Review Article

Leptospira and Inflammation

C. F. Gonçalves-de-Albuquerque, P. Burth, A. R. Silva, M. Younes-Ibrahim, H. C. Castro-Faria-Neto, and M. V. Castro-Faria

1 Laboratório de Imunofarmacologia, Fundação Oswaldo Cruz, FIOCRUZ, Rio de Janeiro 21040-900, Brazil
2 Departamento de Biologia Celular e Molecular, Instituto de Biologia, Universidade Federal Fluminense, Niterói 24020-150, Brazil
3 Departamento de Medicina Interna, Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro 20550-900, Brazil

Correspondence should be addressed to C. F. Gonçalves-de-Albuquerque, cassianofg@gmail.com and M. V. Castro-Faria, mcastrofaria@gmail.com

Received 26 July 2012; Revised 25 September 2012; Accepted 27 September 2012

1. Introduction

Leptospirosis is a zoonosis of global importance caused by several species and more than 200 different serovars of pathogenic Leptospira spp. The disease affects both animals and humans and has veterinary, economic, and medical relevance [1, 2]. Leptospirosis is still a major public health problem in tropical countries, with epidemic outbreaks occurring in the rainy season and after floods [3–5]. The annual incidence of this disease is estimated at 10–100 per 100,000 in tropical regions and 0.1–1.0 per 100,000 in temperate areas [6]. In recent years, leptospirosis outbreaks have occurred all over the world; thus, an adequate disease notification system would be useful to create surveillance networks [7]. Leptospirosis is transmitted to humans primarily by water contaminated with the urine of either wild or domestic mammals that have been chronically colonized by Leptospira spp [8]. It has recently been reported that Leptospira can persist in certain organs, indicating that people themselves can act as hosts [9].

In developed countries, the transmission mechanism is mainly associated with occupational and recreational activities [10–14]. The infection may be nonsymptomatic or may result in different clinical conditions ranging from a mild “flu-like” disease to a severe form known as Weil’s disease [15–19]. Icterohemorrhagic syndrome is a severe form of leptospirosis in which symptoms comprise hepatitis, hemorrhage, acute lung injury, and renal failure [3, 18, 20, 21].

The leptospiral genome is greater than that of other spirochetes such as Treponema sp, which may explain the ability of Leptospira to live in several different environments and hosts [22, 23]. Leptospira species were recently grouped according to their genetic homology [24, 25], and studies...
aimed at the development of an efficacious vaccine are underway [26, 27].

After reaching the blood stream, spirochetes preferentially colonize the liver and kidney [28]. These organs can offer a large lipid supply because fatty acids are an essential requirement for leptospiral growth [29, 30]. There is evidence that leptospiras form a biofilm during kidney colonization in the proximal renal tubule lumen of rabbit norevigericus [31]. Leptospiras can, however, also be found in other organs such as the lung and central nervous system [29, 30].

2. Pathogenesis

Toxin production and/or the host immune response seem to be the main pathogenic mechanisms in leptospirosis. Like other spirochetes, leptospiras have a distinctive double membrane architecture that shares characteristics of both Gram-positive and Gram-negative bacteria [32].

A large proportion of the structural and functional outer membrane proteins (OMPs) is either lipoproteins such as LipL 32, LipL 21, and LipL 41 [33] or integral membrane proteins such as the porin OmpL1 [34]. In particular, OMPs may play key roles in pathogenesis by acting as adhesion or antigenic targets for bactericidal antibodies, receptors for various host molecules, and/or porins. Recent studies using five independent experimental methods have identified four novel surface-exposed and membrane-integrated leptospiral proteins (OmpL36, OmpL37, OmpL47, and OmpL54), although no functional roles have been described for them [35]. OmpA70 was identified in L. interrogans serovar Copenhageni [36] and the Lsa66 is a novel OmpA-like protein with dual activity that may promote the attachment of Leptospira to host tissues and may contribute to leptospiral invasion [37], indicating that OmpA-like proteins may have a role in leptospirosis pathogenesis.

Virulence, characterized by mobility and the ability to invade tissues, may be associated with some lipopolysaccharides and adhesins [38-40]. Bacterial mobility likely plays a major role in the disease process of multiple spirochetes [41]. The ability to move rapidly in a sticky environment could contribute to the ability of the spirochete to cross through epithelial cells [38]. In vitro, pathogenic leptospiras penetrate the intercellular junction of endothelial cells while saprophytic L. biflexa do not [39]. The ability of leptospiras to penetrate and disseminate in mammalian tissue also depends on their ability to attach to cells and to the extracellular matrix. In vitro, L. interrogans binds to a variety of cell lines including fibroblasts, endothelial cells, and kidney epithelial cells [42].

Some proteins are potential virulence factors and have a role in bacterial adhesion to host tissues, such as the Lig protein and the leptospiral endostatin-like (Len) outer membrane proteins [43, 44]. Pathogenic leptospiras also express surface-exposed proteins that possess bacterial immunoglobulin-like domains such as LigA, LigB, and LigC, which are adhesin candidates [45]. Recent work has shown that LigB binds fibrinogen and inhibits fibrin formation [46]. Several groups have reported that immunization with the LigA-unique region conferred protection from lethal infection in both a mouse model [47] and a hamster model [48, 49] of leptospirosis. In addition, resistance in hamsters seems to depend on an immunity against a conformational epitope of Lig A that includes domains 11 and 12 and a third flanking domain (either 10 or 13) that may be required for proper conformational folding [50]. Moreover, the endostatin-like protein A (Len A) was shown to bind to the host component laminin [51] and to human plasminogen [52].

Comparative studies of different serovar genomes have suggested that other components such as integrin alpha-like protein (also an adhesin candidate), lipopolysaccharides, cell surface capsular polysaccharides, and exopolysaccharides may also play a role in bacterial survival in specific host organs [22]. The OmpA-like protein Loa22 was reported to be essential for leptospiral virulence [53] and to promote inflammatory responses in cultured rat renal cells [54]. The virulence factor Loa22 is a highly conserved lipoprotein with a peptidoglycan-binding motif similar to OmpA that is upregulated during acute leptospira infection [19]. Hemoxygenase, FltY (flagellar motor switch protein), and LPS are other recognized virulence factors [32].

Other molecules that could play a part in leptospira infection include potential toxins such as the hemolysin SphH, a pore-forming protein without sphingomyelinase or phospholipase activities [55], and the enzyme catalase (KatE), which is produced only by pathogenic strains and is involved in resistance to oxidative killing [22, 56].

3. Leptospira Metabolism and Endotoxins

Leptospiras are strictly aerobic spirochetes. In their culture medium, they require ammonia as the nitrogen source [57] and long chain fatty acids as the sole carbon and fuel sources [58], and they obtain energy through the fatty acid β-oxidation pathway [29]. The most commonly used culture medium is Ellinghausen-McCullough/Johnson-Harris medium, which contains oleic acid, bovine serum-albumin, and polysorbate [19].

The biological activity of the lipopolysaccharide-like substance (LLS) extracted from the L. interrogans serovar canicola was weaker than the lipopolysaccharide (LPS) obtained from other gram-negative bacteria [59]. Lipid A is the active component of LPS and is responsible for its toxic activity. The lipid A of leptospiral LPS has an unusual fatty acid composition and, more strikingly, a unique methylated phosphate residue [60]. Leptospiral lipid A is structurally and functionally different than the lipid A of E. coli [61]. The glycolipoprotein fraction (GLP) is another leptospiral component that has cytopathic activity [62].

Due to their peculiar metabolism, leptospiras are able to store lipids such as fatty acids [62, 63]. Some lipids are stored associated with GLP (palmitovacenic, linoleic, and oleic acids) [62], while others are stored associated with LPS and LLS (hydroxylauric, palmitic, and oleic acids) [64, 65]. These reports indicate that leptospiras are able to store and associate fatty acids with their endotoxins (LPS and GLP).
This ability may have important pathophysiological consequences.

**4. Toll-Like Receptors and Immune Response in Leptospirosis**

The innate immune response is based on the recognition of pathogen-associated molecular patterns (PAMPs) [66, 67]. Immune cells express proteins called pathogen recognition receptors (PRRs) that allow them to recognize conserved microbial motifs such as peptidoglycans and LPS [68–70].

TLR4 was the first PRR to be described and was identified in 1997 [71]. TLR4 shows a highly orchestrated usage of coreceptors to discriminate between ligands. This receptor signals the presence of LPS in association with the CD14 [72] and MD-2 proteins [73]. This multifaceted receptor system additionally plays a role in triggering several signal transduction pathways [74]. For example, LPS binding to TLR4 activates transcription factors such as the nuclear factor NF-κB, which induces the production of inflammatory interleukins (IL-1β, IL-6, IL-8) and tumor necrosis factor (TNF) [69].

Another TLR, TLR2, is essential for the recognition of Gram-positive bacterium components such as the macrophage-activating lipopeptide 2 (MALP-2) and lipoarabinomannan, the main glycolipid of *Mycobacterium tuberculosis* [75]. In association with another TLR (TLR6), TLR2 triggers intracellular signaling through the mitogen-activated protein kinases (MAPKs) and NF-κB [70].

During leptospirosis, bacterial recognition by host is under disclosure, but *Leptospira* presence may be sensed through TLR4 and TLR2 receptors [76].

It is well known that LPS from Gram-negative bacteria activates the TLR4 signaling cascade. Paradoxically, *L. interrogans* LPS binds both CD14 and TLR2 but does not generate intracellular signaling through TLR4 activation [77]. The lipid A from *Leptospira* LPS apparently stimulates mouse cells through the TLR4-MD2 complex but does not induce signaling in human cells [61], indicating that there are species-specific aspects of LPS signaling that differ between mouse and human cells.

In recent years, considerable research has been conducted on the outer membrane proteins expressed by *Leptospira* spp. during infection. LipL32 is the major leptospiral outer membrane lipoprotein expressed during infection and is the immune-dominant antigen recognized in humoral responses against leptospirosis in humans [78, 79]. This lipoprotein is highly conserved among pathogenic *Leptospira* species [79] and signals through TLR2 [77], as recently confirmed by data showing the LipL32 binding to TLR2 in renal cells [80]. However, LipL32 was not required either for the development of acute leptospirosis in hamsters or for renal colonization in a rat model [81]. LipL21, the second major outer membrane protein of the *Leptospira interrogans* serovar Lai, exhibits potent immunogenic activity [82].

It has been reported that the *Leptospira santarosai* serovar Shermani activates the production of proinflammatory chemokines induced by p38 MAPK phosphorylation through TLR2 activation in proximal tubule epithelial cells in mice [83]. These same investigators also observed that OMPs and LipL32 increased TLR2 expression in human embryonic kidney cells (HEK 293). In addition, LipL32 augmented iNOS and CCL2/MCP1 mRNA expression and protein secretion via TLR2 binding [84].

The infection of guinea pigs with the *L. interrogans* serovar Icterohemorrhagiae increased the levels of IL-6 and TNFα mRNA in the lungs [85], and uveitis of leptospiral origin was associated with an increased production of the cytokines IL-6 and IL-8 [86]. An increase in cytokine production was also linked to a lethal outcome in leptospirosis patients [87].

C3H/HeJ mice have deficient LPS signaling and only respond to high doses of LPS [88]. Animals unable to detect LPS appropriately are susceptible to infection by Gram-negative bacteria [66]. When C3H/HeJ mice were infected with the *Leptospira interrogans* serovar icterohemorrhagiae, they presented with a lethal infection with morphological changes in the kidney and lungs [89] as well as sustained expression of CCL2/MCP-1 and CXCL1/KC in the lungs, which were correlated to the severity and progression of disease [90]. Another strain of mice, C57BL/10ScCr, carries a null TLR4 mutation, does not express TLR4 protein, and is resistant to high doses of LPS [88]. These animals do not express the receptor to IL-12p40. Both C3H/HeJ and C3H/SCID mice presented with a lethal outcome when infected with the *Leptospira interrogans* serovar Copenhageni [91]. The C3H/HeJ animals died after an intraperitoneal injection of *Leptospira interrogans* serovar icterohemorrhagiae, presenting with liver disease and lung hemorrhage [92].

Virulent leptospiras can protect themselves against components of the host’s innate immune system, such as phagocytic cells and the complement system. Pathogenic leptospiras escape from phagocytosis and are resistant to intracellular killing mechanisms [93, 94]. To establish a successful leptospirosis infection, the leptospiras must be able to evade the complement system. In contrast, non-pathogenic leptospiras are killed after exposure to the human complement system [95]. It has been shown that the acquisition of factor H (FH) and other complement modulators displayed on the *Leptospira* surface is crucial for bacterial survival in serum. Leptospiras isolated from patients can bind the complement system inhibitor FH, a regulatory complement protein that prevents complement activation, and can restrict the deposition of the late complement components on their surfaces [96]. Thus, binding of this major alternative complement pathway inhibitor is related to serum resistance in *Leptospira* spirochetes. Interestingly, FH binding was shown to be dependent upon Lig proteins [97]. The multifunctional LigB protein also binds to C3b and C4b and interferes with complement activation [98]. Lsa30, a novel leptospiral adhesion protein, may help pathogenic *Leptospira* to escape the immune system by interfering with the complement cascade through interaction with the C4bp regulator [99]. Lsa33 also bind to C4bp and may be important in immune evasion [100]. The recently described LcpA (leptospiral complement regulator-acquiring protein A) also binds to C4bp [101].
Acquired immunity that is protective against reinfection by Leptospira does occur, but this has been shown in animal models to be dependent on the specific Leptospira serovar [102]. Specific antibodies to Leptospira membrane proteins may play a role in host defense [103] in animal vaccination models. Vaccines prepared with the LipL21 antigen protected guinea pigs from leptospiral infection [82], but there is currently no consensus regarding which signaling pathway is involved. Recent work showed that murine B cells were crucial to clearing Leptospira, through both early IgM production against LPS, which depends on TLR4, and protective IFNγ production, which depends on TLR2 and TLR4 activation [104]. It has also been shown that cattle immunized with a killed Leptospira vaccine develop protective immunity associated with CD4+ T cells and y6T cells [105]. Nevertheless, patients who have recovered from leptospirosis do not seem to generate memory T cells that can be activated by in vitro stimulation with Leptospiral protein antigens [106].

5. New Insights

When humans come in contact with contaminated water or soil, pathogenic leptospires enter the blood stream either via skin lesions or by actively penetrating the mucosa and colonizing organs such as the kidney and liver (Figure 1). Meanwhile, the immune system induces bacterial lysis, releasing many antigens, including the glycolipoprotein GLP and LPS.

The hypothesis that Leptospira produces an endotoxin released after bacterial lysis due to the host immune response was investigated and is supported by clinical and histopathological observations [107]. Nevertheless, the severity of Weil’s syndrome seems to be related not only to the virulence and toxin liberation from the infective serovar but also to the intensity and the speed of the host immune response [3, 108]. The production of specific antibodies is essential to protect mice from Leptospira infection because macrophages can only efficiently phagocyte leptospirosis in the presence of a specific antibody [109]. The L. interrogans GLP is also released by bacterial lysis and can activate inflammatory cells, such as peripheral blood mononuclear cells (PBMC), leading to an increased production of TNFα and IL-6 [16], an increased expression of the adhesion molecule CD69, and an augmented secretion of prostaglandin E2, leukotriene B4, and nitric oxide [110].

Acute lung injury (ALI) is characterized by cytokine release and the loss of epithelium/endothelium integrity. The increased permeability leads to protein extravasation and edema. This is the hallmark of all ALI/ARDS [111]. The presence of leptospirosis and leptospiral antigens in lung endothelial cells is thought to be evidence that pulmonary lesions are triggered by bacteria and their toxic products [3, 112, 113]. Patients with fatal leptospirosis generally suffer extensive pulmonary hemorrhage [114]. Leptospira infections in monkeys mimic the features of severe human leptospirosis, including pulmonary hemorrhage [115]. The pulmonary hemorrhage is thought to be linked to the deposition of immunoglobulin and complement in the alveolar septa [116]. Pulmonary hemorrhage is a serious life-threatening disorder and is the major cause of death due to leptospirosis in Brazil [18].

In the lung, the enzyme adenosine triphosphatase is activated by Na+, K+, and Mg2+ (Na/K-ATPase) and removes sodium from alveolar fluid, contributing to edema clearance and acting as a homeostatic mechanism to maintain lung integrity [117–119]. Inhibition of the Na/K pump in this organ may contribute significantly to lung failure in severe cases [120]. The kidney is another important leptospiral target, and acute kidney injury is an early manifestation of leptospirosis [121]. Inhibition of the Na/K pump in the kidney leads to loss of potassium and to hypokalemia [122]. Indeed, acute renal failure in leptospirosis is initially characterized by hypokalemia [123, 124]. Dysfunctional Na+ transporters in the kidney and lung have already been observed in the context of this disease [125]. Interestingly, engulfed GLP has been detected in phagocytes in the kidney [126] and, as we have demonstrated, is a specific Na/K-ATPase inhibitor [127].

The liver is another organ that is affected in leptospirosis infections. Inhibition of Na/K-ATPase in liver contributes to liver functional disorder and causes decreased albumin and increased nonesterified fatty acids (NEFA) and bilirubin in the plasma [127]. We also showed that this inhibition may be caused by nonesterified monounsaturated fatty acids (NEUFA) such as oleic and linoleic acids, which are GLP components and are substantially augmented in the plasma of patients with severe leptospirosis [128]. High NEFA levels are characteristic of patients with severe leptospirosis and other inflammatory conditions [128]. Increased circulating levels of NEFA also occur in some respiratory diseases, and as NEFA are known to be immune-stimulatory agents [129], this increase may directly contribute to systemic inflammation and more severe disease by stimulating the production of inflammatory mediators [130]. High levels of circulating NEFA can either inhibit or activate TLR4, triggering the inflammatory response [131]. Similar to LPS, saturated fatty acids can induce inflammatory responses in dendritic cells [132], although polyunsaturated fatty acids negatively modulate TLR4 [133]. Fatty acids such as lauric, palmitic, and oleic acids activate TLR4 in adipocytes and macrophages, leading to augmented IL-6 and TNFα production [130]. Furthermore, NEFA binding to free fatty acid receptors stimulates intracellular responses, augmenting the formation of inflammatory mediators [134, 135] via the activation of NF-κB and AP-1, as demonstrated in human endothelial cells [136].

Recently, Na/K-ATPase has been described as a receptor for intracellular signaling cascades. In this novel role, the enzyme functions as a receptor for nanomolar ouabain concentrations and other cardiac glycosides and triggers intracellular signaling cascades without changing the intracellular Na+ and K+ concentrations [137, 138]. Protein interactions with Na/K-ATPase have an important role in membrane rafts, which are linked to calcium signaling [139], and can be released through IP3 receptor binding [140]. In the presence of ouabain, calcium oscillations lead to NF-κB activation [141] and ERK/MAPK activation, which may
Figure 1: Severe leptospirosis: from the infection to immunological target. Due to their mobility, leptospiras are able to penetrate mucosal tissues and injured skin. Transported by the blood stream, they reach target organs, mainly the kidney and liver. The host immune response kills the bacteria, promoting endotoxin release. The innate immune system of both human and mouse recognizes endotoxins through specific receptors. This immune cell response is mediated by Toll-like receptors and Na/K-ATPase, which sense antigen molecules and trigger intracellular signaling pathways driving the translocation of transcription factors, leading to increased inflammatory mediator production. This scenario creates an inflammatory microenvironment that can lead to organ dysfunction. Another important observation in this disease is the increased NEFA levels in the systemic circulation (mainly oleic acid). Augmented albumin unbound-NEFA may play an important role in multiorgan dysfunction by acting on endothelium and immune cells. TLR2: Toll-like receptor 2; TLR4: Toll-like receptor 4; NKA: Na/K-ATPase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NEFA: nonesterified fatty acid; LIPL32: major outer membrane leptospiral lipoprotein; GLP: leptospiral glycolipoprotein.
lead to the activation of the transcription factor AP-1 [142]. The ouabain effects in signal transduction occur through a pool of Na/K-ATPase without interfering with pump activity [143]. In this regard, we demonstrated that ouabain acts on lymphocytes without depolarizing the membrane, suggesting a mechanism that is independent of classic pump inhibition [144].

Na/K-ATPase binding triggers intracellular pathways that lead to the production of proinflammatory mediators [136, 137]. The binding of ouabain to Na/K-ATPase induces mononuclear cells to secrete TNF-α and IL-1 [145]. In the context of inflammatory leptospirosis, monocytes stimulated by leptospirosis and their extracts respond by activating intracellular pathways, phosphorylating p38, activating NF-κB, and releasing cytokines and nitric oxide [94, 146]. The relevance of inflammatory mediators to the physiopathology of experimental and clinical leptospirosis is well known. Hamsters infected with *L. interrogans* sororvar Icterohemorrhagiae that exhibit lung injury had increased mRNA levels of TNF and IL-6 [85]. Components of *Leptospira* are able to induce TNF release [147]. The *L. interrogans* GLP, a bacterial fraction that inhibits Na/K-ATPase [122, 127, 148], is able to induce inflammatory cell activation and increase TNFα and IL-6 production [16]. Increased TNF production is a predictor of poor clinical outcome in patients with leptospirosis [149]. Furthermore, the uveitis seen in leptospirosis is associated with a rise in IL-6, IL-8, TNF-α, and IL-10 production [86]. Increased cytokine production is associated with increased patient mortality during the disease progression [87]. IL-1β and IL-18 are produced by inflammasome activation [150]. The inflammasome consists of several proteins, of which NLRP3 is involved in the recognition of bacterial RNA, ATP, uric acid, and low intracellular potassium concentrations [151]. A recent report showed that *Leptospira* induces production of the cytokine IL1β through synergy between LPS signaling via TLRs and leptospiral GLP, which inhibits the Na/K ATPase, triggers a decrease in intracellular potassium levels, and activates the NLRP3 inflammasome [152]. Thus, it is possible that the increased production of inflammatory mediators in leptospirosis is related both to recognition mechanisms involving TLR4 and fatty acid receptors and to a mechanism dependent on Na/K-ATPase signaling. In this way, both GLP and ouabain inhibit Na/K-ATPase and induce the production of inflammatory mediators directly involved in the pathophysiology of leptospirosis.

We cannot dismiss the hypothesis that GLP, also a specific Na/K-ATPase inhibitor, and the increased NEFA concentrations observed in the plasma of leptospirosis patients, represent a novel mechanism of triggering the inflammatory cascade, leading to the exacerbation of the immune response associated with the multiorgan dysfunction observed in this disease.

### 6. Final Remarks

In summary, the existing data still form an incomplete picture. TLR4 seems to be a crucial effector in the fight against *Leptospira* and is directly involved in the development of resistance to leptospiral infection. TLR2 also has an important role in leptospiral protein and LPS recognition. Furthermore, both TLR4 and TLR2 seem to be involved in the protection against pathogenic *Leptospira* antigens. Although TLR4 and TLR2 are directly implicated in the immune response to this disease, other mechanisms could be involved in the recognition of leptospiral molecular patterns. Some candidates are now emerging.

*Leptospira* components that are directly released after bacterial lysis may be involved in the pathophysiology of this disease either by causing direct injury or by triggering inflammation. In this respect, Na/K-ATPase alterations caused by GLP binding or by increased plasma levels of NEFA can trigger direct or indirect damage through the exacerbation of the inflammatory response.

### Abbreviations

TLR: Toll-like receptor  
LipL: Leptospiral outer membrane lipoprotein  
OMP: Outer membrane protein  
GLP: Glycolipoprotein fraction  
FliY: Flagellar motor switch protein  
KAE: Enzyme catalase  
PRR: Pathogen recognition receptor  
LLS: Lipopolysaccharide-like substance  
LPS: Lipopolysaccharide  
IL: Interleukin  
NF: Nuclear factor kappa-light-chain-enhancer of activated B cells  
MAPK: Mitogen-activated protein kinase  
NEUFA: Nonesterified monounsaturated fatty acids  
ALI: Acute lung injury  
NEFA: Nonesterified fatty acids  
MAPK: Mitogen-activated protein kinase  
AP-1: Activator protein

### Conflict of Interests

The authors declare no conflict of interests.

### References

[1] B. Adler and A. de la Peña Mocetuzuma, “Leptospira and leptospirosis,” *Veterinary Microbiology*, vol. 140, no. 3-4, pp. 287–296, 2010.

[2] A. R. Bharti, J. E. Nally, J. N. Ricaldi et al., “Leptospirosis: a zoonotic disease of global importance,” *The Lancet Infectious Diseases*, vol. 3, no. 12, pp. 757–771, 2003.

[3] M. Dolnikoff, T. Mauad, E. P. Bethlem, and C. R. R. Carvalho, “Pathology and pathophysiology of pulmonary manifestations in leptospirosis,” *Brazilian Journal of Infectious Diseases*, vol. 11, no. 1, pp. 142–148, 2007.

[4] R. B. Reis, G. S. Ribeiro, R. D. M. Felzemburgh et al., “Impact of environment and social gradient on *Leptospira* infection in urban slums,” *PLoS Neglected Tropical Diseases*, vol. 2, no. 4, article e228, 2008.
[5] A. I. Ko, M. Galvão Reis, C. M. Ribeiro Durado, W. D. Johnson, and L. W. Riley, "Urban epidemic of severe leptospirosis in Brazil," The Lancet, vol. 354, no. 9181, pp. 820–825, 1999.

[6] W.H.O., Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control, W.H. Organization and I.L. Society, WHO library Cataloguing-in-Publication Data Malta, 2003.

[7] G. Pappas, P. Papadimitriou, V. Siozopoulou, L. Christou, and N. Akritidis, "The globalization of leptospirosis: worldwide incidence trends," International Journal of Infectious Diseases, vol. 12, no. 4, pp. 351–357, 2008.

[8] M. T. D. Faria, M. S. Calderwood, D. A. Athanazio et al., "Carriage of Leptospira interrogans among domestic rats from an urban setting highly endemic for leptospirosis in Brazil," Acta Tropica, vol. 108, no. 1, pp. 1–5, 2008.

[9] C. A. Ganoza, M. A. Matthias, M. Saito, M. Cespedes, E. Gotuzzo, and J. M. Venet, "Asymptomatic renal colonization of humans in the Peruvian Amazon by Leptospira," PLoS Neglected Tropical Diseases, vol. 4, no. 2, article e612, 2010.

[10] R. U. M. Palaniappan, S. Ramanujam, and Y. F. Chang, "Leptospirosis: pathogenesis, immunity, and diagnosis," Current Opinion in Infectious Diseases, vol. 20, no. 3, pp. 284–292, 2007.

[11] J. G. Songer and A. R. Thiermann, "Leptospirosis," Journal of the American Veterinary Medical Association, vol. 193, no. 10, pp. 1250–1254, 1988.

[12] P. N. Levett, "Leptospirosis: a forgotten zoonosis?" Clinical and Applied Immunology Reviews, vol. 4, no. 6, pp. 435–448, 2004.

[13] G. Baranton and D. Postic, "Trends in leptospirosis epidemiology in France. Sixty-six years of passive serological surveillance from 1920 to 2003," International Journal of Infectious Diseases, vol. 10, no. 2, pp. 162–170, 2006.

[14] R. Van Crevel, P. Speelman, C. Gravekamp, and W. J. Terpstra, "Leptospirosis in travelers," Clinical Infectious Diseases, vol. 19, no. 1, pp. 132–134, 1994.

[15] M. A. Martinez García, A. De Diego Damia, R. M. Villanueva, and J. L. López Hontagás, "Pulmonary involvement in leptospirosis," European Journal of Clinical Microbiology and Infectious Diseases, vol. 19, no. 6, pp. 471–474, 2000.

[16] F. Dorigatti, M. K. C. Brunalti, E. C. Romero, E. G. Kallas, and R. Salomão, "Leptospira interrogans activation of peripheral blood monocyte glycoal protein demonstrated in whole blood by the release of IL-6," Brazilian Journal of Medical and Biological Research, vol. 38, no. 6, pp. 909–914, 2005.

[17] A. I. A. McBride, D. A. Athanazio, M. G. Reis, and A. I. Ko, "Leptospirosis," Current Opinion in Infectious Diseases, vol. 18, no. 5, pp. 376–386, 2005.

[18] E. L. Gouveia, J. Metcalfe, A. L. F. De Carvalho et al., "Leptospirosis-associated severe pulmonary hemorrhagic syndrome, Salvador, Brazil," Emerging Infectious Diseases, vol. 14, no. 3, pp. 505–508, 2008.

[19] K. V. Evangelista and J. Coburn, "Leptospirosis as an emerging pathogen: a review of its biology, pathogenesis and host immune responses," Future Microbiology, vol. 5, no. 9, pp. 1413–1425, 2010.

[20] A. Dobrina, E. Nardon, E. Vecile, M. Cinco, and P. Patriarca, "Leptospira icterohaemorrhagiae and leptospire peptidolgy-cans induce endothelial cell adhesiveness for polymorphonuclear leukocytes," Infection and Immunity, vol. 63, no. 8, pp. 2995–2999, 1995.

[21] Y. Sekkach, H. Qaçif, M. Jira, M. El qatni, N. El omri, and D. Ghafir, "Acute respiratory distress revealed a sever pulmonary leptospirosis," Revue de Medecine Interne, vol. 28, no. 1, pp. 48–51, 2007.

[22] A. L. T. O. Nascimento, A. I. Ko, E. A. L. Martins et al., "Comparative genomics of two Leptospira interrogans serovars reveals novel insights into physiology and pathogenesis," Journal of Bacteriology, vol. 186, no. 7, pp. 2164–2172, 2004.

[23] S. X. Ren, G. Fu, X. G. Jiang et al., "Unique physiological and pathogenic features of Leptospira interrogans revealed by whole-genome sequencing," Nature, vol. 422, no. 6934, pp. 888–893, 2003.

[24] P. Vijayachari, A. P. Sugunan, and A. N. Shiriram, "Leptospirosis: an emerging global public health problem," Journal of Biosciences, vol. 33, no. 4, pp. 557–569, 2008.

[25] K. Nalam, A. Ahmed, S. M. Devi et al., "Genetic affinities within a large global collection of pathogenic Leptospira: implications for strain identification and molecular epidemiology," PLoS ONE, vol. 5, no. 8, Article ID e12637, 2010.

[26] N. Koizumi and H. Watanabe, "Identification of a novel antigen of pathogenic Leptospih spp. that reacted with convalescent mice sera," Journal of Medical Microbiology, vol. 52, no. 7, pp. 585–589, 2003.

[27] M. Gamberini, R. M. Gómez, M. V. Atzingen et al., "Whole-genome analysis of Leptospira interrogans to identify potential vaccine candidates against leptospirosis," FEMS Microbiology Letters, vol. 244, no. 2, pp. 305–313, 2005.

[28] D. A. Athanazio, E. F. Silva, C. S. Santos et al., "Rattus norvegicus as a model for persistent renal colonization by pathogenic Leptospira interrogans," Acta Tropica, vol. 105, no. 2, pp. 176–180, 2008.

[29] J. B. Baseman and C. D. Cox, "Intermediate energy metabolism of Leptospira," Journal of Bacteriology, vol. 97, no. 3, pp. 992–1000, 1969.

[30] N. Stern, E. Shenberg, and A. Tietz, "Studies on the metabolism of fatty acids in Leptospira: the biosynthesis of delta 9- and delta 11-monoensaturated acids," European Journal of Biochemistry, vol. 8, no. 1, pp. 101–108, 1969.

[31] P. Ristow, P. Bourhy, S. Kerneis et al., "Biofilm formation by saprophytic and pathogenic leptospires," Microbiology, vol. 154, no. 5, pp. 1309–1317, 2008.

[32] T. R. Fraga, A. S. Barbosa, and L. Isaac, "Leptospirosis: aspects of innate immunity, immunopathogenesis and immune evasion from the complement system," Scandinavian Journal of Immunology, vol. 73, no. 5, pp. 408–419, 2011.

[33] P. A. Cullen, X. Xu, J. Matsunaga et al., "Surfaceome of Leptospira spp., Infection and Immunity, vol. 73, no. 8, pp. 4853–4863, 2005.

[34] E. S. Shang, M. E. Exner, T. A. Summers et al., "The rare outer membrane protein, OmpL1, of pathogenic Leptospira species is a heat-modifiable porin," Infection and Immunity, vol. 63, no. 8, pp. 3174–3181, 1995.

[35] M. Pinne and D. A. Haake, "A comprehensive approach to identification of surface-exposed, outer membrane-spanning proteins of Leptospira interrogans," PLoS ONE, vol. 4, no. 6, Article ID e6071, 2009.

[36] T. R. Fraga, R. M. Chura-Chambi, A. P. Gonçalves et al., "Refolding of the recombinant protein OmpA70 from Leptospira interrogans whole-genome sequencing, revealed novel insights into physiology and pathogenesis of Leptospira interrogans from inclusion bodies using high hydrostatic pressure and partial characterization of its immunological properties," Journal of Biotechnology, vol. 148, no. 2–3, pp. 156–162, 2010.
that binds extracellular matrix molecules and plasminogen, "PLoS ONE, vol. 6, no. 7, Article ID e21962, 2011.

[38] T. Ito and R. Yaegawa, "Leptospiral attachment to extracellular matrix of mouse fibroblast (L929) cells," Veterinary Microbiology, vol. 15, no. 1-2, pp. 89-96, 1987.

[39] D. D. Thomas and L. M. Higbie, "In vitro association of leptospires with host cells," "Infection and Immunity, vol. 58, no. 3, pp. 581-585, 1990.

[40] Y. Liu, W. Zheng, L. Li, Y. Mao, and J. Yan, "Pathogenesis of leptospirosis: interaction of Leptospira interrogans with in vitro cultured mammalian cells," Medical Microbiology and Immunology, vol. 196, no. 4, pp. 233-239, 2007.

[41] N. W. Charon and S. F. Goldstein, "Genetics of motility and chemotaxis of a fascinating group of bacteria: the spirochetes," Annual Review of Genetics, vol. 36, pp. 47-73, 2002.

[42] D. D. Breiner, M. Fahey, R. Salvador, J. NovaKova, and J. Coburn, "Leptospira interrogans binds to human cell surface receptors including proteoglycans," Infection and Immunity, vol. 77, no. 12, pp. 5528-5536, 2009.

[43] H. A. Choy, M. M. Kelley, T. L. Chen, A. K. Meller, J. Matsunaga, and D. A. Haake, "Physiological osmotic induction of Leptospira interrogans adhesion: LigA and LigB bind extracellular matrix proteins and fibrinogen," Infection and Immunity, vol. 75, no. 5, pp. 2441-2450, 2007.

[44] A. S. Barbosa, P. A. E. Abreu, F. O. Neves et al., "A newly identified leptospiral adhesin mediates attachment to laminin," Infection and Immunity, vol. 74, no. 11, pp. 6356-6364, 2006.

[45] J. Matsunaga, M. A. Barocchi, J. Croda et al., "Pathogenic Leptospira species express surface-exposed proteins belonging to the bacterial immunoglobulin superfamily," Molecular Microbiology, vol. 49, no. 4, pp. 929-945, 2003.

[46] H. A. Choy, M. M. Kelley, J. Croda et al., "The multifunctional LigB adhesin binds homoeostatic proteins with potential roles in cutaneous infection by pathogenic Leptospira interrogans," PLoS ONE, vol. 6, no. 2, Article ID e16879, 2011.

[47] N. Koizumi and H. Watanabe, "Leptospiral immunoglobulin-like proteins elicit protective immunity," Vaccine, vol. 22, no. 11-12, pp. 1545-1552, 2004.

[48] É. F. Silva, M. A. Medeiros, A. J. A. McBride et al., "The terminal portion of leptospiral immunoglobulin-like protein LigA confers protective immunity against lethal infection in the hamster model of leptospirosis," Vaccine, vol. 25, no. 33, pp. 6277-6286, 2007.

[49] S. M. Faisal, W. Yan, C. S. Chen, R. U. M. Palaniappan, S. P. McDonough, and Y. F. Chang, "Evaluation of protective immunity of Leptospira immunoglobulin like protein A (LigA) DNA vaccine against challenge in hamsters," Vaccine, vol. 26, no. 2, pp. 277-287, 2008.

[50] M. L. Coutinho, H. A. Choy, M. M. Kelley et al., "A LigA three-domain region protects hamsters from lethal infection by Leptospira interrogans," PLoS Neglected Tropical Diseases, vol. 5, no. 12, Article ID e1422, 2011.

[51] B. Stevenson, H. A. Choy, M. Pinne et al., "Leptospira interrogans endostatin-like outer membrane proteins bind host fibronectin, laminin and regulators of complement," PLoS ONE, vol. 2, no. 11, Article ID e1188, 2007.

[52] A. Verma, C. A. Brissette, A. A. Bowman, S. T. Shah, P. F. Zipfel, and B. Stevenson, "Leptospiral endostatin-like protein a is a bacterial cell surface receptor for human plasminogen," Infection and Immunity, vol. 78, no. 5, pp. 2053-2059, 2010.

[53] P. Ristow, P. Bourhy, F. W. da Cruz McBride et al., "The OmpA-like protein Loa22 is essential for leptospiral virulence," PLoS Pathogens, vol. 3, no. 7, p. e97, 2007.

[54] Y. Zhang, L. Bao, H. Zhu, B. Huang, and H. Zhang, "OmpA-like protein Loa22 from Leptospira interrogans serovar Lai is cytotoxic to cultured rat renal cells and promotes inflammatory responses," Acta Biochimica et Biophysica Sinica, vol. 42, no. 1, pp. 70-79, 2010.

[55] S. H. Lee, S. Kim, S. C. Park, and M. J. Kim, "Cytotoxic activities of Leptospira interrogans hemolysin SphH as a pore-forming protein on mammalian cells," Infection and Immunity, vol. 70, no. 1, pp. 315-322, 2002.

[56] M. Picardeau, D. M. Bulach, C. Bouchier et al., "Genome sequencing of the saprophyte Leptospira biflexa provides insights into the evolution of Leptospira and the pathogenesis of leptospirosis," PLoS ONE, vol. 3, no. 2, Article ID e1607, 2008.

[57] R. C. Johnson and P. Rogers, "Metabolism of leptospirae. I. Utilization of amino acids and purine, and pyrimidine bases," Archives of Biochemistry and Biophysics, vol. 107, no. 3, pp. 459-470, 1964.

[58] R. C. Johnson and J. K. Walby, "Cultivation of leptospires: fatty acid requirements," Applied Microbiology, vol. 23, no. 5, pp. 1027-1028, 1972.

[59] T. Shimizu, E. Matussaka, and K. Takayanagi, "Biochemical activities of lipopolysaccharide-like substance (LLS) extracted from Leptospira interrogans serovar canicola strain Moulton," Microbiology and Immunology, vol. 31, no. 8, pp. 727-735, 1987.

[60] N. L. S. Que-Gewirth, A. A. Ribeiro, S. R. Kalb et al., "A methylated phosphate group and four amide-linked acyl chains in Leptospira interrogans lipid A: the membrane anchor of an unusual lipopolysaccharide that activates TLR2," Journal of Biological Chemistry, vol. 279, no. 24, pp. 25420-25429, 2004.

[61] M. A. Nahori, E. Fourmié-AMazouz, N. S. Que-Gewirth et al., "Differential TLR recognition of leptospiral lipid A and lipopolysaccharide in murine and human cells," Journal of Immunology, vol. 175, no. 9, pp. 6022-6031, 2005.

[62] T. Vinh, B. Adler, and S. Faine, "Glycolipoprotein cytotoxin from Leptospira interrogans serovar copenhageni," Journal of General Microbiology, vol. 132, no. 1, pp. 111-123, 1986.

[63] Y. Arimitsu, A. Moribayashi, and N. Goto, "Skin reaction to lipids from avirulent strain Shibaura of Leptospira interrogans serovar copenhageni," Canadian Journal of Microbiology, vol. 35, no. 11, pp. 1009-1014, 1989.

[64] T. Vinh, B. Adler, and S. Faine, "Ultrastructure and chemical composition of lipopolysaccharide extracted from Leptospira interrogans serovar copenhageni," Journal of General Microbiology, vol. 132, no. 1, pp. 103-109, 1986.

[65] T. Shimizu, E. Matussaka, and N. Nagakura, "Chemical properties of lipopolysaccharide-like substance (LLS) extracted from Leptospira interrogans serovar canicola strain Moulton," Microbiology and Immunology, vol. 31, no. 8, pp. 717-725, 1987.

[66] B. Butler, "Innate immune sensing of microbial infection: the mechanism and the therapeutic challenge," Wiener Medizinische Wochenschrift, vol. 152, no. 21-22, pp. 547-551, 2002.

[67] E. M. Creagh and L. A. J. O'Neill, "TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity," Trends in Immunology, vol. 27, no. 8, pp. 352-357, 2006.
[68] T. Kawai and S. Akira, “TLR signaling,” *Seminars in Immunology*, vol. 19, no. 1, pp. 24–32, 2007.

[69] B. Verstak, P. Hertzog, and A. Mansell, “Toll-like receptor signalling and the clinical benefits that lie within,” *Inflammation Research*, vol. 56, no. 1, pp. 1–10, 2007.

[70] D. M. Underhill and A. Ozinsky, “Toll-like receptors: key mediators of microbe detection,” *Current Opinion in Immunology*, vol. 14, no. 1, pp. 103–110, 2002.

[71] R. Medzhitov, P. Preston-Hurlburt, and C. A. Janeway, “A human homologue of the *Drosophila* toll protein signals activation of adaptive immunity,” *Nature*, vol. 388, no. 6640, pp. 394–397, 1997.

[72] T. E. Mollnes, D. Christiansen, O. L. Brekke, and T. Espevik, ”Hypothesis: combined inhibition of complement and cd14 as treatment regimen to attenuate the inflammatory response," *Advances in Experimental Medicine and Biology*, vol. 632, pp. 253–263, 2008.

[73] S. Akashi, R. Shimazu, H. Ogata et al., “Cutting edge: cell surface expression and lipopolysaccharide signaling via the Toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages,” *Journal of Immunology*, vol. 164, no. 7, pp. 3471–3475, 2000.

[74] R. Ostuni, I. Zanoni, and F. Granucci, “Deciphering the complexity of Toll-like receptor signaling,” *Cellular and Molecular Life Sciences*, vol. 67, no. 24, pp. 4109–4134, 2010.

[75] J. Asong, M. A. Wolfert, K. K. Maiti, D. Miller, and G. J. Boons, “Binding and cellular activation studies reveal that toll-like receptor 2 can differentially recognize peptidoglycan from gram-positive and gram-negative bacteria,” *Journal of Biological Chemistry*, vol. 284, no. 13, pp. 8643–8653, 2009.

[76] I. Lesur, J. Textoris, B. Loriod et al., “Gene expression profiles characterize inflammation stages in the acute lung injury in mice,” *PLoS ONE*, vol. 5, no. 7, Article ID e11485, 2010.

[77] C. Werts, R. I. Tapping, and C. A. Janeway, “A human homologue of the *Drosophila* toll protein signals activation of adaptive immunity,” *Nature*, vol. 388, no. 6640, pp. 394–397, 1997.

[78] D. A. Haake, G. Chao, R. L. Zuerner et al., “The leptospiral lipoprotein, the C terminus is the primary immunogenic domain and mediates interaction with collagen IV and plasma fibronectin,” *Infection and Immunity*, vol. 76, no. 6, pp. 2642–2650, 2008.

[79] D. A. Haake, G. Chao, R. L. Zuerner et al., “The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection,” *Infection and Immunity*, vol. 68, no. 4, pp. 2276–2285, 2000.

[80] S. H. Hsu, Y. Y. Lo, J. Y. Tung et al., “LipL32, the major outer membrane lipoprotein, the C terminus is the primary immunogenic domain and mediates interaction with collagen IV and plasma fibronectin,” *Infection and Immunity*, vol. 76, no. 6, pp. 952–958, 2009.

[81] G. L. Murray, A. Srikram, D. E. Hoke et al., “Major surface protein LipL32 is not required for either acute or chronic infection with *Leptospira interrogans*,” *Infection and Immunity*, vol. 77, no. 3, pp. 952–958, 2009.

[82] H. He, W. Wang, Z. Wu, Z. Lv, J. Li, and L. Tan, “Protection of guinea pigs against *Leptospira interrogans* serovar Lai by lipL21 DNA vaccine,” *Cellular and Molecular Immunology*, vol. 5, no. 5, pp. 385–391, 2008.

[83] C. C. Hung, C. T. Chang, Y. C. Tian et al., “Leptospiral membrane proteins stimulate pro-inflammatory chemokines secretion by renal tubule epithelial cells through toll-like receptor 2 and p38 mitogen activated protein kinase,” *Nephrology Dialysis Transplantation*, vol. 21, no. 4, pp. 898–910, 2006.

[84] C. W. Yang, C. C. Hung, M. S. Wu et al., “Toll-like receptor 2 mediates early inflammation by leptospiral outer membrane proteins in proximal tubule cells,” *Kidney International*, vol. 69, no. 5, pp. 815–822, 2006.

[85] M. Marinho, I. S. Oliveira-Júnior, C. M. R. Monteiro, S. H. Perri, and R. Salomão, “Pulmonary disease in hamsters infected with *Leptospira interrogans*: histopathologic findings and cytokine mRNA expressions,” *American Journal of Tropical Medicine and Hygiene*, vol. 80, no. 5, pp. 832–836, 2009.

[86] C. G. Priya, S. R. Rathnam, and V. Mutthukkaruppan, “Evidence for endotoxin as a causative factor for leptospiral uveitis in humans,” *Investigative Ophthalmology and Visual Science*, vol. 49, no. 12, pp. 5419–5424, 2008.

[87] J. E. P. Wagenaar, M. G. A. Goris, M. H. Gasem et al., “Long pentraxin PTX3 is associated with mortality and disease severity in severe Leptospirosis,” *Journal of Infection*, vol. 58, no. 6, pp. 425–432, 2009.

[88] A. Poltorak, X. He, I. Smirnova et al., “Defective LPS signaling in *C3H/HeJ* and *C57BL/10ScCr* mice: mutations in *Tlr4* gene,” *Science*, vol. 282, no. 5396, pp. 2085–2088, 1998.

[89] M. M. Pereira, J. Andrade, R. S. Marchevsky, and R. Ribeiro Dos Santos, “Morphological characterization of lung and kidney lesions in *C3H/HeJ* mice infected with *Leptospira interrogans* serovar icterohaemorrhagiae: defect of CD4+ and CD8+ T-cells are prognosticators of the disease progression,” *Experimental and Toxicologic Pathology*, vol. 50, no. 3, pp. 191–198, 1998.

[90] J. B. D. Silva, T. M. V. Ramos, M. de Franco et al., “Chemokines expression during *Leptospira interrogans* serovar *copenhageni* infection in resistant BALB/c and susceptible *C3H/HeJ* mice,” *Microbial Pathogenesis*, vol. 47, no. 2, pp. 87–93, 2009.

[91] J. E. Nally, M. C. Fishbein, D. R. Blanco, and M. A. Lovett, “Lethal infection of *C3H/HeJ* and *C3H/SCID* mice with an isolate of *Leptospira interrogans* serovar *copenhagenii*,” *Infection and Immunity*, vol. 73, no. 10, pp. 7014–7017, 2005.

[92] S. Viriyakosol, M. A. Matthias, M. A. Swancutt, T. N. Kirkland, and J. M. Vinetz, “Toll-like receptor 4 protects against *Leptospira interrogans* infection and contributes to in vivo control of leptospiral burden,” *Infection and Immunity*, vol. 74, no. 2, pp. 887–895, 2006.

[93] R. Murgia, R. Garcia, and M. Cinco, “Leptospires are killed in vitro by both oxygen-dependent and -independent reactions,” *Infection and Immunity*, vol. 70, no. 12, pp. 7172–7175, 2002.

[94] M. Cinco, “New insights into the pathogenicity of leptospires: evasion of host defences,” *New Microbiologica*, vol. 33, no. 4, pp. 283–292, 2010.

[95] M. Cinco and E. Banfi, “Activation of complement by leptospires and its bactericidal activity,” *Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene A*, vol. 254, no. 2, pp. 261–265, 1983.

[96] T. Meri, R. Murgia, P. Stefanell, S. Meri, and M. Cinco, “Regulation of complement activation at the C3-level by serum resistant leptospires,” *Microbial Pathogenesis*, vol. 39, no. 4, pp. 139–147, 2005.

[97] M. M. Castiblanco-Valencia, T. R. Fraga, L. B. D. Silva et al., “Leptospiral immunoglobulin-like proteins interact with human complement regulators factor H, FHL-1, FH1-1, and C4BP,” *Journal of Infectious Diseases*, vol. 205, no. 6, pp. 995–1004, 2012.
[98] H. A. Choy, “Multiple activities of lipopolysaccharide virulence of *Leptospira interrogans* inactivation of alternative and classical pathways of complement,” *PLoS ONE*, vol. 7, no. 7, Article ID e41566, 2012.

[99] N. M. Souza, L. M. Vieira, I. J. Alves, Z. M. de Morais, S. A. Vasconcellos, and A. L. T. O. Nascimento, “Lsa30, a novel adhesin of *Leptospira interrogans* binds human plasminogen and the complement regulator C4bp,” *Microbial Pathogenesis*, vol. 53, no. 3–4, pp. 125–134, 2012.

[100] R. F. Domingos, L. M. Vieira, E. C. Romero et al., “Features of two proteins of *Leptospira interrogans* with potential role in host-pathogen interactions,” *BMC Microbiology*, vol. 12, article 50, 2012.

[101] A. S. Barbosa, D. Monaris, L. B. Silva et al., “Functional characterization of LcpA, a surface-exposed protein of *Leptospira spp.* that binds the human complement regulator C4BP,” *Infection and Immunity*, vol. 78, no. 7, pp. 3207–3216, 2010.

[102] B. Adler and S. Faine, “Host immunological mechanisms in the resistance of mice to leptospiral infections,” *Infection and Immunity*, vol. 17, no. 1, pp. 67–72, 1977.

[103] D. A. Haake, M. K. Mazel, A. M. McCoy et al., “Leptospiral outer membrane proteins OmpL1 and LipL41 exhibit synergistic immunoprotection,” *Infection and Immunity*, vol. 67, no. 12, pp. 6572–6582, 1999.

[104] C. Chassin, M. Picardeau, J. M. Goujon et al., “TLR4- and TLR2-mediated B cell responses control the clearance of the bacterial pathogen, *Leptospira interrogans*,” *Journal of Immunology*, vol. 183, no. 4, pp. 2669–2677, 2009.

[105] B. M. Naiman, D. Alt, C. A. Bolin, R. Zuerner, and C. L. Baldwin, “Protective killed *Leptospira borgpetersenii* vaccine induces potent Th1 immunity comprising responses by CD4 and γδ T lymphocytes,” *Infection and Immunity*, vol. 69, no. 12, pp. 7550–7558, 2001.

[106] I. Tuero, J. M. Vinetz, and G. R. Kumpel, “Lack of demonstrable memory T cell responses in humans who have spontaneously recovered from leptospirosis in the peruvian amazon,” *Journal of Infectious Diseases*, vol. 201, no. 3, pp. 420–427, 2010.

[107] D. R. Finco and D. G. Low, “Endotoxin properties of *Leptospira canicola*,” *American Journal of Veterinary Research*, vol. 28, no. 127, pp. 1863–1872, 1967.

[108] R. C. R. M. Abdulkader, E. F. Daher, E. D. Camargo, C. Spinosa, and M. V. Da Silva, “Leptospirosis severity may be associated with the intensity of humoral immune response,” *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 44, no. 2, pp. 79–83, 2002.

[109] V. Yu, B. Adler, and S. Faine, “The role of macrophages in the protection of mice against leptospirosis: in vitro and in vivo studies,” *Pathology*, vol. 14, no. 4, pp. 463–468, 1982.

[110] D. Diamant, M. K. C. Brunialti, E. C. Romero, E. G. Kallas, and R. Salomao, “Peripheral blood mononuclear cell activation induced by *Leptospira interrogans* glycolipoprotein,” *Infection and Immunity*, vol. 70, no. 4, pp. 1677–1683, 2002.

[111] L. Nieuwenhuizen, P. G. De Groot, J. C. Grutters, and D. H. Biesma, “A review of pulmonary coagulopathy in acute lung injury, acute respiratory distress syndrome and pneumonia,” *European Journal of Haematology*, vol. 82, no. 6, pp. 413–425, 2009.

[112] E. Marchiori, S. Lourenço, S. Setúbal, G. Zanetti, T. D. Gasparetto, and B. Hochhegger, “Clinical and imaging manifestations of hemorrhagic pulmonary leptospirosis: a state-of-the-art review,” *Lung*, vol. 189, no. 1, pp. 1–9, 2011.

[113] H. I. Chen, S. J. Kao, and Y. H. Hsu, “Pathophysiological mechanism of lung injury in patients with leptospirosis,” *Pathology*, vol. 39, no. 3, pp. 339–344, 2007.

[114] V. M. Arean, “The pathologic anatomy and pathogenesis of fatal human leptospirosis (Weil’s disease),” *The American Journal of Pathology*, vol. 40, pp. 393–423, 1962.

[115] M. M. Pereira, J. J. Pereira Da Silva, M. A. Pinto et al., “Experimental leptospirosis in marmoset monkeys (Calithrix jacchus): a new model for studies of severe pulmonary leptospirosis,” *American Journal of Tropical Medicine and Hygiene*, vol. 72, no. 1, pp. 13–20, 2005.

[116] J. Croda, A. N. D. Neto, R. A. Brasil, C. Pagliari, A. C. Nicodemo, and M. I. S. Duarte, “Leptospirosis pulmonary haemorrhage syndrome is associated with linear deposition of immunoglobulin and complement on the alveolar surface,” *Clinical Microbiology and Infection*, vol. 16, no. 6, pp. 593–599, 2010.

[117] J. I. Szinajder, K. M. Ridge, Z. L. Harris et al., “Alveolar type II cell Na,K-ATPase is upregulated during mechanical ventilation-induced pulmonary edema,” *Chest*, vol. 105, no. 3, supplement, pp. 116S–117S, 1994.

[118] J. I. Szinajder, “Alveolar edema must be cleared for the acute respiratory distress syndrome patient to survive,” *American Journal of Respiratory and Critical Care Medicine*, vol. 163, no. 6, pp. 1293–1294, 2001.

[119] M. A. Matthay, “Alveolar fluid clearance in patients with ARDS: does it make a difference?” *Chest*, vol. 122, no. 6, supplement, pp. 340S–345S, 2002.

[120] I. Vadász, R. E. Morty, M. G. Kohstall et al., “Oleic acid inhibits alveolar fluid reabsorption: a role in acute respiratory distress syndrome?” *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 5, pp. 469–479, 2005.

[121] C.-W. Yang, M.-S. Wu, and M.-J. Pan, “Leptospirosis renal disease,” *Nephrology Dialysis Transplantation*, vol. 16, supplement 3, pp. 73–77, 2001.

[122] M. Younes-Ibrahim, B. Buffin-Meyer, L. Cheval et al., “Na,K-ATPase: a molecular target for *Leptospira interrogans* endotoxin,” *Brazilian Journal of Medical and Biological Research*, vol. 30, no. 2, pp. 213–223, 1997.

[123] A. I. Ko, G. Goarant, and M. Picardeau, “*Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen,” *Nature Reviews Microbiology*, vol. 7, no. 10, pp. 736–747, 2009.

[124] A. C. Seguro, A. V. Lomar, and A. S. Rocha, “Acute renal failure of leptospirosis: nonoliguric and hypokalemic forms,” *Nephron*, vol. 55, no. 2, pp. 146–151, 1990.

[125] L. Andrade, A. C. Rodrigues, T. R. C. Sanches, R. B. Souza, and A. C. Seguro, “Leptospirosis leads to dysregulation of sodium transporters in the kidney and lung,” *American Journal of Physiology*, vol. 292, no. 2, pp. F586–F592, 2007.

[126] M. M. Pereira, J. Andrade, M. D. Lacerda, N. M. Batoru, R. S. Marchevsky, and R. Ribeiro Dos Santos, “Demonstration of leptospiral antigens on tissues using monoclonal antibodies and avidin-biotin peroxidase staining,” *Experimental and Toxicologic Pathology*, vol. 49, no. 6, pp. 505–511, 1997.

[127] M. Younes-Ibrahim, P. Burth, M. V. Castro Faria et al., “Inhibition of Na,K-ATPase by an endotoxin extracted from *Leptospira interrogans*: a possible mechanism for the physiopathology of leptospirosis,” *Comptes Rendus de l’Academie des Sciences III*, vol. 318, no. 5, pp. 619–625, 1995.

[128] P. Burth, M. Younes-Ibrahim, M. C. B. Santos, H. C. Castro-Faria Neto, and M. V. De Castro Faria, “Role of nonesterified unsaturated fatty acids in the pathophysiological processes of
leptospiral infection,” *Journal of Infectious Diseases*, vol. 191, no. 1, pp. 51–57, 2005.

[129] G. Boden, P. She, M. Mozzoli et al., “Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-κB pathway in rat liver,” *Diabetes*, vol. 54, no. 12, pp. 3458–3465, 2005.

[130] L. G. Wood, H. A. Scott, M. L. Garg, and P. G. Gibson, “Innate immune mechanisms linking non-esterified fatty acids and respiratory disease,” *Progress in Lipid Research*, vol. 48, no. 1, pp. 27–43, 2009.

[131] T. Martins De Lima, R. Gorjão, E. Hatanaka et al., “Cell signaling microdomains with Na,K-ATPase and inositol 1,4,5-trisphosphate receptor generates calcium oscillations,” *Journal of Immunology*, vol. 174, no. 9, pp. 5390–5397, 2005.

[132] J. Y. Lee, J. Ye, Z. Gao et al., “Reciprocal modulation of toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids reciprocally modulate dendritic cell functions mediated through TLR4,” *Journal of Biological Chemistry*, vol. 278, no. 39, pp. 37041–37051, 2003.

[133] S. S. Chi, I. G. Tikhonova, S. Neumann et al., “Identification of residues important for agonist recognition and activation in GPR40,” *Journal of Biological Chemistry*, vol. 282, no. 40, pp. 29248–29255, 2007.

[134] S. Costanzi, S. Neumann, and M. C. Gershengorn, “Seven transmembrane-spanning receptors for free fatty acids as therapeutic targets for diabetes mellitus: pharmacological, phylogenetic, and drug discovery aspects,” *Journal of Biological Chemistry*, vol. 283, no. 24, pp. 16269–16273, 2008.

[135] M. Toborek, Y. W. Lee, R. Garrido, S. Kaiser, and B. Hennig, “Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cell,” *American Journal of Clinical Nutrition*, vol. 75, no. 1, pp. 119–125, 2002.

[136] A. Y. Bagrov, J. I. Shapiro, and O. V. Fedorova, “Endogenous cardiotonic steroids: physiology, pharmacology, and novel therapeutic targets,” *Pharmacological Reviews*, vol. 61, no. 1, pp. 9–38, 2009.

[137] Z. Xie and T. Cai, “Na+-K+-ATPase-mediated signal transduction: from protein interaction to cellular function,” *Mol Interv.*, vol. 3, no. 3, pp. 157–168, 2003.

[138] J. Tian and Z. J. Xie, “The Na-K-ATPase and calcium-signaling microdomains,” *Physiology*, vol. 23, no. 4, pp. 205–211, 2008.

[139] A. Aperia, “New roles for an old enzyme: Na,K-ATPase emerges as an interesting drug target,” *Journal of Internal Medicine*, vol. 261, no. 1, pp. 44–52, 2007.

[140] A. Miyakawa-Naito, P. Uhlen, M. Lal et al., “Cell signaling microdomain with Na,K-ATPase and inositol 1,4,5-trisphosphate receptor generates calcium oscillations,” *Journal of Biological Chemistry*, vol. 278, no. 50, pp. 50355–50361, 2003.

[141] M. Nesher, U. Shpolansky, H. Rosen, and D. Lichtstein, “The digitalis-like steroid hormones: new mechanisms of action and biological significance,” *Life Sciences*, vol. 80, no. 23, pp. 2093–2107, 2007.

[142] M. Liang, J. Tian, L. Liu et al., “Identification of a pool of non-pumping Na/K-ATPase,” *Journal of Biological Chemistry*, vol. 282, no. 14, pp. 10585–10593, 2007.

[143] S. Rodrigues-Mascarenhas, A. D. S. De Oliveira, N. D. Amoedo, O. R. Affonso-Mitidieri, F. D. Rumjanek, and V. M. Rumjanek, “Modulation of the immune system by ouabain,” *Annals of the New York Academy of Sciences*, vol. 1153, pp. 153–163, 2009.

[144] A. D. Foey, A. Crawford, and N. D. Hall, “Modulation of cytokine production by human mononuclear cells following impairment of Na,K-ATPase activity,” *Biochimica et Biophysica Acta*, vol. 1355, no. 1, pp. 43–49, 1997.

[145] E. Blasi, A. Ardizzone, B. Colombani et al., “NF-kB activation and p38 phosphorylation in microglial cells infected with Leptospira or exposed to partially purified leptospiral lipoproteins,” *Microbial Pathogenesis*, vol. 42, no. 2-3, pp. 80–87, 2007.

[146] M. Cinco, E. Vecile, R. Murgia, P. Dobrina, and A. Dobrina, “Leptospira interrogans and Leptospira peptidoglycans induce the release of tumor necrosis factor α from human monocytes,” *FEMS Microbiology Letters*, vol. 138, no. 2-3, pp. 211–214, 1996.

[147] P. Burth, M. Younes-Ibrahim, F. H. F. S. Gonzalez, E. R. Costa, and M. V. C. Faria, “Purification and characterization of a Na+-K+ ATPase inhibitor found in an endotoxin of Leptospira interrogans,” *Infection and Immunity*, vol. 65, no. 4, pp. 1557–1560, 1997.

[148] M. H. Tajiki and R. Saloniö, “Association of plasma levels of tumor necrosis factor α with severity of disease and mortality among patients with leptospirosis,” *Clinical Infectious Diseases*, vol. 23, no. 5, pp. 1177–1178, 1996.

[149] F. Martinon, K. Burns, and J. Tschopp, “The Inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β,” *Molecular Cell*, vol. 10, no. 2, pp. 417–426, 2002.

[150] S. Akira, S. Uematsu, and O. Takeuchi, “Pathogen recognition and innate immunity,” *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

[151] S. Lacroix-Lamandé, M. F. D’Andon, E. Michel et al., “Downregulation of the Na/K-ATPase pump by leptospiroplasm glycoprotein activates the NLRP3 inflammasome,” *Journal of Immunology*, vol. 188, no. 6, pp. 2805–2814, 2012.