gland

Once at the salivary gland, *B. burgdorferi* is transmitted with the tick saliva during feeding. Tick feeding is a dynamic process that involves penetrating the epidermis, digesting tissue, dilating capillaries, preventing coagulation and dampening immune responses at the

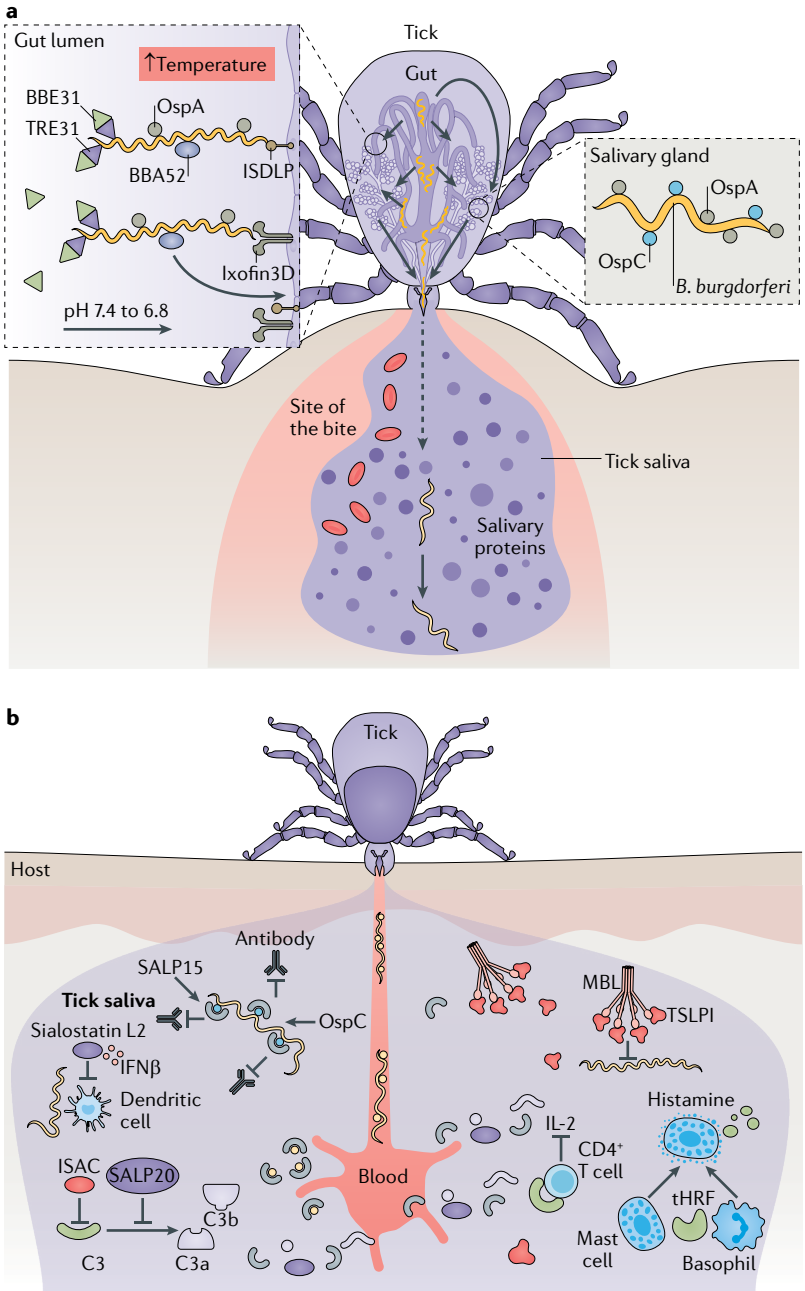

Fig. 4 | **Transmission of *Borrelia burgdorferi* to a vertebrate host. a** | Several environmental changes that occur at the onset of tick feeding are cues for spirochaetes in the gut to transition to a form that is infectious for vertebrates and to initiate migration to the salivary glands. Outer surface proteins important for this process include BBA52, which is upregulated during the early stages of feeding[72], and BBE31, which interacts with the tick receptor TRE31 to enable the spirochaetes to exit the gut epithelial layer and migrate through the haemocoel to the salivary glands[71]. Ixofin3D and ISDLP are other proteins expressed by epithelial cells that bind spirochaetes and are thought to assist in exit from the gut[32,79]. Spirochaetes outside the gut express OspA and OspC, which promotes binding to tick salivary glands and early dissemination in the vertebrate host[10,197]. **b** | Transmission of *Borrelia burgdorferi* to a mammalian host is enhanced by the activity of several tick salivary proteins. As the tick feeds, several proteins are secreted into the host to modulate the host environment and to obtain a complete bloodmeal. These proteins also assist *B. burgdorferi* transmission. Complement is an important immune defence mechanism that restricts *B. burgdorferi*, as well as tick feeding. The tick salivary proteins ISAC, SALP20 and TSLPI inhibit activation of complement and increase *B. burgdorferi* transmission[164,179,180]. Sialostatin L2 also modulates the immune response against the tick bite by impairing cytokine secretion by dendritic cells on exposure to *B. burgdorferi*[167]. Tick histamine release factor (tHRF) is a salivary protein secreted during the late stage of tick feeding and triggers the release of histamine, presumably from mast cells or basophils[185]. The best studied salivary protein is SALP15, which enhances *B. burgdorferi* transmission. *B. burgdorferi* expresses OspC on its surface during migration from the gut to the salivary glands. SALP15 binds OspC and can shield the spirochaete from antibody-mediated killing[190]. Additionally, SALP15 suppresses CD4+ T cell function and IL-2 secretion[161].

bite site[138]. These processes are mediated by proteins injected into the host through the tick saliva[139]. Tick saliva contains at least several hundred proteins, possibly thousands[24]. Saliva composition varies between life stages of the tick, the type of host it is feeding on and also during feeding to adapt to the changing conditions encountered during feeding[23,140–142]. For example, the tick secretes an adhesive cement-like substance shortly after insertion of the mouthparts to anchor itself in the skin. Subsequently, saliva composition shifts to proteins that facilitate acquisition of the bloodmeal and immune evasion, and eventually wound healing and detachment from the host[23,143,144]. One study found that the composition of *I. scapularis* saliva changed every 24 hours, although it is likely that salivary proteome changes occur at shorter intervals[23]. Although the primary function of these proteins is to facilitate acquisition of a bloodmeal, they can also benefit *B. burgdorferi* transmission[145] (FIG. 4). For example, the immune response against *B. burgdorferi* is highly dependent on tick salivary proteins. Mice infected with *B. burgdorferi* developed a type 2 T helper cell response, whereas mice inoculated with a syringe developed a mixed type 2 T helper cell and type 1 T helper cell response[146]. These differences are likely mediated, at least in part, by the proteins in saliva. Saliva and salivary gland extract can downregulate IFNγ and IL-2 production in T cells stimulated with concanavalin A and inhibit T cell proliferation[147,148]. By contrast, *B. burgdorferi* alone induces IFNγ production[149]. In the following subsections, we will discuss key salivary proteins that facilitate a successful bloodmeal and how they impact *B. burgdorferi* transmission and/or acquisition.

Salivary proteins important for tick feeding. Innate defences in the skin are a major hurdle for ticks to overcome to obtain a complete bloodmeal. In short, ticks digest tissue, insert their hypostome and take in blood, which pools at the wound[138]. Injury to skin normally triggers wound healing, which consists of three overlapping phases — inflammation, tissue formation and tissue repair[144]. Wound healing could be detrimental to tick feeding; therefore, ticks have evolved strategies to modulate host response. Initial damage to the skin disrupts blood vessels, resulting in release of extracellular

Type 2 T helper cell response
Immune response by effector CD4+ T cells characterized by production of cytokines promoting B cell proliferation and antibody production.

Type 1 T helper cell response
Immune response by effector CD4+ T cells characterized by production of proinflammatory cytokines involved in the killing intracellular pathogens.

Hypostome
A component of a tick's mouthpart apparatus that serves to anchor the tick in the host's skin during feeding.

adenosine triphosphate and adenosine diphosphate, which stimulates platelet aggregation and inhibits blood flow[150]. Ticks use several strategies to maintain blood flow. The saliva of *Ixodes* spp. ticks contains an apyrase enzyme that degrades adenosine diphosphate to adenosine monophosphate and thus inhibits platelet aggregation[151]. Similarly, saliva proteins in *I. scapularis* such as SALP14, TIX, Ixolaris, and Penthalaris have anticoagulation properties[152–154]. Knockdown of SALP14 by RNAi reduced the anticoagulation activity of saliva, as measured by the activity of complement factor Xa, which is important for thrombin activation. SALP14 knockdown also inhibited tick feeding and reduced engorgement by 50–70% (REF.[152]). Several additional proteins, such as ISL 929, and metalloproteinases also have important roles in controlling wound healing in response to the tick bite[155–158].

Skin contains several resident immune cells, such as macrophages, mast cells, dendritic cells, Langerhans cells and T cells, that are important for protection against invading pathogens[159]. Evasion of the immune response is crucial for the tick as feeding involves injection of foreign proteins into the host over the course of several days. Ticks have evolved several strategies to control recruitment of inflammatory cells and cells involved in tissue repair. Tick saliva and salivary gland extract inhibit angiogenesis at the bite site[157,160]. Additionally, saliva dampens the inflammatory immune response by directly targeting immune cells and complement activation at the tick bite site[161–164]. Several proteins in saliva inhibit T cell proliferation and decrease type 1 T helper cell cytokine production[165,166]. For example, sialostatin L2 suppresses the immune response by interfering with activation of the JAK–STAT pathway and IFNβ production in murine dendritic cells[167]. In addition to controlling inflammation at the bite site, sialostatin L2 has an important role during pathogen transmission[168,169]. Sialostatin L2 inhibits the formation of the NLRC4 inflammasome during transmission of *A. phagocytophilum* by binding annexin A2, which is required for NLRC4 oligomerization and inflammasome formation[168,170]. Immunization of guinea pigs against sialostatin L2 resulted in decreased engorgement, suggesting that the immunosuppressive function of sialostatin L2 is important for tick feeding[171].

Salivary proteins that facilitate Borrelia transmission. The complement system is an important component of the innate immune system involved in clearance of invading pathogens. There are three major pathways, lectin, classical and alternative, that can activate complement[172]. On activation, effector pathways result in opsonization and phagocytosis, generation of proinflammatory anaphylatoxins or direct lysis of the pathogen by the formation of membrane attack complexes[172]. Complement activation has an important role in controlling *B. burgdorferi* dissemination in mice[173,174]; therefore, *B. burgdorferi* has evolved mechanisms to counter the effects of the complement cascade through expression of complement inhibitor proteins on the outer membrane, such as complement regulatory-acquiring surface proteins (CRASPS), which directly interfere with complement activation[175–177]. Several studies have reported that tick salivary proteins can be important in controlling complement activation to enhance *B. burgdorferi* transmission[163,164]. Multiple salivary proteins from *I. scapularis* can inhibit the complement pathway, such as Isac, TSLPI and SALP20 (REFS[163,164,178,179]). For example, SALP20 from *I. scapularis* inhibits the alternative complement pathway by preventing cleavage of C3 to C3a and C3b[178]. Furthermore, SALP20 partially protected *B. burgdorferi* from complement mediated lysis[180].

Knockdown of TSLPI in ticks impaired *B. burgdorferi* transmission to mice[164]. Similarly, infected ticks initiated less pathogen transmission to mice immunized against TSLPI than non-immunized mice. In vitro, *B. burgdorferi* was resistant to complement-mediated killing in the presence of recombinant TSLPI. TSLPI binds mannose-binding lectin, a pattern recognition protein involved in activation of the lectin pathway, and thereby TLSPI inhibits the lectin–complement cascade[164,181]. Additionally, mannose-binding lectin is involved in controlling *B. burgdorferi* infection in the skin[182].

Controlling the host's immune response during the different phases of tick feeding is critical for obtaining a successful bloodmeal. Histamine released from degranulated basophils and mast cells has a negative impact on tick feeding within the first 24 hours from attachment[183,184]. Therefore, ticks secrete several histamine-binding proteins to reduce the detrimental effects of histamine on feeding and attachment; however, ticks also encode proteins to stimulate degranulation during the late phase of tick feeding[185]. Tick histamine release factor (tHRF) is upregulated in the salivary glands of infected *I. scapularis* nymphs during the late stages of feeding[185]. tHRF stimulates the release of histamine from basophils in mice, possibly as a mechanism to increase blood flow and pooling. Knockdown of tHRF impaired tick feeding on mice, and mice that received passive transfer of tHRF antiserum also showed decreased *B. burgdorferi* transmission.

Neutrophils are the first cells to be recruited to the site of acute inflammation. SALP25D is a secreted salivary protein important for combating neutrophils at the bite site[186,187]. Recombinant SALP25D protected spirochaetes from activated neutrophils compared with control protein. Knockdown of SALP25D in *I. scapularis* impaired *B. burgdorferi* acquisition in ticks[187]. Additionally, the study authors demonstrated that SALP25D is an antioxidant protein that quenches reactive oxygen species generated by activated neutrophils. Although the *B. burgdorferi* genome encodes enzymes that remove reactive oxygen species, SALP25D is a clear example of *B. burgdorferi* co-opting a vector protein to help maintain its life cycle[186]. Furthermore, two secreted salivary proteins, ISL929 and ISL1373, which belong to a family of disintegrin-like proteins, reduced neutrophil recruitment in vivo[158], and this function provided a substantial advantage for *B. burgdorferi* at the vector–host interface, representing yet another example of saliva-assisted transmission[188]. SALP15 is a unique example of a salivary protein that promotes *B. burgdorferi* transmission by binding to the spirochaete. SALP15 also binds to $CD4^+$ T cells and inhibits T cell activation and IL-2 signalling[161,189]. SALP15 interacts directly with

Opsonization
The process of coating a foreign surface with antibodies and complement to facilitate phagocytosis.

Anaphylatoxins
Proinflammatory complement fragments C3a, C4a and C5a produced during activation of complement.

B. burgdorferi during migration out of the tick, and is able to enhance transmission to naive mice[190]. Mice inoculated with spirochaetes preincubated with recombinant SALP15 had a higher *B. burgdorferi* burden compared with mice preincubated with a control protein. SALP15 binds directly to OspC on the spirochaetes, protecting them from antibody-mediated killing[163,190]. Knockdown of SALP15 in ticks strongly reduced transmission to mice. Mice actively immunized with SALP15 were also partially protected against infection with tick-borne *B. burgdorferi*[191].

Conclusions

B. burgdorferi has evolved complex mechanisms to infect both vertebrates and arthropods. An expanding set of research tools is facilitating an increasingly deeper understanding of these features. The recent sequencing of the *I. scapularis* genome and bioinformatics work has improved our understanding of *Borrelia*–tick interactions[21,25,26]. These studies have helped to expand our understanding of the tick innate immune defence pathways and interactions with *B. burgdorferi*. This work has also provided a useful resource for comparative genomics and arthropod evolutionary biology. Additionally, development of tick cell lines has led to major advances in our ability to study tick–pathogen interactions for *I. scapularis* in vitro[192–194], including isolation of tick-borne pathogens, expression of RNA and proteins, genetic manipulation and knockout studies. As described herein, *B. burgdorferi* relies on interactions with the tick for successful colonization, persistence and transmission. With the recent advancements in our understanding of the tick immune system and microbiota, further analysis is required to identify how gene expression changes in *B. burgdorferi* help to modulate the tick environment. Several recent studies clearly demonstrate that the tick IMD and JAK–STAT pathways have roles in controlling infection. However, further studies are required to determine how *B. burgdorferi* can avoid clearance and identify the proteins involved. Additionally, studies to restore components of the JAK–STAT, IMD and Toll signalling pathways absent in ticks would be an interesting research direction to explore. Similarly, studies are required to examine how the tick microbiota can impact metabolic functions of the tick and *B. burgdorferi*. Further characterization of these interactions, as well as interactions at the tick bite site, could help to identify potentially druggable targets to disrupt infection and transmission. For example, salivary proteins enhance transmission of *B. burgdorferi* and *A. phagocytophilum* to the vertebrate host. Targeting these interactions in the salivary glands has become an exciting approach for vaccine development[195,196]. Developing immunity against tick salivary proteins can be a strategy to prevent tick bites, as well as infection with the pathogens they transmit.

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