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REVIEW

Toll-like receptor cascade and gene polymorphism in host–pathogen interaction in Lyme disease

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Abstract: Lyme disease (LD) risk occurs in North America and Europe where the tick vectors of the causal agent *Borrelia burgdorferi* sensu lato are found. It is associated with local and systemic manifestations, and has persistent posttreatment health complications in some individuals. The innate immune system likely plays a critical role in both host defense against *B. burgdorferi* and disease severity. Recognition of *B. burgdorferi*, activation of the innate immune system, production of proinflammatory cytokines, and modulation of the host adaptive responses are all initiated by Toll-like receptors (TLRs). A number of *Borrelia* outer-surface proteins (eg, OspA and OspB) are recognized by TLRs. Specifically, TLR1 and TLR2 were identified as the receptors most relevant to LD. Several functional single-nucleotide polymorphisms have been identified in *TLR* genes, and are associated with varying cytokines types and synthesis levels, altered pathogen recognition, and disruption of the downstream signaling cascade. These single-nucleotide polymorphism-related functional alterations are postulated to be linked to disease development and posttreatment persistent illness. Elucidating the role of TLRs in LD may facilitate a better understanding of disease pathogenesis and can provide an insight into novel therapeutic targets during active disease or postinfection and posttreatment stages.

Keywords: Lyme disease, Toll-like receptors, *Borrelia* lipoproteins, genetic polymorphisms, host–pathogen interaction

Introduction

Lyme disease (LD) is a tick-borne disease caused by spirochetes of the bacterial species complex *Borrelia burgdorferi* sensu lato.[1] Risk from LD, also known as Lyme borreliosis, occurs primarily in forested areas across North America, Asia, and parts of Europe where the tick vectors (black-legged ticks *Ixodes scapularis* and *I. pacificus*) and natural wild-animal hosts (eg, mice, chipmunks, gray squirrels, opossums, and raccoons) of the ticks and bacterium are found. A number of species of *B. burgdorferi* sensu lato cause LD in humans, including *B. burgdorferi* sensu stricto, *B. afzelii*, *B. bissettii*, *B. bavariensis*, and *B. garinii*. Among these, *B. burgdorferi* sensu stricto (hitherto termed *B. burgdorferi*) is the primary confirmed cause of LD in North America, while the others are responsible for LD in Europe and Asia.[2]

In the US, there were over 36,000 confirmed and probable cases in 2013,[3,4] with an estimated number of approximately 300,000 true cases every year.[4] Risk of infection is found where populations of black-legged ticks occur. These ticks are found in distinct regions of eastern, midwestern, and western North America, although the geographic footprint of risk is expanding: northward geographic expansion of the geographic range

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of infected ticks has recently driven LD expansion in Canada, and this has been attributed to climate change.[5–8] Although the incidence of LD is not as high in Canada compared to the USA, there was a twofold rise in cases reported in Canada from 315 in 2012 to 682 in 2013.[9,10]

Early LD can be manifested as the characteristic erythema migrans rash, with or without a range of common symptoms, such as fatigue, chills, fever, headache, muscle and joint pain, swollen lymph nodes, and numbness or tingling.[11] The skin infection can be localized close to the site of the tick bite or become disseminated systemically within days or weeks.[12] However, as the diagnostic erythema migrans rash occurs in ~70%–80% of infected subjects, various other early and late symptoms may arise without the rash.[12,13] If untreated or not treated adequately, the early form of the disease can progress to the disseminated phase, which is characterized by neurologic complications of the central or peripheral nervous system, cardiac manifestations, and Lyme arthritis.[14,15] A small percentage of patients can also be affected by posttreatment LD syndrome despite antibiotic treatment, in which such symptoms as fatigue, neurological problems, disabling musculoskeletal aches, paralysis, and persistent Lyme arthritis are observed and can last from months to years.[13]

Exposure to foreign microbial, chemical, or physical agents causes the activation of the host innate immune system. This is the first line of defense, and results in a wide range of inflammatory reactions to allow for the repair of incurred damage, isolation, or elimination of the infectious agent and reestablishment of homeostasis.[16,17] In order to initiate inflammatory reactions following infection, host innate immunity relies on pattern-recognition receptors (PRRs), such as the Toll-like receptors (TLRs). TLRs are type I transmembrane proteins that have an extracellular domain containing leucine-rich repeats and a cytoplasmic tail with a conserved Toll/IL-1 receptor (TIR) domain, and they are expressed by such cells as macrophages and dendritic cells.[18] PRRs recognize structurally conserved pathogen-derived molecules, eg, triacetylated lipoproteins, peptidoglycans, and lipopolysaccharides,[19,20] triggering a downstream proinflammatory signaling cascade leading to the nuclear translocation of the transcription factor NFκB. This process initiates cytokine production, the expression of an array of adhesion molecules and generation of reactive oxygen species.[21–24] Of the ten human TLRs, TLR2 and TLR4 were the first members of the PRRs to be recognized as involved in antibacterial defense.[25]

Interaction of TLRs with the immunogenic *B. burgdorferi* outer-surface proteins (Osps) plays an important role in the initial stages of LD pathogenesis.[2,26–28] This interaction was proposed to mediate both short-lasting and severe Lyme arthritis and Lyme carditis in animal models.[29–31] More recent evidence, however, suggests that a number of single-nucleotide polymorphisms (SNPs) in the *TLR* genes may modulate the host response to infection with *B. burgdorferi*. For example, SNPs in *TLR8* can lead to immunodeficiency syndromes and cause an increased risk of severe clinical manifestations associated with *B. burgdorferi* infection.[32,33]

This review explores our current knowledge of the interaction between *B. burgdorferi* surface lipoproteins and the TLR-related signaling cascade in humans. We used such terms as "Lyme disease", "toll-like receptors", "*Borrelia* lipoproteins", and "genetic polymorphisms" to identify key studies relevant to this article from the Ovid and PubMed databases. Special emphasis is directed to understanding the critical function of *TLR* genetic polymorphisms in LD manifestations, and their possible role as mediators of pathogen–host interaction in disease symptomatology and severity.

Borrelia lipoproteins in diseases pathogenesis

B. burgdorferi expresses a wide variety of surface lipoproteins that allow for adaptation to hosts and tick vectors throughout the bacterium's life cycle, including infectivity and dissemination within hosts. *B. burgdorferi* is a Gram-negative bacterium that lacks the highly immunogenic surface glycolipid lipopolysaccharide (LPS) present in most other Gram-negative bacteria. Therefore, immune-system modulation can be mediated primarily via the bacterial surface lipoproteins.[34] These lipoproteins can be categorized into three main subclasses: 1) Osps interacting with the innate immune system via PRRs,[34] 2) lipoproteins that mediate attachment to the extracellular matrix (ECM),[35] and 3) lipoproteins that influence complement and acquired immune-response inactivation to prolong bacterial survival.[36]

Outer-surface proteins in *B. burgdorferi*

When bacteria enter the tick, the new environment activates the Rrp2–RpoN–RpoS pathway, a novel regulatory network that governs the differential expression of numerous genes of *B. burgdorferi*, including lipoproteins and the two major virulence genes *OSPA* and *OSPC*, allowing for bacterial survival within the arthropod vector.[37] Following infection of the tick

after feeding on an infected host, *B. burgdorferi* persists in the tick gut, at which time OspA and OspB are expressed, allowing attachment to the midgut endothelium.[2] Expression of surface lipoproteins BptA74[38] and BB690[39] is also critical for bacterial colonization and prolonged residence in the tick midgut. Feeding activates bacterial translocation from the midgut to the tick's salivary glands, which is facilitated by interaction between such lipoproteins as BBE31[40] and BBA52.[41] At this stage, OspA is downregulated and replaced with OspC.[42] This allows *B. burgdorferi* to migrate to the salivary glands and bind the Salp15 protein, which has immunosuppressive properties and likely aids bacterial survival during the early stage of infection of vertebrate hosts.[43] OspC has been shown to play a significant role in invading the salivary glands and thus the subsequent transmission of *B. burgdorferi* from the tick to the vertebrate host.[44] OspC has plasminogen-binding activity, which likely facilitates the migration of bacteria within the host by destroying intercellular junctions.[45] OspC is not the only *B. burgdorferi* lipoprotein that has plasminogen-binding activity: OspA, ErpA/C/P[46] and CRASP1[47] have also exhibited varying binding affinities to human plasminogen. Hijacking of the plasminogen proteolytic system leads to the secretion of urokinase, which is thought to be synthesized as a result of TLR-related signaling activation.[48] Plasminogen activation mediates a downstream cascade of activation of several host proteases that digest the ECM.[49]

While OspA expression diminishes during the early stages of infection, anti-OspA antibodies have been observed in patients with rheumatoid arthritis, suggesting that OspA may also play a role in the manifestation of persistent disease outcomes.[50] Scheckelhoff et al[51] reported that when recognized by *B. burgdorferi*, host adrenergic stress hormones lead to OspA expression for what was hypothesized to be a reentry stage into a tick vector during the bacterial life cycle. The role of OspA in persistent disease was in part determined by its homology to LFA1, an adhesion molecule mediating cell–cell interactions during inflammation and found only in *B. burgdorferi* sensu stricto. This homology results in autoimmune reactions responsible for some posttreatment LD syndromes.[52] IgG antibody titers to OspA in persistent Lyme arthritis patients correlate with the severity of this condition.[53]

B. burgdorferi attachment to extracellular matrix

In order to establish initial infection, *B. burgdorferi* binds to various components of the host ECM. One of the pathways involves bacterial binding to glycosaminoglycans (GAGs) on the host cell surface. The GAG-binding protein Bgp attaches to host cells and inactivates toxic metabolites, allowing bacterial growth.[35] The DbpA and DbpB proteins bind decorin, which is a type I collagen-associated GAG.[54] Since the Bgp and Dbp proteins complement each other during establishment of infection within the host, constant expression of either is not necessary.[35]

The P66 adhesin protein has been shown to bind the cell-surface integrins $\alpha II\beta_3$ and $\alpha_v\beta_3$, causing upregulation of surface-adhesion molecules.[55] Fibronectin, another component of the ECM, has been identified as an additional ligand for the *B. burgdorferi* lipoproteins BBK32[56] and RevA/RevB. Furthermore, RevA,[57] along with ErpX[58] and Bmp,[59] bind laminin within the host ECM.[60] BmpA can be directly involved in Lyme arthritis, due to its spatial and temporal upregulation in the joints, which coincides with the development of inflammation.[60] Not all adhesin proteins are expressed to the same extent, and lack of one type is usually compensated for by upregulated expression of another.[61]

Complement inactivation in Lyme disease

The complement system is a major component of the innate immune system, which acts as a first defense against pathogenic invasion. Complement comprises a complex of more than 30 proteins that through different pathways lead to the conversion of C3 to C3a and C3b by C3-convertase proteins. C3b aids in opsonization of pathogens, thereby attracting immune cells that directly kill pathogens. The complement system is tightly regulated by employing several C3-convertase regulators that either directly dissociate C3 convertase or degrade the reaction products.[62] *B. burgdorferi* uses such complement C3-convertase regulators to its own advantage, where various surface lipoproteins bind factor H, which is responsible for mediating cleavage of C3b.[63,64] By binding *B. burgdorferi* factor H, the bacteria facilitate further cleavage of C3b and downregulate the overall activation of the immune system.[36] CRASP1 or CspA has been identified as a critical component in immune-system evasion by *B. burgdorferi*, where complement sensitivity was increased in human sera with CspA-knockout bacteria.[36] Although binding of host complement regulators is the main mechanism of bypassing complement-mediated killing, CspA has also been found to bind late complement products like C7, C8, and C9 in the absence of regulators and interfere with assembly of the membrane attack complex.[65] Furthermore, CspA has also been shown to take part in host cell adhesion

by binding to various ECM proteins. This seems to occur, however, at a different binding site, and thereby CspA can simultaneously bind factor H, downregulate host defenses, and enhance tissue invasion.[47]

B. burgdorferi and corresponding TLR signaling

There are ten different human TLRs (Table 1),[16,17,32] but only a few of these are capable of recognizing and mounting a response to *B. burgdorferi*. TLR3, TLR7, and TLR8 do not respond to *B. burgdorferi* lipoproteins, as they are intracellular sensors that recognize viral single- and double-stranded nucleic acids. Nonetheless, some studies have indicated a link between *B. burgdorferi* and TLR7[66] and TLR8[67] that induce the synthesis of type I IFNs.[68] TLR9 recognizes bacterial CpG DNA, and might play a role in *B. burgdorferi* infection when the bacterial DNA is made accessible via lysis or degradation.[26] TLR2 and TLR4 are extracellular receptors that recognize bacterial ligands, and have been demonstrated to play a role in *B. burgdorferi* infection.[68]

TLR2 has a broad range of ligands, due to its ability to form heterodimer receptors with other TLRs. When TLR2 associates as a heterodimer with TLR6, it can recognize peptidoglycan and diacylated lipopeptides. The TLR2/1 heterodimer, on the other hand, recognizes bacterial lipoproteins, such as OspA. However, an innate immune response to *B. burgdorferi* was still observed in TLR1- and TLR2-knockout mice,[69] indicating an alternative mechanism(s) for *B. burgdorferi* recognition. TLR4 normally responds to the highly immunogenic surface glycolipid LPS of Gram-negative bacteria (see Table 1) that is lacking in *B. burgdorferi*. TLR2 and TLR4 downstream cascades are similar, where TIRAP is involved in the MyD88-dependent pathway via both receptors,[16] which may account for the similarity in innate immune response to *B. burgdorferi* lipoproteins and response to LPS. Both TLR2 and TLR4 employ the CD14 co-receptor to enhance the detection of LPS and recognize other pathogen-associated molecular patterns (PAMPs), such as lipoteichoic acid.[34] CD14 is a surface receptor on neutrophils and monocytes/macrophages, binds to and facilitates signaling by LPS, and is also the signaling receptor for *B. burgdorferi* lipoproteins and can bind to both OspA and OspC.[70] A loss of this CD14 or its function was found to result in a severe inflammatory response to *B. burgdorferi* infection.[34] The role of TLR5 in *Borrelia* infection is somewhat unclear. Although this TLR is known to respond to the flagellin protein (Table 1), *B. burgdorferi* has endoflagella that are not surface-exposed. In other similar pathogenic spirochetes, eg, *Treponema phagedenis*, TLR5 activation could occur due to transient gaps in the outer membrane.[71] Despite the fact that the presence of these gaps is yet to be fully characterized in *B. burgdorferi*, they might provide an explanation for the reported involvement of TLR5 in modulating innate immune responses to the pathogen.[72]

TLR cascade in Lyme disease

The innate immune system can be activated by a wide range of pathogenic molecules, due to its ability to recognize PAMPs by PRRs (Table 1). PAMPs are molecular signatures of pathogens that are conserved for infectivity and/or survival across various classes of microorganisms that are not expressed by host cells.[73] Although their downstream cascades and terminal effectors are quite similar, the ten human TLRs respond differently to different PAMPs.[68]

Each TLR has an extracellular domain, which contains binding sites for ligands, and a cytoplasmic domain involved in signal transmission. However, the cytoplasmic domains of TLRs are too short to transduce signals, and require additional proteins to carry out this function.[16] The TIR component of cytoplasmic domains functions by recruiting adapter proteins like MyD88, TIRAP, TRIF, or TRAM, and facilitates dimerization of different TLRs.[16,17] The first step in signal transduction is recruitment of the MyD88 adapter, which triggers the assembly of several IRAK scaffold proteins to form an MyD88–IRAK complex (myddosome). The IRAK complex subsequently recruits TRAF6 and TAKs to phosphorylate IKK.[16] Upon phosphorylation, IKK dissociates from the NFκB transcription factor, allowing for its translocation into the nucleus and activation of a number of genes that code for a variety of proinflammatory cytokines, chemokines, and other effectors.[17,74] Expression of TNF and IL-1β lead to activation of the endothelium and local upregulation of cell surface-adhesion molecules, allowing for tethering of immune cells to the site of infection.[17] IL-1β and IL-6 act together in liver cells to produce acute-phase reactants, which in turn activate the complement cascade and enhance opsonization of the pathogen. Signaling through TLRs also leads to direct killing of the pathogen via production and secretion of antimicrobial peptides into the intracellular phagocytic compartments of immune cells.[75] The following subsections emphasize on and explain the role of TLRs and related signaling, as well as pathogen–host interaction along this pathway, in LD susceptibility and early and long-term complications.

Table 1 Characteristics of the Toll-like receptors and their gene polymorphism-related infectious diseases

TLR	Immune-cell expression	Ligands	Pathogen-associated molecular patterns	Disease-related SNPs	SNP-linked infectious disease
TLR1	Cell surface of monocytes, macrophages, dendritic cells, and B-cells	Multiple triacyl lipopeptides Heat-shock proteins High-mobility group proteins Proteoglycans	Triacylated lipoproteins Peptidoglycans Lipopolysaccharides	Asn248Ser Ile602Ser Arg80Thr G-7202A[a]	Sepsis Leprosy Candidemia Lyme disease
TLR2	Cell surface of monocytes, macrophages, dendritic cells, and B-cells	Multiple triacyl lipopeptides Heat-shock proteins High-mobility group proteins Proteoglycans Lipoteichoic acid β-Glucan	Diacylated lipoproteins Triacylated lipoproteins Peptidoglycans Lipopolysaccharides	Arg677Trp Arg753Gln Arg395Stop Ser249Pro	Leprosy Tuberculosis Lyme disease Urinary tract infection Staphylococcal infection
TLR3	Endosomes of B-cells, T-cells, natural killer cells, dendritic cells, neutrophils, and mast cells	mRNA tRNA	dsRNA (poly[I:C]) tRNA siRNA	Asn284Ile Tyr307Asp Leu412Phe Pro554Ser Ser737Thr	HIV infection Herpes simplex encephalitis
TLR4	Cell surface and endosomes of monocytes, macrophages, myeloid dendritic cells, mast cells, intestinal epithelium, and B lymphocytes	Lipopolysaccharide Heat-shock proteins Opioid drugs Fibrinogen Proteoglycans Nickel Fibronectin Tenascin C	Glycolipid lipopolysaccharides Paclitaxel	Asp299Gly Thr399Ile	Risk of infection
TLR5	Cell surface of monocytes, macrophages, dendritic cells, and intestinal epithelium	Bacterial flagellin Profilin	Flagellin	Arg392Stop	Pneumonia Systemic lupus erythematosus Lyme disease (probable)
TLR6	Cell surface of monocytes, macrophages, dendritic cells, and B-cells	Multiple triacyl lipopeptides Heat-shock proteins High-mobility group proteins Proteoglycans Lipoteichoic acid β-Glucan	Mycoplasma Diacylated lipoproteins Triacylated lipoproteins Peptidoglycans Lipopolysaccharides	Ser249Pro	Aspergillosis infection Lyme disease (probable)
TLR7	Endosomes of monocytes, macrophages, plasmacytoid dendritic cells, and B-cells	ssRNA Imidazoquinoline Bropirimine	ssRNA Imidazoquinolines Guanosine analogs	Gln11Leu	Hepatitis C infection HIV infection Systemic lupus erythematosus
TLR8	Endosomes of monocytes, macrophages, dendritic cells, and mast cells	Small synthetic compounds ssRNA Phagocytized bacterial RNA	ssRNA Imidazoquinolines	Met1Val	Hepatitis C infection Congo hemorrhagic fever Tuberculosis
TLR9	Endosomes of monocytes, macrophages, plasmacytoid dendritic cells, B-cells, and T-cells	Unmethylated CpG oligodeoxynucleotide DNA	Bacteria DNA viruses	Pro99Leu Pro545Pro T-1237C[b] G1174A[b]	Cerebral malaria Lupus nephritis
TLR10	Endosomes of monocytes, macrophages, and dendritic cells	Remains the only TLR without a defined ligand	Profilin-like proteins (probable)	Pro344Pro Iso775Val	Influenza viral infection (probable)

Notes: [a]5′-Flanking region; [b]transition substitution, intronic SNPs. Data from Takeda et al,[16] Beutler,[17] and Lin et al.[32]
Abbreviations: TLR, Toll-like receptor; SNP, single-nucleotide polymorphism; mRNA, messenger RNA; tRNA, transfer RNA; dsRNA, double-stranded RNA; siRNA, small interfering RNA; ssRNA, single-stranded RNA.

TLRs relevant to Lyme disease

A number of TLRs have been shown to play a significant role in the susceptibility and risk of LD. The involvement of TLRs in LD pathogenesis occurs through: 1) proinflammatory reactions mediated by the endosomal TLRs (TLR3, TLR7, TLR8, and TLR9) upon phagocytosis of the bacterium, or 2) the interaction of *B. burgdorferi* lipoproteins with plasma membrane TLRs (TLR1, TLR2, TLR4, TLR5, and TLR6).[67,76]

The ensuing inflammatory reactions induced by these processes differ both quantitatively and qualitatively.[67,76] For example, internalization of live spirochetes leads to a higher synthesis of different proinflammatory cytokines than when the pathogen lipoproteins interact with cell-surface TLRs.[77] Furthermore, different cytokines can be induced by different host TLRs.[67] Taken together, these observations may at least partially explain interindividual variability in response to *B. burgdorferi* infection and in LD symptomatology.

Numerous studies have examined the involvement of TLR2 in the response to *B. burgdorferi* independently or as a heterodimer with TLR1 and TLR6.[78,79] TLR2 recognizes *B. burgdorferi* OspA, as demonstrated in human peripheral blood mononuclear cells (PBMCs)[66] and in OspA-vaccinated mice,[68] leading to the expression of NFκB-dependent cytokines. Neutrophils from patients with LD exhibited elevated expression of *TLR2* messenger RNA and protein together with an excessive synthesis of IL-6 and IL-1β.[80] In contrast, TLR2-deficient mice had an impaired innate immune response to *B. burgdorferi* OspA and a diminished cytokine synthesis from their bone marrow-derived macrophages.[81,82] Moreover, *B. burgdorferi*-infected *TLR2*$^{-/-}$ mice had 50- to 250-fold higher numbers of spirochetes and severe arthritis[81,83,84] and inflammatory carditis[85] compared to their wild-type homozygous or heterozygous counterparts. This effect was also noted to a lesser extent in *TLR2*$^{-/+}$ mice,[86] demonstrating that one nonfunctional allele of *TLR2* may be enough to impair cytokine synthesis and influence disease susceptibility upon exposure to *B. burgdorferi*. Some evidence suggests that TRIF and MYD88 adapters are essential to the inflammatory function of TLR2, and may have a more critical role than TLR2 in LD pathogenesis. TRIF-deficient mice had lower cytokine production in response to *B. burgdorferi* compared to their wild-type counterparts,[87] whereas MyD88 had a more marked effect on phagocytosis of the bacterium than TLR2.[67,82]

Other members of the TLR family were also implicated in the susceptibility to LD and its complications. A status of enhanced pathogen lipoprotein recognition was elicited upon the heterodimerization of TLR1 with TLR2.[88] In addition, TLR5 exhibits a transcriptional upregulation upon *B. burgdorferi* phagocytosis, although a receptor for bacterial flagellin is lacking in *B. burgdorferi*.[26] Moreover, the expression of endosomal TLR7 and TLR9, mediators of type I IFN synthesis in dendritic cells, was downregulated upon exposure to *B. burgdorferi*,[66] together with the synthesis of IFNα,[89] IFNγ_1,[66] and the transcription of IFN-induced genes.[89] Similarly, downregulation of TLR8, which influences the synthesis of IFNβ, inhibits cytokine release in cells that have phagocytosed *B. burgdorferi*.[67,90]

Genetic polymorphisms of TLRs in Lyme disease etiology

A number of SNPs have been identified in the *TLR* genes and their downstream factors[91,92] (Table 1). These polymorphisms are known to alter TLR-signaling patterns, and have been linked to changes in clinical manifestations caused by bacterial, fungal, and viral infections.[76] For example, *TLR1* Ile602Ser was linked to elevated synthesis of proinflammatory cytokines and a more effective T_H1-like response in patients with LD.[72] On the other hand, *TLR2* Arg677Trp and Arg753Gln were associated with lepromatous leprosy[93] and tuberculosis.[94–96] In LD, however, the *TLR2* Arg753Gln polymorphism provides protection against the development of late-stage disease.[86] Compared to wild-type PBMCs from healthy individuals, cells with *TLR1* Arg80Thr, Asn248Ser, Ile602Ser, and *TLR6* Ser249Pro had a significantly lower synthesis of proinflammatory cytokines when heterodimerized with *TLR2*.[97]

TLR1 T1805G (Ile602Ser), *TLR2* G2258A (Arg753Gln), and *TLR5* C1174T (Arg395Stop) were examined in patients with different LD-associated clinical symptoms, including erythema migrans, antibiotic-responsive arthritis, and antibiotic-refractory arthritis.[72] These SNPs were associated with an impairment of downstream signaling and reduced cytokine production by decreasing the number of plasma membrane TLRs (*TLR1* T1805G and *TLR2* G2258A) or with abrogation of the cellular flagellin-signaling pathway (*TLR5* C1174T). Subjects with antibiotic-refractory arthritis had approximately twofold-higher frequency of *TLR1* Ile602Ser (T1805G) compared to subjects with erythema migrans (odds ratio [OR] 1.9, P=0.05)[72] (Table 2). The status of antibiotic-refractory Lyme arthritis occurs when there is persistence of synovitis for at least 3 months after antibiotic treatment, despite expulsion of viable *B. burgdorferi* from the affected area.[98]

In contrast to the possible increased risk of Lyme arthritis associated with *TLR1* Ile602Ser (T1805G), *TLR2* Arg293Gln (A2258G) was shown in one study to be protective.[86] The arginine residue at the 753 position of *TLR2*, located within the signal-transduction domain of the receptor, is critical for the stable formation of the *TLR1–TLR2* heterodimer.[86,91] The frequency of *TLR2* Arg753Gln (A2258G) was lower in LD patients compared to matched controls (OR 0.39, 95% confidence interval 0.17–0.89; P=0.03). In this study, patients with stage III LD (late persistent disease with Lyme arthritis) had a further lower frequency of Arg753Gln (A2258G) compared to their matched controls (OR 0.15, 95% confidence interval 0.03–0.65; P=0.003)[86] (Table 2). These findings substantiated a protective effect of *TLR2* Arg293Gln against Lyme arthritis, a clinical symptom in the late stages of LD.

Table 2 Impact of *TLR1* T1805G (Ile602Ser) and *TLR2* A2258G (Arg753Gln) alleles on late-stage Lyme disease

Gene	SNP	Findings[a]				
TLR1	T1805G (Ile602Ser)		**Control**	**Erythema migrans**	**Antibiotic-responsive arthritis**	**Antibiotic-refractory arthritis**
		n	505	71	76	101
		Frequency (%)	50	51	47	62
		OR[b]				1.9[c]
		P				<0.05
TLR2	A2258G (Arg753Gln)		**Matched control**	**Stage I[d]**	**Stage II**	**Stage III**
		n	155	27	40	88
		Frequency (%)	13.5	0	17.5	2.3
		OR (95% CI)				0.15[e] (0.03–0.65)
		P				0.003

Notes: [a]Only statistically significant *P*-values reported; [b]95% CI was not clearly mentioned in the original article;[72] [c]versus erythema migrans group; [d]stage I, early localized Lyme disease (1–4 weeks); stage II, early disseminated infection (1–4 months); stage III, late persistent Lyme disease (eg, Lyme arthritis); [e]versus matched control group. Data from Strle et al[72] and Schröder et al.[86]

Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Functional effects of TLR polymorphisms

A number of genotype–phenotype matching studies were carried out to elucidate the functional effects of gene polymorphisms in *TLRs* on host cytokine synthesis in response to *B. burgdorferi* infection (Table 3). Patients showing erythema migrans who were carriers of the *TLR1* T1805G allele and infected with the RST1 strain of *B. burgdorferi* had higher levels of IL-6 and the IFNγ-inducible chemokines CXCL9 and CXCL10 in their PBMCs compared to wild-type carriers.[72] These subjects also exhibited elevated serum cytokine and chemokine levels than patients infected with the RST2 or RST3 strains. Similar findings were observed for the *TLR2* Arg753Gln and *TLR5* Arg395Stop SNPs (Table 3). The RST1 strain of *B. burgdorferi*-carrying genes for OspC major group A is known to be associated with stimulation of macrophages to release higher levels of IL-6, IL-8, CCL3, CCL4, TNF, and IL-1β than RST2 and RST3 strains.[99,100]

Similarly to patients with erythema migrans, subjects with Lyme arthritis exhibited elevated T_H1-like adaptive cytokines and chemokines in the synovial fluid of individuals with the *TLR1* T1805G compared to wild-type carriers.[72] Evidence for the association of the *TLR1* T1805G with this stage of

Table 3 Summary of *TLR*-gene polymorphisms' functional significance in Lyme disease and their role in host–pathogen interactions

TLR	SNP	Functional significance
TLR1	Arg80Thr Asn248Ser Ile602Ser	• Lower cytokine production in PBMCs homozygous for the three SNPs after Pam_3Cys exposure • Live *Borrelia burgdorferi* led to lower IL-1β synthesis in cells homozygous for Arg80Thr • Significantly lower IL-1β production in cells with TLR1 Asn248Ser and Ser602Ile following exposure to live *B. burgdorferi* • Induction with Pam_3Cys lipopeptide and live pathogen led to a lower production of IFNγ • Significant differences in the synthesis of IL-6, IL-8, TNFα, and IL-10 after Pam_3Cys and *B. burgdorferi* stimulation • Patients with erythema migrans more likely to have symptoms and elevated serum levels of IL-6, CXCL9, and CXCL10 • Patients with antibiotic-refractory arthritis exhibited high IL-6, CXCL9, and CXCL10 compared to erythema migrans or patients with antibiotic-responsive Lyme arthritis
TLR2	Arg753Gln	• Decreased synthesis of TNFα and IFNγ following exposure to *B. burgdorferi* lysates in human kidney embryo epithelial HEK293 cells • No significant difference in cytokines/chemokines was observed in PBMCs from patients with Lyme arthritis from basal levels after exposure to *B. burgdorferi*
TLR5	Arg395Stop	• No significant difference in cytokines/chemokines observed from basal levels after exposure to *B. burgdorferi*
TLR6	Ser249Pro	• No significant difference in IL-1β, IL-6, IL-8, IL-10, or TNFα cytokine levels upon FSL1 exposure • Stimulation with live *B. burgdorferi* inhibited IL-1β, IL-6, and IL-8 synthesis • No effect on IFNγ or IL-17 compared to wild type

Note: Data from Strle et al,[72] Schröder et al,[86] and Oosting et al.[97]

Abbreviations: SNP, single-nucleotide polymorphism; PBMCs, peripheral blood mononuclear cells.

LD was substantiated from observations demonstrating that patients with antibiotic-refractory arthritis had approximately 15- and twofold-higher CXCL9 and CXCL10 chemokine levels, respectively, than subjects with erythema migrans and antibiotic-responsive arthritis.[72] CXCL9 and CXCL10 chemokines act as chemoattractants for $CD4^+$ and $CD8^+$ T-cells, the main infiltrating cell types that cause chronic synovial inflammation in antibiotic-refractory Lyme arthritis,[101] suggesting a role for these factors in symptom persistence despite antibiotic therapy at the late disease stages. Further studies on three different *TLR1* polymorphisms in PBMCs from individuals homozygous for Arg80Thr, Asn248Ser, and Ser602Ile demonstrated that stimulation with the Pam_3Cys ligand can significantly lower cytokine production compared to that in the wild-type carrier cells.[97] Arg80Thr influenced the synthesis of IL-1β, whereas Asn248Ser and Ile602Ser affected that of IL-6, IL8, TNFα, and IL-10. The three *TLR1* SNPs affected the production of IFNγ and IL-17.[72,97]

The effect of the *TLR2* Arg753Gln SNP on the inflammatory responses in blood samples from heterozygous and wild-type carrier subjects has been studied.[86] Upon stimulation with *B. burgdorferi* lysate, the SNP carriers exhibited decreased synthesis of TNFα and IFNγ compared to their wild-type counterparts.[86] This observation was thought to be related to the influence of the nonfunctional allele on stabilizing the TLR2–TLR1 heterodimer that interacts with triacylated *B. burgdorferi* lipoproteins. Further studies on the human kidney embryo epithelial cell line HEK293 (heterozygous for *TLR2* Arg293Gln) stimulated with the synthetic diacylated lipopeptide Pam_2Cys or with *B. burgdorferi* lysate demonstrated the ability of *TLR2* Arg293Gln to induce ~50% lowering in cytokine synthesis and downregulate the innate immune response compared to the wild-type allele.[86] The nonsynonymous Ser249Pro in *TLR6*, another coreceptor of TLR2, causes a malfunction of its extracellular domain.[97] Although stimulation with the specific TLR2–TLR6 ligand FSL1 in PBMCs from Ser249Pro carriers did not change the levels of IL-1β, IL-6, IL-8, IL-10, or TNFα, lower levels were observed with *B. burgdorferi* treatment.[97] This observation indicates a limited role of *TLR6* in cytokines response to *B. burgdorferi* infection and LD risk, severity, and complications.

Conclusion

Although several in vitro and animal studies have demonstrated a role of TLRs 1, 2, and 4–9 in LD, the consensus of available information particularly implicates TLRs 1 and 2 in the process of disease risk and severity. The nature of TLR interaction with pathogen lipoproteins and the ensuing pattern of cytokine synthesis differs depending on the cellular location of the TLRs[67,77] and the presence or absence of a polymorphism in their regulating genes.[72,86] This effect may be linked to the involvement of receptors and their downstream signaling at different stages of LD.

A number of functional *TLR* SNPs were recently reported to include differential cytokine synthesis and level, altered pathogen recognition, and disruption of the downstream signaling cascade.[72,97] However, contradictory results may preclude a definitive conclusion about the effect of a particular *TLR* SNP(s) on host–pathogen interaction in LD etiology. For example, following cell exposure to *B. burgdorferi* lysate, *TLR2* Arg753Gln did not significantly affect basal chemokine/cytokine levels in one study,[72] although it was linked to a marked reduction in the synthesis of these factors in another report.[86] The effects of the *TLR6* Ser-249Pro polymorphism on cytokine synthesis in response to *B. burgdorferi* were similarly inconsistent.[97] While this SNP results in disruption of the extracellular TLR6 domain and influences the TLR2–TLR6 heterodimeric structure, it does not affect cytokine synthesis in cells exposed to FSL1 (a TLR2–TLR6 ligand), but only in those treated with live *B. burgdorferi*.[97] Although the contradictory findings for each of *TLR2* Arg753Gln[72,86] and *TLR6* Ser249Pro[97] may be simply attributed to the differences in the types of treated cells and/or administered *B. burgdorferi* components, it highlights the need to develop further studies to elucidate the genotype–phenotype relationship of various polymorphisms along the TLR cascade and their function in LD risk, severity, and complications.

Several approaches can be considered to identify *TLR* SNPs and their functional effect in LD. For instance, the candidate-gene approach could be employed, given its ability to evaluate directly the effects of specific gene variants (even those with small effects) in disease causation and progression.[102] This approach can be limited by the small number of relevant SNPs that have been already identified in LD in human, animal, and cell-culture models. However, evaluating *TLR* SNPs examined for their role in other infectious diseases (eg, those mentioned in Table 3) can provide an initial guide for these types of studies. Genome-wide association studies can be similarly considered as a feasible approach to identify novel candidate *TLRs* and other SNPs throughout the human genome that may play a role in disease risk and severity.[103] Developing these types of studies however, can be hampered by the requirement for large sample sizes in order to identify associations between SNPs and disease outcomes. Ideally,

meta-analysis would have been used here to study the role of *TLR* SNPs in LD as an alternative to candidate-gene studies or genome-wide association-study approaches. However, in our literature search we found only three studies addressing the role of TLR polymorphisms in human disease, and there were inconsistent findings and significant limitations in methodologies in these studies.[72,86,97] This inadequate number of reports is not sufficient or comprehensive enough for meta-analyses at this time.

A recombinant OspA Lyme vaccine was approved by the US Food and Drug Administration in 1998, and had approximately 80% efficacy in adults. In 2002, the manufacturer voluntarily withdrew this vaccine product for fears of vaccine side effects and declining sales. Currently, no vaccine is available for LD. Antibiotics and extended-treatment plans are the available options now for mitigating early and chronic LD, respectively. Indeed, before attempting to utilize TLRs in the prevention of LD, large-scale studies addressing the role of *TLR* SNPs in host–pathogen interaction should be generated. In this respect, it is necessary to consider a systematic and more robust research approach in human subjects. One possibility can be adopted from studies that recently defined the role of *TLR* SNPs in influencing the inflammatory response in obstructive pulmonary disease.[104] In that report, nine different *TLR2* and 17 *TLR4* SNPs were identified in a longitudinal study by employing SNP tagging while analyzing their effect on lung function and inflammatory reactions. In this manner, the roles of the polymorphisms in disease severity and progression were determined. In parallel, it will be imperative to develop prospective cohort studies nationally and/or internationally of patients at different stages of LD. These would enable the design of multiple candidate-gene studies (aided by animal or cell-culture models) or genome-wide association studies to identify particular *TLR* polymorphisms related to disease risk, severity, and complications. These studies have the potential to provide not only a better understanding of disease pathogenesis but also an insight into novel, therapeutic targets during active disease or postinfection and posttreatment complications, as well as disease-preventive targets aiding in vaccine development.

Author contributions

AB conceived the study idea and design. AB, SR, and MS prepared the first draft of the manuscript. NHO and RL helped in drafting the article and contributed to study design. All authors critically reviewed the manuscript, approved the final draft, and agreed to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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