INVITED REVIEW

A joint effort: The interplay between the innate and the adaptive immune system in Lyme arthritis

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Abstract

Articular joints are a major target of *Borrelia burgdorferi*, the causative agent of Lyme arthritis. Despite antibiotic treatment, recurrent or persistent Lyme arthritis is observed in a significant number of patients. The host immune response plays a crucial role in this chronic arthritic joint complication of *Borrelia* infections. During the early stages of *B. burgdorferi* infection, a major hinder in generating a proper host immune response is the lack of induction of a strong adaptive immune response. This may lead to a delayed hyperinflammatory reaction later in the disease. Several mechanisms have been suggested that might be pivotal for the development of Lyme arthritis and will be highlighted in this review, from molecular mimicry of matrix metallopeptidases and glycosaminoglycans, to autoimmune responses to live bacteria, or remnants of *Borrelia* spirochetes in joints. Murine studies have suggested that the inflammatory responses are initiated by innate immune cells, but this does not exclude the involvement of the adaptive immune system in this dysregulated immune profile. Genetic predisposition, via human leukocyte antigen-DR isotype and microRNA expression, has been associated with the development of antibiotic-refractory Lyme arthritis. Yet the ultimate cause for (antibiotic-refractory) Lyme arthritis remains unknown. Complex processes of different immune cells and signaling cascades are involved in the development of Lyme arthritis. When these various mechanisms are fully been unraveled, new treatment strategies can be developed to target (antibiotic-refractory) Lyme arthritis more effectively.

KEYWORDS
Borrelia burgdorferi, innate and adaptive immune system, Lyme arthritis, T-helper cells

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1 | INTRODUCTION AND CLINICAL BACKGROUND

In 1976, Lyme disease was first described in the town of Old Lyme, Connecticut, USA. The disease was recognized as a tick-transmitted disease due to the geographical clustering of children with what was initially thought to be juvenile rheumatoid arthritis (RA).[1,2] A couple of years later in 1981, the causal pathogen was identified as *Borrelia burgdorferi*.[3] The early localized form of the disease is diagnosed by identifying the pathognomonic erythema migrans (EM), often described as a bull's-eye rash around the tick bite.[3,4] In a longitudinal observational study, about 60% of 55 untreated EM patients developed at least one attack of arthritis over the course of 4 years.[5] Since these cases, Lyme arthritis has been characterized as a mono- or oligoarthritis, typically presenting as a monoarthritis of the knee. Other signs of Lyme arthritis include joint swelling, synovial hypertrophy, vascular proliferation, and infiltration of immune cells.[6]

The occurrence of Lyme arthritis differs between geographical regions due to the presence of various species of *B. burgdorferi* sensu lato, which are more likely to disseminate to a certain organ depending on their surface protein expression.[7] Since *Borrelia garinii* and *Borrelia afzelii* are more common in Europe, and *B. burgdorferi* sensu stricto, the strain more likely to disseminate to the joints, is predominantly observed in North America, Lyme arthritis is more common in this latter region.[8-13]

Lyme disease can be difficult to diagnose when clear pathognomonic signs, such as a typical EM, or the presence of a tick bite, have not been observed. Also, serological testing for Lyme disease has its limitations. Shortly after infection, antibodies can still be undetectable, and serological responses can be broken off due to antibiotic therapy.[14,15] On the other hand, once formed, immunoglobulin G (IgG) antibodies can be detectable for years, even after the infection has passed.[16,17] Incorrect diagnosis increases the risk of further advancement of Lyme disease in the patient while the infection could be effectively treated with antibiotics such as doxycycline.[18,19] However, in a small percentage of patients, symptoms persist, even after antibiotic treatment, probably due to differences in disease development and recurrent inflammation (Figure 1).[1]

Lyme arthritis can manifest itself as early as 4 days or as late as 4 years after an EM. In untreated patients, it affects not only the knee joint but also other large or small joints. If left untreated, synovitis can continue for months up to years.[5] Usually, Lyme arthritis symptoms resolve after appropriate antibiotic therapy. In one study, resolution of arthritis has been observed in 80% of patients treated with doxycycline.[20] However, more recent studies describe residual synovitis after the first course of antibiotics in 34% and even up to 40% in patients treated with doxycycline.[21,22] Even after repeated
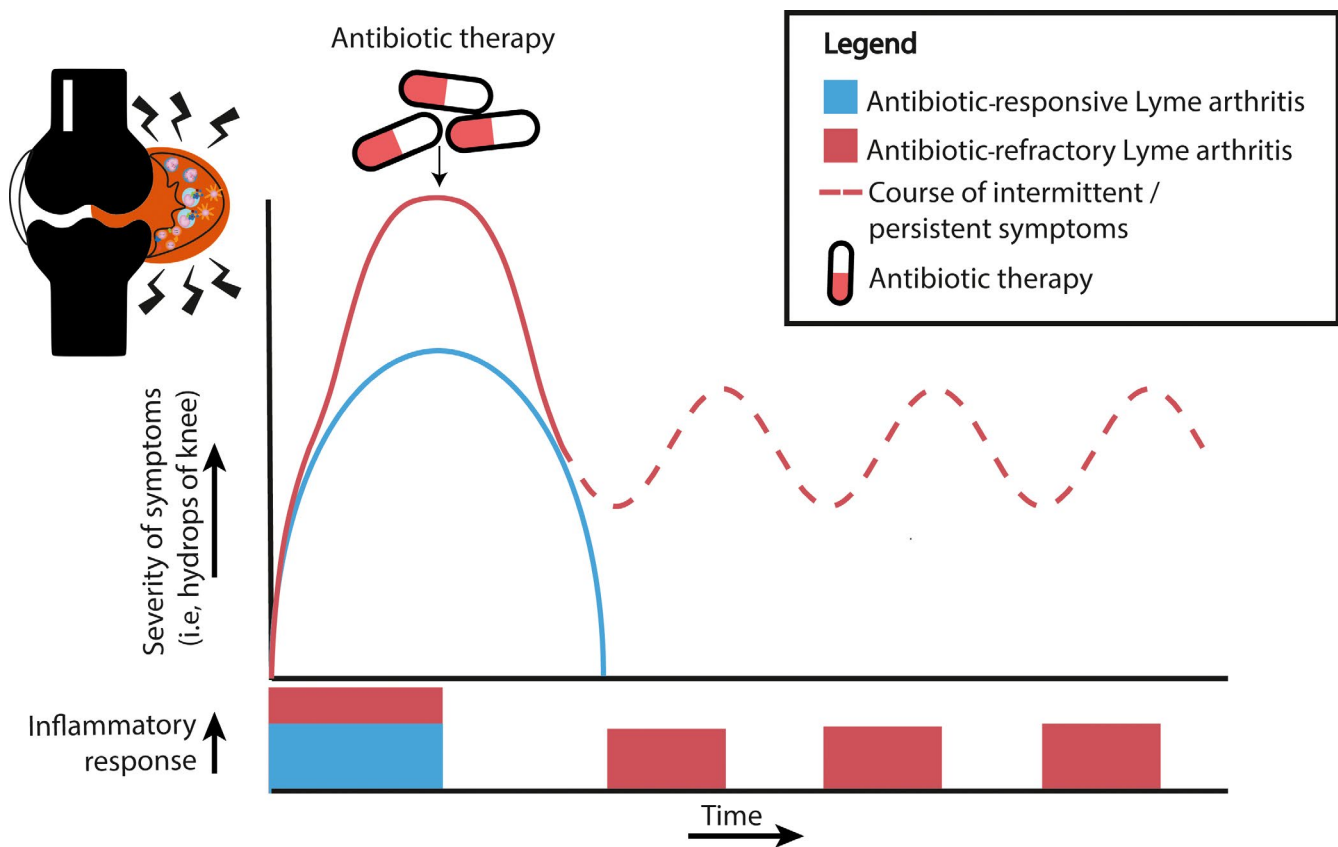
FIGURE 1 Schematic representation of the possible course of (antibiotic-refractory) Lyme arthritis symptoms over time. In the majority of patients, arthritis symptoms resolve when antibiotic therapy is given. However, arthritis can persist in a subset of cases. Usually, these symptoms are present intermittently as is presented in this graph. A depiction of the (maladaptive) immune response is given below

courses of antibiotic therapy, symptoms persist in some patients. This condition is called antibiotic-refractory Lyme arthritis.

The most imperative question for both healthcare providers and patients remains if this is due to persistent infection and if additional courses of antibiotics should be prescribed. Persistence of *B. burgdorferi* infection as a cause for antibiotic-refractory Lyme arthritis seems unlikely based on several observations.[23] Firstly, polymerase chain reaction (PCR) for *B. burgdorferi* DNA in the synovial fluid is often negative in antibiotic-refractory Lyme arthritis patients, while it is positive at the onset of disease.[24-26] Likewise, a study on synovial samples collected by arthroscopic synovectomy in 26 antibiotic-refractory Lyme arthritis patients observed negative PCR results in all samples.[27] Secondly, in most cases cultivation of *B. burgdorferi* in synovial fluid cannot be performed or shows non-motile spirochetes.[28-30] Finally, recurrent or persistent Lyme arthritis often improves upon anti-inflammatory therapy.[31]

In this review, we will discuss the role and interaction of *B. burgdorferi* with the innate and the adaptive immune response. We will describe this relationship during early infection, dissemination, and the development of persistent inflammatory reaction in some patients, resulting in antibiotic-refractory Lyme arthritis. This overview may generate directions for future research on the pathogenesis of Lyme arthritis.

2 | FROM SKIN INVASION TO ARTICULAR JOINTS: INITIATION OF LYME ARTHRITIS

The dermis is the first tissue that the *B. burgdorferi* bacteria encounter once they enter the skin after tick inoculation.[32] It consists of a broad range of extracellular matrix (ECM) proteins and polysaccharide components and is particularly rich in collagen type I.[33] Tick saliva supports the spirochete to survive in the host tissues. Various tick salivary factors accommodate in the localized disruption of host tissues and immune responses.[34-38] Outer surface proteins (Osps) are widely expressed by the spirochete once established in the host and support the bacteria to evade the immune system through suppression of several immune recognition pathways.[39] Following skin inoculation, in some patients *B. burgdorferi* may spread throughout the human body, targeting specific tissue sites. This can cause a diverse range of symptoms, from chronic neurological complications to carditis, skin abnormalities and damage to the host's joints.[5,7-13]

Borrelia species dissemination may occur through two routes: hematogenous and non-hematogenous.[40-43] Non-hematogenous spread transpires via the lymphatic system. Upon hematogenous dissemination, *Borrelia* species translocate through the endothelium and ECM of the vasculature, crossing the last remaining tissue barriers to arrive at the target site.[44-47] *Borrelia* species preferentially target the heart or articular joints or cross the blood-brain barrier.[1,7]

Microvascular interactions of *Borrelia* spirochetes in murine skin were visualized using epifluorescence and spinning disk confocal intravital microscopy. These images showed that following inoculation the bacterium was localized in the capillaries, postcapillary venules, and large veins. After encountering the vascular endothelium, *Borrelia* bacteria tethered to the endothelial cells and underwent dragging interactions in the direction with the blood flow, followed by stationary adhesion.[45] Plentiful *Borrelia* lipoproteins bind to ECM components and may be involved in the tethering of *Borrelia* bacteria to the vascular endothelium.[46-48] Fibronectin and glycosaminoglycans (GAGs) potentially mediate these interactions.[49] Spirochaetal lipoproteins have also been observed to interact with integrins of primary human chondrocytes, triggering myeloid differentiation primary response 88 (MyD88)–independent inflammatory pathways and eventually leading to the development of Lyme arthritis.[50,51]

Fibronectin binding may generate enough time for integrins to bind to the vascular wall, thereby supporting the contact of pathogen and host.[52] BBK32 is a *B. burgdorferi* surface protein that binds to fibronectin,[44,49,53,54] possibly through the utilization of lectin binding sites or other molecules. Several other *B. burgdorferi* proteins have also been identified as fibronectin-binding proteins, including RevA, RevB, and BB0347.[55,56] *Borrelial* lipoprotein decorin-binding protein A (DbpA) can bind decorin, heparin, and dermatan sulfate GAGs [48,57,58] and is widely expressed in skin and cartilage tissue.[59,60] *B. burgdorferi* dissemination to the joints has been correlated with the expression of this DbpA gene, and the presence of spirochetes has been associated with the amount of decorin found in host tissues.[61] Moreover, decorin in the host was observed to be required for the development of Lyme arthritis in mice.[62]

While decorin is important in *B. burgdorferi* adhesion and invasion, it has also been suggested that the spirochete is able to directly bind to intact collagen. A study using hydrated collagen type I lattices of *B. burgdorferi* adhesion without decorin showed that the bacteria were still able to bind to the collagen matrix, even if glycosaminoglycan chain degrading enzymes were added to the matrix or if an agar matrix, bovine serum albumin, gelatin, or pepsinized type I collagen was utilized. In contrast, *B. burgdorferi* adhesion to collagen was shown to diminish in the absence of flagella and by proteinase K treatment, demonstrating that *B. burgdorferi* surface proteins play an important role in collagen binding.[63] GAGs seem to further promote *Borrelia* spirochete adhesion to the collagen.[63] Therefore, these above-mentioned molecules and structures may be important in *B. burgdorferi* association with intact type I collagen matrices. *B. burgdorferi* also expresses peptidoglycans, which may be recognized by the human immune system. Recently, human IgG responses against *B. burgdorferi* peptidoglycan have been related to worsening of Lyme arthritis in the long term.[64]

After adhering to the endothelium, *B. burgdorferi* traverses the endothelial cell monolayers through tight junctions.[65,66] *Borrelia* P66 protein can form an OM β-barrel porin that adheres to host β1 and β3 chain integrins. The β1 chain is crucial in *Borrelial* internalization and transmigration across cellular junctions. P66 may assist in the intracellular invasion of *B. burgdorferi* as well.[67-69] Furthermore, P66 is known to associate with OspA and OspB.[70] High-temperature requirement protease A is a well-known family of adenosine triphosphate-independent serine proteases that can break down ECM components. *B. burgdorferi* encodes such a protease, supporting *Borrelial* tissue invasion through ECM degradation.[71]

Another *Borrelia* host target is aggrecan, a proteoglycan commonly found in cartilage ECM.[62,72] The enzymes responsible for the degradation of aggrecan, called aggrecanases, have been shown to be induced in human chondrocytes infected with *B. burgdorferi*.[72,73] One of these aggrecanases is ADAMTS-4. Active forms of ADAMTS-4 were significantly increased in the synovial fluid samples of patients with active Lyme arthritis and *Borrelia*-infected mice.[72] ADAMTS-5 was not elevated in Lyme arthritis, but is prominent in osteoarthritis.[72,74] ADAMTS-4 may therefore be an aggrecanase induced differentially from ADAMTS-5. This differential expression may be specifically associated with cartilage damage in Lyme arthritis.

B. burgdorferi also activates the host enzyme plasmin via Osp binding.[55,75-77] By binding plasminogen, *B. burgdorferi* promotes its conversion to plasmin by a host-derived activator, thereby further promoting the degradation of ECM components, through the activation of matrix metalloproteinases (MMPs).[55,75-80] Plasmin activates pro-MMP forms of MMP-1, MMP-3, MMP-10, and MMP-13, enzymes involved in degrading the ECM.[81-86] This degradation may support the host to clear the infection, but conversely, it may also promote spirochete dissemination or the development of Lyme arthritis.[78,87-89] The intricate balance in the immune and tissue repair responses seems to play an important role in whether the infection may develop into a hyperinflammatory response that damages the joints or helps the host in tackling the infection. *B. burgdorferi* can directly induce the production of several MMPs by host cells as well. In human chondrocyte cultures with the bacterium, an increased production of MMP-1, MMP-3, MMP-13, and MMP-19 was demonstrated,[87,90-92] whereas *Borrelia*-stimulated monocytes produced MMP-9 and MMP-10.[78,93,94] In blister fluid from human EM lesions, MMP-9 expression was selectively increased compared with blister fluid from normal-appearing skin.[95] Moreover, antibiotic-refractory Lyme arthritis patients have higher levels of MMP-3 in their serum and synovial fluid, though antibodies against these enzymes were less common. Furthermore, synovial fluid concentrations of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), as well as T-cell chemoattractants CXCL9 and CXCL10, were significantly higher.[96-98] The expression of these cytokines and chemokines may stimulate tissue-resident cells to express degrading enzymes such as MMP-10.

These studies give an overview of the broad range of host and *B. burgdorferi* molecules, proteins, and tissues involved early in infection, further complicating *Borrelia* infection and the immune responses against this invading pathogen. Some of these host and bacterial factors have been shown to be involved in the initiation of Lyme arthritis or even lead to such a hyperinflammatory reaction that antibiotic therapy is no longer an effective treatment strategy (Figure 2).

3 | INITIATION AND DEVELOPMENT OF ANTIBIOTIC-REFRACTORY LYME ARTHRITIS

Over the last years, several hypotheses on the precise mechanism behind the development of antibiotic-refractory Lyme arthritis have been introduced. Both autoimmune responses and remaining bacteria or bacterial antigens have been suggested to contribute to this process.[25,99]

Borrelia DNA has been shown to be present in the synovial tissue of Lyme arthritis patients.[25] A recent study described persistence of flagellin B DNA and uncultivable *B. burgdorferi* in a range of murine heart muscle, joint, and muscle tissues and at the inoculation site up to 12 months after antibiotic treatment.[100] Also, spirochete remnants were found within joint entheses and adjacent to cartilage after ceftriaxone and doxycycline treatment. This was studied in MyD88 knockout mice that lack the crucial adapter protein for several innate recognition receptors called Toll-like receptors (TLRs), thereby allowing *Borrelia* spirochetes to survive. Interestingly, these spirochete remnants induced IgG antibodies to *B. burgdorferi* in naive mice and triggered macrophages to produce TNF-α in vitro.[101] In contrast to mice, pathogen loads in human Lyme arthritis are low.[24] However, these studies suggest that the persistence of dead spirochetes in the joint may cause an immunogenic response, resulting in prolonged inflammation in these tissues.

Autoimmune responses have been suggested to contribute to the pathogenesis of Lyme arthritis. The observation that specific human leukocyte antigen-DR isotype (HLA-DR) alleles such as DRB1*0401, crucial in binding of *B. burgdorferi* OspA, are more frequent in patients with antibiotic-refractory Lyme arthritis than in those responding to treatment.[102]

Molecular mimicry is another autoimmune mechanism that is potentially involved in the development of antibiotic-refractory Lyme arthritis. Partial sequence homology has been observed between OspA and human lymphocyte function–associated antigen 1 (LFA-1), MAWD-BP, and human cytokeratin-10.[103,104] Other possible targets for molecular mimicry with *Borrelia* bacteria are MMPs as well as several non-protein antigens and neural proteins.[105-110]

Endothelial cell growth factor (ECGF) is an autoantigen, targeted by T- and B-cell responses in around 20% of the antibiotic-refractory Lyme arthritis patients and in 15% of EM patients.[111,112] These ECGF antibody responses correlated directly with the extent of obliterative lesions and the amount of vascularity in the tissue. ECGF is an IFN-inducible protein and is highly expressed in the synovial fluid of patients suffering from antibiotic-refractory Lyme arthritis compared to antibiotic-responsive Lyme arthritis.[112,113] In archival serum samples from 27 untreated patients with Lyme arthritis, 26% of the samples showed ECGF antibody responses. The total duration of active arthritis attacks in these patients was significantly longer than in those who lacked ECGF reactivity. Compared to other rheumatological chronic inflammatory diseases, synovial tissue from Lyme arthritis patients consisted of significantly greater layer thickness and cellular infiltration. Obliterative microvascular lesions were observed in patients with Lyme arthritis only, whereas these lesions were not reported in RA.[112] The presence of these lesions was correlated with autoantibody responses to ECGF. ECGF autoantibodies are produced early in Lyme infection, months before the onset of arthritis and obliterative microvascular lesions.[111,112] This implies that autoantibodies play a role in the development of these
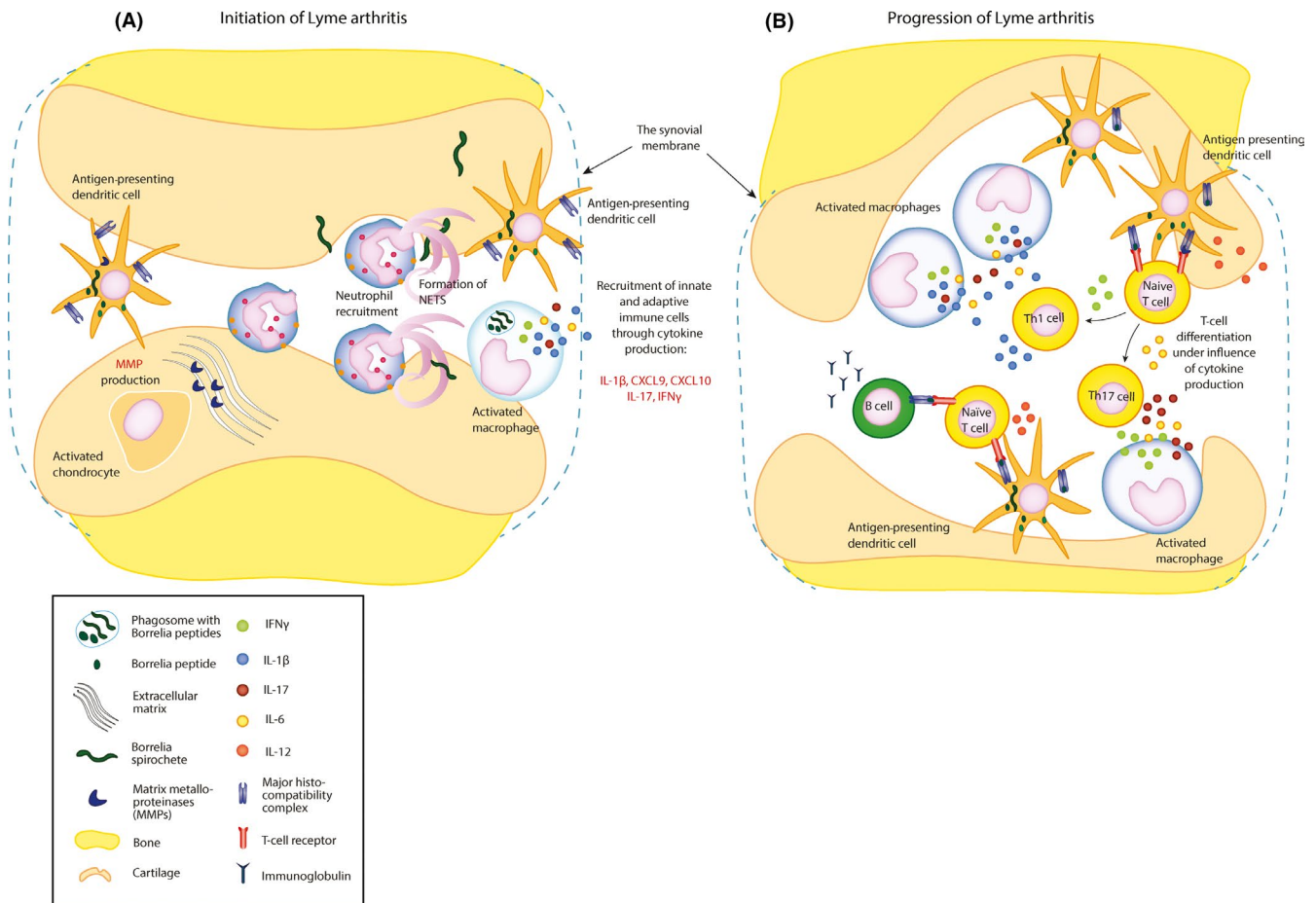
FIGURE 2 Lyme arthritis: a joint effort. A, The initiation of Lyme arthritis through enhanced cytokine and chemokine production by both macrophages and dendritic cells, as well as neutrophil formation of extracellular traps (NETs) and tissue-resident cells such as chondrocytes producing matrix metalloproteinases (MMPs). Of these factors IL-1β, IFN-γ, IL-17, CXCL9, CXCL10, and MMPs are shown in red because they play a major role in worsening the disease development and whose levels are often enhanced in patients with antibiotic-refractory Lyme arthritis. B, The progression of Lyme arthritis, causing a more chronic phenotype with high numbers of (effector) T cells, initiated via dendritic cell stimulation of T-cell receptors using either Borrelia or self-antigens, without the necessary presence of live Borrelia spirochetes, and antibody production from activated B cells. The continued presence of activated innate immune cells further heightens the number of activated adaptive immune cells and cytokines and chemokine levels

microvascular lesions and thereby in antibiotic-refractory Lyme arthritis. Non-human primate studies have shown that obliterative microvascular lesions may not just be specific for Lyme arthritis, but may be a more general consequence of spirochaetal infections.[114-117]

Lyme arthritis patients are prone to enhanced inflammatory responses. Patients carrying a particular TLR1 polymorphism develop a strong innate and adaptive immune reaction, resulting in high TNF-α, interleukin (IL)-1β, and IFN-γ concentrations in the joints.[97,98] IFN-γ stimulates ECGF production by macrophages and other cells, leading to vascular proliferation and fibroblast activation. As mentioned above, T- and B-cell responses to ECGF were found in only a small number of EM patients.[111,112] However, if these ECGF responders were more prone to develop Lyme arthritis was not further investigated.[112]

Not only ECGF, but also apolipoprotein B-100, annexin A2, N-acetylglucosamine-6-sulfatase, and filamin A–derived peptides may induce T-cell responses in Lyme arthritis patients, usually

already early in the infection, when EM is apparent.[99,111,118,119] Antibiotic-refractory Lyme arthritis patients have higher levels of MMP-3 in their serum and synovial fluid, though antibodies against these enzymes were less common. Furthermore, synovial fluid concentrations of IFN-γ and TNF-α, as well as T-cell chemoattractants CXCL9 and CXCL10, were significantly higher.[96-98] The expression of these cytokines and chemokines may stimulate tissue-resident cells to express degrading enzymes such as MMP-10.

Furthermore, a MMP-10-derived peptide was isolated from an antibiotic-refractory Lyme arthritis patient's synovial tissue. This peptide could also be bound by HLA-DR sequences. Subsequently, the role of MMP-10 was studied in various Lyme patient groups. A marked T-cell response was observed in 25% of the antibiotic-refractory patients and MMP-10 autoantibody levels were correlated with a worse phenotype of Lyme arthritis. However, as the authors suggest, this single autoantibody response is likely not solely responsible for the development of (antibiotic-refractory) Lyme arthritis.[99]

4 | THE ROLE OF THE INNATE IMMUNE SYSTEM DURING THE EARLY STAGES OF *BORRELIA* INFECTION

After initial *Borrelia* infection of the skin, an influx of immune cells from the peripheral blood occurs. Polymorphonuclear leukocytes (PMNs) play an important role in infections. Freshly isolated human PMNs are shown to eradicate spirochetes mostly through antibody-independent extracellular killing processes such as reactive oxygen species (ROS) production, whereas differentiated monocyte-derived macrophages ingest the bacteria and kill the spirochetes without the necessity of opsonization.[120,121] When the tick feeds on a human host, both the bacteria and the tick saliva are released and encounter the accumulating immune cells, influencing the host defense responses.[34,35,122]

Neutrophils are recruited very early at the site of infection. They are able to kill pathogens by a range of defense mechanisms such as phagocytosis, ROS production, and extrusion of their nuclear DNA with elastase and myeloperoxidase, leading to formation of neutrophil extracellular traps (NETs), to capture invading pathogens.[123-125] In vitro incubation of human neutrophils and in vivo infection of mice with *B. burgdorferi* combined with saliva or salivary gland extracts induced NET formation. NETs can trap and kill *Borrelia* spirochetes.[126] Human neutrophils are directly activated by *B. burgdorferi* through interaction with OspA.[127] *B. burgdorferi* is sensitive to ROS and granule enzymes in an in vitro setting, in the absence of tick saliva.[128] However, tick salivary factors impair neutrophil actions at the site of infection [34,129] and there is a lack of neutrophils in EM lesions.[130] Moreover, *Borrelia* spirochetes have developed a range of neutrophil evasion mechanisms, including the inhibition of IL-8 secretion, a cytokine involved in neutrophil recruitment, by Evasin-E3,[131] inhibition of neutrophil chemotaxis through *N*-formylmethionyl-leucyl-phenylalanine activation, and interference of OspB with neutrophil phagocytosis, possibly through direct interaction with the complement receptor 3 (CR3) and oxidative bursts via unknown mechanisms.[132,133] Using rapid movements, the spirochetes can also escape neutrophil interaction. Neutrophil migration is inhibited by binding of the tick saliva leukotriene B4, enhancing bacterial propagation and formation of EM.[134] Tick saliva further interferes with neutrophil function through downregulation of neutrophil integrin expression, inhibition of superoxide anions production and elastase inhibition.[135,136] Previous studies have shown that a diminished neutrophil response may increase the hosts' susceptibility to *Borrelia* infection, whereas increased recruitment of neutrophils early in infection may reduce dissemination of *Borrelia* to the joint.[130]

During infections, neutrophils produce IL-1β, TNF-α, IL-8, and IL-6[137-139] and IL-15[140] in vitro. IL-15 is involved in adaptive responses and vital in natural killer (NK)-cell homeostasis.[141] Depletion of IL-15 in *Borrelia*-vaccinated and *Borrelia*-challenged mice hampered the development of arthritis. Furthermore, anti-IL-15 antibody or recombinant IL-15 receptor alpha reduced infiltration of neutrophils into the synovium.[142]

A role for NK cells in the initiation of Lyme arthritis is postulated by several studies. C57BL/6 mice genetically deficient in granulocytes and NK cells developed less severe arthritis than their wildtype counterparts.[143] However, depletion of NK cells did not influence the course of arthritis,[144] suggesting NK cells augment the development of Lyme arthritis. OspA augments the activation of NK cells,[145] which can drive the induction of various inflammatory mediators, including TNF-α.[146] Anti-TNF-α therapy has been described to be effective in antibiotic-refractory Lyme arthritis.[147] Therefore, these NK cell–derived mediators may contribute to the development and persistence of Lyme arthritis.

Mast cells are also important for the development of immune response in early infection, while the spirochetes still reside in the skin. They patrol the skin to detect new pathogens and play a role in other hyperinflammatory reactions such as allergies.[148] The primary mast cell function in the early stages of Lyme disease was recently studied in C57BL/6 mice. While OspC induced degranulation of mast cells, this degranulation was not observed upon stimulation with whole *Borrelia* spirochetes. In contrast, the addition of tick salivary gland extract (SGE) to the spirochetes enhanced the degranulation. The absence of mast cells in KitWsh−/− mice did not significantly affect the replication rate of *B. burgdorferi* in the skin but promoted *Borrelia* dissemination to the joints. Moreover, the expression of cytokines production by *B. burgdorferi*–activated mast cells was significantly inhibited in the presence of SGE. The authors postulated that the modulation of mast cell function by *B. burgdorferi* in combination with tick saliva may affect long-term and/or repeated infections and protect the host against Lyme borreliosis.[149]

Spread of *B. burgdorferi* is hampered by the ability of macrophages and dendritic cells to bind and phagocytize *B. burgdorferi*.[150-152] Spirochetes ingested by macrophages are quickly localized in endosomes and lysosomes.[153] However, in severe combined immunodeficiency (SCID) and C3H/HeN murine studies with peritoneal macrophages, OspC was shown to protect the spirochete against phagocytosis.[154] Depletion of mononuclear phagocytes in these mice permitted non-functional OspC *Borrelia* mutants to initiate infection in vivo. Increased OspC expression also reduced spirochete uptake by murine peritoneal macrophages, suggesting a role for OspC in anti-phagocytic function.[154] Furthermore, OspC was shown to interfere in plasminogen function and promote spirochete invasiveness.[75,155,156] Thus, *B. burgdorferi* effectively uses its Osps to avoid phagocytosis.

In EM lesions, large populations of monocytes, macrophages, and dendritic cells are observed.[157] Interaction of murine macrophages with *Borrelia* antigens induces production of nitric oxide, IL-1, TNF-α, IL-6, and IL-12,[145] and MMP-9.[95] Intriguingly, LSH hamsters injected with macrophages that were previously exposed to *B. burgdorferi* developed severe, destructive arthritis. In contrast, animals that received unprimed macrophages did not develop arthritis.[158] Furthermore, the combination of primed macrophages and T cells accelerated the development of arthritis in *Borrelia*-infected ICR mice compared to either one alone.[159,160] This highlights the importance of immune cells capable of presenting *Borrelia* antigens to T cells for the progression to Lyme arthritis. Moreover, this suggests that previous exposure to *B. burgdorferi* might increase pro-inflammatory responses of macrophages.

Type I IFN production by macrophages and myostatin expression has been correlated with *B. burgdorferi* arthritis–associated locus 1 (Bbaa1), in a study on loci associated with Lyme arthritis. Monoclonal antibody blockade of IFN-β resulted in reduced severity of Lyme arthritis in infected B6.C3-Bbaa1 mice. Bone marrow–derived macrophages stimulated with *B. burgdorferi* enhanced IFN-β expression. Inhibition of myostatin in vivo suppressed Lyme arthritis in reduced interval congenic Bbaa1 mice, implicating that myostatin is a downstream mediator in Lyme arthritis.[161] Receptor-blocking antibodies and receptor ablation showed the crucial role of type I IFN production by macrophages in Lyme arthritis severity.[162,163]

Increased IFN-γ signaling in macrophages also enhances indoleamine-pyrrole 2,3-dioxygenase levels.[164] This is a molecule regulating ROS, collagenase, elastase, and stromelysin activity,[165] thereby increasing ECM degradation. Destabilization in the membrane structure of murine cells results in adherence of spirochetes remnants to the cartilage.[101] These *Borrelia* remnants may, in turn, initiate an inflammatory reaction and stimulate TNF-α production by macrophages.[164]

Many studies of Lyme arthritis are performed in mice, due to the limited number of Lyme arthritis patients and available tissue specimens. Murine models are not completely comparable to human Lyme arthritis, and large differences in disease expression are observed between different mouse strains. C3H/HeN mice, for example, are highly susceptible to *Borrelia*-induced arthritis once the bacteria are injected into the joint. These mice have a defect in IL-12 production, which hampers the induction of IFN-γ through IL-12/IL-18 signaling. This possibly explains why these mice are more susceptible to invading bacteria.[166] In contrast, C57BL/6 mice do produce IFN-γ upon *Borrelia* exposure.[167]

TLRs are cell receptors that recognize conserved molecular patterns on microbial components, such as lipoproteins, lipopolysaccharide, proteoglycans, flagellin, and nucleic acids. Binding of TLRs with these components results in NF-κB-mediated expression of pro-inflammatory cytokines. TLR2-deficient mice were shown to have a significant increase in the number of spirochetes in their joints after infection compared to WT mice.[157,168,169] TLR2-deficient C3H mice demonstrated a T cell–dependent increase in mononuclear cell infiltration. The number of T cells attracted to the joint tissue was enhanced, possibly through augmented production of T-cell chemokines CXCL9 and CXCL10. These T cells, in turn, enhanced mononuclear cell migration via the increased release of other inflammatory factors.[170,171] A recent study in C3H/HeN mice showed that this increase may be attributed to CD8 T-cell activation of synoviocytes.[171] Moreover, transcripts for the IFN-inducible gene IFN-γ-induced GTPase were elevated.[170] Intriguingly, recent studies have shown that TLR2 can function as a co-receptor on activated T cells, influencing their responses and function.[172] However, when compared to wildtype control mice, TLR2-deficient mice had higher *Borrelial* load but no significant difference in joint swelling,[169] while increases in swelling and arthritis have been reported in *Borrelia*-infected TLR2-deficient C57BL/6 mice in another study.[168]

The co-receptor CD14 is crucial in *Borrelia* recognition in collaboration with TLR2.[173-176] Interestingly, a study by Sahay et al[177] suggests that CD14 may be important in *Borrelia* clearance and tolerization of macrophages, through SOCS1 and SOCS3 signaling, whose pathways have also been linked to MMP production and degradation of collagen.[178,179] CD14 deficiency in mice reduced these effects significantly and led to higher bacterial burdens and more severe and persistent inflammation.[177,180] However, the murine studies demonstrated a possible link between CD14 signaling and persistent inflammation in Lyme arthritis. Yet, it is still unclear whether this is caused by inhibition of TLR2 signaling or by the direct function of CD14 itself.

The intracellular NOD2 receptor has also been shown to be involved in *Borrelia* recognition.[181] Remarkably, *B. burgdorferi* infection of NOD2 deficiency C57BL/6 mice led to increased inflammation in joints and cardiac tissue when compared to wildtype mice, suggesting that NOD2 tolerization normally protects these mice against dissemination, possibly through the induction of multiple cytokines and interferons.[182] A study by Oosting et al[183] demonstrated that apoptosis-associated speck-like protein containing CARD (ASC)/caspase-1 induction was crucial for IL-1β production of in murine Lyme arthritis. The activation and secretion of IL-1β occurred independently from NOD2/RICK, as well as NOD-like receptor-family member protein 3 (NLRP3) activation, while the TLR2-MyD88 pathway was crucial for IL-1β production. *Borrelia*-exposed NOD1-, NOD2-, and RICK-deficient murine cells still produced IL-6 and TNF-α. In contrast to previous in vitro data, NLRP3 did not seem to be involved in Lyme arthritis development in a murine model.[183-186] ASC was pivotal in Lyme arthritis initiation as an important adapter protein in the inflammasome, involved in antigen presentation, lymphocyte migration, NF-κB activation, and messenger RNA (mRNA) stability and via dedicator of cytokinesis 2 (Dock2) expression and Rac activation.[187] Lyme arthritis patients with high levels of IL-1Ra and a low concentration of IL-1β in their synovial fluid demonstrated rapid resolution of the disease. The crucial role of IL-1Ra in the attenuation of inflammatory diseases was supported by the observation that IL-1Ra-deficient mice spontaneously developed arthritis (Figure 3), while IL-1 receptor–deficient mice had significantly less inflammatory disorders, such as Lyme arthritis. Caspase-1 also plays a role in the induction of an anti-*Borrelia* response in the joint, but over time becomes less important in controlling the progression of the disease.[183] It would be interesting to study whether NOD signaling is involved in IL-1Ra production and thereby in the attenuation of Lyme arthritis.

Another innate regulatory mechanism in Lyme arthritis is the expression of microRNAs (miRNAs). miRNAs are small, non-coding RNA molecules that can bind mRNA and inhibit its translation. They are expressed by various innate immune cells, such as monocytes, macrophages, DCs, granulocytes, and NK cells. miRNAs are crucial in the development and function of innate immune cells and play a role in adaptive immune cell regulation.[188,189] Dysfunction of miRNAs can induce inflammatory diseases and autoimmunity.[190] In mouse studies, several miRNAs are reported to modulate experimental models
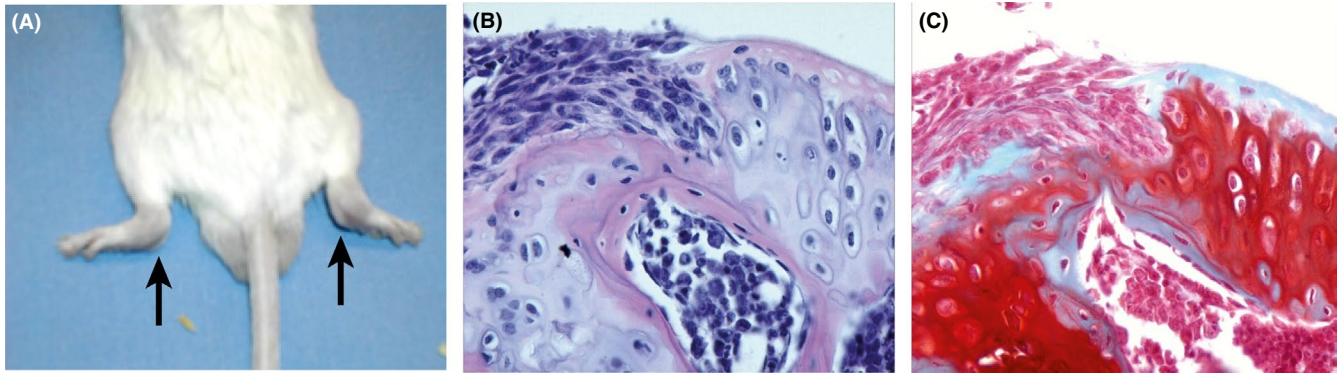
FIGURE 3 Uncontrolled inflammation in the knee joint of a 16-wk-old IL-1Ra−/− mouse. Depicted from left to right: (A) inflamed ankle joints of the IL-1Ra-deficient mice (swelling and redness) indicated by arrows; (B) HE staining of the knee joint—note the pannus-like tissue destroying the bone and cartilage; and (C) Safranin O staining of the same knee joint, showing the severe cartilage destruction (loss of red staining in the cartilage layer). HE, hematoxylin and eosin; IL-1RA, interleukin-1 receptor antagonist

of arthritis, including miR-155,[191] miR-223,[192] and miR-146a.[193] This is consistent with the role of miRNAs in RA.[194] Intriguingly, mice lacking either miR-146a or miR-155 were shown to develop more severe Lyme arthritis or carditis.[195,196]

Recently, in groups of patients with different stages of Lyme arthritis, alternate miRNA profiles were observed. Elevated levels of miR-223 and low levels of miRNAs such as miR-146a, miR-155, and miR-142 were found in synovial fluid of Lyme arthritis patients prior to antibiotic therapy (n = 5). Also, elevated levels of white blood cells, specifically PMNs, were found in SF of these patients.[197] In contrast to postantibiotic persistent Lyme arthritis patients (n = 13), who had higher percentages of lymphocytes and mononuclear cells, and a high expression of miR-146a, miR-155, miR-142, miR-233, and miR-17-92. Let-7a family levels were reduced.[197] Of note, arthritis in two of these 13 patients resolved after intravenous administration of antibiotics. miRNA expression in patients with postinfectious Lyme arthritis was most similar to the expression in RA patients, where miR-155, miR-146a, and miR-223 were prominent in the synovial fluid, synovial tissue, and synovial fibroblasts.[198] Higher levels of miR-146a, miR-17, and miR-233 are also correlated with increased duration of Lyme arthritis.[197] Thus, the miRNA pattern in SF changes during stages of Lyme arthritis, possibly representing the altering nature of Lyme arthritis after bacterial killing.

Strle et al[98] studied single nucleotide polymorphisms (SNPs) with a significant effect on the host immune cell function. In general, SNPs in TLR1 (1805GG), TLR2 (2258GA), and TLR5 (1174CT) seem to be crucial in immune function. However, when studying the frequency of these SNPs in a Lyme arthritis cohort, TLR1-1805GG was enhanced in antibiotic-refractory Lyme arthritis patients compared to responsive patients. EM patients carrying this SNP had higher serum levels of CXCL9 and CXCL10, especially if infected with the *B. burgdorferi* 16S-23S ribosomal spacer RNA intergenic type 1 (RST1) strains. CXCL9 and CXCL10 are well-known chemoattractants for CD4 and CD8 effector T cells, the most prominent infiltrating cells in the joint lesions of antibiotic-refractory Lyme arthritis patients.[97] EM patients were also more likely to have symptomatic infection and had greater inflammatory responses than EM patients carrying the

1805TG/TT polymorphism, despite having similar number of spirochetes in EM skin lesions and similar frequency of disseminated infection. Furthermore, antibiotic-refractory Lyme arthritis patients with the 1805GG polymorphism had higher CXCL10 concentrations in joint fluid than antibiotic-responsive patients. The frequency and function of TLR2 and TLR5 polymorphisms did not vary between the patient groups. However, previous murine studies have suggested that TLR2 may play a role in the severity of Lyme arthritis.[170,199] This may be partially explained by the fact that this TLR2 polymorphism does not abolish TLR2 function, as only one allele is affected. Moreover, this allele is present in only a low proportion of patients with a wide range of manifestations of Lyme disease, including EM, ACA, and Lyme arthritis.[200] Nevertheless, this is of interest, since the TLR1/TLR2 heterodimer recognizes *Borrelia* lipopeptides as a complex.[201,202] It is unclear how *Borrelia* infection in humans with the 1805GG polymorphism or in mice with TLR2 deficiency results in a heightened Th1 response.[98] The cells with the SNP might recognize different peptides, activate the adaptive immune cells using a different mechanism, or affect a whole recognition pathway. In murine macrophages stimulated with *B. burgdorferi*, TLR1/2 complex deficiency resulted in persistent augmented levels of inflammatory cytokines and chemokines due to a significant reduction in p38 activation and SOCS expression.[177] Interestingly, IFN-responsive gene induction was shown to occur independently of TLR2, TLR4, TLR9, and MyD88, suggesting that *B. burgdorferi* activates another type I IFN transcription pathway through a different receptor or sensing molecule.[162]

5 | FROM INNATE INITIATION TO ADAPTIVE DYSREGULATION

The innate immune system is the key initiator of Lyme arthritis (Figure 2). It recruits, activates, and drives the adaptive immune response, leading to further dysregulation and worsening of the disease. Studies in SCID mice have shown that in the absence of the adaptive immune system, innate immune responses are the primary

drivers for *B. burgdorferi* tissue damage and Lyme arthritis. In contrast, the adaptive responses control the pathogen, primarily using antibody production, and may in a later stage contribute to immune dysregulation in the joints.[162,203] The predominant innate immune cells seem to differ in several manifestations of Lyme disease, and so the innate pathways may differ.[204] The importance of innate immune cells was studied in mice without both B and T cells. The authors observed that innate immune cells are crucial in Lyme arthritis commencement and adaptive immune cells are not the direct cause of Lyme arthritis initiation and probably only worsen the disease symptoms.[99,162] Moreover, innate immune cells are responsible for the production of MMPs, HLA-DR subsets, and signaling involved in the disease, as well as for induction of IFN production, through other IFNs, IL-12, and IL-18.[81,102,162,183] In synovial tissue from patients with Lyme arthritis, DCs were observed in areas with T- and B-cell aggregates. This demonstrates the collaboration of these immune cell subsets in the joints. While innate immune cells initiate the immune response, adaptive immune cells further contribute to worsening of the disease phenotype, either by formation of autoantibodies or excessive inflammation. The same study showed how plasma cells were abundantly present around the lymphoid aggregates, demonstrating the local production and secretion of (auto)antibodies as well as a joint effort of both innate and adaptive immune cells in the development of Lyme arthritis.[112]

Interestingly, T cell–independent antibodies have been shown to be protective against Lyme arthritis.[205] Splenic B-cell compartments expanded marginal zone B cells during *B. burgdorferi* infection. No changes were observed in the follicular B-cell subset.[206] In contrast, peripheral blood plasmablasts and CD27− memory B-cell populations were elevated in untreated Lyme arthritis. Higher plasmablast numbers and robust serum reactivity were also correlated with a more rapid resolution of the disease.[207] Consequently, the balance in B cell–independent and T cell–independent antibody responses may affect disease development and patient prognosis of Lyme arthritis.

Beyond these observations on how innate immune recognition and activation of adaptive immune responses result in a more severe phenotype of Lyme arthritis, the initiation of Lyme arthritis in *Borrelia*-infected C3H SCID mice occurred independently of both B- and T-cell infiltration in the joint tissue. Moreover, the severity of Lyme arthritis seemed directly related to type I IFN induction in innate immune cells, since blocking its receptor, IFNAR1, reduced the ankle swelling in mice significantly,[162] while anti-IFN-γ had no effect. In addition, other studies in mice have shown that IFN-γ production was not required for the development of Lyme arthritis.[144,208-210] However, the synovial fluid of patients with antibiotic-refractory Lyme arthritis contains significant levels of IFN-γ and related chemokines. Thus, IFN-γ may be crucial in further development of persistent arthritis and/or as a consequence of initiator of the disease.[96-98]

Next to Th1 cell–oriented responses, Th17 cell responses have been shown to play a role in Lyme arthritis. The Th1 and Th17 cell phenotypes point toward the involvement of both IFN-γ and IL-17 in Lyme arthritis. The role of IL-17 has been elaborated in several studies. In an experimental mouse study, IL-17 inhibition prevented the development of Lyme arthritis.[211] In patients with Lyme arthritis, Th17 cells were observed in the synovial fluid.[212] Moreover, *B. burgdorferi* or its lipoproteins were shown to be able to induce the production of IL-17 in vitro.[213,214] In addition, synovial T cells, extracted from patients with Lyme arthritis, produced IL-17 in response to *Borrelial* antigen neutrophil-activating protein A.[215] IL-17 production was shown to be caspase-1- and IL-1β-dependent, through the activation of IL-17-producing T cells. In the absence of IL-1β, a defective IL-17 production was observed, while IL-18, a cytokine which is also caspase-1-dependent, was vital for IFN-γ production in Lyme arthritis. Caspase-1-dependent IL-33 did not play a role in *Borrelia*-induced IL-1β, IFN-γ, or IL-17 secretion.[167]

Patients with antibiotic-refractory Lyme arthritis have remarkably high levels of the innate mediator IL-6 in their synovial fluid, which is vital for Th17 lineage specification.[216] IL-23 levels are also enhanced, which may polarize Th17 cells toward a more pathogenic phenotype throughout the course of the disease, due to enhanced infiltration of inflammatory myeloid cells into the tissue.[217,218] In some patients, a chronic inflammatory environment, with particularly elevated IL-23 levels and autoantigens present in the joints, contributes to the development of pathogenic and Th17-related autoimmune responses. In mice, neutralizing antibodies against both IL-17 and IL-23 delayed the onset of joint swelling, reduced IL-17-producing CD4 T cells, and enhanced regulatory CD4 CD25 T-cell numbers.[219]

IL-23 is vital for the development and maintenance of Th17 cells. Oosting et al[220] showed that both IL-17 and IL-23 were produced upon *B. burgdorferi* exposure. However, in contrast to murine studies, when the IL-23 bioactivity was inhibited in human peripheral blood mononuclear cell (PBMC) cultures or when PBMCs from human subjects who carried a loss of function SNP in the gene coding for the IL-23 receptor were stimulated with *B. burgdorferi*, IL-17 production was significantly suppressed, while IFN-γ production was not affected. Though IL-17 production was decreased, the IL-23R gene polymorphism had no consequences for the development and pathogenesis of persistent symptoms attributed to Lyme disease. Both IL-17 and IL-22 were produced upon PBMC stimulation with *B. burgdorferi*.[220] However, whereas IL-17 levels remained quite low, IL-22 was significantly upregulated within 10 hours of exposure, related to monocyte-induced caspase-1 and IL-1 bioactivity.[221] Thus, Bachmann et al[221] suggest IL-1β as a single stimulus for the induction of IL-22 but not for IL-17 in human PBMCs. Of high interest, *Borrelia*-induced IFN-γ and IL-22, but not IL-17, production in humans is shown to be age-dependent. This might explain why elderly subjects are more susceptible to infection, including Lyme disease.[222]

In collaboration with antigen-presenting cells, T cells function between the innate and adaptive immune responses, promoting each other's function and manifesting cytolytic activity. Caspases are also involved in cell proliferation and death. Their signaling in T cells is more efficient if T-cell receptors (TCRs) are activated. Analysis of active caspases in human T cells showed that caspase-3, but not its upstream initiator caspase-8 or caspase-9, is elevated in Lyme

arthritis.[223] These active caspases seem to appear in lipid rafts at the cell membrane during T-cell cycling[224,225]; thereby, their access to cytoplasmic substrates involved in cell death is denied. The prominent caspase-3 activity may be caused by more intense TCR signaling in T cells, resulting in a rapid effector T-cell response. Moreover, this activation may be related to the Fas-associated death domain-like IL-1 expression.[223] Of high interest, recent research proposes that Fas:FasL signaling between CD4 T cells, DCs, and γδ T cells may be part of the inflammatory reaction in (antibiotic-refractory) Lyme arthritis.[226]

The synovium from Lyme arthritis patients contains high levels of CXCL10, a chemokine specific for Th1 cells, and CCL2, important for both Th1 and Th17 recruitment via CXCR3 expression.[97] PMNs are well known for producing these chemokines in response to LPS or IFN-γ[227-229] and may therefore be involved in T-helper cell recruitment. Codolo et al[6] demonstrated that CCL20 is also present in the synovial cavity of Lyme arthritis patients, which attracts lymphocytes and possibly dendritic cells through its association with CCR6, showing the vital cytokine and chemokine signals involved in attracting various immune cell subsets to the joint.

In the synovial fluid of patients with Lyme arthritis, RA, and other types of inflammatory arthritis, Th1 cells are prominent. Previous studies have shown that the Th1/Th2 cell ratio directly correlates with the severity of Lyme arthritis, suggesting a protective effect of Th2 cells on the Th1-driven inflammatory reaction.[212] Some of the Th1 cells identified were specifically targeting *Borrelial* OspA. Active CD4 regulatory T cells (Tregs) were more abundant in the synovial fluid of antibiotic-refractory Lyme arthritis than in the peripheral blood. An increase in CD4 effector/Treg cell ratio is known to directly correlate with the development of antibiotic-refractory Lyme arthritis.[96] Patients with fewer Tregs had suboptimal responses to disease-modifying antirheumatic drugs (DMARDs), required longer antibiotic treatment, and often required synovectomies for successful resolution of their Lyme arthritis.[212] Another study showed that the synovial fluid of antibiotic-refractory Lyme arthritis patients consisted of a CD4$^+$CD25^{hi} population with fewer FOXP3$^+$ Treg cells and more FOXP3$^-$ effector T cells compared to patients with antibiotic-responsive Lyme arthritis. Antibiotic-refractory Lyme arthritis patient cells also showed significantly greater expression of glucocorticoid-induced TNFR-related protein (GITR) and OX-40, two co-receptors that augment T-cell function. Their T cells did not effectively suppress T-cell activation and proliferation. They showed enhanced secretion of IFN-γ or TNF-α. Moreover, in the refractory patients, higher ratios of CD25^{hi}FOXP3$^-$/CD25^{hi}FOXP3$^+$ cells were associated with longer post-treatment duration of Lyme arthritis symptoms.[96]

6 | THERAPY OF LYME ARTHRITIS

As is the case in localized early Lyme disease, timely antibiotic treatment is recommended for Lyme arthritis.[230-232] This is based on several double-blinded randomized trials. Efficacy was first investigated in a parental penicillin trial.[233] In this study, 35% of patients had complete resolution compared to none of the placebo-treated patients. The percentage of patients with resolution of Lyme arthritis was higher in trials on doxycycline therapy.[20,23] Oral therapy was shown to be as effective as IV therapy, but safer and less expensive.[234] Furthermore, antibiotic treatment shortens the duration of Lyme arthritis considerably. The clinical course of 21 patients with EM and Lyme arthritis treated with non-steroidal anti-inflammatory drugs and intra-articular steroids without antibiotic treatment was described in the 1980s.[5] These individuals had attacks of arthritis, ranging from short attacks of arthritis to continuous synovitis, for a median total time of 43 months,[5] whereas the duration of arthritis in antibiotic-treated patients ranged from a median of 4 months in an antibiotic-responsive group (n = 50) to 16 months in an antibiotic-refractory disease group (n = 62).[23] In most patients with Lyme arthritis, symptoms resolved after a single course of antibiotic therapy, but this proportion varies from 48% to 90% in both Europe[22,235,236] and North America.[20,23,237]

In the case of persistent arthritis, a second course of antibiotics could be given. Often, in persistent severe or worsening cases IV therapy with ceftriaxone is chosen because of its reported effectiveness.[238] Another option is to wait for 3 months before retreatment is commenced,[230] because resolution of Lyme arthritis might occur months after a first course of antibiotic therapy.[20,23]

Other therapeutic approaches for antibiotic-refractory Lyme arthritis may be considered, for example treatment strategies employed against other forms of inflammatory arthritis (ie, RA). Unfortunately, randomized trials with DMARDs are lacking. In vitro studies suggest an effect of hydroxychloroquine against *B. burgdorferi*,[239,240] and there are studies reporting the potential benefits of this drug as an antirheumatic drug[241,242]; however, no data are available in patients with Lyme arthritis. While randomized controlled clinical trials are lacking for systemic anti-inflammatory regimens, DMARDs have been shown to reduce symptoms of inflammation and patients benefit from this therapy in clinical practice. TNF inhibitors (ie, tocilizumab) are suggested to have a clinical beneficial effect as well, although there have been concerns about the risk of infection with such immune suppressive therapy.[243-245] Arthroscopic synovectomy is another therapeutic option, when the response to a DMARD is incomplete and the arthritis is limited to one joint.[246] Intra-articular corticosteroids are not recommended during the second period of antibiotic therapy,[230] since contradicting reports on steroid efficacy in Lyme arthritis have been published.[23,247-249] Prognosis of Lyme arthritis is generally favorable after antibiotic therapy. However, in case of antibiotic-refractory Lyme arthritis systemic anti-inflammatory regimens seem to have a more positive effect on the course of the disease in clinical practice.

7 | FUTURE PERSPECTIVES

Over the last few years, our knowledge of Lyme disease has massively increased. The current research shows that the induction of a

proper immune response toward *Borrelia* bacteria early in infection may fail, resulting in hyperinflammatory reaction in different tissues. The interaction of *Borrelia* with different immune cell subsets shows the diverse range of immune evasion mechanisms: from suppressing early immune cell recruitment to reduction of DC migration, and lack of T-cell activation. Later, severe immune responses are strongly correlated with the development of Lyme arthritis.

However, there are still gaps in our understanding of the weak induction of both innate and adaptive immune responses and the development of the disease, during early infection and in the late stages of Lyme disease. Most of the studies have only been performed in mice, and give potential clues to the human in vivo situation, but findings may not be translatable directly to the human with Lyme arthritis. Furthermore, many murine studies assess only one or a few aspects of Lyme disease and therefore lack the complete overview of what is occurring during *B. burgdorferi* infection in humans. Therefore, models that closely resemble the in vivo situation in humans during *Borrelia*-induced inflammation need to be elaborated to further investigate the interaction of *B. burgdorferi* with the innate and adaptive immune networks. When a better understanding of the mechanisms involved in Lyme arthritis is achieved, both innate and adaptive immune responses could be targeted. This might improve treatment strategies for (antibiotic-refractory) Lyme arthritis.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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