Spirochetal Lipoproteins and Immune Evasion

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Spirochetes are a major threat to public health. However, the exact pathogenesis of spirochetal diseases remains unclear. Spirochetes express lipoproteins that often determine the cross talk between the host and spirochetes. Lipoproteins are pro-inflammatory, modulatory of immune responses, and enable the spirochetes to evade the immune system. In this article, we review the modulatory effects of spirochetal lipoproteins related to immune evasion. Understanding lipoprotein-induced immunomodulation will aid in elucidating innate pathogenesis processes and subsequent adaptive mechanisms potentially relevant to spirochetal disease vaccine development and treatment.

Keywords: spirochetes, lipoproteins, evasion mechanism, immune system, immunity

INTRODUCTION

Spirochetes cause many human diseases such as syphilis, Lyme disease, and leptospirosis that pose major threats to public health (1). Epidemiological studies have shown that the incidence of Lyme disease (2–4), syphilis (5–7), and leptospirosis (8, 9) have increased, both within United States and globally (10, 11). However, the immunopathogenesis of spirochetal diseases remains unclear (12–14). Despite the apparent immune response generated following spirochete infection (i.e., tissue inflammation) (15), spirochetes are known to persist in their host (16) through a wide variety of mechanisms ranging from a dynamic outer membrane capable of antigenic variation in the presence of outer-surface proteins capable of inhibiting macrophage facilitated phagocytosis (17, 18).

A critical question is what cellular components can trigger the strong immune responses that are characteristic of spirochetal infections. Spirochetal membranes play a pivotal role in interacting with a host's immune system (19, 20). Bacterial components such as lipopolysaccharides (LPSs) often play a major role in the induction of inflammation in bacterial infections (21, 22). Interestingly, aggressive immune responses are often observed despite the lack of LPS (endotoxin) in particular spirochetes, such as Borrelia burgdorferi (19, 23–25). Certain spirochetes such as Treponema pallidum, the spirochete responsible for syphilis, rely greatly on their ability to express adhesins over the surface of their membrane as a tool with which they can invade various tissues (26). Lipids compose 25–30% of a cell's dry weight (19, 20). Detergent treatments of spirochetal membranes have confirmed that lipoproteins are the most abundant in number out of all proteins expressed by spirochetes (27–32) and are major integral spirochetal membrane proteins (27, 33). For example, B. burgdorferi species express >100 lipoproteins (34) and Leptospira spp. have >140 lipoprotein genes (35). Although numerous examples of spirochetal lipoproteins can be listed, a few prominent ones include OspA from B. burgdorferi, Tp47 from T. pallidum, and Lip32 from the Leptospira species (36–38). The number of bacterial lipoproteins that have been studied parallels the myriad of roles that lipoproteins play in bacteria such as envelope biogenesis, stress responses, pathogenicity, and nutrient transport (39–41).
However, there is limited evidence regarding the interplay between lipoproteins and human immune responses, partly due to the fact that in vitro studies do not accurately reflect human models. Understanding lipoprotein-induced immunomodulation will aid in elucidating innate pathogenesis processes and subsequent adaptive mechanisms potentially relevant to spirochetal disease vaccine development and treatment. In this article, we review the scientific evidence regarding the modulatory effects of spirochetal lipoproteins related to immune activation and evasion.

**MODULATORY EFFECTS OF SPIROCHETAL LIPOPROTEINS RELATED TO ACTIVATION OF THE IMMUNE SYSTEM**

Understanding the dualistic roles (activation vs inhibition) of lipoproteins in their interaction with the immune system is pivotal (42). Thus, before we explore mechanisms of spirochetal immune evasion, a better understanding of all the regulatory mechanisms (such as pro-inflammatory effects and immune activation) of spirochetal lipoproteins is needed. Better understanding of spirochetal lipoproteins and their regulatory mechanisms may provide insight into clinical outcomes arising from spirochetal infections. For example, spirochetal infections may increase the risk of Alzheimer’s disease (43).

**Spirochetal Lipoproteins Induce Pro-inflammatory Effects**

One of the primary manifestations of spirochetal infection is tissue inflammation that is the mainstay of spirochetal diseases such as Lyme neuroborreliosis (22, 29). Spirochetal lipoproteins are known to induce strong pro-inflammatory responses in their hosts (27, 33, 34, 44–52) that comprise the initial innate immune response to the invading pathogen (49). Components of the inflammatory infiltrate include keratinocytes, macrophages, leukocytes, and cells capable of responding to the presence of lipoproteins (53–55). A better understanding of the modulatory effects of spirochetal lipoproteins in myeloid and non-myeloid immune cells is needed.

**Spirochetal Lipoproteins Have Modulatory Effects on Neutrophils**

Neutrophils have a major role in the immunopathogenesis of acute bacterial infections. Spirochetal lipoproteins, such as OspB, have been documented to inhibit neutrophil function and prevent oxidative burst in a variety of tissues, to prolong host infection (56–58). However, other lipoproteins can promote neutrophil activation. For example, Ospa, even when presented at picomolar concentrations, has been shown to play a role in the activation of neutrophils and their chemotactic capabilities (51, 59). Subsequent to neutrophil activation, neutrophil tissue infiltration contributes to localized tissue inflammation that is pre-dominant in inflamed arthritic joints and in myocarditis (associated with spirochetal infections) (50, 51, 60). In addition to mediating inflammatory responses, spirochetes, such as *Leptospira*, may induce neutrophils extracellular traps, which are a relatively novel pathogen-killing mechanism for extracellular microbes independent of phagocytic uptake and degranulation (61). Thus, spirochetal lipoproteins can modulate the function of neutrophils that are recruited early in acute inflammatory responses.

**Spirochetal Lipoproteins Have Pleotropic Modulatory Effects on Monocytes and Macrophages (M/M) That Are Mediated through Several Pathways**

Except for neutrophils, M/M also play a major role in spirochetal immunopathogenesis. Lipoproteins bind CD14 in the membrane of M/M at the CD14 site that also interacts with LPS (62–64). This interaction activates the NF-κB pathway and induces pro-inflammatory responses (62, 63, 65). In addition, unlike the membrane-bound CD14, soluble CD14 also allows the activation of non-myeloid cells (66). Furthermore, the pro-inflammatory effects of spirochetal lipoproteins are often mediated by toll-like receptors (TLR) (67–69). TLR signaling leads to increased production of numerous cytokines that induce pro-inflammatory responses (25, 47). Interestingly, TLR-deficient mice had exacerbated inflammation and increased spirochetal burdens, both of which were attenuated by impairing T cell responses (70). As a bodily response to the vast amounts of pro-inflammatory cytokines produced upon spirochetal lipoprotein presence, monocytes have also been seen to produce IL-10 upon being presented with *B. burgdorferi* lipoproteins (71–75). IL-10, unlike cytokines such as IL-1 and IL-12, is known to reduce inflammation via TLR-pathway downregulation and can therefore assist in combatting the spirochetal infection as well as any possible chronic effects such as arthritis (76, 77). The above was confirmed in recent mice studies that utilized a TLR2 agonist, Pam3CSK4, to induce IL-10 production which attenuated inflammatory response to *Leptospira* (78). Thus, spirochetal lipoproteins exert their pro-inflammatory effects through several pathways including CD14, TLR, and NF-κB signaling and induce both pro-inflammatory (such as IL-1) and anti-inflammatory cytokines (IL-10) production in myeloid cells such as M/M.

**Spirochetal Lipoproteins Induce Activation of Dendritic Cells**

Similar to the activation of neutrophils, M/M, spirochetes also maintain the ability to activate other myeloid cells such as dendritic cells, key components in linking both the innate and adaptive immune system. Spirochetes activate cell adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), which then facilitate T-cell interactions and subsequent dendritic cell migration to lymph nodes for the mounting of an immune response (79, 80). In early stages of inflammation, lipoproteins in *T. pallidum* upregulate ICAM-1 and activate dendritic cells to mount immune responses (25, 46, 49, 81–84). Immune activation can also be induced upon spirochetal death or phagocytosis of spirochetes, both processes of which lead to further introduction of lipoproteins to the surrounding environment (80). The modulatory effects of spirochetal lipoproteins on dendritic cells are particularly important since dendritic cells play a major role in vaccine responses (discussed below).
Chronic Modulatory Effects of Spirochetal Lipoproteins and Effects on Adaptive Immunity May Drive Pathogenesis of Spirochetal Diseases

Spirochetal lipoproteins may also play a role in the transition from the acute immune responses to the more chronic effects that characterize spirochetal diseases such as arthritis, peripheral neuropathy, numerous neurologic manifestations, and the vascular endothelial damage thought to underlie a significant portion of the chronic symptoms in spirochetal diseases (85–89). Although the exact mechanism of transition may not be well understood, lipoproteins may activate B-cells and T-cells, both of which are known to play major roles in long-term adaptive immunity (46, 47, 49–52). Further understanding of the exact transition process has major potential in terms of possibly delaying, or inhibiting, many of the debilitating chronic effects characteristic of numerous spirochetal infections.

MODULATORY EFFECTS OF SPIROCHETAL LIPOPROTEINS RELATED TO FACILITATION OF IMMUNE EVASION

Spirochetes evade a host’s immune system through mechanisms such as antigenic variation, which is capable of producing myriads of variants (90). Spirochetal interference of the innate immune system presents one more mechanism, in a list of many, to allow for the persistence of spirochetes in their host (16, 91). Spirochetes use multiple mechanisms of immune evasion that are related to spirochetal lipoproteins. Indeed, except for pro-inflammatory effects, lipoproteins are also responsible for modulatory effects such as immune evasion. Spirochetes may limit the expression of membrane lipoproteins and their access to antibodies (92, 93) or induce antigenic variation of surface lipoproteins (19, 90, 94–100). Spirochetal lipoproteins may also interact with, and inhibit, components of innate immunity such as the complement (63, 68, 88, 101–108), neutrophils, and serum lipoproteins (109). Major pathways of spirochetal immune evasion are discussed below (see also Table 1 and Figure 1) (110–130).

Differential Dynamics of Spirochetal Lipoprotein Expression As a Mechanism of Immune Evasion

The expression of lipoproteins on the outer leaflet of the membrane allows the spirochete to interact with tissues and the host’s immune system (110). Naturally, the vast abundance of lipoproteins a given spirochete can express are not all necessary at a given time point, and their expression is time sensitive (111). Although more work is needed to elucidate the time-sensitive expression of surface lipoproteins, studies have hinted at the possibility of a temperature-sensitive mechanism to underlie expression patterns (112). For example, OspA in B. burgdorferi is not needed upon host infection and is therefore downregulated upon infection of a host via a temperature-sensitive alteration in membrane composition (111). Coupled closely with the need of a lipoprotein to be expressed on the exterior of the cell for interactions to occur, the lipoprotein must maintain its N-terminus as it has been documented that it is this region specifically to which immune system–spirochete interactions occur (113, 114). In line with the above statement, removal of the N-terminus disrupts the aforesaid interactions while synthesis of N-terminus analogs restored immune cell activation (114, 115). The limitation of outer-membrane lipoprotein expression in spirochetes may also act as a mechanism to facilitate host humoral defense evasion. Antibody recognizable lipoproteins may be scarcely expressed on the exterior leaflets, as opposed to the relatively more lipoprotein dense cytoplasmic leaflet (92, 93, 116). Further studies are needed to elucidate the role of differential dynamics of spirochetal lipoprotein expression in spirochetal immunopathogenesis.

TABLE 1 | Mechanisms of immune evasion of major spirochetal lipoproteins.

| Bacteria         | Role in immune evasion                                                                                                                                                                                                 |
|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Borrelia burgdorferi | Antigenic variation [VlsE proteins (118, 120, 131–134), OspC (133)] Evasion of complement-mediated lysis [OspE, Erp (135–138), OspA (139)] Impairment of neutrophil function (BBA67) (143) |
| Oral treponemes (ex. Treponema denticola) | C3b inactivation (various lipoproteins) (141)                                                                                                                                                                           |
| Borrelia recurrentis | Antigenic variation (variable large and small protein genes and Vmp variants) (19, 110) Bind to complement regulatory proteins, i.e., CFH and CFHR-1 (FhBA, BnCRASP-1, and HcpA (142–145)) |
| Borrelia turicatae | Antigenic variation (variable large and small protein genes and Vmp variants) (19, 110) Inhibit C4bp and C1-Inh, the major inhibitors of the classical and lectin pathway of complement activation (C3b) (146) |
| Borrelia hermsii | Antigenic variation (variable large and small protein genes and Vmp variants) (19, 110) Bind to complement regulatory proteins, i.e., CFH and CFHR-1 (FhBA, BnCRASP-1, and HcpA (142–145)) |
| Leptospira interrogans | Impairment of neutrophil function (LIC11207) (147) Bind to complement regulators [FcRnA, FcRnB, Len A, Len B] (148)                                                                                                                                 |

Antigenic variation in borrelas may result from recombination of variable large and small protein genes. Lipoproteins may also impair mechanisms of innate immunity such as neutrophil function and complement activation. These mechanisms allow the spirochete to evade the host’s immune response and persist in the mammalian host. BBA57, Borrelia burgdorferi A57 protein; BnCRASP-1, Borrelia hermsi complement regulator-acquiring surface protein 1; C1-Inh, human C1 esterase inhibitor; C4bp, C4b-binding protein; CspA, complement regulator-acquiring surface protein 1; Erp, OspE-F-related lipoprotein; FhBA, complement factor H-binding protein; HcpA, human complement regulator and plasminogen-binding protein; LIC11207, L. interrogans serovar Copenhageni (LIC) protein 11207; LigA, leptospiral immunoglobulin-like protein A; LigB, leptospiral immunoglobulin-like protein B; OspC, outer-surface protein C; OspE, outer-surface protein E; VlsE, variable major protein-like sequence E; Vmp, variable major lipoprotein.
Spirochetes also undergo a process of antigenic variation in terms of expressed outer-leaflet lipoproteins (96, 118). Studies in immunocompromised hosts have suggested that the host immune responses have a major role in producing spirochetal antigenic variants (96). Antigenic variation in borrelias may result from recombination of variable large and small protein genes (98) and the diversity of variable major lipoprotein lipoproteins allows these pathogens to evade the host immune response (19, 23, 119). Moreover, outer-leaflet lipoprotein variation also allows spirochetal adherence to a wide variety of host cells, as studies of *T. pallidum* TP0435 isoforms have recently shown (26). The antigenic variation of major surface lipoproteins is described in Table 1 (19, 90, 94–100).

The ability to vary surface lipoprotein expression has been studied in *B. burgdorferi*, where it has been shown that prolonged infections are due to the embodiment of a *vls* locus that is capable of random segmental variation in the surface-exposed lipoprotein it encodes (118, 120). The *vls* locus variation specifically allows for the variation in the encoded variable major protein-like sequence lipoprotein which has been documented to allow for persistence of *B. burgdorferi* in its host (120). The antigenic variation of spirochetes leads to evasion of the immune system and ultimately to the phenomenon of host relapsing (121). Most interestingly, antigenic variation characteristic of *B. burgdorferi* is only seen during host infection. Spirochetal antigenic variation has not been described *in vitro*. Thus, the cross talk between host cellular responses and *B. burgdorferi* is needed for development of antigenic variation (perhaps through downregulation of OspA) (96). Elimination of the ability to undergo antigenic variation, as was done in *Borrelia hermsii*, may greatly reduce host infectivity/persistence (119). Understanding the exact mechanisms behind a spirochete's ability to elicit immune evasion via antigenic variation could set the basis for targeted interventions to inhibit infections (122).

**Inhibition of Neutrophil Function by Spirochetes**

Neutrophil-mediated phagocytosis of pathogens is a major host immune response to infection. Thus, spirochetes evade immune responses by inactivating neutrophil function (56). The most prominent examples of the above can be seen with the *B. burgdorferi* surface protein OspB, which may prevent phagocytosis of the spirochete and inhibit respiratory/oxidative burst in a variety of tissues, such as the skin (56–58). It should be noted that *B. burgdorferi* also contains outer-surface protein C which plays a role in inhibiting phagocytosis by macrophages (18). Similar to OspB that impairs neutrophil function, the novel lipoprotein *Leptospira interrogans* serovar Copenhageni (LIC) protein 11207 from *Leptospira*, promotes apoptotic pathways in neutrophils (123). Thus, spirochetal lipoproteins can both activate and impair neutrophils.

**Lipoprotein Inhibition of Complement Activation**

One of the major components of a host's innate immune system is the complement system that plays a role in the phagocytosis/elimination of a pathogen and is a target of spirochetes upon infection (124). Activation of the complement system is known to occur through the recognition of surface-exposed lipoproteins as well as other antigens such as oligosaccharides (124). The multi-stage process of complement activation presents spirochetes (such as *B. burgdorferi*) with the opportunity to attack at multiple phases. For example, *B. burgdorferi* binds and inhibits the C1 initiation
complex and accelerates C3b inactivation (91, 125). Furthermore, B. burgdorferi can bind either Factor H or FHL-1, two important complement regulators which upon being bound by CRASP-2 and CRASP-1 (B. burgdorferi membrane-bound lipoproteins), respectively, are inactivated and inhibit formation of complement system activation products (126, 127). B. burgdorferi also maintains the ability to bind factor H, via particular Osp, such as outer-surface protein E, accomplishing the same outcome as with CRASP-2 binding (128). Hijacking of the complement system is a conserved mechanism of immune evasion among numerous pathogens (such as Plasmodium falciparum) (129). Therefore, understanding the mechanisms behind complement hijacking in spirochetes could potentially contribute to understanding conserved pathways in other pathogens.

Lipoprotein Inhibition of Natural Killer T (NKT) Cells

Natural killer (NK) cells act to bridge the innate and adaptive immune responses to pathogenic infections; however, it is their ability to respond to a variety of lipid antigens that allows them to maintain a functional presence during combat of spirochetal infections (130). Spirochetes are capable of interfering with the NKT cells that respond to CD1d glycolipids on the surface of spirochetes such as B. burgdorferi (149). Although the exact biochemical pathway of interference is not well understood, patients with syphilis have been known to exhibit low NKT numbers (150). Further studies are needed to understand the possible interaction between spirochetal lipoproteins and NK cells.

UNDERSTANDING LIPOPROTEIN-MEDIATED PATHWAYS OF IMMUNE EVASION MAY PAVE THE WAY FOR DEVELOPMENT OF STRATEGIES TO TREAT SPIROCHETAL INFECTIONS

Understanding the pleotropic modulatory effects of lipoproteins may contribute to the development of new approaches to combat a plethora of diseases (151–154). Use of adjuvants in vaccines can help target various diseases including cancer (155, 159). On the other hand, incorporation of a lipid moiety in peptide-based vaccines may induce TLR2 signaling in dendritic cells and subsequent protection against viral and bacterial infections (156). Finally, the use of lipopeptide-based antibiotics such as daptomycin, that can cause both immunomodulation (160) and also target spirochetes (161), remains to be studied as a therapeutic option for patients with spirochetal infections.

CONCLUSION

Lipoproteins play a significant role in the various stages of a spirochete’s ability to infect a host and survive, through pleotropic effects involving transfer from vector to host, immune activation, or even immune evasion. Further studies are needed to understand the molecular basis and mechanisms that underpin the numerous modulatory effects (both acute and chronic) of spirochetal lipoproteins. The payout from such targeted research can be significant considering the sheer amount of spirochetal infections occurring on a yearly basis as well as the morbidity associated with chronic spirochetal infections in humans. Ultimately, the use of knowledge surrounding spirochetal lipoproteins can be put toward the development of vaccines or, perhaps shed light on the pathogenesis of other vector-based pathogens.

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13. Hook EW III, Peeling RW. Spirochetal lipoproteins – a continuing challenge. N Engl J Med (2004) 351(2):122–4. doi:10.1056/NEJMep048126

14. Duray PH. Histopathology of clinical phases of human Lyme disease. Rheum Dis Clin North Am (1989) 15(4):691–710.

15. Chaaya G, Jaller-Char JJ, Ali SK. Beyond the bull’s eye: recognizing Lyme disease. J Fam Pract (2016) 66(6):373–9.

16. Radolf JD, Deka RK, Anand A, Smajs D, Norgard MV, Yang XF. Treponema pallidum, the syphilis spirochete: making a living as a stealth pathogen. Nat Rev Microbiol (2016) 14(12):744–59. doi:10.1038/nrd.2016.141

17. Radolf JD, Desrosiers DC. Treponema pallidum, the stealth pathogen, changes, but how? Mol Microbiol (2009) 72(5):1081–6. doi:10.1111/j.1365-2958.2009.06711.x

18. Carrasco SE, Truzzell B, Yang Y, Brandt SL, Li H, Sandusky GE, et al. Outer surface protein OspA is an antiphagocytic factor that protects Borrelia burgdorferi from phagocytosis by macrophages. Infect Immun (2015) 83(3):4848–60. doi:10.1128/IAI.01215-15

19. Haake DA. Spirochetal lipoproteins and pathogenesis. Microbiology (2000) 146(Pt 7):1491–504. doi:10.1099/0221287-146-7-1491

20. Hossain H, Wellensiek H, Geyer R, Lochnit G. Structural analysis of glycolipids from Borrelia burgdorferi. Biochimie (2001) 83(7):683–92. doi:10.1016/S0300-9326(01)00129-2

21. Uvelicht RJ, Tobias PS. Recognition of gram-negative bacteria and endotoxin by the innate immune system. Curr Opin Immunol (1999) 11(1):19–22. doi:10.1016/S0952-7915(99)80004-1

22. Ramesh G, Meisner OC, Philipp MT. Anti-inflammatory effects of dexamethasone and meloxicam on Borrelia burgdorferi lipids from phagocytosis by macrophages. Front Immunol (2016) 7:1404. doi:10.3389/fimmu.2016.01404

23. Schröder NW, Eckert J, Stübs G, Schumann RR. Immune responses induced by spirochetal outer membrane lipoproteins and glycolipids. Immunobiology (2008) 213(3–4):329–40. doi:10.1016/j.imbio.2007.11.003

24. Rietschel ET, Schletter J, Weidemann B, El-Samalouti V, Mattern T, Zähringer U, et al. Lipopolysaccharide and peptidoglycan: CD14-dependent bacterial inducers of inflammation. Curr Opin Immunol (2001) 13(6):793–803. doi:10.1016/S0952-7915(00)00131-1

25. Morrison TB, Weis JH, Weis JJ. Regulation of toll-like receptor expression in monocyte-derived Langerhans cells. Int Immunol (2003) 15(6):721–30. doi:10.1093/intimm/dxg068

26. Setubal JC, Reis M, Matsumura J, Haake DA. Lipoprotein computational prediction in spirochaetal genomes. Microbiology (2006) 152(Pt 1):113–21. doi:10.1099/mic.0.28331-0

27. Norgard MV, Riley BS, Richardson JA, Radolf JD. Dermal inflammation by spirochetal outer membrane lipoproteins and glycolipids. Immunobiology (2001) 206(6):1–11. doi:10.1078/1434-9741.s12974-015-0461-y

28. Norgard MV, Riley BS, Richardson JA, Radolf JD. Dermal inflammation by spirochetal outer membrane lipoproteins and glycolipids. Immunobiology (2001) 206(6):1–11. doi:10.1078/1434-9741.s12974-015-0461-y

29. Norgard MV, Riley BS, Richardson JA, Radolf JD. Dermal inflammation by spirochetal outer membrane lipoproteins and glycolipids. Immunobiology (2001) 206(6):1–11. doi:10.1078/1434-9741.s12974-015-0461-y

30. Braun V, Hantke K. Biochemistry of bacterial cell envelopes. Arch Microbiol (1974) 94(0):89–121. doi:10.1007/BF00428232

31. Norgard MV, Riley BS. Lipopolysaccharide and peptidoglycan: CD14-dependent bacterial inducers of inflammation. Curr Opin Immunol (2001) 13(6):793–803. doi:10.1016/S0952-7915(00)00131-1

32. Norgard MV, Riley BS. Lipopolysaccharide and peptidoglycan: CD14-dependent bacterial inducers of inflammation. Curr Opin Immunol (2001) 13(6):793–803. doi:10.1016/S0952-7915(00)00131-1

33. Brandt ME, Riley BS, Radolf JD, Norgard MV. Immunogenic integral membrane protein immunogens of Borrelia burgdorferi are lipoproteins. Infect Immun (1990) 58(4):983–91.

34. Fraser CM, Norris SJ, Weinstock GM, White O, Sutton GG, Dodson R, et al. Complete genome sequence of Treponema pallidum, the syphilis spirochete. Science (1998) 281(5375):375–88. doi:10.1126/science.281.5375.375

35. Brandt ME, Riley BS, Radolf JD, Norgard MV. Immunogenic integral membrane protein immunogens of Borrelia burgdorferi are lipoproteins. Infect Immun (1990) 58(4):983–91.

36. Ma Y, Weis JJ. Borrelia burgdorferi lipoprotein genes. Mol Microbiol (2000) 35(3):490–516. doi:10.1046/j.1365-2958.2000.01698.x

37. Wooten RM, Modur VR, McIntyre TM, Weis JJ. Borrelia burgdorferi outer membrane protein A induces nuclear translocation of nuclear factor-kappa B. Infect Immun (1996) 64(9):3845–52.

38. Weis JJ, Yang X, Li Y, Li J, Zhao X, Huang WM, et al. Borrelia burgdorferi lipoprotein genes. Mol Microbiol (2000) 35(3):490–516. doi:10.1046/j.1365-2958.2000.01698.x

39. Casjens S, Palmer N, van Vugt R, Huang WM, Stevenson B, Rosa P, 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(3):490–516. doi:10.1046/j.1365-2958.2000.01698.x

40. Vazquez TB, Weis JJ, Weis JH. Regulation of toll-like receptor expression in monocyte-derived Langerhans cells. Int Immunol (2003) 15(6):721–30. doi:10.1093/intimm/dxg068

41. Gondolf KB, Mihatsch M, Curschellas E, Dunn JJ, Batsford SR. Induction of experimental allergic arthritis with outer surface proteins of Borrelia burgdorferi. Arthritis Rheum (1999) 42(7):1707–10. doi:10.1002/1529-0170(199907)42:7<1707::AID-ART2>3.0.CO;2-3

42. Kupper TS, Fulbright RC. Immune surveillance in the skin: mechanisms and clinical consequences. Nat Rev Immunol (2004) 4(3):211–22. doi:10.1038/nri1310

43. Iwatsuki Y, Sone T, Muto T, Nakajima S, Kondo K, Sakai M, et al. Expression and function of toll-like receptors 2 and 4 in human keratinocytes. Int Immunol (2003) 15(6):721–30. doi:10.1093/intimm/dxg068

44. Takeuchi O, Watari E, Shinya E, Norose Y, Matsumoto M, Seya T, et al. Down-regulation of toll-like receptor expression in monocyte-derived Langerhans
cell-like cells: implications of low-responsiveness to bacterial components in the epidermal Langerhans cells. Biochem Biophys Res Comm (2003) 306(3):674–9. doi:10.1016/S0006-291X(02)00852-8
56. Hartila J, Hynynen J, Suhonen I, Leipärinta O, Tuominen-Gustafsson H, Viljanen MK. Borrelia burgdorferi inhibits human neutrophil functions. Microbes Infect (2008) 10(1):60–8. doi:10.1016/j.micinf.2007.10.004
57. von Burgel ND, Kraiczky TJ, Zipfel PF, van Dam AP. Identification and functional characterisation of complement regulator acquiring surface protein-1 of resistant Borrelia garinii Ospa type 4. BMC Microbiol (2010) 10:43. doi:10.1186/1471-2100-10-43
58. Sadziene A, Thomas DD, Barbour AG. Early induction of interferon-γ in response to Borrelia burgdorferi. Infect Immun (1995) 63(4):1572–80.
59. Benach JL, Coleman JL, Garcia-Monco JC, Deponte PC. Biological activity of Borrelia burgdorferi antigens. Ann N Y Acad Sci (1988) 539:115–25. doi:10.1111/j.1749-6632.1988.tb3845.x
60. Detmer SE, Bouljihad M, Hayden DW, Schefers JM, Arminen A, Wünschmann A. Fatal pyogranulomatous myocarditis in 10 Boxer puppies. J Vet Diagn Invest (2016) 28(2):144–9. doi:10.1177/1040638716652486
61. Scharrig E, Carestia A, Ferrer MF, Cédola M, Pretre G, Drut R, et al. Role of CD14 in signaling mediated by outer membrane lipoproteins of Borrelia burgdorferi and T. pallidum. J Immunol (2016) 196(11):5455–64.
62. 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. Infect Immun (1998) 66(1):548–92.
63. Wright SD, Ramos RA, Tobias PS, Ulевич RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science (1990) 249(4975):1431–3. doi:10.1126/science.1659311
64. Giambartolomei GH, Dennis VA, Lasater BL, Philipp MT. Induction of pro- and anti-inflammatory cytokines by Borrelia burgdorferi lipoproteins in synthesis activates monocytes via CD14-dependent pathway distinct from that used by lipopolysaccharide. J Immunol (1999) 160(11):5455–64.
65. Mason LM, Wagemakers A, van’t Veer C, Oei A, van der Pot WJ, Ahmed K, et al. Borrelia burgdorferi induces TLR2-mediated migration of activated dendritic cells in an ex vivo human skin model. PLoS One (2016) 11(10):e0164040. doi:10.1371/journal.pone.0164040
66. Bois DA, Popova TG, Takashima A, Norgard MV. Dendritic cells phagocyte and are activated by Treponema pallidum. Infect Immun (2001) 69(5):1518–28.
67. Akins DR, Purcell BK, Mitra MM, Norgard MV, Radolf JD. Lipid modification of the 17-kilodalton membrane immunogen of Treponema pallidum determines macrophage activation as well as amphotiphility. Infect Immun (1993) 61(4):1202–10.
68. DeGnaye L, Pramanik BC, Arndt LL, Jones JD, Rush J, Slaughter CA, et al. Solid-phase synthesis of biologically active lipopeptides as analogs for spirochetal lipopeptides. Pept Res (1994) 7(2):91–7.
69. Ranoa DR, Kelley SL, Tapping RI. Human lipopolysaccharide-binding protein (LBP) and CD14 independently deliver triacylated lipoproteins to toll-like receptor 1 (TLR1) and TLR2 and enhance formation of the ternary signaling complex. J Biol Chem (2013) 288(14):9729–41. doi:10.1074/jbc.M112.436251
70. Hirschfeld M, Kirschning CJ, Schwandner R, Wesche H, Weis JH, Wooten RM, et al. Cutting edge: inflammatory signaling by Borrelia burgdorferi lipoproteins is mediated by toll-like receptor 2. J Immunol (1999) 163(3):2382–6.
71. Maleev VV, Datta LS, Maleev AV, et al. [Follow-up of patients with tick-borne borrelioses]. Ter Arkh (2016) 84(12):3350–7. doi:10.1128/IAI.00708-16
72. Devenish RI, Barbour AG, Restrepo BI, Schwan TG. Population dynamics of Borrelia burgdorferi. J Vet Diagn Invest (2015) 27(6):639–48. doi:10.1177/1040638715626486
73. Cox DL, Chang P, McDowall AW, Radolf JD. The outer membrane, not a coat protein, stimulates monocytes. FEMS Immunol Med Microbiol (1997) 19(1):15–23. doi:10.1111/j.1574-695X.1997.tb01068.x
74. Christodoulides et al. | March 2017 | Volume 8 | Article 364
75. Arminen A, Wünschmann A, van’t Veer C, Oei A, van der Pot WJ, Ahmed K, et al. Borrelia burgdorferi induces TLR2-mediated migration of activated dendritic cells in an ex vivo human skin model. PLoS One (2016) 11(10):e0164040. doi:10.1371/journal.pone.0164040
76. Loach Head RB, Zachary JF, Dalla Rosa L, Ma Y, Weis JJ, O’Connell RM, et al. Antagonistic interplay between microRNA-155 and IL-10 during Lyme carditis and arthritis. PLoS One (2015) 10(8):e0135142. doi:10.1371/journal.pone.0135142
77. Zhang W, Wang N, Xie X, Guo J, Jin X, Xue F, et al. Toll-Like receptor 2 agonist Pam3CSK4 alleviates the pathology of leptospirosis in hamster. Infect Immun (2016) 84(13):3530–7. doi:10.1128/IAI.00708-16
78. Mason LM, Wagemakers A, van’t Veer C, Oei A, van der Pot WJ, Ahmed K, et al. Borrelia burgdorferi induces TLR2-mediated migration of activated dendritic cells in an ex vivo human skin model. PLoS One (2016) 11(10):e0164040. doi:10.1371/journal.pone.0164040
79. Bois DA, Popova TG, Takashima A, Norgard MV. Dendritic cells phagocyte and are activated by Treponema pallidum. Infect Immun (2001) 69(5):1518–28.
80. Akins DR, Purcell BK, Mitra MM, Norgard MV, Radolf JD. Lipid modification of the 17-kilodalton membrane immunogen of Treponema pallidum determines macrophage activation as well as amphotiphility. Infect Immun (1993) 61(4):1202–10.
81. DeGnaye L, Pramanik BC, Arndt LL, Jones JD, Rush J, Slaughter CA, et al. Solid-phase synthesis of biologically active lipopeptides as analogs for spirochetal lipopeptides. Pept Res (1994) 7(2):91–7.
82. Riley BS, Oppenheimer-Marks N, Hansen DJ, Radolf JD, Norgard MV. Virulent Treponema pallidum activates human vascular endothelial cells. J Infect Dis (1992) 165(3):484–93. doi:10.1093/infdis/165.3.VMP484
83. Maleev VV, Datta LS, Maleev AV, et al. [Follow-up of patients with tick-borne borrelioses]. Ter Arkh (2016) 84(12):3350–7. doi:10.1128/IAI.00708-16
84. Salazar JC, Pope CD, Moore MW, Pope J, Kiely TG, Radolf JD. Lipoprotein-dependent and -independent immune responses to spirochetal infection. Clin Diag Lab Immun (2005) 12(8):949–58.
polymerorphism of two multigene families that encode immunogenic outer surface lipoproteins. Insect Immun (1998) 66(2):432–40.
95. Zhang JR, Norris SJ. Genetic variation of the Borrelia burgdorferi gene vlsE involves cassette-specific, segmental gene conversion. Infect Immun (1998) 66(8):3698–704.
96. Zhang JR, Norris SJ Kinetics and in vivo induction of genetic variation of vlsE in Borrelia burgdorferi. Infect Immun (1998) 66(8):3689–97.
97. Rogovskyy AS, Bankhead T. Variable VlsE is critical for host reinfestation by the Lyme disease spirochete. PLoS One (2013) 8(4):e61226. doi:10.1371/journal.pone.0061226.
98. Vidal V, Cutler S, Scragg IG, Wright DJ, Kwiatkowski D. Characterisation of silent and active genes for a large variable protein of Borrelia recurrentis. BMC Infect Dis (2002) 2:25. doi:10.1186/1471-2334-2-25.
99. Schwanz TG, Hinnebusch BJ. Bloodstream- versus tick-associated variants of a relapsing fever bacterium. Science (1998) 280(5371):1938–40. doi:10.1126/science.280.5371.1938.
100. Carter CJ, Bergström S, Norris SJ, Barbour AG. A family of surface-exposed proteins of 20 kilodaltons in the genus Borrelia. Infect Immun (1994) 62(7):2792–9.
101. Dennis VA, Dixit S, O’Brien SM, Alvarez X, Pahar B, Philipp MT. Live Borrelia burgdorferi spirochetes elicit inflammatory mediators from human monocytes via the toll-like receptor signaling pathway. Infect Immun (2009) 77(3):1238–45. doi:10.1128/IJAI.01078-08.
102. Bolz DJ, Sundabat RS, Ma Y, Akira S, Kirschning CJ, Zachary JF; et al. MyD88 plays a unique role in host defense but not arthritis development in Lyme disease. J Immunol (2004) 173(3):2003–10. doi:10.4049/jimmunol.173.3.2003.
103. Benhnia MR, Wroblewski D, Akhtar MN, Patel RA, Lavezzi W, Gangloff to.
104. 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. J Immunol (2005) 174(3):1539–48. doi:10.4049/jimmunol.174.3.1539.
105. 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 CH/HeJ mice. FEMS Microbiol Lett (2004) 231(2):219–25. doi:10.1016/S0378-1097(03)00690-1.
106. Wooten RM, Weis JJ. Host-pathogen interactions promoting inflammatory arthritis: use of mouse models for dissection of disease processes. Curr Opin Microbiol (2001) 4(3):274–9. doi:10.1016/S1369-5274(00)00202-2.
107. Lien E, Sellati TJ, Yoshimura A, Flo TH, Rawadi G, Finberg RW; et al. Borrelia burgdorferi activation by Heterozygous Arg753Gln polymorphism of human TLR-2 impairs immune defense to infection and toll-like receptor 2-independent killing of Borrelia burgdorferi. J Immunol (2005) 174(7):2792–800. doi:10.4049/jimmunol.174.7.2792.
108. Hsu SH, Lo YY, Tung JY, Ko YC, Sun YJ, Hung CC; et al. Leptospiral outer membrane lipoprotein LipL32 binding on toll-like receptor 2 of renal mesangial cells as determined with an atomic force microscope. Biochemistry (2010) 49(26):5408–17. doi:10.1021/bi100058w.
109. Hoffmann P, Heinle S, Schade UF, Loppnow H, Ulmer AJ, Flad HD; et al. Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues. Immunobiology (1998) 177(2):158–70. doi:10.1006/imbi.1997.2911.
110. Radolf JD, Norgard MV, Schulz WW. Outer membrane ultrastructure explains the limited antigenicity of virulent Treponema pallidum. Proc Natl Acad Sci U S A (1989) 86(6):2051–5. doi:10.1073/pnas.86.6.2051.
111. Hoffmann P, Heinle S, Schade UF, Loppnow H, Ulmer AJ, Flad HD; et al. Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues. Immunobiology (1998) 177(2):158–70. doi:10.1006/imbi.1997.2911.
112. Patel S. Drivers of bacterial genomes plasticity and roles they play in pathogen virulence, persistence and drug resistance. Infect Genet Evol (2016) 45:151–64. doi:10.1016/j.meegid.2016.08.030.
113. Hoffmann P, Heinle S, Schade UF, Loppnow H, Ulmer AJ, Flad HD; et al. Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues. Immunobiology (1998) 177(2):158–70. doi:10.1006/imbi.1997.2911.
114. Hoffmann P, Heinle S, Schade UF, Loppnow H, Ulmer AJ, Flad HD; et al. Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues. Immunobiology (1998) 177(2):158–70. doi:10.1006/imbi.1997.2911.
115. Hoffmann P, Heinle S, Schade UF, Loppnow H, Ulmer AJ, Flad HD; et al. Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues. Immunobiology (1998) 177(2):158–70. doi:10.1006/imbi.1997.2911.
coli heat-labile enterotoxin. Clin Vaccine Immunol (2012) 19(5):740–5. doi:10.1128/CVI.05270-11

134. Luo D, Xue F, Ojcius DM, Zhao J, Mao Y, Li L, et al. Protein typing of major outer membrane lipoproteins from Chinese pathogenic Leptospira spp. and characterization of their immunogenicity. Vaccine (2009) 28(1):243–55. doi:10.1016/j.vaccine.2009.09.089

135. Rossmann E, Krakczyn P, Herzberger P, Skerka C, Kirschfink M, Simon MM, et al. The complement regulator factor H binds to the surface protein OspE of Borrelia burgdorferi. J Biol Chem (2001) 276(11):8427–35. doi:10.1074/jbc.M007994200

136. Guo BP, Brown EL, Dorward DW, Hook M. Decorin-binding proteins of Borrelia burgdorferi. Mol Microbiol (1998) 30(4):711–23. doi:10.1046/j.1365-2958.1998.01103.x

137. Hauk P, Macedo F, Romero EC, Vasconcellos SA, de Moraes ZM, Barbosa AS, et al. In LipL32, the major leptomis lipoprotein, the C terminus is the primary immunogenic domain and mediates interaction with collagen IV and plasma fibronectin. Infect Immun (2008) 76(6):2642–50. doi:10.1128/IAI.01639-07

138. Choy HA, Kelley MM, Chen TL, Moller AK, Matsunaga J, Haake DA. Physiological osmotic induction of Leptospira interrogans adhesion: LigA and LigB bind extracellular matrix proteins and fibrinogen. Infect Immun (2007) 75(5):2441–50. doi:10.1128/IAI.01639-07

139. Hovis KM, Jones JP, Sadlon T, Raval G, Gordon DL, Marconi RT. Molecular analyses of the interaction of Borrelia hermsii FhbA with the complement regulatory proteins factor H and factor H-like protein 1. Infect Immun (2006) 74(4):2007–14. doi:10.1128/IAI.74.4.2007-2014.2006

140. Bankhead T, Chaconas G, The role of VlsE antigenic variation in Borrelia burgdorferi infection. J Immunol (2007) 178(11):7292–301. doi:10.4049/jimmunol.178.11.7292

141. Hellwage J, Meri T, Heikkilä T, Alitalo A, Panelius J, Lahdenne P, et al. The complement regulator factor H binds to the surface protein OspE of Borrelia burgdorferi. J Biol Chem (2001) 276(11):8427–35. doi:10.1074/jbc.M007994200

142. Luo D, Xue F, Ojcius DM, Zhao J, Mao Y, Li L, et al. Protein typing of major outer membrane lipoproteins from Chinese pathogenic Leptospira spp. and characterization of their immunogenicity. Vaccine (2009) 28(1):243–55. doi:10.1016/j.vaccine.2009.09.089

143. Werts C, Tapping RI, Mathison JC, Chuang TH, Kravchenko V, Saint Bankhead T, Chaconas G. The role of VlsE antigenic variation in Borrelia burgdorferi infection. J Immunol (2007) 178(11):7292–301. doi:10.4049/jimmunol.178.11.7292

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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