

## Insight into the Pathogenesis of Lyme Disease

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Lyme disease is the most common vector-borne disease in the United States and Europe, caused by a tick-borne spirochete, *Borrelia burgdorferi*. Life cycle alternation between arthropod and mammals enhanced *B. burgdorferi* to adapt to two diverse niches. Although *B. burgdorferi* infection in these reservoir hosts appears asymptomatic, infection in human can typically cause inflammation in the skin, nervous system, musculoskeletal system and heart. In this review, we discuss the basic molecular characteristics and cell biology of *B. burgdorferi* and provide an overview of spirochete-induced activation of innate and adaptive immunity, resulting in particular immunopathology. Advancing understanding of the immune evasion mechanisms of *B. burgdorferi* provides important implications for ongoing research and clinical practice of Lyme disease.

**Key Words:** Lyme disease, *B. burgdorferi*, Pathogenesis

### Introduction

Lyme disease was first described in Lyme, Connecticut, after an outbreak of what was thought to be "juvenile rheumatoid arthritis" (1). Since juvenile rheumatoid arthritis does not occur in outbreaks, researchers studied these patients, which led to the identification of Lyme arthritis. Later, it was founded that Lyme disease affects different organs of Lyme disease patients during different stages of the infection (2). Despite improvements in diagnostic tests and public awareness of Lyme disease, there remain still approximately 30,000 cases of Lyme disease patients per year in the United States (3).

Lyme disease is diagnosed clinically based on symptoms, objective physical findings (such as erythema migrans, facial

palsy, or arthritis), a history of possible exposure to infected ticks, as well as serological tests. The clinical manifestations of Lyme disease are complex and can be divided into three different stages: acute-localized, acute-disseminated and chronic. When a *B. burgdorferi*-infected tick feeds on a human, it inoculates the spirochetes into the skin. During an acute localized stage, the spirochetes are localized in the skin, where they cause an inflammatory rash, known as erythema migrans (EM). In the weeks or months after infection, the spirochetes disseminate through blood and lymph, reaching other organs, such as the heart, joints and nervous system. Chronic infection reflects the establishment in tissues, where the spirochetes persist even in the face of the specific host immune response. When left untreated with antibiotics, infection with *B. burgdorferi* can result in chronic arthritis that may progress to a severe, erosive

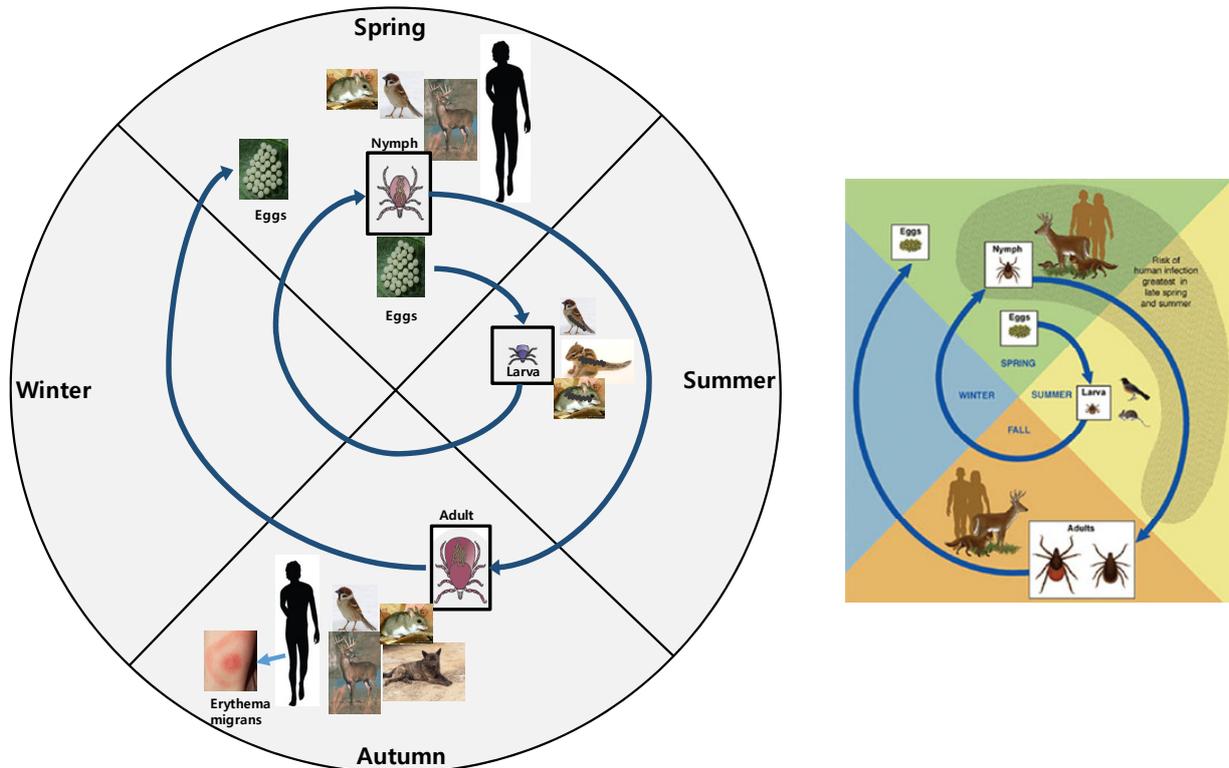
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**Figure 1. Life cycle of *Borrelia burgdorferi*.** *Ixodes* ticks have three life stages that require blood: larvae, nymphs, and adults. Because *B. burgdorferi* is not vertically transmitted by ticks to their offspring, larvae become infected when feeding on infected reservoir animals and transmit the infection to new animals during their next bloodmeal. Nymphal ticks appear to be responsible for the majority of human transmission in the eastern U.S.; the majority of human cases occur in late spring and summer when this stage is most commonly encountered. Many mammals and birds have been implicated as reservoirs for *B. burgdorferi* in the U.S., although in most of the sites where transmission is intense, white-footed mice appear to most frequently contribute to the spirochetal life cycle (3).

arthritis with histopathologic similarities to rheumatoid arthritis. In most people, treatment with antibiotics is very effective in eliminating symptoms, preventing progression to later manifestations of the disease, and curing the infection. Some symptoms improve rapidly with this treatment, whereas other symptoms gradually improve over weeks to months.

### Epidemiology of *B. burgdorferi*

The epidemiology of human Lyme disease is determined by the geographic distribution and life cycle of its tick vector, described in Fig. 1. *B. burgdorferi* is transmitted by the bite of infected *Ixodes* ticks (*I. scapularis* in the northern U.S., *I. pacificus* in the western U.S. and Canada, *I. ricinus*

and *I. persulcatus* in Europe and *I. persulcatus* in Asia). *Ixodes* ticks have three life stages that require blood: larvae, nymphs, and adults, feeding only once during every active stage. At any stage, ticks can be infected with *B. burgdorferi*. Male ticks rarely feed and never engorge but female ticks can feed on host animals. After feeding for a few days (about 3 days for larvae, 5 for nymphs, and 7 days for adult females), the ticks drop off their host and locate on or near the soil surface and take several months to develop into their next developmental stage, or, in the case of adult females, lay about 2000 eggs. The length of a tick's life cycle varies between 2 years and 6 years, depending on climate, or host availability.

*B. burgdorferi sensu stricto* (the sole genospecies transmitted in the eastern U.S.) is not vertically transmitted

by ticks to their offspring, larvae become infected when feeding on infected reservoir animals and transmit the infection to new animals during their next blood meal. Nymphal ticks appear to be responsible for the majority of human transmission in the eastern U.S.; the majority of human cases occur in late spring and summer when this stage is most commonly encountered. Many mammals and birds have been implicated as reservoirs for *B. burgdorferi* in the U.S., although in most of the sites where transmission is intense, white-footed mice appear to most frequently contribute to the spirochetal life cycle. Other rodents such as chipmunks and Norway rats may locally contribute to transmission.

### Microbiology of *B. burgdorferi*

*B. burgdorferi* was first identified as a causative organism for Lyme disease by Dr. Willy Burgdorfer in 1982 (4). *B. burgdorferi* belongs to a species of bacteria of the class spirochetes, which include *Treponema Pallidum*, and *Leptospira*. *B. burgdorferi* exhibit a characteristic morphology, with inner and outer membranes surrounding periplasmic flagella and a flexible cell wall. The outer membrane is rich in lipoproteins, including the highly immunogenic outer-surface proteins (Osp), but does not contain lipopolysaccharide (LPS). The unique style of spirochete motility results from endoflagella contained within the periplasmic space between a semi rigid peptidoglycan helix and a multi-layer, flexible outer membrane sheath. When the filaments rotate within this space, spirochetes move in a cork-screw fashion (3).

*B. burgdorferi* was the first spirochete for which the complete genome was sequenced and the genome of *B. burgdorferi* strain B31 was published in 1997 (5, 6). There are several unique aspects to its composition. For example, the *B. burgdorferi* genome is composed of a small linear chromosome of approximately 900 kb and >20 different plasmids ranging in size from 5 to 56 kb. Some plasmids are unstable during *in vitro* propagation, but are required for infectivity *in vivo* (7). The most remarkable aspect of the *B. burgdorferi* genome is the large number of sequences

encoding predicted or known lipoproteins.

*B. burgdorferi* genome encodes for over 160 lipoproteins that become expressed during different stages of its life cycle. Because *B. burgdorferi* has unusually large numbers of lipoproteins, many scientists focused on investigating the function of these lipoproteins. It is believed that *B. burgdorferi* changes surface protein expression to enable a successful infection and survive in two different environments, the tick and the mammalian host. Among many outer surface lipoproteins, well-characterized paradigm in the protein expression shift is that of OspA and OspC. OspA is highly up-regulated by *B. burgdorferi* within the midgut of unfed ticks but is down-regulated in *B. burgdorferi* as ticks take a blood meal and are transmitted to the mammalian host (8). In contrast, in unfed ticks, OspC is not expressed by *B. burgdorferi* (8, 9), however, OspC is quickly up-regulated upon entry to the mammalian host (10, 11).

### Role of innate immunity

Both innate and adaptive immunity are important for controlling the pathogenesis of *B. burgdorferi*. Before the discovery of toll-like receptors (TLRs), the main focus of immunological research in Lyme disease was on the role of adaptive immunity. However, with the discovery of TLRs, many studies pointed to the importance of innate immune cells, such as neutrophils and macrophages, in initiating an inflammatory response and controlling the clearance of the organisms.

*B. burgdorferi* lipoproteins are potent stimulants that can activate pro-inflammatory products in many different cell types, including macrophages, endothelial cells, neutrophils and B lymphocytes (12~17). It has been shown that recognition of *B. burgdorferi* lipoprotein OspA is mediated through activation of TLR1/2 heterodimers, leading to nuclear translocation of the transcription factor NFκB, which is crucial for initiation of inflammatory responses (18). Ligation of TLR2 by *B. burgdorferi* lipoproteins has been shown *in vitro* to result in activation of peripheral blood mononuclear cells (PBMCs) and release of pro-inflammatory cytokines and chemokines (18). Although it was believed

that inflammatory manifestations of *B. burgdorferi* infection were mediated by TLR signaling pathways, mice deficient in CD14, TLR2, or MyD88 still developed arthritis and harbored a higher organism burden than their wild-type counterparts (19~23). Specifically, MyD88 deficiency in mice resulted in 100 fold higher replication of spirochetes in the joints, heart and ears, whereas TLR2 deficiency in mice increased pathogen burden by 10 fold, compared with wild type mice at 2, 4, and 8 weeks post infection (19, 22~24). Joint inflammation in *B. burgdorferi*-infected MyD88-deficient mice has been variously reported by three different groups as either unchanged from wild-type or increased (19, 22, 23). It was expected the absence of TLRs would result in the reduced induction of major inflammatory signaling pathways, however surprisingly, these mice showed increased inflammation and increased cytokine/chemokine production in arthritic joints. This increased inflammation is ineffective at clearing the organisms and the bacterial loads in the joints are 1~2 logs higher than in wild-type mice (19, 22~24). Carditis, which is the other major manifestation of *B. burgdorferi* infection in mice, was unchanged from wild-type levels in MyD88-deficient mice (23), but myositis was greatly increased (22). In conclusion, there might be additional receptors that are required for TLR-independent activation of inflammatory symptoms in mice deficient in TLR2 or MyD88.

Recently, it was discovered that in addition to TLR2, other TLRs, such as TLR5, TLR7, TLR8 and TLR9, cooperate with TLR2-TLR1 to induce pro-inflammatory molecules, including type I interferons (IFNs) (25~27). Besides TLRs, NOD-like receptors (NLRs) also play an important role in sensing *B. burgdorferi* on dendritic cells and macrophages within the dermis (Fig. 2) (28~30). NLRs sense the presence of intracellular muropeptides derived from bacterial peptidoglycans and NOD1 and NOD2 are mainly expressed by epithelial cells and antigen-presenting cells (APCs) such as macrophages and dendritic cells (31). It was shown that *B. burgdorferi* up-regulates NOD2 on astrocytes after exposure to several TLR-ligands (32). In addition, NOD2 is involved in the release of several different inflammatory cytokines induced by *B. burgdorferi* infection, such as IL-6

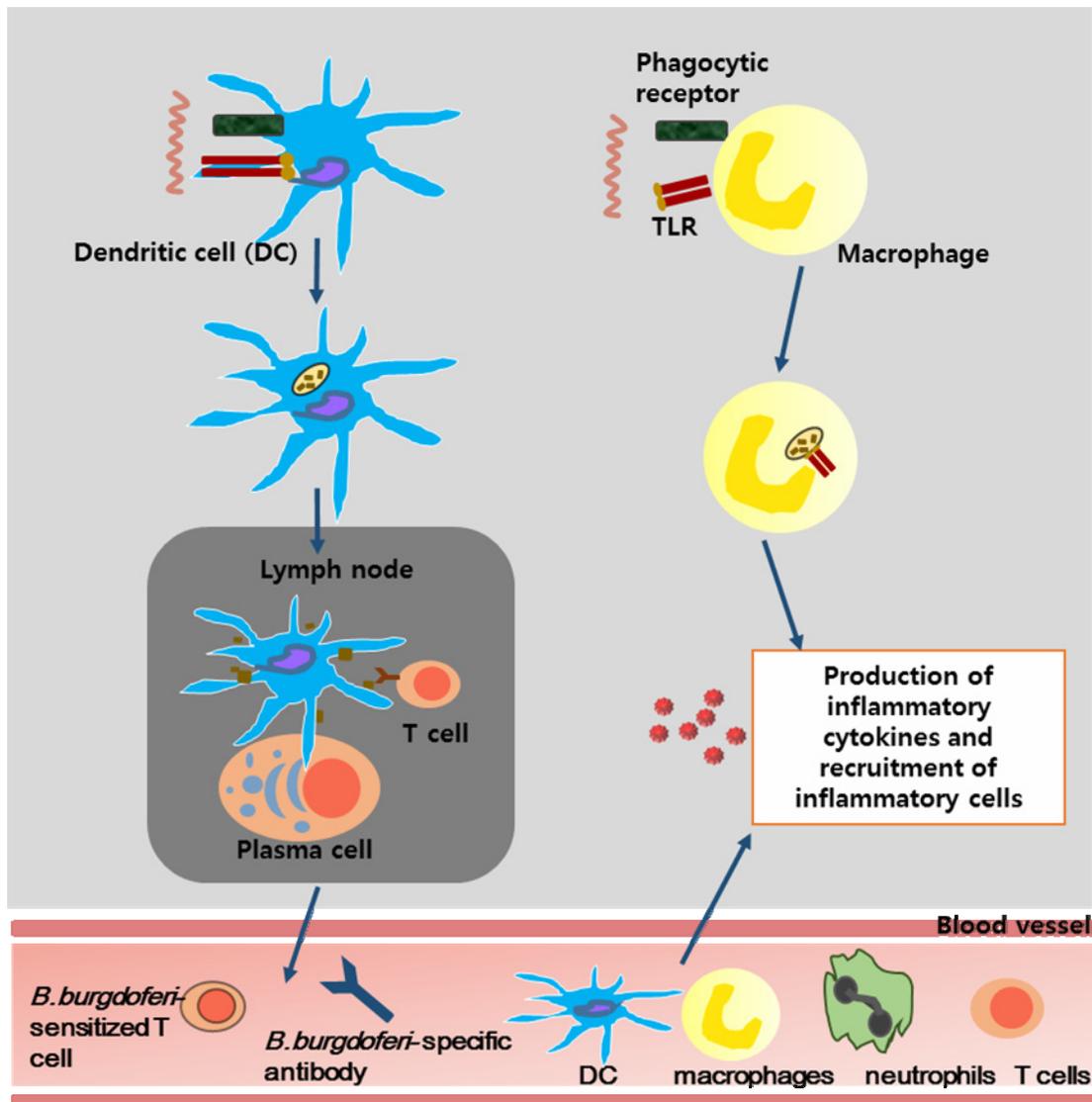
(33, 34). Association of inflammasome in sensing *B. burgdorferi* was also reported that *B. burgdorferi*-induced production of IL-1 $\beta$  require activation of the inflammasome components ASC and caspase-1. Furthermore, it was found that *B. burgdorferi* induces inflammasome-mediated caspase-1 activation, although the inflammasome activation does not appear to be mediated via NLRP3 receptor (28, 35, 36). However, more details of how TLRs and NLRs interact and sense *B. burgdorferi* in the pathogenesis of Lyme disease remains unknown.

In addition to TLRs and NLRs, integrins are also key signaling molecules that can activate TLR-independent signaling pathways for matrix metalloproteinase (MMP) production (37). Previous work showed that *B. burgdorferi* binds to integrins  $\alpha$ IIb $\beta$ 3,  $\alpha$ v $\beta$ 3,  $\alpha$ 5 $\beta$ 1 and  $\alpha$ 3 $\beta$ 1 (38~40). Furthermore, at least two different integrins,  $\alpha$ M $\beta$ 2 integrin (also known as CR or CD18-CD11b) and  $\alpha$ 3 $\beta$ 1 integrin mediate internalization of spirochetes in the absence of antibodies (41, 42).

Although persistently elevated numbers of spirochetes in tissues from these mice were thought to be due to defective development of antibody response, surprisingly, the antibody response appeared normal and functional, suggesting TLR-independent activation of B lymphocytes could function efficiently in the absence of TLRs (19). Although Liu *et al* reported that deletion of MyD88 in *B. burgdorferi*-infected mice led to a shift in a humoral response to Th2-associated antibody isotypes with an increase in the production of Th2 associated antibody isotypes, IgG1, in MyD88 deficient mice (23), *B. burgdorferi*-specific antibody production in TLR2 or MyD88 deficient mice does not lead to efficient clearance of spirochetes, suggesting that inability to control numbers of spirochetes in the absence of MyD88 can be due to a defect in effector cells involved in innate immune response (19, 24). In summary, the recognition of *B. burgdorferi* components by TLR2 or MyD88 is not essential for the development of the early adaptive immune response.

### **Role of adaptive immunity**

Previous studies suggested that the development of



**Figure 2. Innate and adaptive immune activation in response to *B. burgdorferi* infection.** *B. burgdorferi* are initially recognized by innate immune effector cells such as dendritic cells (DCs), neutrophils and macrophages; activation of these cells increases following internalization and degradation of spirochetes within phagolysosomes. DCs that have taken up spirochetes migrate to the lymph nodes, where they present processed *B. burgdorferi* antigens to T cells and B cells. While T cells enter the circulation and are recruited to the site of infection, B cells can be differentiated into plasma cells that can secrete specific antibodies that can kill *B. burgdorferi* via complement-dependent and -independent pathways. Production of pro-inflammatory cytokines by activated macrophages results in the recruitment of additional neutrophils, T cells, macrophages and DCs to the bite site, and eventually the development of erythema migrans (3).

specific humoral immunity plays a major role in the control of spirochete burden. B cells have been shown to be very important in the resolution of arthritis and control of spirochete burden, whereas T cells appear to be necessary for inflammation, but are questionably important in control of spirochete burdens. Studies of *B. burgdorferi* infection

of severe combined immunodeficiency (SCID) and RAG-deficient mice, which lack both T and B cells, or of mice deficient in B cells alone, resulted in higher pathogen burdens but severity of arthritis was similar to wild type mice (43~47).

In humans, clinical symptoms during early stage of Lyme

disease, specifically, biopsies of erythema migrans lesions show infiltration by T cells (CD8<sup>+</sup> cells as well as CD4<sup>+</sup> cells), macrophages, plasmacytoid and monocytoïd dendritic cells, and neutrophils. T cells also appear to be directly involved in the development of inflammatory symptoms such as carditis and arthritis, but are not critical for the resolution of the disease (48). It has been shown that Th1 cells dominate the immune response in the synovial fluid of patients with Lyme disease and that the severity of arthritis correlates with the ratio of Th1 cells to Th2 cells in the synovial fluids (49). C3H/HeJ mice whose predominant CD4<sup>+</sup> T cell response to *B. burgdorferi* is Th1 type show more severe arthritis than mice whose response is Th2 type (BALB/C mice) (50~52). However, it is likely that other genetic factors also play a role and recent data have pointed away from a key role for Th1/Th2 responses (53).

Recent studies have indicated potential roles of other T cell types, including natural killer T cells (NKT) and Th17 cells. Glycolipids of *B. burgdorferi* can be recognized by NKT cells and antigen-specific activation of NKT cells prevents persistent joint inflammation and promotes a spirochetal clearance (54, 55). Furthermore, studies of the role of a newly discovered member of T cell subsets, Th17, suggested that neutrophils can drive the differentiation of Th17 cells in response to *B. burgdorferi* (56).

Unlike T cell's role in inflammation control, B cell-mediated response is important for clearing the pathogen (57). IgM, T cell independent antibodies, is crucial for the initial reduction of spirochetal burdens, whereas T cell-dependent production of IgG by B cells is typically detectable by the second week of infection (58, 59).

### Mouse models of Lyme arthritis

Animal models have been very important in the understanding of the immune response to *B. burgdorferi*. Although both dogs and monkeys develop arthritis in response to *B. burgdorferi* infection, the vast majority of studies have focused on mice due to availability and the potential for genetic manipulations. Murine Lyme arthritis is characterized by early onset of arthritis (typically < 2

weeks) followed by spontaneous resolution of the ankle swelling/arthritis with the development of a specific immune response (typically ~8 weeks). However, low levels of spirochetes appear to persist for prolonged periods of time. Histologically, during acute infection, there is evidence of inflammation of joints, tendons, ligaments and peri-articular connective tissue (60). This is characterized by neutrophilic infiltration and synovial hyperplasia. Arthritis appears to correlate with spirochete burden in the joint in certain strains of mice (BALB/C) but not others (C57BL/6 or C3H). It is important to note that all immuno-competent mice resolve their arthritis spontaneously regardless of antibiotic therapy, although mild recurrent arthritis may occur in a small percentage.

There are significant differences in the response to *B. burgdorferi* among strains of mice. Numerous studies have indicated that genetic regulation of inflammatory responses control the severity of Lyme arthritis. Among the inbred strains, C3H mice develop the most significant levels of arthritis while C57BL/6 mice are relatively resistant (43, 61). BALB/C mice develop variable arthritis depending upon the numbers of spirochetes present. Studies have focused on identifying unique pathways associated with the differential severity of Lyme arthritis (62~64). Although this difference is not dependent on major histocompatibility complex (MHC) alleles, it has been linked to quantitative trait loci (QTL) on chromosomes 1, 4, 5, 11, and 12 (62). Another factor that influences disease severity in mice is the levels of pro- and anti-inflammatory immunomodulators produced by host cells. C57BL/6 mice are better able to regulate inflammation in response to *B. burgdorferi* infection than C3H mice, because C57BL/6 macrophages produce larger amounts of the anti-inflammatory cytokine, interleukin-10 (IL-10), than did C3H macrophages, whereas there are increased production of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and interferon- $\gamma$  (IFN- $\gamma$ ) in macrophages from C3H mice (65). Consequently, the deficiency of IL-10 results in more severe Lyme arthritis in mice, suggesting an essential role of IL-10 in the regulation of severity of Lyme arthritis (65).

## Inflammatory signaling pathways in Lyme disease

*B. burgdorferi* stimulates a robust inflammatory response at sites of localization. Infection with *B. burgdorferi* results in the activation of inflammatory signaling pathways that lead to the release of cytokines and chemokines and an influx of inflammatory cells that contribute to many of the clinical manifestations of Lyme disease. Therefore, it is important to understand receptors and signaling pathways involved in the generation of inflammation and identify host signaling pathways, which modulate the inflammatory pathology that is characteristic of Lyme disease.

The pathology at sites such as the skin, heart, central nervous system and joints occurs as a result of a host inflammatory response to the organism that results in the induction and release of cytokines, chemokines and proteases such as matrix metalloproteinases (MMPs) that destroy tissue (66~72). Specifically, Lyme arthritis appears to be caused by the host inflammatory system in response to invasion of joints by the spirochete and is characterized by edema, synovial thickening, tendonitis, and a leukocytic infiltration consisting mainly of neutrophils and mononuclear cells. *B. burgdorferi* produces many outer membrane lipoproteins which possess potent inflammatory potential and are believed to be responsible for the inflammatory properties attributed to this spirochete (16). These lipoproteins (such as OspA) are capable of activating a wide variety of cell types, including macrophages, neutrophils, and endothelial cells, which results in the production of a broad spectrum of pro- and anti-inflammatory mediators that have been linked to inflammatory response by *B. burgdorferi* (12~17).

The induction of pro-inflammatory molecules is mediated through the activation of specific signaling pathways. The signaling pathways involved are typically specific to the stimulus and to the cell type. Several laboratories have studied the role of major signaling pathways in the development of Lyme arthritis. Among them are mitogen activated protein kinase (MAPK), and janus kinase/signal transducer and activator of transcription (JAK/STAT). Behera

*et al.* have shown that the addition of *B. burgdorferi* to cultures of human chondrocytes results in the activation of at least two arms of the MAPK pathway, p38 and JNK, and components of the JAK/STAT pathway including STAT-3 and STAT-6, but not STAT-1 (69). Inhibition of each of these signaling pathways results in a diminution of the expression of specific groups of cytokines, chemokines and MMPs (69). Anguita *et al.* have demonstrated the importance of the MAPK pathways *in vivo* using mice that are deficient in MAP kinase kinase-3 (MKK-3) which is an upstream activator of p38 (73, 74). These mice showed significantly reduced production of pro-inflammatory cytokines and a reduction in arthritis compared to matched controls. Consistent with *in vitro* studies of chondrocyte stimulation with *B. burgdorferi* showing lack of STAT-1 activation (69), Brown *et al.* have recently shown using STAT-1 deficient mice that STAT-1 is involved in carditis in response to *B. burgdorferi* but not arthritis (75).

## Phagocytosis of *B. burgdorferi*

The presence of phagocytes, such as neutrophils and macrophages, at the sites of *B. burgdorferi* infection indicates that the phagocytosis may play an important role in efficient clearance of the organisms. It has previously been shown that *B. burgdorferi* are efficiently phagocytosed by macrophages, neutrophils and dendritic cells (23, 76~78) and recruited to a lysosome within 20 min post *B. burgdorferi* infection. Specifically, macrophages *in vitro* rapidly ingest and kill *B. burgdorferi* in large numbers, with or without opsonization and participation of Fc receptors (79). The role of TLR signaling for phagocytosis of *B. burgdorferi* was studied using bone marrow-derived macrophages and peritoneal macrophages. MyD88-mediated effect on uptake of *B. burgdorferi* is thought to be through TLR signaling-mediated activation of phosphoinositide 3 kinase (PI3K) and recruitment of actin-related protein complexes (Arp2/3), which result in actin polymerization to initiate phagocytosis (80, 81). Phagocytosis is not only required for efficient clearance of the organisms, it is also found to be important for generating signals for host inflammatory

response. The phagocytosis of *B. burgdorferi* and subsequent degradation within phagolysosomes further were shown to amplify the release of inflammatory cytokines through activation of TLR signaling within phagolysosome (26).

### Immune evasions by *B. burgdorferi*

As discussed above, a robust humoral and cellular immune response occurs upon *B. burgdorferi* infection, however, infection with *B. burgdorferi* can persist. While the exact mechanism by which *B. burgdorferi* evades the immune system is not known, several mechanisms have been hypothesized. One possibility is that *B. burgdorferi* evades immune response by binding to various components of the extracellular matrix and migrating rapidly intracellular compartments and the extracellular matrix. After *B. burgdorferi* reaches the dermis, it expresses binding proteins called adhesins to facilitate its dissemination. In particular, decorin-binding adhesins (DbpA and DbpB) seem to play an important role by binding to decorin, a collagen-associated proteoglycan (82).

Another possibility is that *B. burgdorferi* frequently changes its surface antigens to evade immune detection. Once *B. burgdorferi* enter the skin, the spirochetes are thought to downregulate lipoproteins, such as OspC, which are no longer required to establish or maintain infection (83). Expression of OspC plays an essential part in the establishment of infection in a mammalian host, although the mechanism by which OspC promotes *B. burgdorferi* infectivity is unknown. *B. burgdorferi* also uses a system of antigenic variation to evade antibodies. VlsE is a 35 kDa lipoprotein that undergoes antigenic variation through the recombination of sequences from silent cassettes into the expressed *vlsE* locus (9, 83, 84). Lastly, *B. burgdorferi* may induce autoimmunity in the host following the initial infection, which could produce chronic infection. This hypothesis was suggested by the findings of the structural homology between OspA and human gene called leukocyte function-associated antigen (LFA-1) (85, 86). These and other findings indicate that the *B. burgdorferi* alter antigen

expression during infection in order to evade B cell-mediated antibody response and do not elicit effective memory responses to protective antigens. Thus, it is challenging to identify target antigens that can induce protective immunity and this needs further investigation.

### Conclusion

*B. burgdorferi* infections and the diseases that they cause are a growing public health problem, and there is currently no available vaccine in use. In 2012, a first patient diagnosed with Lyme disease was for the first time officially confirmed by the national surveillance system in Korea (87). Because of wide distribution of *Ixodes* ticks among mountains in Korea, which carry *B. burgdorferi*, it is necessary to perform continuous surveillance system for Lyme disease and alert the public about how to protect themselves from getting tick bites.

Despite continued advances in our understanding of the pathogenesis of Lyme disease, there remains much to learn about how host immune defenses react to the organism. How host immune system modulates sensing of the pathogens and activates various signaling pathways requires more attention. In addition, host genes responsible for Lyme disease susceptibility in humans is largely unknown and require more studies because we need to answer the question of why some infected patients have only subclinical disease, whereas others develop overt manifestations. These and other advances will hopefully lead to a better understanding of the determinants of vector and host specificity for *B. burgdorferi*, and to the manipulation of these determinants to interrupt the cycle of transmission.

Lastly, it will be essential to develop a new vaccine for Lyme disease. An OspA-based human vaccine to protect against *B. burgdorferi* infection was developed and approved by the US Food and Drug Administration (FDA). However, due to low demand, the vaccine was removed from the market in early 2002 by the manufacturer, GlaxoSmith-Kline (GSK). Limitations and failed public acceptance of a human vaccine led to its demise, yet current research involving new paradigms for future vaccine design that

would include elements of both the vector and the pathogen will be interesting to follow.

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