REVIEW ARTICLE

Activation of innate host defense mechanisms by *Borrelia*

Anneleen Berende[1,3], Marije Oosting[1,2], Bart-Jan Kullberg[1,2], Mihai G. Netea[1,2], Leo A.B. Joosten[1,2]

[1] Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen
[2] Nijmegen Institute for Infection, Inflammation and Immunity (N4i)
[3] Department of Internal Medicine, Jeroen Bosch Hospital, 's Hertogenbosch, The Netherlands

Correspondence: L.A.B. Joosten, Department of Medicine (463), Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
<L.Joosten@aig.umcn.nl>

Accepted for publication September 15, 2009

ABSTRACT. *Borrelia* is the causative agent of Lyme disease, a widespread disease with important health consequences. Immune-mediated mechanisms are believed to play a major role in both host defense and in late complications of Lyme disease. Recognition of *Borrelia* and the initial activation of the innate immune system are important for host defense, as well as modulation of adaptive responses. Several classes of pattern recognition receptors (PRRs) have been suggested to be involved in the recognition of *Borrelia*: Toll-like receptors (TLRs), NOD-like receptors (NLRs) and C-type lectin receptors (CLRs). TLR2 has been found to be the most important receptor of the TLRs. The intracellular receptor NOD2, a member of the NLRs, might also play an important role in recognition. Mannose receptor is also involved in *Borrelia* recognition, but little is known about other CLRs such as dectin-1. After PRRs have recognized *Borrelia*, a signaling cascade is induced that leads to transcription of NF-κB, resulting in the production of pro-inflammatory cytokines. Understanding these pathways provides not only a better insight into the pathogenesis, but also provides potential, novel, therapeutic targets during active disease or post-infection complications.

Keywords: Lyme disease, *Borrelia*, innate host defense

Lyme disease is caused by a spirochete of the *Borrelia* genus, *Borrelia burgdorferi* sensu lato (herein referred to as *Borrelia*), which can be further classified into three human pathogenic species: *Borrelia burgdorferi sensu stricto* (s.s.), *Borrelia afzelii*, and *Borrelia garinii*. In the United States, only *B. burgdorferi* can be found, while *B. afzelii* and *B. garinii* cause most cases of Lyme disease in Europe and Asia [1]. Lyme disease is the most frequent arthropod-borne disease in the Northern hemisphere [2, 3]. In the United States, where Lyme disease is a notifiable disease, the US Centers for Diseases Control and Prevention have reported a steady increase of cases, with 19,931 cases reported in 2006. The incidence in the different states varies significantly: from almost no cases in Montana to 73.6 per 100,000 inhabitants in Connecticut [4]. In Europe, the highest frequency occurs in Central Europe and Scandinavia, especially in forested areas, with an incidence of 111 per 100,000 inhabitants in Germany [3].

The clinical manifestations of Lyme disease can be divided into three stages: early infection, disseminated infection and persistent infection [5, 6]. In the first stage, a localized infection of the skin, so-called erythema migrans (EM), can be seen in approximately 70 to 80% of patients [2, 7, 8]. If the pathogen disseminates through the blood and lymphatics, it can localize in places such as the heart, eyes, joints, and peripheral or central nervous system (CNS). This can lead to the second stage of the disease, the so-called early disseminated Lyme disease, which is arrived at after several weeks to a few months post-infection [1]. Lyme arthritis develops in approximately 50% of patients with untreated EM, this being the most frequent symptom of disseminated disease in the US. It is characterized by recurrent, intermittent attacks of inflammation, usually in the large joints and most often the knee [9]. In addition to arthritis, CNS involvement called neuroborreliosis can develop at this stage, with manifestations such as aseptic meningitis, radiculoneuritis, cranial neuritis and meningoradiculitis (also called Bannwarth syndrome) [10, 11]. The third stage, persistent infection or late stage Lyme disease, can develop months to years after the initial tick bite. It can be characterized by acrodermatitis chronica atrophicans (ACA), which is frequently accompanied by sensory peripheral polyneuropathy, and is almost exclusively caused by *B. afzelii* [12]. Persistent infection can also include neuroborreliosis and chronic arthritis [9, 13]. Some of these symptoms occur despite long-term antibiotic treatment. There are two hypotheses for the chronic arthritis: one hypothesizes that the complication is due to persistent infection, the other that an infection-induced autoimmune process is involved [1]. There is variation

doi: 10.1684/ecn.2009.0179

in the clinical presentation in Europe and the US, which is partly due to the relationship between the *Borrelia* species and the type of clinical manifestation. For example, *B. burgdorferi* sensu stricto is commonly associated with arthritis, while *B. afzelii* causes mainly skin manifestations, and *B. garinii* often gives rise to neuroborreliosis [14].

Many aspects of the pathophysiology of Lyme disease remain unexplained, and the nature of the immune response to the pathogen is only partly understood. One important aspect of spirochetal-host interaction is represented by the spirochetal recognition of the host. How spirochetes are recognized by the innate immune system and how they cause inflammation remains incompletely understood. Because the activation of the innate immune system is also responsible for the further modulation of the secondary adaptive immune responses, the recognition of *Borrelia* and the initial triggering of innate immunity are important for understanding both host defense and immune-mediated, late complications. In this review, we shall present a summary of what is known about the recognition of *Borrelia* species by the innate immune system, and discuss which aspects need further investigation.

THE PATHOGEN AND ITS VECTOR

Borrelia is a thin (0.2-0.5 μm), elongated (20 μm), helically-coiled, Gram-negative bacterium that belongs to the phylum Spirochaetes [15]. It has a protoplasmic cylinder surrounded by a fluid outer membrane and a peptidoglycan layer. The outer cell membrane contains many lipoproteins, including the outer surface proteins (Osps) A through F [16]. In the periplasmic space, situated between the outer cell membrane and the peptidoglycan layer, seven to eleven flagella are attached to and wound around the protoplasmic cylinder. These flagella are responsible for the shape and motility of the pathogen [17]. A flagellum consist of a helical filament made of 41-kDa flagellin, a basal body and a hook that is attached to the protoplasmic cylinder [18].

The genome of *B. burgdorferi* sensu stricto (strain B31) has been sequenced and seems quite small, with approximately 1.5 megabases. It consists of an unusual, small, linear chromosome of 950 kilobases and 21 plasmids, of which 12 are linear (lp) and nine circular (cp) [19, 20]. *Borrelia* distinguishes itself from other spirochetes by the fact that 40% of its genetic material, including genes encoding for certain outer-membrane proteins, is encoded by these plasmids [19]. Some of the plasmids can be lost during *in vitro* cultivation, indicating that they are not all stable and may not be essential [21]. Other plasmids are necessary, since they encode for proteins that are essential for the survival of *Borrelia*, such as the Osps and other lipoproteins, which will be discussed extensively later.

Borrelia is transmitted by ticks of the *Ixodes* complex, with *I. ricinus* and *I. persulcatus* being the primary vectors in Europe and Asia. In general, *I. scapularis* (or *I. dammini*) is considered, next to *I. pacificus*, to be the most important vector in North America [22, 23]. However, some groups argue that *I. scapularis* is a vector to humans, although this tick is infected naturally with *B. burgdorferi* and is an efficient experimental vector [22]. At any stage (larval, nymphal, adult) of their two-year lifespan, ticks can be infected with *Borrelia*. The percentage of infected ticks varies from 9% to 55% [24, 25]. Once infected, ticks transmit *Borrelia* by injection of *Borrelia*-containing saliva into the skin upon feeding [22]. This is achieved primarily by nymphs since they are small and consequently less noticed, which is important since transmission of *Borrelia* to a mammalian host only takes place when the tick is attached for longer than 48 hours [26]. Ticks feed on a large range of animals, and although many do not act as a reservoir, they are important for the survival of the tick since they supply nutrients. In Europe, rodents such as the Apodemus mice and voles, shrews, hares and several birds are significant reservoirs [27, 28]. In the US, mostly rodents and deer are involved as reservoirs [1].

MECHANISMS THROUGH WHICH *BORRELIA* PROMOTES TRANSMISSION AND DISSEMINATION

Outer surface proteins OspA, OspB and OspC

Borrelia utilizes different mechanisms to establish adequate transmission *(figure 1)*. Outer surface proteins (Osps) play an important role. To be able to survive inside the tick, *Borrelia* expresses outer surface proteins OspA and OspB. They help *Borrelia* to attach to the tick midgut by binding to the tick receptor for OspA (TROSPA) [29-31]. In this way, *Borrelia* can stay in the tick midgut as long as the tick is unfed. Upon the tick feeding on a mammal, *Borrelia* travels to the salivary glands. At this time, OspA is downregulated and OspC is upregulated. Regulation of this expression is mediated by temperature and pH [32]. OspC binds a tick salivary protein, Salp15, that contributes to transmission in mammals by its immunosuppressive properties, one of which is the inhibition of antibody-mediated killing and inhibition of CD4+ T-cell activation [21, 33]. Evidence that OspC is of key importance in transmission, was provided by OspC-deficient *Borrelia* that were unable to colonize ticks [34] and establish infection in mice [35]. Furthermore, OspC might be important for dissemination, since disseminated disease is associated with only certain OspC variants of *B. burgdorferi* strains [36].

Adhesins

After *Borrelia* has reached the dermis, it expresses binding proteins on the surface (adhesins) to facilitate its dissemination [37]. Adhesion to the extracellular matrix is one way to accomplish this, and in particular, decorin-binding adhesins (DbpA and DbpB) seem to play an important role by binding to decorin, a collagen-associated proteoglycan [38, 39] *(figure 1)*. Decorin is usually linked to glycosaminoglycans (GAGs) and both seem to be needed for optimal binding [40]. The binding protein BBK32 is also important for binding to the extracellular matrix since it binds to fibronectin, an extracellular matrix protein

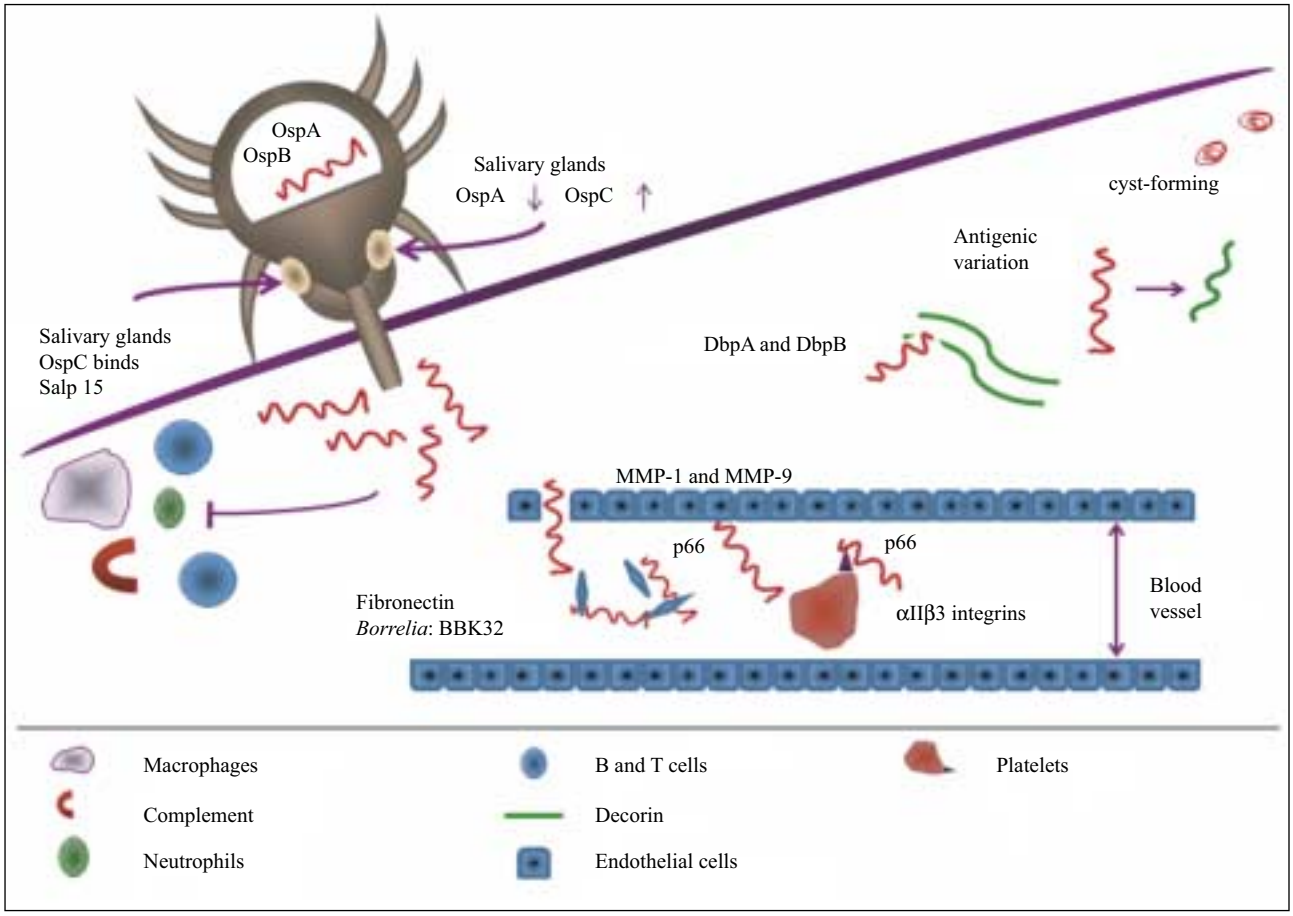

Figure 1

Transmission and dissemination of *Borrelia*. *Borrelia* bacteria are able to inhibit their destruction by host cells via several different mechanisms. This can be done by inhibition of innate immune cells such as macrophages and the complement system. In addition to binding to endothelial cells in blood vessels via p66, spirochetes are also able to enter the host tissues, form cysts to hide from attacking cells or change their outer surface antigens to protect themselves.

[41, 42]. Expression of BBK32 is dependent not only on the type of *Borrelia* strain, but also on the culture conditions *in vitro*.

Another mechanism through which *Borrelia* promotes dissemination is by penetrating the matrix and the endothelial monolayers. This is mediated by binding of *Borrelia* to plasminogen, leading to plasmin formation and the induction of proteolytic activity [43-45]. Matrix metalloproteinase-1 (MMP-1) and MMP-9, whose expression and release is induced by *Borrelia*, also enhance the penetration of tissue barriers *(in vitro)* [46, 47].

Spirochetes do not only use adhesins for binding to the extracellular matrix, but for binding to cells as well. This is done by binding to non-decorin GAGs, which are produced by a wide variety of cells [48, 49]. The binding capacity is dependent on the cell type and the spirochete strain [50]. One example is the *Borrelia* glycosaminoglycan protein (Bgp) that binds to heparin sulfate present on the surface of endothelial cells [49]. The p66 outer-surface protein also binds to endothelial cells (and macrophages) by binding the integrin αVβ3 that is present on their cell surface [51, 52]. Integrins are heterodimeric receptors, and are the most important metazoan receptors involved in adhesion of cells to the extracellular matrix and other cells [53]. In addition to binding endothelial cells, p66 also binds platelets through the integrin αIIbβ3 [54]. Consequently, p66 seems to be very important for colonization of the blood vessel wall *(figure 1)*.

RECOGNITION OF *BORRELIA* BY THE INNATE IMMUNE RESPONSE

The task of the innate immune system is to control the infection until the more specific adaptive response is developed. The innate immune system defends the host from infection in a non-specific way, without eliciting immunological memory. It involves the epithelium, the complement system, phagocytic cells (neutrophils and macrophages), NK cells and several cytokines that coordinate the actions of the above-mentioned cells.

Complement-mediated killing of Borrelia

The complement system plays a crucial role in the first line of defense against micro-organisms, by either direct lysis of the pathogen, or recruitment of leukocytes to the site of infection. Approximately thirty plasma and cellular proteins are known to be involved in the complement system that is divided in the classical and alternative

pathway. The main step in the alternative complement activation is the cleavage of C3 into C3a and C3b by C3-convertases. C3b will cover the outer surface of pathogens followed by opsonization and formation of the membrane-attack complexes [55]. To protect the host from damage by C3b deposition, vertebrates express proteins on their cell membranes that convert C3b into an inactive protein. These proteins belong to the family of complement regulatory proteins or regulators of complement activator (RCA). Factor H and factor H-like protein 1 are prominent members of this family. Micro-organisms often use similar proteins that down-regulate complement activation to avoid killing by the host complement system. The pathogenicity of *Borrelia* species is determined by their ability to interfere with the complement system leading to serum resistance [56].

Pathogen-associated molecular patterns and their pattern recognition receptors

Pathogen-associated molecular patterns (PAMPs) play a very important role in the activation of the innate immune system. PAMPs are conserved structures or components from micro-organisms that cannot be found in host cells. They are shared by groups of micro-organisms and show little variation among a given class. Their expression can be essential for the survival of the micro-organism [57]. This last characteristic prevents extensive changes in structure and gives the innate immune system a chance to recognize the micro-organism. Examples of PAMPs include hypomethylated DNA with CpG motifs, peptidoglycans, lipopeptides, flagellins and double-stranded RNA [58]. Gram-negative bacteria cause a major inflammatory response through the stimulatory properties of lipopolysaccharide (LPS) [59, 60]. *Borrelia* does not contain LPS in the structure of its cell wall, but it does express many membrane-associated lipoproteins. Several of these have been shown to stimulate the innate immune response, such as OspA and OspB [61-63].

The innate immune response is initiated when PAMPs are recognized by pattern recognition receptors (PRRs), which are expressed by cells of the innate immune system. Each PRR has broad specificities for the various conserved and non-variant structures of several micro-organisms [64]. Three types of PRRs on immune cells exist: secreted PRRs such as the LPS-binding protein (LBP), cell surface PRRs such as Toll-like receptors (TLRs), and PRRs that are only found intracellularly, such as nucleotide-binding oligomerization domain proteins (NOD) [65, 66]. TLRs are the best-characterized PRR class so far. In the case of *Borrelia*, several types of PRRs have been suggested to be involved in its initial recognition: TLRs, NOD1 and NOD2, and C-type lectin receptors such as the mannose receptor (MR) and dectin-1 *(figure 2)*.

TLRs

Toll-like receptors have been found to play an important role in the innate immunity and inflammation of the host, in response to several different microbial components. They are expressed by mucosal epithelial cells, as well as professional phagocytes. TLRs are type 1 integral membrane glycoproteins, which are characterized by a single, trans-membrane domain and an intra-cytoplasmic domain, also called the TOLL/interleukin-1 receptor homology domain (TIR domain). An important characteristic that distinguishes TLRs from interleukin-1 receptors is the extracellular domain consisting of 19 to 25 leucine-rich repeats (LRR) *(figure 2)*. Although the LRR domains of the several family members of TLRs share homology, different TLRs are able to recognize structurally unrelated proteins [58, 67]. Eleven mammalian TLRs have been reported: TLR1 through TLR11; the ligand for TLR10 has not yet been determined [58], and TLR11 is a truncated molecule in humans [68]. The localization of the various TLRs differs: TLR3, TLR7, TLR8 and TLR9 are only found in intracellular compartments, whereas TLR1, TLR2, TLR4, TLR5 and TLR6 are expressed mainly on the surface of the cell membrane, and can be recruited into the phagosomes [58, 69-71].

TLR2

The expression of TLR2 is restricted to antigen-presenting cells, epithelial and endothelial cells [72]. TLR2 has a very broad range of ligands: ranging from peptidoglycan from Gram-positive bacteria, to bacterial lipoproteins and mycobacterial cell-wall lipoarabinomannan [58]. This broad range of ligands might be explained by the fact that TLR2 can form a functionally active heterodimer receptor with other TLRs such as TLR6 or TLR1 [73-75], but also with other PRRs such as dectin-1 or CD36 [76, 77]. TLR1 and TLR6 discriminate between bacterial lipoproteins that are triacylated or diacylated at the amino-terminal cysteine residue [78]. It was recently demonstrated that TLR2 requires TLR6 to transduce efficiently signals in TLR2-transfected endothelial cells and macrophages [73, 74, 79]. The role of TLR2 in the pathogenesis of Lyme disease has been studied extensively and has proved to be important. Wooten and colleagues demonstrated that macrophages from TLR2-deficient mice were unable to induce an immune response after stimulation with the *Borrelia* lipoprotein OspA [62]. Another study showed that neutrophils of patients with Lyme disease have an upregulation of TLR2 mRNA and protein in combination with an elevated production of IL-6 and IL-1β after recognition of *Borrelia* [80]. Peripheral blood monocytes (PBMCs) of patients with a Arg753Gln mutation in TLR2 show impaired cytokine induction after stimulation with *Borrelia* lysates [81]. However, it remains unclear whether intact *Borrelia* spirochetes induce this cytokine response through TLR2 alone, or whether other TLRs might cooperate with TLR2. For example, in a study with TLR2 knock-out mice, macrophages do respond to lysates of whole spirochetes, indicating that there is also a TLR2-independent mechanism [62].

Whether TLR2 is important only for host defense against *Borrelia*, or whether it also induces deleterious inflammatory reactions in the pathogenesis of Lyme disease remains unclear. In a study using TLR2 knock-out mice, a 100-fold increase in the load of spirochetes was seen in tissues, including ankle joints, ears and hearts of TLR2 knock-out mice compared to wild-type mice, who

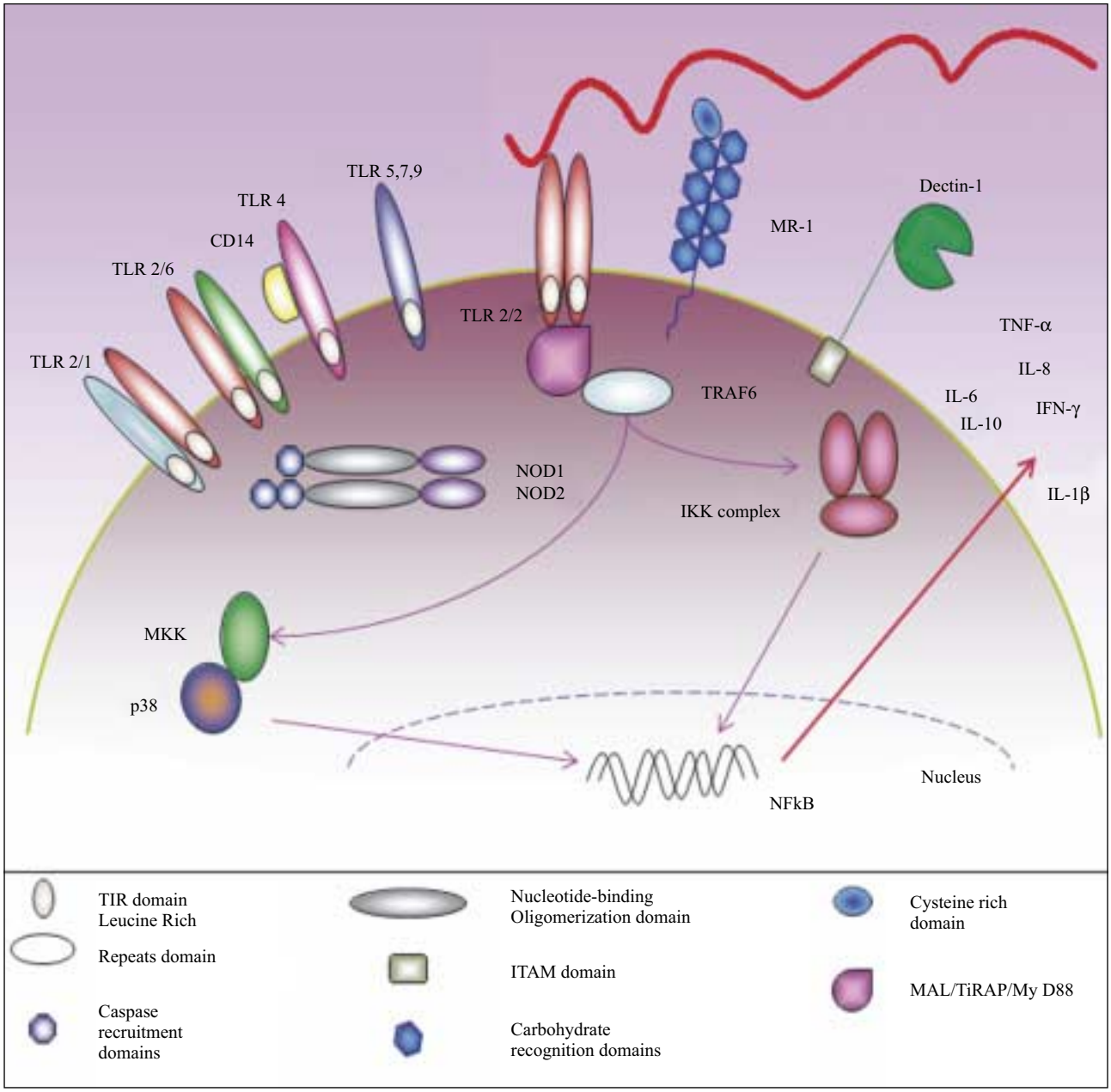

Figure 2

Involvement of PRRs in the recognition of *Borrelia* and intracellular signaling pathways.
Borrelia spirochetes can be recognized via several different pathogen-recognition receptors (PRRs) of the host. Both extracellular and intracellular receptors are involved. After recognition of *Borrelia* species by a PRR, both pro- and anti-inflammatory cytokine production is induced through transcription of NF-κB. NF-κB is located in the cell nucleus and can be activated through proteins of signaling pathways such as the MKK-p38 complex. Which pathway is induced depends on the type of receptor that recognizes *Borrelia*.

developed a relative milder, inflammatory carditis [79]. The number of spirochetes in tissues after four weeks of infection was comparable between the knock-out mice and their wild-type littermates, which suggests that TLR2 probably plays an important role in the innate immune response against *Borrelia*. This observation was able to be confirmed in humans by Schröder *et al.* [81]. This group found that a heterozygous mutation in TLR2 (Arg753Gln), may protect against the development of late stage Lyme disease, since smaller amounts of inflammatory cytokines such as TNF-α and IFN-γ were produced.

Most lipoproteins contain a Pam_3Cys-modified cysteine that harbors the stimulatory effect [63, 82-84]. Lipoproteins which contain Pam_3Cys are known to be expressed on *B. burgdorferi* during tick feeding and inflammation in mammals, and OspA is a well known example of such a lipoprotein [85, 86]. The response elicited by lipoproteins is very similar to that of LPS, and this is probably due to the similarities between TLR2 and TLR4 signaling pathways [87, 88].

TLR4

TLR4 is expressed by cells of the immune system, mostly by macrophages and dendritic cells [64]. The main ligand for TLR4 is lipopolysaccharide (LPS) from Gram-negative bacteria [89]. *Borrelia* spirochetes do not express LPS on their outer surface [90]. Not surprisingly,

a role for TLR4 in Lyme disease has not been demonstrated. Nonetheless, an elevated expression of TLR4 was seen in primary microglia after uptake of *B. burgdorferi*, and upregulation of TLR4 was found on dendritic cells and macrophages of healthy volunteers that were stimulated with synthetic lipopeptides corresponding to OspC of *B. burgdorferi*, although TLR1 and TLR2 were upregulated as well [91, 92].

CD14

CD14 is a co-receptor of both TLR2 and TLR4 that is able to recognize a variety of microbial compounds, thereby enhancing the activity of TLR4 [87, 88]. Wooten and colleagues were the first to report that the lipoproteins of *Borrelia* (OspA and OspC) could activate cells via pathways mediated by CD14 [88]. CD14 knock-out mice were found to have a more severe inflammatory response after infection with *Borrelia*, and the numbers of spirochetes in tissue were higher compared to their wild-type littermates [87]. Human neutrophil and human umbilical vein endothelial cell (HUVECs) sensitivity was decreased twenty-fold after blocking CD14 molecules. However, these cell types were still activated when CD14 molecules were absent or blocked, indicating that CD14 is able to facilitate signaling, but is not the ligand-specific receptor. In patients with acute Lyme disease, CD14 was found to be upregulated in serum, indicating that this protein may play a role in the pathogenesis [93].

TLR5

Bacteria derive motility from flagella and TLR5 recognizes flagellin, the main component of flagella. Flagellin is a potent, pro-inflammatory inducer which acts by inducing degradation of Iκβ [94], and thereby the induction of the NFκB-pathway, which will be discussed later. It was hypothesized that TLR5 does not play a major role in the recognition of *Borrelia* species, since the flagella of the spirochetes are located between the outer and inner membrane. However, in other spirochetes, for example *Treponema pallidum*, it was shown that TLR5 can recognize flagellin because of transient gaps in the membrane of the spirochete [95]. Whether these gaps are present in *Borrelia* spirochetes has not yet been demonstrated, and the role of TLR5 in the recognition of *Borrelia* is an important area of investigation for the future.

TLR9

TLR9 recognizes unmethylated CpG motifs in bacterial DNA. This is preceded by internalization of CpG DNA into late endosomal or lysosomal compartments [96]. CpG DNA is a component of sonicated *B. burgdorferi*, and has been shown to activate murine cells through TLR9 [97]. In addition, *B. burgdorferi* was shown to release DNA in culture, which could provide ligands for induction of signaling pathways via TLR9 [98]. However, a definite role for TLR9 in the recognition of *Borrelia* has not yet been reported. Shin and colleagues were unable to find differences in cytokine induction after stimulation of cells of TLR9-deficient mice [98]. Furthermore, when astrocytes and microglia were stimulated with *Borrelia* spirochetes, upregulation of TLR9 mRNA expression was not seen, suggesting that TLR9 does not play a major role in the pathogenesis of Lyme neuroborreliosis [91].

TLR3, TLR7 and TLR8

TLR3, TLR7 and TLR8 are probably not involved in the innate recognition of *Borrelia*. These TLRs have ligands for fragments of viruses, such as double-stranded RNA (TLR3), and single-stranded RNA (TLR7 and TLR8), and also other small antiviral compounds are recognized by these TLRs [66]. Nonetheless, cooperation between several TLRs has been seen before, and the activation of non-involved TLRs by other activated TLRs as well [62, 74, 91, 99, 100].

NOD-like receptors

Another class of PRR receptors are the NOD-like receptors, also called nucleotide-binding domain and leucine-rich repeat-containing molecules (NLRs). NLRs sense the presence of intracellular muropeptides derived from bacterial peptidoglycans. Several members of this family have been shown to induce signaling pathways by acting as PRRs [101]. NOD1 and NOD2 are mainly expressed by epithelial cells and antigen-presenting cells (APCs) such as macrophages and dendritic cells [100]. Cell walls of Gram-positive and Gram-negative bacteria contain peptidoglycans that are responsible for providing shape and mechanical rigidity. Peptides derived from peptidoglycans, such as muramyl dipeptide (MDP) and γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP) are found to be the NOD1 and NOD2 ligands respectively [102-107].
Several groups have reported a role for NOD1 in the recognition and induction of signaling pathways of inflammation in a variety of Gram-negative bacteria such as *Chlamydia* and *E. coli* [108, 109]. *B. burgdorferi* was reported to upregulate NOD-proteins on astrocytes after exposure to several TLR-ligands [110]. Sterka and colleagues found that NOD2, and not NOD1, was highly upregulated on primary murine microglia after stimulation with *B. burgdorferi* [111]. This may suggest that NOD-proteins are involved in the induction of inflammation. Indeed, in preliminary studies we have shown that NOD2 is involved in the release of several different inflammatory cytokines induced by *Borrelia*, such as IL-6. Persons with a non-functional NOD2 express lower cytokine levels after stimulation with *Borrelia* spirochetes (unpublished data). However, the exact role of NOD2 in the pathogenesis of Lyme disease remains unknown.

C-type lectin receptors

C-type lectin receptors (CLR) comprise a family of proteins that contain one or more structurally-related, C-type lectin-like domains. In vertebrates, 17 subgroups have been identified, which can be further divided in soluble lectins and cell-associated (transmembrane) C-type lectins, such as dectin-1 and mannose receptor. Many transmembrane CLRs are expressed by antigen-presenting cells. They function as PRRs by recognizing polysaccharide PAMPs of micro-organisms [112].

Mannose receptor

Mannose is found in glycoproteins on the surface of many micro-organisms. It is recognized by the mannose receptor family, a subgroup of the C-type lectin superfamily, consisting of the M-type phospholipase A_2 receptor, DEC-205/gp200-MR-6, Endo180/uPARAP, and macrophage mannose receptor [113]. The mannose receptor (MR) is a transmembrane protein that is involved in the recognition of several micro-organisms including *Candida albicans, Pneumocystis carinii, Leishmania donovani, Mycobacterium tuberculosis*, and *Klebsiella pneumoniae* via distinct domains [114-119]. The MR is expressed on several cells of the innate immune system, such as tissue macrophages, dendritic cells and endothelial cells [120]. Ezekowitz and colleagues demonstrated that the MR plays a role in the endocytosis and phagocytosis of bound ligands of *Candida albicans* by macrophages [114]. Similar results were seen for several different strains of *Mycobacterium tuberculosis* [118].
The MR possibly plays a role in the host defense against *Borrelia* infection by facilitating the phagocytosis of the bacteria by monocytes and macrophages. *Borrelia* spirochetes in the dermis and epidermis can be processed by Langerhans cells and dendritic cells. The MR on dendritic cells is highly upregulated after activation by spirochetes, and *B. burgdorferi* can be recognized and bound by it [120]. It was also reported that the MR is able to induce the release of IL-1β, IL-6 and IL-12 after triggering by other micro-organisms [121, 122]. Whether the MR is also able to induce the secretion of cytokines after triggering with *Borrelia* remains to be investigated.

Dectin-1

Dectin-1 is the best-known member of the natural killer (NK)-cell-receptor-like C-type lectin family, and is the only PRR that is able to transduce its own intracellular signals without the help of TLRs [123], through pathways involving CARD9 on one hand, and Raf-1 on the other [124, 125]. The main ligands for dectin-1 are β-(1,3)-glucans. So far, there has been no evidence to support a role for dectin-1 in the recognition of *Borrelia*. In addition, chemical analysis of *Borrelia* did not reveal any potential ligands for dectin-1 in its cell wall [126].

SIGNALING PATHWAYS INDUCED BY RECOGNITION OF *BORRELIA*

Recognition of *Borrelia* by the PRRs, induces a cascade of signals that ultimately activates the cell. In the TLR signaling pathway, TLR2 and TLR4 dimerize after binding the ligand, which allows the intracellular domain to form a TIR-TIR interface with the TIR domain of the MyD88 adaptor molecule (MAL, also known as TIRAP), and, in turn, with the myeloid differentiation factor-88 (MyD88). The amino-terminal death domain of MyD88 then induces phosphorylation of IL-1R-associated kinase 4 (IRAK4) and IRAK1, allowing formation of a complex with tumor-necrosis-factor-receptor-associated factor 6 (TRAF6), a ubiquitin ligase. TRAF6 induces activation of TGF-beta-activated kinase (TAK1). Finally, these events activate the nuclear transcription factor (NF-κB) by degrading the IKK complex (inhibitor of NF-κB kinase complex). NFκB is thereafter able to translocate to the nucleus and induce transcription of inflammatory genes [58, 67] *(figure 2)*.
The role of MyD88-dependent signals in cell activation by *Borrelia* has recently been shown. In MyD88-deficient mice, the number of spirochetes in tissues was considerably higher than in wild-type mice [127]. Furthermore, MyD88 seems to be necessary for efficient clearance of *Borrelia* [128]. However, MyD88-independent pathways are induced by *Borrelia* as well. MyD88-deficient mice were shown to have arthritis similar to wild-type mice [128] or even more severe [127]. This suggests that there are other pathways involved in inflammation other than through TLRs alone.
Once intracellular signals are induced by *Borrelia*, there is a central role for p38 mitogen-activated protein (MAP) kinase activity in the generation of the pro-inflammatory response. The p38 MAP kinase phosphorylates mitogen- and stress-activated protein kinase 1 (MSK1), which in turn phosphorylates NF-κB, resulting in transcription of pro-inflammatory and host defense genes [129].

LEUKOCYTE EFFECTOR MECHANISMS AGAINST *BORRELIA* INFECTION

Production of cytokines and chemokines

Borrelia has potent stimulatory activities, one of the most important being cytokine induction. The expression of the pro-inflammatory cytokines IL-6, IL-1β, IL-12, TNF-α and IFN-γ is increased *in vitro* when different cells such as PBMCs and mast cells are stimulated with *Borrelia* [130-138]. This response is elicited by the outer surface lipoproteins that induce translocation of NF-κB through PRR signaling [63, 84, 132, 139]. *Borrelia* is able to induce, not only the production of pro-inflammatory cytokines, but also anti-inflammatory cytokines such as IL-10 [140]. In addition, chemokines (e.g. IL-8) and adhesion molecules (such as E-selectin, VCAM-1 and ICAM-1 by OspA) are expressed in response to *Borrelia* [63, 141, 142]. Together, these molecules direct the recruitment of macrophages and neutrophils, which can eliminate the spirochetes by producing oxygen radicals such as nitric oxide [82, 83]. Finally, the innate immune system plays a role in inducing the adaptive immune system; co-stimulatory molecules on antigen-presenting cells being upregulated through PRR signaling [66]. Furthermore, proliferation of B-cells and production of immunoglobulin are induced by *Borrelia* [143]. The several different mechanisms through which TLRs signal might provide an explanation for the variation in inflammation duration and severity of Lyme disease [66].

Escape mechanisms of Borrelia *from host response*

Although the innate immune system tries to prevent *Borrelia* from harming the host, the spirochete has its own mechanisms to avoid the host defense system [60]

(figure 1). We mentioned earlier that *Borrelia* benefits from Salp15, because it suppresses the host immune response. Besides Salp15, the *I. scapularis* tick saliva also contains *I. scapularis* salivary anti-complement (Isac), which inhibits the complement system by suppressing the C3-C5 convertase enzyme [144]. *Borrelia* can also inactivate the host complement system by binding host complement regulatory proteins Factor H and Factor-H-like protein with complement regulator-acquiring surface proteins (CRASPs) and OspE-related proteins (Erps). Through this mechanism C3b is inactivated, and consequently the complement cascade at the surface of the spirochete is inhibited [145-149]. Subtypes of *Borrelia* have a different susceptibility to complement: *B. afzelli* is complement-resistant, *B. garinii* is complement-sensitive, while *B. burgdorferi* s.s. is intermediately sensitive. Both the classical pathway and the alternative pathway are activated by all *Borrelia* species [145, 150].

Another mechanism employed by *Borrelia* to escape the immune response is to use antigenic variation. The variable, major protein-like sequence gene locus (vlsE) on plasmid 28-1 undergoes extensive variation, which is stimulated by tick feeding [151-153]. vlsE Gene expression is induced when the mammalian host is infected [154, 155]. Evidence for the importance of vlsE is provided by the fact that loss of the plasmid 28-1 results in reduced infectivity [156]. Besides vlsE, also OspA, OspB and OspC are subject to antigenic variation [157].

A different immune escape mechanism induced by *Borrelia* consists of using lateral gene transfer. Bacteriophages play an important role in this by transmission of the 32-kb circular plasmids between the different *Borrelia* species [158]. Also, transfer of OspC genes has been reported [159, 160].

Furthermore, the fact that *Borrelia* does not need iron for growth *in vitro* might help in escaping the host defense mechanism of iron deprivation [161]. The pathogen is dependent on its host for nutrition though, since the genome encodes for very few proteins with biosynthetic activity [20]. However, *Borrelia* can avoid this dependence by changing its morphology by forming cysts that are serum-independent [59]. Lastly, elimination by phagocytic cells seems possible for certain strains of *Borrelia* [162]. As with many aspects of *Borrelia* infection pathogenesis, the mechanism by which this is achieved remains to be discovered.

CONCLUSION

Pattern recognition receptors play an important role in the recognition of *Borrelia*. Amongst the Toll-like receptors, TLR2 is the most important receptor for the recognition of the spirochete. The intracellular receptor NOD2 also seems to play an important role in recognition, while little is known about the role of C-type lectins in the recognition of *Borrelia*, with the exception of the macrophage mannose receptor, which can mediate *Borrelia* recognition. The precise role played by PRRs in host defense against *Borrelia*, as well as their potential effect on the immune-driven, late complications, are likely to represent a fruitful area of research in the coming years, and with the potential of providing novel, therapeutic targets against Lyme disease.

Disclosure. *None of the authors has any conflict of interest to disclose.*

REFERENCES

1. Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease. *J Clin Invest* 2004; 113: 1093-101.
2. Berglund J, Eitrem R, Ornstein K, *et al.* An epidemiologic study of Lyme disease in southern Sweden. *N Engl J Med* 1995; 333: 1319-27.
3. Huppertz HI, Bohme M, Standaert SM, Karch H, Plotkin SA. Incidence of Lyme borreliosis in the Wurzburg region of Germany. *Eur J Clin Microbiol Infect Dis* 1999; 18: 697-703.
4. Bacon RM, Kugeler KJ, Mead PS. Surveillance for Lyme disease–United States, 1992-2006. *MMWR Surveill Summ* 2008; 57: 1-9.
5. Duray PH, Steere AC. Clinical pathologic correlations of Lyme disease by stage. *Ann N Y Acad Sci* 1988; 539: 65-79.
6. Stanek G, Strle F. Lyme borreliosis. *Lancet* 2003; 362: 1639-47.
7. Gerber MA, Shapiro ED, Burke GS, Parcells VJ, Bell GL. Lyme disease in children in southeastern Connecticut. Pediatric Lyme Disease Study Group. *N Engl J Med* 1996; 335: 1270-4.
8. Steere AC, Sikand VK. The presenting manifestations of Lyme disease and the outcomes of treatment. *N Engl J Med* 2003; 348: 2472-4.
9. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. *Ann Intern Med* 1987; 107: 725-31.
10. Pachner AR, Steere AC. The triad of neurologic manifestations of Lyme disease: meningitis, cranial neuritis, and radiculoneuritis. *Neurology* 1985; 35: 47-53.
11. Pfister HW, Rupprecht TA. Clinical aspects of neuroborreliosis and post-Lyme disease syndrome in adult patients. *Int J Med Microbiol* 2006; 296 (Suppl. 40): 11-6.
12. Asbrink E, Hovmark A. Early and late cutaneous manifestations in Ixodes-borne borreliosis (erythema migrans borreliosis, Lyme borreliosis). *Ann N Y Acad Sci* 1988; 539: 4-15.
13. Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. *N Engl J Med* 1990; 323: 1438-44.
14. Balmelli T, Piffaretti JC. Association between different clinical manifestations of Lyme disease and different species of Borrelia burgdorferi sensu lato. *Res Microbiol* 1995; 146: 329-40.
15. Paster BJ, Dewhirst FE. Phylogenetic foundation of spirochetes. *J Mol Microbiol Biotechnol* 2000; 2: 341-4.
16. Lam TT, Nguyen TP, Montgomery RR, Kantor FS, Fikrig E, Flavell RA. Outer surface proteins E and F of Borrelia burgdorferi, the agent of Lyme disease. *Infect Immun* 1994; 62: 290-8.
17. Rosa PA. Microbiology of Borrelia burgdorferi. *Semin Neurol* 1997; 17: 5-10.
18. Wallich R, Moter SE, Simon MM, Ebnet K, Heiberger A, Kramer MD. The Borrelia burgdorferi flagellum-associated 41-kilodalton antigen (flagellin): molecular cloning, expression, and amplification of the gene. *Infect Immun* 1990; 58: 1711-9.

19. Casjens S, Palmer N, van VR, *et al.* A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete Borrelia burgdorferi. *Mol Microbiol* 2000; 35: 490-516.
20. Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, *et al.* Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi. *Nature* 1997; 390: 580-6.
21. Fikrig E, Narasimhan S. Borrelia burgdorferi--traveling incognito? *Microbes Infect* 2006; 8: 1390-9.
22. Lane RS, Piesman J, Burgdorfer W. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. *Annu Rev Entomol* 1991; 36: 587-609.
23. Steere AC. Lyme disease. *N Engl J Med* 2001; 345: 115-25.
24. Jouda F, Perret JL, Gern L. Density of questing Ixodes ricinus nymphs and adults infected by Borrelia burgdorferi sensu lato in Switzerland: spatio-temporal pattern at a regional scale. *Vector Borne Zoonotic Dis* 2004; 4: 23-32.
25. Junttila J, Peltomaa M, Soini H, Marjamaki M, Viljanen MK. Prevalence of Borrelia burgdorferi in Ixodes ricinus ticks in urban recreational areas of Helsinki. *J Clin Microbiol* 1999; 37: 1361-5.
26. des VF. Piesman J, Heffernan R, Schulze TL, Stafford KC, III, Fish D. Effect of tick removal on transmission of Borrelia burgdorferi and Ehrlichia phagocytophila by Ixodes scapularis nymphs. *J Infect Dis* 2001; 183: 773-8.
27. Huegli D, Hu CM, Humair PF, Wilske B, Gern L. Apodemus species mice are reservoir hosts of Borrelia garinii OspA serotype 4 in Switzerland. *J Clin Microbiol* 2002; 40: 4735-7.
28. Gylfe A, Bergstrom S, Lundstrom J, Olsen B. Reactivation of Borrelia infection in birds. *Nature* 2000; 403: 724-5.
29. Fikrig E, Pal U, Chen M, Anderson JF, Flavell RA. OspB antibody prevents Borrelia burgdorferi colonization of Ixodes scapularis. *Infect Immun* 2004; 72: 1755-9.
30. Yang XF, Pal U, Alani SM, Fikrig E, Norgard MV. Essential role for OspA/B in the life cycle of the Lyme disease spirochete. *J Exp Med* 2004; 199: 641-8.
31. Pal U, Li X, Wang T, Montgomery RR, Ramamoorthi N, Desilva AM, *et al.* TROSPA, an Ixodes scapularis receptor for Borrelia burgdorferi. *Cell* 2004; 119: 457-68.
32. Tokarz R, Anderton JM, Katona LI, Benach JL. Combined effects of blood and temperature shift on Borrelia burgdorferi gene expression as determined by whole genome DNA array. *Infect Immun* 2004; 72: 5419-32.
33. Ramamoorthi N, Narasimhan S, Pal U, *et al.* The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature* 2005; 436: 573-7.
34. Pal U, Yang X, Chen M, *et al.* OspC facilitates Borrelia burgdorferi invasion of Ixodes scapularis salivary glands. *J Clin Invest* 2004; 113: 220-30.
35. Grimm D, Tilly K, Byram R, *et al.* Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. *Proc Natl Acad Sci U S A* 2004; 101: 3142-7.
36. Seinost G, Dykhuizen DE, Dattwyler RJ, *et al.* Four clones of Borrelia burgdorferi sensu stricto cause invasive infection in humans. *Infect Immun* 1999; 67: 3518-24.
37. Coburn J, Fischer JR, Leong JM. Solving a sticky problem: new genetic approaches to host cell adhesion by the Lyme disease spirochete. *Mol Microbiol* 2005; 57: 1182-95.
38. Brown EL, Wooten RM, Johnson BJ, *et al.* Resistance to Lyme disease in decorin-deficient mice. *J Clin Invest* 2001; 107: 845-52.
39. Guo BP, Brown EL, Dorward DW, Rosenberg LC, Hook M. Decorin-binding adhesins from Borrelia burgdorferi. *Mol Microbiol* 1998; 30: 711-23.
40. Guo BP, Norris SJ, Rosenberg LC, Hook M. Adherence of Borrelia burgdorferi to the proteoglycan decorin. *Infect Immun* 1995; 63: 3467-72.
41. Probert WS, Johnson BJ. Identification of a 47 kDa fibronectin-binding protein expressed by Borrelia burgdorferi isolate B31. *Mol Microbiol* 1998; 30: 1003-15.
42. Probert WS, Kim JH, Hook M, Johnson BJ. Mapping the ligand-binding region of Borrelia burgdorferi fibronectin-binding protein BBK32. *Infect Immun* 2001; 69: 4129-33.
43. Coleman JL, Sellati TJ, Testa JE, Kew RR, Furie MB, Benach JL. Borrelia burgdorferi binds plasminogen, resulting in enhanced penetration of endothelial monolayers. *Infect Immun* 1995; 63: 2478-84.
44. Coleman JL, Gebbia JA, Piesman J, Degen JL, Bugge TH, Benach JL. Plasminogen is required for efficient dissemination of B. burgdorferi in ticks and for enhancement of spirochetemia in mice. *Cell* 1997; 89: 1111-9.
45. Klempner MS, Noring R, Epstein MP, *et al.* Binding of human plasminogen and urokinase-type plasminogen activator to the Lyme disease spirochete, Borrelia burgdorferi. *J Infect Dis* 1995; 171: 1258-65.
46. Gebbia JA, Coleman JL, Benach JL. Borrelia spirochetes upregulate release and activation of matrix metalloproteinase gelatinase B (MMP-9) and collagenase 1 (MMP-1) in human cells. *Infect Immun* 2001; 69: 456-62.
47. Perides G, Tanner-Brown LM, Eskildsen MA, Klempner MS. Borrelia burgdorferi induces matrix metalloproteinases by neural cultures. *J Neurosci Res* 1999; 58: 779-90.
48. Isaacs RD. Borrelia burgdorferi bind to epithelial cell proteoglycans. *J Clin Invest* 1994; 93: 809-19.
49. Leong JM, Morrissey PE, Ortega-Barria E, Pereira ME, Coburn J. Hemagglutination and proteoglycan binding by the Lyme disease spirochete, Borrelia burgdorferi. *Infect Immun* 1995; 63: 874-83.
50. Parveen N, Robbins D, Leong JM. Strain variation in glycosaminoglycan recognition influences cell-type-specific binding by lyme disease spirochetes. *Infect Immun* 1999; 67: 1743-9.
51. Coburn J, Barthold SW, Leong JM. Diverse Lyme disease spirochetes bind integrin alpha IIb beta 3 on human platelets. *Infect Immun* 1994; 62: 5559-67.
52. Coburn J, Leong JM, Erban JK. Integrin alpha IIb beta 3 mediates binding of the Lyme disease agent Borrelia burgdorferi to human platelets. *Proc Natl Acad Sci USA* 1993; 90: 7059-63.
53. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002; 110: 673-87.
54. Coburn J, Chege W, Magoun L, Bodary SC, Leong JM. Characterization of a candidate Borrelia burgdorferi beta3-chain integrin ligand identified using a phage display library. *Mol Microbiol* 1999; 34: 926-40.
55. Gros P, Milder FJ, Janssen BJ. Complement driven by conformational changes. *Nat Rev Immunol* 2008; 8: 48-58.
56. Ekdahl KN, Henningsson AJ, Sandholm K, Forsberg P, Ernerudh J, Ekerfelt C. Immunity in borreliosis with special emphasis on the role of complement. *Adv Exp Med Biol* 2007; 598: 198-213.
57. Teixeira MM, Almeida IC, Gazzinelli RT. Introduction: innate recognition of bacteria and protozoan parasites. *Microbes Infect* 2002; 4: 883-6.
58. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004; 4: 499-511.

59. Alban PS, Johnson PW, Nelson DR. Serum-starvation-induced changes in protein synthesis and morphology of Borrelia burgdorferi. *Microbiology* 2000; 146: 119-27.

60. Singh SK, Girschick HJ. Molecular survival strategies of the Lyme disease spirochete Borrelia burgdorferi. *Lancet Infect Dis* 2004; 4: 575-83.

61. Alexopoulou L, Thomas V, Schnare M, *et al.* Hyporesponsiveness to vaccination with Borrelia burgdorferi OspA in humans and in. *Nat Med* 2002; 8: 878-84.

62. Wooten RM, Ma Y, Yoder RA, *et al.* Toll-like receptor 2 is required for innate, but not acquired, host defense to Borrelia burgdorferi. *J Immunol* 2002; 168: 348-55.

63. Wooten RM, Modur VR, McIntyre TM, Weis JJ. Borrelia burgdorferi outer membrane protein A induces nuclear translocation of nuclear factor-kappa B and inflammatory activation in human endothelial cells. *J Immunol* 1996; 157: 4584-90.

64. Medzhitov R, Preston-Hurlburt P, Janeway Jr CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997; 388: 394-7.

65. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature* 2007; 449: 819-26.

66. Singh SK, Girschick HJ. Toll-like receptors in Borrelia burgdorferi-induced inflammation. *Clin Microbiol Infect* 2006; 12: 705-17.

67. Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001; 1: 135-45.

68. Balenga NA, Balenga NA. Human TLR11 gene is repressed due to its probable interaction with profilin expressed in human. *Med Hypotheses* 2007; 68: 456.

69. Heil F, hmad-Nejad P, Hemmi H, *et al.* The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. *Eur J Immunol* 2003; 33: 2987-97.

70. hmad-Nejad P, Hacker H, Rutz M, Bauer S, Vabulas RM, Wagner H. Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. *Eur J Immunol* 2002; 32: 1958-68.

71. Matsumoto M, Funami K, Tanabe M, *et al.* Subcellular localization of Toll-like receptor 3 in human dendritic cells. *J Immunol* 2003; 171: 3154-62.

72. Muzio M, Bosisio D, Polentarutti N, *et al.* Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. *J Immunol* 2000; 164: 5998-6004.

73. Bulut Y, Faure E, Thomas L, Equils O, Arditi M. Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. *J Immunol* 2001; 167: 987-94.

74. Ozinsky A, Underhill DM, Fontenot JD, *et al.* The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci USA* 2000; 97: 13766-71.

75. Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, *et al.* Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol* 2002; 169: 10-4.

76. Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* 2003; 197: 1107-17.

77. Hoebe K, Georgel P, Rutschmann S, Du X, Mudd S, Crozat K, *et al.* CD36 is a sensor of diacylglycerides. *Nature* 2005; 433: 523-7.

78. Takeuchi O, Kawai T, Muhlradt PF, *et al.* Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol* 2001; 13: 933-40.

79. Wang G, Ma Y, Buyuk A, McClain S, Weis JJ, Schwartz I. Impaired host defense to infection and Toll-like receptor 2-independent killing of Borrelia burgdorferi clinical isolates in TLR2-deficient C3H/HeJ mice. *FEMS Microbiol Lett* 2004; 231: 219-25.

80. Jablonska E, Marcinczyk M. TLR2 expression in relation to IL-6 and IL-1beta and their natural regulators production by PMN and PBMC in patients with Lyme disease. *Mediators Inflamm* 2006; 2006: 32071.

81. Schroder NW, Diterich I, Zinke A, *et al.* Heterozygous Arg753Gln polymorphism of human TLR-2 impairs immune activation by Borrelia burgdorferi and protects from late stage Lyme disease. *J Immunol* 2005; 175: 2534-40.

82. Ma Y, Seiler KP, Tai KF, Yang L, Woods M, Weis JJ. Outer surface lipoproteins of Borrelia burgdorferi stimulate nitric oxide production by the cytokine-inducible pathway. *Infect Immun* 1994; 62: 3663-71.

83. Morrison TB, Weis JH, Weis JJ. Borrelia burgdorferi outer surface protein A (OspA) activates and primes human neutrophils. *J Immunol* 1997; 158: 4838-45.

84. Norgard MV, Arndt LL, Akins DR, Curetty LL, Harrich DA, Radolf JD. Activation of human monocytic cells by Treponema pallidum and Borrelia burgdorferi lipoproteins and synthetic lipopeptides proceeds via a pathway distinct from that of lipopolysaccharide but involves the transcriptional activator NF-kappa B. *Infect Immun* 1996; 64: 3845-52.

85. Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA. Induction of an outer surface protein on Borrelia burgdorferi during tick feeding. *Proc Natl Acad Sci USA* 1995; 92: 2909-13.

86. de Silva AM, Fikrig E. Arthropod- and host-specific gene expression by Borrelia burgdorferi. *J Clin Invest* 1997; 99: 377-9.

87. Benhnia MR, Wroblewski D, Akhtar MN, *et al.* Signaling through CD14 attenuates the inflammatory response to Borrelia burgdorferi, the agent of Lyme disease. *J Immunol* 2005; 174: 1539-48.

88. Wooten RM, Morrison TB, Weis JH, Wright SD, Thieringer R, Weis JJ. The role of CD14 in signaling mediated by outer membrane lipoproteins of Borrelia burgdorferi. *J Immunol* 1998; 160: 5485-92.

89. Hoshino K, Takeuchi O, Kawai T, *et al.* Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 1999; 162: 3749-52.

90. Takayama K, Rothenberg RJ, Barbour AG. Absence of lipopolysaccharide in the Lyme disease spirochete, Borrelia burgdorferi. *Infect Immun* 1987; 55: 2311-3.

91. Bernardino AL, Myers TA, Alvarez X, Hasegawa A, Philipp MT. Toll-like receptors: insights into their possible role in the pathogenesis of lyme neuroborreliosis. *Infect Immun* 2008; 76: 4385-95.

92. Salazar JC, Pope CD, Moore MW, Pope J, Kiely TG, Radolf JD. Lipoprotein-dependent and -independent immune responses to spirochetal infection. *Clin Diagn Lab Immunol* 2005; 12: 949-58.

93. Zhao Z, Fleming R, McCloud B, Klempner MS. CD14 mediates cross talk between mononuclear cells and fibroblasts for upregulation of matrix metalloproteinase 9 by Borrelia burgdorferi. *Infect Immun* 2007; 75: 3062-9.

94. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001; 410: 1093-9.

95. Blanco DR, Radolf JD, Lovett MA, Miller JN. The antigenic interrelationship between the endoflagella of Treponema phagedenis biotype Reiter and Treponema pallidum Nichols strain. I. Treponemicidal activity of cross-reactive endoflagellar antibodies against T. pallidum. *J Immunol* 1986; 137: 2973-9.

96. Hacker H, Mischak H, Miethke T, *et al.* CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. *EMBO J* 1998; 17: 6230-40.

97. Hemmi H, Takeuchi O, Kawai T, *et al.* A Toll-like receptor recognizes bacterial DNA. *Nature* 2000; 408: 740-5.

98. Shin OS, Isberg RR, Akira S, Uematsu S, Behera AK, Hu LT. Distinct roles for MyD88 and Toll-like receptors 2, 5, and 9 in phagocytosis of Borrelia burgdorferi and cytokine induction. *Infect Immun* 2008; 76: 2341-51.

99. Hugot JP, Chamaillard M, Zouali H, *et al.* Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; 411: 599-603.

100. Strober W, Murray PJ, Kitani A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol* 2006; 6: 9-20.

101. Inohara N, Nunez G. NODs: intracellular proteins involved in inflammation and apoptosis. *Nat Rev Immunol* 2003; 3: 371-82.

102. Tattoli I, Travassos LH, Carneiro LA, Magalhaes JG, Girardin SE. The Nodosome: Nod1 and Nod2 control bacterial infections and inflammation. *Semin Immunopathol* 2007; 29: 289-301.

103. Chamaillard M, Hashimoto M, Horie Y, *et al.* An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat Immunol* 2003; 4: 702-7.

104. Girardin SE, Boneca IG, Carneiro LA, *et al.* Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science* 2003; 300: 1584-7.

105. Girardin SE, Boneca IG, Viala J, *et al.* Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* 2003; 278: 8869-72.

106. Inohara N, Ogura Y, Fontalba A, *et al.* Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 2003; 278: 5509-12.

107. Kanneganti TD, Lamkanfi M, Nunez G. Intracellular NOD-like receptors in host defense and disease. *Immunity* 2007; 27: 549-59.

108. Kim JG, Lee SJ, Kagnoff MF. Nod1 is an essential signal transducer in intestinal epithelial cells infected with bacteria that avoid recognition by toll-like receptors. *Infect Immun* 2004; 72: 1487-95.

109. Opitz B, Forster S, Hocke AC, *et al.* Nod1-mediated endothelial cell activation by Chlamydophila pneumoniae. *Circ Res* 2005; 96: 319-26.

110. Sterka Jr D, Rati DM, Marriott I. Functional expression of NOD2, a novel pattern recognition receptor for bacterial motifs, in primary murine astrocytes. *Glia* 2006; 53: 322-30.

111. Sterka Jr D, Marriott I. Characterization of nucleotide-binding oligomerization domain (NOD) protein expression in primary murine microglia. *J Neuroimmunol* 2006; 179: 65-75.

112. Robinson MJ, Sancho D, Slack EC, LeibundGut-Landmann S, Reis e Sousa. Myeloid C-type lectins in innate immunity. *Nat Immunol* 2006; 7: 1258-65.

113. East L, Isacke CM. The mannose receptor family. *Biochim Biophys Acta* 2002; 1572: 364-86.

114. Ezekowitz RA, Sastry K, Bailly P, Warner A. Molecular characterization of the human macrophage mannose receptor: demonstration of multiple carbohydrate recognition-like domains and phagocytosis of yeasts in Cos-1 cells. *J Exp Med* 1990; 172: 1785-94.

115. Stahl PD, Ezekowitz RA. The mannose receptor is a pattern recognition receptor involved in host defense. *Curr Opin Immunol* 1998; 10: 50-5.

116. Ezekowitz RA, Williams DJ, Koziel H, Armstrong MY, Warner A, Richards FF, *et al.* Uptake of Pneumocystis carinii mediated by the macrophage mannose receptor. *Nature* 1991; 351: 155-8.

117. Athamna A, Ofek I, Keisari Y, Markowitz S, Dutton GG, Sharon N. Lectinophagocytosis of encapsulated Klebsiella pneumoniae mediated by surface lectins of guinea pig alveolar macrophages and human monocyte-derived macrophages. *Infect Immun* 1991; 59: 1673-82.

118. Schlesinger LS. Macrophage phagocytosis of virulent but not attenuated strains of Mycobacterium tuberculosis is mediated by mannose receptors in addition to complement receptors. *J Immunol* 1993; 150: 2920-30.

119. Chakraborty P, Das PK. Role of mannose/N-acetylglucosamine receptors in blood clearance and cellular attachment of Leishmania donovani. *Mol Biochem Parasitol* 1988; 28: 55-62.

120. Cinco M, Cini B, Murgia R, Presani G, Prodan M, Perticarari S. Evidence of involvement of the mannose receptor in adhesion of Borrelia burgdorferi to monocyte/macrophages. *Infect Immun* 2001; 69: 2743-7.

121. Shibata Y, Metzger WJ, Myrvik QN. Chitin particle-induced cell-mediated immunity is inhibited by soluble mannan: mannose receptor-mediated phagocytosis initiates IL-12 production. *J Immunol* 1997; 159: 2462-7.

122. Yamamoto Y, Klein TW, Friedman H. Involvement of mannose receptor in cytokine interleukin-1beta (IL-1beta), IL-6, and granulocyte-macrophage colony-stimulating factor responses, but not in chemokine macrophage inflammatory protein 1beta (MIP-1beta), MIP-2, and KC responses, caused by attachment of Candida albicans to macrophages. *Infect Immun* 1997; 65: 1077-82.

123. Brown GD. Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat Rev Immunol* 2006; 6: 33-43.

124. Gross O, Gewies A, Finger K, Schafer M, Sparwasser T, Peschel C, *et al.* Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* 2006; 442: 651-6.

125. Gringhuis SI, den DJ, Litjens M, *et al.* Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. *Nat Immunol* 2009; 10: 203-13.

126. Schroder NW, Eckert J, Stubs G, Schumann RR. Immune responses induced by spirochetal outer membrane lipoproteins and glycolipids. *Immunobiology* 2008; 213: 329-40.

127. Bolz DD, Sundsbak RS, Ma Y, *et al.* MyD88 plays a unique role in host defense but not arthritis development in Lyme disease. *J Immunol* 2004; 173: 2003-10.

128. Liu N, Montgomery RR, Barthold SW, Bockenstedt LK. Myeloid differentiation antigen 88 deficiency impairs pathogen clearance but does not alter inflammation in *Borrelia burgdorferi*-infected mice. *Infect Immun* 2004; 72: 3195-203.

129. Olson CM, Hedrick MN, Izadi H, Bates TC, Olivera ER, Anguita J. p38 mitogen-activated protein kinase controls NF-kappaB transcriptional activation and tumor necrosis factor alpha production through RelA phosphorylation mediated by mitogen- and stress-activated protein kinase 1 in response to Borrelia burgdorferi antigens. *Infect Immun* 2007; 75: 227-70.

130. Defosse DL, Johnson RC. *In vitro* and *in vivo* induction of tumor necrosis factor alpha by Borrelia burgdorferi. *Infect Immun* 1992; 60: 1109-13.

131. Haupl T, Landgraf S, Netusil P, *et al.* Activation of monocytes by three OspA vaccine candidates: lipoprotein OspA is a potent stimulator of monokines. *FEMS Immunol Med Microbiol* 1997; 19: 15-23.

132. Ma Y, Weis JJ. Borrelia burgdorferi outer surface lipoproteins OspA and OspB possess B-cell mitogenic and cytokine-stimulatory properties. *Infect Immun* 1993; 61: 3843-53.

133. Miller LC, Isa S, Vannier E, Georgilis K, Steere AC, Dinarello CA. Live *Borrelia burgdorferi* preferentially activate interleukin-1 beta gene expression and protein synthesis over the interleukin-1 receptor antagonist. *J Clin Invest* 1992; 90: 906-12.

134. Radolf JD, Norgard MV, Brandt ME, Isaacs RD, Thompson PA, Beutler B. Lipoproteins of *Borrelia burgdorferi* and *Treponema pallidum* activate cachectin/tumor necrosis factor synthesis. Analysis using a CAT reporter construct. *J Immunol* 1991; 147: 1968-74.

135. Radolf JD, Goldberg MS, Bourell K, Baker SI, Jones JD, Norgard MV. Characterization of outer membranes isolated from *Borrelia burgdorferi*, the Lyme disease spirochete. *Infect Immun* 1995; 63: 2154-63.

136. Rasley A, Anguita J, Marriott I. *Borrelia burgdorferi* induces inflammatory mediator production by murine microglia. *J Neuroimmunol* 2002; 130: 22-31.

137. Tatro JB, Romero LI, Beasley D, Steere AC, Reichlin S. *Borrelia burgdorferi* and *Escherichia coli* lipopolysaccharides induce nitric oxide and interleukin-6 production in cultured rat brain cells. *J Infect Dis* 1994; 169: 1014-22.

138. Talkington J, Nickell SP. *Borrelia burgdorferi* spirochetes induce mast cell activation and cytokine release. *Infect Immun* 1999; 67: 1107-15.

139. Gondolf KB, Mihatsch M, Curschellas E, Dunn JJ, Batsford SR. Induction of experimental allergic arthritis with outer surface proteins of *Borrelia burgdorferi*. *Arthritis Rheum* 1994; 37: 1070-7.

140. Giambartolomei GH, Dennis VA, Lasater BL, Philipp MT. Induction of pro- and anti-inflammatory cytokines by *Borrelia burgdorferi* lipoproteins in monocytes is mediated by CD14. *Infect Immun* 1999; 67: 140-7.

141. Burns MJ, Sellati TJ, Teng EI, Furie MB. Production of interleukin-8 (IL-8) by cultured endothelial cells in response to *Borrelia burgdorferi* occurs independently of secreted [corrected] IL-1 and tumor necrosis factor alpha and is required for subsequent transendothelial migration of neutrophils. *Infect Immun* 1997; 65: 1217-22.

142. Ebnet K, Brown KD, Siebenlist UK, Simon MM, Shaw S. Borrelia burgdorferi activates nuclear factor-kappa B and is a potent inducer of chemokine and adhesion molecule gene expression in endothelial cells and fibroblasts. *J Immunol* 1997; 158: 3285-92.

143. Tai KF, Ma Y, Weis JJ. Normal human B lymphocytes and mononuclear cells respond to the mitogenic and cytokine-stimulatory activities of *Borrelia burgdorferi* and its lipoprotein OspA. *Infect Immun* 1994; 62: 520-8.

144. Valenzuela JG, Charlab R, Mather TN, Ribeiro JM. Purification, cloning, and expression of a novel salivary anticomplement protein from the tick, Ixodes scapularis. *J Biol Chem* 2000; 275: 18717-23.

145. Kraiczy P, Skerka C, Kirschfink M, Zipfel PF, Brade V. Mechanism of complement resistance of pathogenic Borrelia burgdorferi isolates. *Int Immunopharmacol* 2001; 1: 393-401.

146. Kraiczy P, Skerka C, Kirschfink M, Brade V, Zipfel PF. Immune evasion of Borrelia burgdorferi by acquisition of human complement regulators FHL-1/reconectin and Factor H. *Eur J Immunol* 2001; 31: 1674-84.

147. Kraiczy P, Skerka C, Brade V, Zipfel PF. Further characterization of complement regulator-acquiring surface proteins of Borrelia burgdorferi. *Infect Immun* 2001; 69: 7800-9.

148. Hellwage J, Meri T, Heikkila T, *et al.* The complement regulator factor H binds to the surface protein OspE of Borrelia burgdorferi. *J Biol Chem* 2001; 276: 8427-35.

149. Kraiczy P, Hellwage J, Skerka C, *et al.* Complement resistance of Borrelia burgdorferi correlates with the expression of BbCRASP-1, a novel linear plasmid-encoded surface protein that interacts with human factor H and FHL-1 and is unrelated to Erp proteins. *J Biol Chem* 2004; 279: 2421-9.

150. van Dam AP, Oei A, Jaspars R, *et al.* Complement-mediated serum sensitivity among spirochetes that cause Lyme disease. *Infect Immun* 1997; 65: 1228-36.

151. Zhang JR, Norris SJ. Genetic variation of the *Borrelia burgdorferi* gene vlsE involves cassette-specific, segmental gene conversion. *Infect Immun* 1998; 66: 3698-704.

152. Zhang JR, Norris SJ. Kinetics and *in vivo* induction of genetic variation of vlsE in *Borrelia burgdorferi*. *Infect Immun* 1998; 66: 3689-97.

153. Ohnishi J, Piesman J, de Silva AM. Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. *Proc Natl Acad Sci USA* 2001; 98: 670-5.

154. Anguita J, Thomas V, Samanta S, *et al. Borrelia burgdorferi*-induced inflammation facilitates spirochete adaptation and variable major protein-like sequence locus recombination. *J Immunol* 2001; 167: 3383-90.

155. McDowell JV, Sung SY, Hu LT, Marconi RT. Evidence that the variable regions of the central domain of VlsE are antigenic during infection with lyme disease spirochetes. *Infect Immun* 2002; 70: 4196-203.

156. Purser JE, Norris SJ. Correlation between plasmid content and infectivity in *Borrelia burgdorferi*. *Proc Natl Acad Sci USA* 2000; 97: 13865-70.

157. Wilske B, Barbour AG, Bergstrom S, *et al.* Antigenic variation and strain heterogeneity in Borrelia spp. *Res Microbiol* 1992; 143: 583-96.

158. Eggers CH, Samuels DS. Molecular evidence for a new bacteriophage of Borrelia burgdorferi. *J Bacteriol* 1999; 181: 7308-13.

159. Wang G, van Dam AP, Dankert J. Evidence for frequent OspC gene transfer between *Borrelia valaisiana sp.* nov. and other Lyme disease spirochetes. *FEMS Microbiol Lett* 1999; 177: 289-96.

160. Balmelli T, Piffaretti JC. Analysis of the genetic polymorphism of *Borrelia burgdorferi* sensu lato by multilocus enzyme electrophoresis. *Int J Syst Bacteriol* 1996; 46: 167-72.

161. Posey JE, Gherardini FC. Lack of a role for iron in the Lyme disease pathogen. *Science* 2000; 288: 1651-3.

162. Georgilis K, Steere AC, Klempner MS. Infectivity of Borrelia burgdorferi correlates with resistance to elimination by phagocytic cells. *J Infect Dis* 1991; 163: 150-5.