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The cross-talk between spirochetal lipoproteins and immunity

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Spirochetal diseases such as syphilis, Lyme disease, and leptospirosis are major threats to public health. However, the immunopathogenesis of these diseases has not been fully elucidated. Spirochetes interact with the host through various structural components such as lipopolysaccharides (LPS), surface lipoproteins, and glycolipids. Although spirochetal antigens such as LPS and glycolipids may contribute to the inflammatory response during spirochetal infections, spirochetes such as *Treponema pallidum* and *Borrelia burgdorferi* lack LPS. Lipoproteins are most abundant proteins that are expressed in all spirochetes and often determine how spirochetes interact with their environment. Lipoproteins are pro-inflammatory, may regulate responses from both innate and adaptive immunity and enable the spirochetes to adhere to the host or the tick midgut or to evade the immune system. However, most of the spirochetal lipoproteins have unknown function. Herein, the immunomodulatory effects of spirochetal lipoproteins are reviewed and are grouped into two main categories: effects related to immune evasion and effects related to immune activation. Understanding lipoprotein-induced immunomodulation will aid in elucidating innate immunopathogenesis processes and subsequent adaptive mechanisms potentially relevant to spirochetal disease vaccine development and to inflammatory events associated with spirochetal diseases.

Keywords: spirochetes, lipoproteins, immunity, *Borrelia*, treponemes, lipopeptides, immunomodulation

INTRODUCTION

Spirochetes are the cause of important human diseases such as syphilis, Lyme disease, and leptospirosis that are major threats to public health (1). However, the immunopathogenesis of these diseases has not been fully elucidated. Tissue inflammation is characteristic of spirochetal diseases such as dermatitis in syphilis and Lyme disease, interstitial nephritis in leptospirosis, and periodontitis caused by oral treponemes (2, 3). Spirochetes such as: *Treponema pallidum* (*T. pallidum*) and *Borrelia burgdorferi* (*B. burgdorferi*), the pathogens for syphilis and Lyme disease, respectively (1), may persist for prolonged periods despite the induced immune responses in the host (4–6). Several mechanisms may explain how spirochetes may evade host defenses such as intracellular sequestration of the spirochetes, the antigenic variation of the spirochetes, manipulation of host defenses to delay, and/or suppress the onset of effective immune responses and structural features of the outer membrane in spirochetes that contribute to immune evasion (7–11).

Spirochetes have unique membrane structure that interacts with the immune system (**Figure 1**) (2, 3, 12–18). Although spirochetal antigens such as lipopolysaccharides (LPS), the main pro-inflammatory component of Gram-negative bacteria (19), and glycolipids may contribute to the inflammatory response during spirochetal infections, spirochetes such as *T. pallidum* and *B. burgdorferi* express abundantly membrane lipoproteins and induce strong immune responses (20–24) despite lack of LPS (2, 3, 17, 18). Thus, lipid–lipid interactions between spirochetes and the lipid rafts in eukaryotic host cells either through glycolipids (3, 25, 26) or lipoproteins (2, 18, 22, 27–31) may occur and these lipid interactions may be an important process that contributes to the immunopathogenesis of spirochetal diseases (3, 25, 26).

In bacteria, membrane lipoproteins are important virulence factors, pro-inflammatory agonists, enzymes, receptors, modular components of ATP binding cassette (ABC) transporters, and protective immune targets that regulate innate immunity (2, 3). In contrast to other bacteria that do not express lipoproteins so abundantly (2, 3, 27), lipoproteins have an important role in the virulence of spirochetes since they are most abundant proteins that are expressed in all spirochetes (2, 3, 18, 22, 27–31). The spirochetes express numerous lipoprotein genes [*T. pallidum* has >20 (24), *B. burgdorferi* has >100 lipoproteins (23), and approximately 8% of *B. burgdorferi* genes may encode lipoproteins (21, 23) and *Leptospira* spp. Have >140 lipoprotein genes (32)]. Examples of abundant lipoproteins in spirochetes include Tp47 of *T. pallidum*, OspA of *B. burgdorferi*, LipL32 of *Leptospira* species, and Vmp proteins of *Borrelia* species (**Table 1**). Finally, spirochetal lipoproteins have more prominent pro-inflammatory effects compared to other bacterial lipoproteins and synthetic lipopeptides (28).

Surface-exposed lipoproteins often determine how spirochetes interact with their environment and immunity (**Figure 1**) (2, 3, 18, 22, 27–31). Lipoproteins may be present in different cellular compartments (2, 70, 84) and their distribution varies among spirochetes (7, 18, 85–88) (**Figure 1**). The NH2-terminal lipopeptide region is the part of the lipoprotein that confers its immunologic activity since removal of this lipid component removed the immunoregulatory properties of these lipoproteins

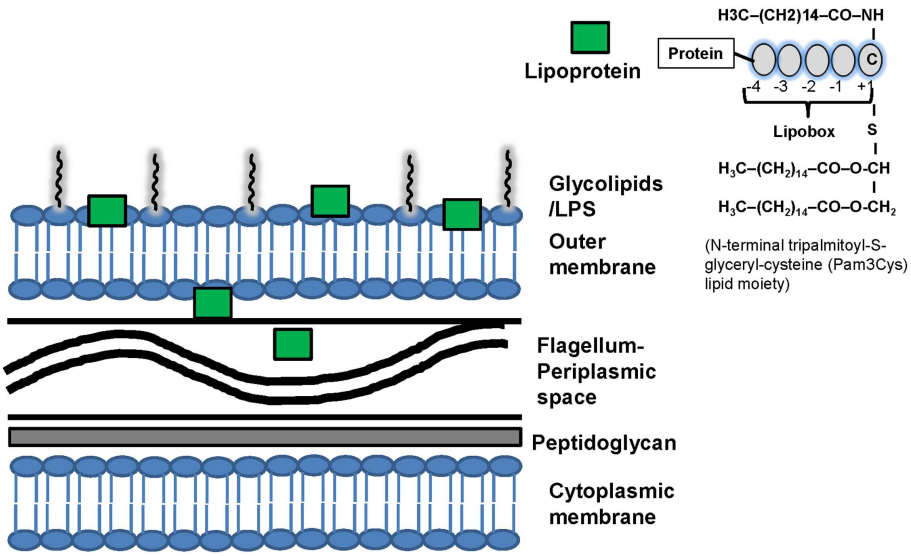

FIGURE 1 | Structure of spirochetal membrane and lipoproteins. Similarly to gram-positive bacteria, the spirochetal cytoplasmic membrane is associated with the cell wall that consists of peptidoglycan. Similarly to Gram-negative bacteria, spirochetes also have an outer membrane, which is not attached to the peptidoglycan layer. Spirochetes differ phylogenetically from Gram-negative bacteria and interact with the host through various structural components such as lipopolysaccharides (LPS), surface lipoproteins and glycolipids that are present mostly in the outer membrane. LPS has not been identified in *Borrelia* and *Treponema*. The periplasmic space contains the flagellum. The distribution of lipoproteins varies among spirochetes and they may be present in different cellular compartments: the outer membrane, the extracellular and the periplasmic spaces. For example the pro-inflammatory lipoproteins of *T. pallidum* are located below its cell surface and thus do not interact directly with the immune system of the host. It has been suggested that uptake and degradation of *T. pallidum* releases lipoproteins and allows their interaction with receptors on immune cells leading to immune cell activation. Computational programs can predict spirochetal protein lipidation but do not determine the location of lipoproteins in the cells. Recently, developed fluorescence activated cell sorting (FACS) and surface proteolysis methods can be used to screen for lipoprotein localization. Right upper corner: structure of spirochetal lipoproteins. The finding of a cysteine residue after a signal peptide (+1) is suggestive evidence that a protein is lipidated. The spirochetal lipoproteins have a lipobox that is four amino acids in length and mediates NH2-terminal lipidation on a conserved cysteine residue. Lipoproteins interact with the phospholipids of membranes via three hydrophobic N-terminal acyl moieties (often palmitate; C16) attached to a N-terminal cysteine residue which may contribute to the localization of spirochetal lipoproteins. An analysis of the fatty acids of *T. pallidum, B. burgdorferi, L. interrogans* phospholipids and lipoproteins found that while fatty acids with different length side chains (C16 and C18) were found in phospholipids, palmitate (C16) predominated in the lipoproteins. The N-terminal tripalmitoyl-*S*-glyceryl-cysteine (Pam3Cys) lipid moiety is the part of the lipoprotein that confers its immunologic activity. C, cysteine; LPS, lipopolysaccharides.

while synthetic lipopeptides based on this lipid component could activate immune cells (**Figure 1**) (7–11, 18, 28, 89–93).

In vitro and *in vivo* studies suggest that spirochetal membrane lipoproteins and lipopeptides are pathogen-associated molecular patterns (PAMPs) that bind to pattern recognition receptors such as toll-like receptors (TLR1, 2). Thus, spirochetal lipoproteins are pro-inflammatory (18, 33, 35–37, 41, 94) by activating endothelial cells (36, 77–80, 95) and cells of innate immunity such as macrophages and dendritic cells (DCs) (7–11, 38, 42–45, 56, 63, 96, 97). Spirochetal lipoproteins also enable the spirochetes to evade the immune system (98, 99) and adhere to the host (100–104) or the tick midgut (105, 106) (**Table 1**). Finally, lipoproteins may be used as vaccine candidates for prevention of spirochetal infections (2, 3). The immunoregulatory effects of spirochetal lipoproteins have mostly been determined for major lipoproteins of *T. pallidum* (e.g., Tp47), *B. burgdorferi* (e.g., OspA, B), and *Leptospira* (e.g., LipL32) (**Table 1**). However, most spirochetal lipoproteins have not been well-studied and their interplay with immunity has recently been elucidated (Table S1 in Supplementary Material). Different methods have been used (**Table 2**) but observations in human models are often different from those obtained *in vitro*.

Lipoproteins are environmentally regulated and may be expressed selectively during spirochetal infection (37, 50, 112–116) (Table 1 in Supplementary Material). For example, the outer-surface protein (Osp) A has a more important role in the pathogenesis of borrelial infection during the tick phase of *B. burgdorferi* and its expression is down regulated during the mammalian phase of *B. burgdorferi* infection (9). Thus, although OspA does not have a major role in regulation of host immunity *in vivo*, since it is not expressed during the later stages of borrelial infection, it has been used as a model to study *in vitro* the immunoregulatory effects of spirochetal lipoproteins (9). Herein, the immunomodulatory effects of spirochetal lipoproteins are reviewed and are grouped into two main categories: effects related to immune evasion and effects related to immune activation (**Figure 2**). Understanding these mechanisms will aid in elucidating the immunopathogenesis of chronic spirochetal diseases.

THE ROLE OF SPIROCHETAL LIPOPROTEINS IN IMMUNE EVASION

Spirochetes may use outer membrane lipoproteins to invade the host but host immunity may also target these lipoproteins.

Table 1 | Immunoregulatory effects of major known spirochetal lipoproteins.

Spirochetal lipoproteins	Endothelial cells	Neutrophils	Complement	Antigen presenting cells: monocytes/ macrophages/DCs	Lymphocytes
Treponema pallidum: mixture of bacterial lipoproteins of various MW [17 kDA (33), 38 kDA (34), 47 kDA (35)] and related synthetic lipopeptides (22, 30)	Activate directly host vascular endothelium which plays important roles in lymphocyte homing and hemostasis (36)	NR	NR	Stimulate macrophage and mDCs function: costimulatory signals (DC-SIGN, CD14) (11, 37, 38) and production of chemokines (CCR5) (39, 40), cytokines such as TNF-a, IL-1 beta, IL-6, and IL-12 (18, 33, 35, 41) through TLRs (42) and mostly TLR2 (43), activated IL-12 p40 promoter (42), NF-KB pathway (37, 38)	Up-regulate CCR5 expression on CD4+ T cells (39, 40)
Borrelia burgdorferi: outer-surface protein A (OspA) and B (OspB) and related synthetic lipopeptides (20, 44–46)	NR	OspB inhibits the phagocytosis and oxidative burst of human neutrophils whereas OspA induces the oxidative burst in neutrophils (47)	Deactivation of host complement by binding to CFH and FHL-1 (47–49)	Stimulate macrophage function and production of nitric oxide (42, 50), chemokines (CXCL13) (51), pro-inflammatory (such as TNF-a, IL-1 beta, IL-6, and IL-12) and anti-inflammatory (IL-10) cytokines (18, 35, 41, 44, 52–55) through TLRs (28, 42, 44, 45, 56, 57), CD14, and NF-kB activation pathway (37, 38, 44, 45). Also increase chemotaxis of circulating pDCs into skin (11) but do not activate pDCs *in vitro* and *in vivo* (58, 59)	Induce memory B cell immune responses (60), B cell proliferation and production of cytokines (61) and Th production of cytokines (IFN-γ and IL-6) (62) and chemokines (CXCL13) (51). OspA may bind TLR 2 and 6, activate NFκB and up-regulate costimulatory molecules as well as of MHC class II, leading to stronger T cell activation (63–65); Possible molecular mimicry for T helper cells between OspA-1 and LFA-1 (66–68). OspA-1 may activate autoreactive T cells against a self-epitope and adaptive immune responses to OspA are implicated in the pathogenesis of antibiotic-refractory Lyme arthritis (68, 69)
Leptospira interrogans LipL32 is the most abundant protein on the outer membrane of *Leptospira* and is expressed at high levels during infection (2, 70–76)	LipL32 interacts with endothelial cells contributing to systemic inflammation (77–80)	NR	NR	The calcium-binding cluster is crucial for the interaction between LipL32 and TLR2, which then triggers the signaling cascade of inflammatory responses (56, 72, 81)	Lipl32 has been used as immunogen for vaccine trials (82, 83)

CFH, complement factor H; FHL-1, factor H-like protein 1; IL, interleukin; kDA, kilodalton; LipL32, 32-kDa lipoprotein of Leptospira; LFA-1, human lymphocyte function associated antigen 1; mDCs, myeloid dendritic cells; MW, molecular weight; NF-kB, NF-kappa B; NR, not reported; OspA, outer-surface protein A; OspB, outer-surface protein B; pDCs, plasmacytoid dendritic cells; TLR, toll-like receptor; Th, T helper; TNF-a, tumor necrosis factor.

Table 2 | Methods used to determine *in vitro* and *in vivo* immunomodulatory properties of spirochetal lipopeptides/lipoproteins.

Method	Comments
Mutagenesis systems	• The lack of mutagenesis systems for *T. pallidum* significantly impairs studies of their immunopathogenesis • Mutagenesis systems for *B. burgdorferi* have recently been developed (107)
Structural studies	• *T. pallidum* cannot be cultured in the laboratory and structural studies have been used to elucidate the function of treponemal lipoproteins (108)
Synthetic lipopeptides	• The immunomodulatory properties of lipopeptides are conferred by the lipid moiety of their N termini and synthetic lipopeptides have been modeled after this structure to study native spirochetal lipoproteins (18, 33, 35–37, 41, 94) • The synthetic lipopeptides have qualitatively similar immunostimulatory properties to those of native lipoproteins (18, 33, 35–37, 41, 94) • Can be isolated in large amounts whereas large amounts of native lipoprotein cannot be isolated from spirochetes that cannot be cultured such as *T. pallidum* • LPS contamination is a major problem when purifying bacterial lipoprotein while synthetic lipopeptides are synthesized under sterile conditions (22, 24, 35)
Skin techniques: injection of the skin with synthetic lipopeptides	• Useful tools to study cellular responses induced by spirochetal lipopeptides within tissues • Can be used instead of immunohistochemistry to characterize cellular infiltrates in target tissues of spirochetal disease (40, 109, 110)
Identification of stereotypical responses to lipopeptides *in vitro* (synthetic lipopeptides) and *in vivo* (presence of similar histopathological abnormalities)	• Often contribute to the understanding of specific immunomodulatory effects of spirochetal lipoproteins (6, 18, 37, 50, 61, 94, 111)

LPS, lipopolysaccharide.

However, all pathogenic spirochetes may cause persistent infections in humans by evading the host immune response through multiple mechanisms such as limiting the expression of membrane lipoproteins (85) and their access to antibodies (15, 117) and antigenic variation of surface lipoproteins (**Table 1**) (2, 98, 118–124). Spirochetal lipoproteins may also interact with and inhibit components of innate immunity such as the complement (10, 43, 56, 57, 97, 125–130), neutrophils, and serum lipoproteins (131).

SPIROCHETES USE ANTIGENIC VARIATION OF SURFACE LIPOPROTEINS TO EVADE IMMUNITY

Antigenic variation in borrelias may result from recombination of variable large and small protein genes (122) and the diversity of Vmp lipoproteins allows these pathogens to evade the host immune response (2, 3). Studies in immunocompromised hosts have suggested that the host immune responses have a major role in producing spirochetal antigenic variants (120).

SPIROCHETAL LIPOPROTEINS INHIBIT COMPLEMENT ACTIVATION

Spirochetes may evade immune responses by inhibiting complement, a major innate immune system of the host (132, 133). Complement activation is caused by pathogen surface antigens such as LPS, antigen–antibody complexes, and binding of lectin to bacterial surfaces (134). Activation is regulated by host regulatory proteins, including factor H (FH) (134). *Borrelia* bind complement regulator FH and/or FH-like protein 1 (FHL-1) by directly interacting with Osp designated complement regulator-acquiring surface proteins (CRASPs) (135). Numerous surface spirochetal lipoproteins (outlined in **Table 1**; Table 1 in Supplementary Material; **Figure 2**) such as OspA, OspE, CspA may contribute to complement inhibition by binding to major complement regulatory proteins such as FH, FHL-1 C4b-binding protein (C4bp) and human C1 esterase inhibitor (C1-Inh) (48, 99, 136–147). Thus, spirochetal lipoproteins contribute to immune evasion through complement inhibition.

SPIROCHETAL LIPOPROTEINS MAY INHIBIT NEUTROPHIL FUNCTION

Except for complement resistance, inhibition of neutrophil function is another mechanism that *B. burgdorferi* uses to evade the immune system. OspB inhibits the phagocytosis and oxidative burst of human neutrophils enabling *B. burgdorferi* to resist phagocytosis and oxidative burst in areas such as joint, skin, and the nervous system (47–49). Other spirochetal lipoproteins such as LIC11207 in *Leptospira* spp. may up-regulate the apoptosis of neutrophils (80). Thus, spirochetal lipoproteins may inhibit neutrophil function which may help the survival of the spirochetes.

THE ROLE OF SPIROCHETAL LIPOPROTEINS IN IMMUNE ACTIVATION

SPIROCHETAL LIPOPROTEINS MAY ACTIVATE NEUTROPHILS

Neutrophils are present in the joint fluid of Lyme arthritis patients (1), suggesting that they contribute to immune responses against *B. burgdorferi*. Although OspA and OspB are similarly regulated in the life cycle of *B. burgdorferi*, they have different functions. Contrary to OspB, OspA may activate human neutrophils (116) induce their oxidative burst (47) and induce neutrophil chemotaxis (148). OspA and OspC up-regulate complement receptor

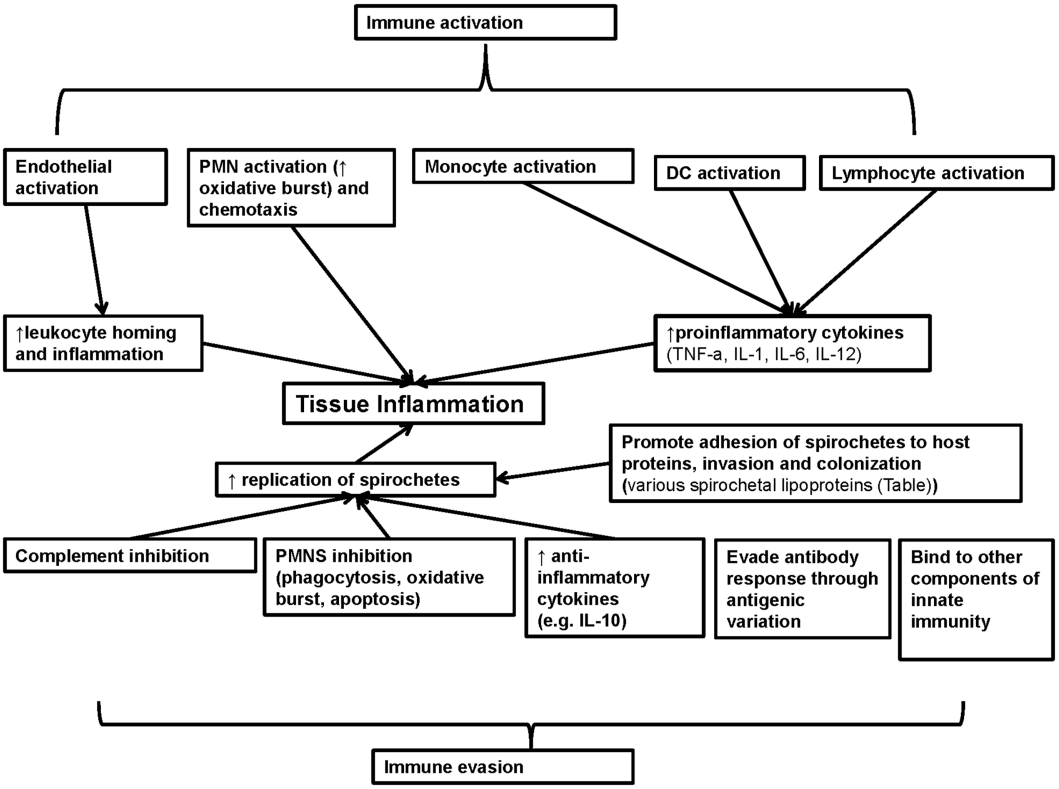

FIGURE 2 | Summary of the role of known spirochetal lipoproteins in regulation of immunity. Spirochetal lipoproteins have two different major effects on immunity: immune evasion and immune activation. These lipoproteins may contribute to immune evasion through inhibition of complement, neutrophils, production of anti-inflammatory cytokines, evasion of antibody responses through antigenic variation and binding to other components of innate immunity (e.g., apolipoproteins). In addition, spirochetal lipoproteins may directly promote spirochetal tissue invasion and colonization and in combination with immune evasion may lead to increased spirochetal replication and tissue inflammation. Spirochetal lipoproteins may also directly and indirectly activate endothelial, epithelial cells, and immune cells that contribute to innate immune responses (neutrophils, monocytes, macrophages, and DCs) or adaptive immune responses (lymphocytes such as B cells and CD4 T helper cells). Collectively, these effects lead to increase inflammation in target tissues (e.g., skin) or adaptive autoimmune responses (e.g., arthritis) that contribute to the clinical manifestations of spirochetal diseases. IL, interleukin; TNF-a, tumor necrosis factor A.

3 (CR3), an adhesion molecule expressed on neutrophils that is involved in the interactions of *Borrelia* species with neutrophils (149), and OspA and OspB may bind to CR3 in a C3bi independent manner (150). Thus, different spirochetal lipoproteins may inhibit neutrophils to evade immune responses but others may activate neutrophils contributing to tissue inflammation.

SPIROCHETAL LIPOPROTEINS ACTIVATE MONOCYTES AND MACROPHAGES TO SECRETE CYTOKINES THROUGH CD14 AND TLR-DEPENDENT MECHANISMS

Bacterial lipoproteins and LPS both have an active lipid moiety and induce similar cell responses in similar cell types (19). CD14 is a protein in the membrane of macrophages that binds LPS and also induces lipoprotein signaling in several cells (38, 97, 151). Cellular membrane CD14 activates myeloid cells such as monocyte, macrophages, and polymorphonuclear white blood cells while soluble CD14 activates non-myeloid (endothelial, epithelial) cells (152). Spirochetal lipoproteins (such *T. pallidum* lipoproteins) bind to CD14 at the site that binds LPS and may activate monocytes and the NF-κB pathway through CD14 (38, 44, 97). In contrast to Gram-negative bacteria, LPS-binding protein (LBP) does not mediate interaction of spirochetal lipoproteins with CD14 (3, 38). However, TLR knockout and overexpression studies have confirmed that lipoproteins drive inflammation in syphilis and Lyme disease through TLR-dependent (TLR1, TLR2) responses (10, 43, 56, 57, 97, 125–130). Thus, during spirochetemia in Lyme disease and syphilis, spirochetal lipoproteins activate cells via TLR1 and TLR2 in contrast to Gram-negative sepsis where cellular activation occurs through TLR4 (45, 56, 63). Integrin α3β1 may co-operate with TLR2/TLR1 in mediating pro-inflammatory responses in human macrophages stimulated with spirochetal lipopeptides such as BBB07 (153, 154). However, TLR-independent receptor responses are also important for spirochetal induced inflammation (125, 154–156). *T. pallidum* lipoproteins, the *B. burgdorferi* OspA lipoprotein and synthetic lipopetides may up-regulate macrophage production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-12 (18, 50). Many different cell types may produce IL-10 in response to stimulation by *B. burgdorferi* lipoproteins (52, 53, 62, 157, 158). On the other hand, endogenously produced and exogenous IL-10

significantly reduced OspA lipoprotein-induced macrophage production of cytokines and chemokines (54, 62, 158–161), consistently with previous studies that have shown that IL-10 may down-regulate the TLR signaling pathway (159). Thus, spirochetal lipoproteins induce immune responses in antigen presenting cells such as monocytes and macrophages through TLR-dependent and -independent mechanisms.

Spirochetal lipoproteins activate dendritic cells

Except for monocytes and macrophages, spirochetal lipoproteins may also activate other antigen presenting cells such as DCs. DCs are a major link between innate and adaptive immunity, since after activation, they up-regulate costimulatory molecules such as CD54 that interact with T cell receptors such as CD11a/CD18 within lymph nodes (162, 163). Consistent with the hypothesis that lipoproteins are key pro-inflammatory mediators in spirochetal diseases, many studies have shown that treponemal lipoproteins and synthetic lipopeptides can up-regulate CD54 and contribute to DC activation (18, 33, 35–37, 41, 94). Phagocytosis of intact spirochetes, activation of TLRs at phagosomes and bacterial cell death may result in the release of treponemal lipoproteins and may also contribute to immune cell activation (163). Also *B. burgdorferi* lipoproteins increase chemotaxis of circulating plasmacytoid dendritic cells (pDCs) into skin (11) but do not activate pDCs *in vitro* and *in vivo* (58, 59).

SPIROCHETAL LIPOPROTEINS INDUCE INFLAMMATORY INFILTRATE INTO TARGET TISSUES AND ADAPTIVE IMMUNE RESPONSES *IN VIVO* THAT CONTRIBUTE TO CLINICAL MANIFESTATIONS OF SPIROCHETAL DISEASES

Although lipoproteins may activate neutrophils, macrophages, endothelial cells *in vitro*, they may also induce inflammatory infiltrate into target tissues during spirochetal infection *in vivo* (37, 50, 61, 94, 115, 116, 164). Lipoproteins may also contribute to the pathogenesis of the Jarisch-Herxheimer reaction, a transient immunological phenomenon that occurs during treatment of spirochetal infections (165, 166). Injection of synthetic lipopeptides into the skin can also be used to study the immunomodulatory effects of spirochetal lipoproteins *in vivo* (94). Cutaneous injection of spirochetal lipopeptides and spirochetal skin infections elicit similar cellular infiltrate supporting the hypothesis that spirochetal lipoproteins recruit diverse leukocytes from peripheral blood into target tissues (10). Lipoprotein-responsive cells (167), such as endothelium (95, 168), keratinocytes (169), and macrophages (170) induce chemotaxis of mixed cellular infiltrate that in combination with extravasating leukocytes further increase the tissue inflammatory response. Spirochetal lipoproteins activate *in vivo* antigen presenting cells (macrophages, DCs, CD4+ T cells) within the inflamed skin in spirochetal diseases (39, 40, 58, 59). Lipopeptides in combination with other antigens from spirochetes facilitate the transition from innate to prolonged adaptive immune responses that contribute to chronic manifestations of spirochetal diseases such as syphilis and Lyme disease (11). Consistent with these data from *in vivo* studies, *in vitro* studies have demonstrated that spirochetal lipoproteins may directly activate both B and T cells (**Table 1**). These lipoprotein-induced adaptive immune responses may trigger autoimmune and vaccine immune responses (**Table 1**). Thus, spirochetal lipoproteins induce initially innate immunity and then adaptive immunity through recruitment of spirochete-specific T cells and tissue inflammation that is associated with clinical manifestations of spirochetal disease such as Lyme arthritis (37, 50, 94, 115, 116, 164).

CONCLUSION

Lipoproteins are widely expressed by many pathogens and have pro-inflammatory effects (171–223). Thus, an understanding of how lipoproteins interact with the immune system will aid in the understanding of the pathogenesis of many infections including spirochetal infections. In addition, elucidating the molecular mechanisms of lipoprotein-induced immunomodulation (summarized in **Figure 2**) will lead to a greater understanding of the inflammatory processes, innate and adaptive immune responses associated with spirochetal diseases that may contribute to spirochetal disease vaccine development (163).

SUPPLEMENTAY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fimmu.2014.00310/abstract

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