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REVIEW

Pathology and Host Immune Evasion During Human Leptospirosis: a Review

Voon Kin Chin[1,2] · R. Basir[3] · S. A. Nordin[2] · M. Abdullah[4] · Z. Sekawi[2]

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Abstract

Human leptospirosis is considered as one of the most widespread and potentially fatal zoonotic diseases that causes high mortality and morbidity in the endemic regions of tropical and subtropical countries. The infection can arise from direct or indirect exposure of human through contaminated environment that contains leptospires or animal reservoirs that carry leptospires. The clinical manifestations during human leptospirosis ranges from asymptomatic, mild infections to severe and life-threatening complications involving multi-organ failures with kidneys, lungs and liver severely affected. Despite much efforts have been put in to unravel the pathogenesis during human leptospirosis, it remains obscure to which extent the host factors or the pathogen itself contribute towards the pathogenesis. Host innate immunity, especially, polymorphonuclear neutrophils and complement system are involved in the first line of defense during human leptospirosis. However, pathogenic *Leptospira* has acquired diverse evasion strategies to evade from host immunity and establish infection in infected hosts. Hence, in this review, we focus on organs pathology during human leptospiral infection and host evasion strategies employed by *Leptospira*. A profound understanding on leptospiral immunity and how *Leptospira* subvert the immune system may provide new insights on the development of therapeutic regimens against this species in future.

Keywords Leptospirosis · Complement system · Host immune evasion · Pathology

Introduction

Human leptospirosis is a neglected tropical disease which remains the cause of high mortality and morbidity seen in patients infected with spirochetes from the genus *Leptospira*. Human leptospirosis often occurs at tropical and subtropical regions where approximately 59,000 deaths and 1.03 million cases were recorded globally (Costa et al. 2015; Torgerson et al. 2015). The high incidence rate has no doubt imposed huge impact on health consequences and economic burden on both affected patients and countries, which requires attention worldwide to handle this infection.

Infection with *Leptospira* spp. can occur via direct or indirect exposure from animal reservoirs or contaminated environments to infected patients. For example, it can be transmitted directly through exposure of wounds with tissues or urine of infected animals, ingestion of foods and water contaminated with infected rats and inhalation of aerosols of contaminated fluids. Meanwhile, it can also be transmitted indirectly via the mucuos membranes (mouth, nose and eyes) with contaminated soil or water (Bharti et al. 2003; Palaniappan et al. 2007). Additionally, pathogenic *Leptospira* spp. are equipped with specific adaptions to enable their survival inside and outside the host and hence make them successful colonizer and invader in both environment and hosts (Levett 2001).

Host immunity, especially innate immunity is the first line of defense against pathogenic leptospires. However, pathogenic leptospires are highly resistant to killing by host immunity and thus resulted in clinical outcomes in varying degrees, ranging from mild symptoms to life-threatening illness. Patients

✉ Voon Kin Chin
VoonKin.Chin@taylors.edu.my

[1] School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, No 1, Jalan Taylor's, 47500 Subang Jaya, Selangor, Malaysia

[2] I Department of Medical Microbiology & Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3] Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[4] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

infected with mild leptospirosis are usually presented with febrile and headache and resolved without complications. However, patients with severe leptospirosis could present with multiple organ failure and complications such as pulmonary hemorrhage, jaundice, renal impairment, or kidney failure (Levett 2001). The underlying pathogenesis under these circumstances remains obscure. However, possible explanations could be due to interplay between epidemiological conditions, pathogen virulence, and host susceptibility. Moreover, pathogenic *Leptospira*spp. have adopted multiple evasion strategies to prevent evasion from host immunity and subsequently establish infection in infected hosts. Hence, in this review, we seek to dissect leptospiral pathogenesis and organ pathology during human leptospirosis and to provide an overview on the anti-leptospiral immunity via complement system and polymorphonuclear neutrophils (PMN). In addition, the evasion strategies recruited by pathogenic *Leptospira* on host complement system will be discussed. The understanding on the capability of pathogenic *Leptospira* to subvert from host immunity is valuable for diagnostic and prognostic applications in future.

Leptospiral Pathogenesis and Organ Pathology During Human Leptospirosis

Leptospira species are highly motile gram-negative bacteria which can be clustered into three major groups, i.e., group I (pathogenic), group II (intermediate pathogenic,) and non-pathogenic (saprophytes), based on molecular phylogenetic analysis of *Leptospira* 16S rRNA (Lehmann et al. 2014; Chin et al. 2018). Group I consists of nine pathogenic *Leptospira* species including *L. santarosai*, *L. kirschneri*, *L. noguchii*, *L. alexendari*, *L. interrogans*, *L. alstoni*, *L. weilii*, *L. borgpetersenii*, and *L. kmetyi*, which can be further differentiated into more than 250 different serotypes, and causing a wide spectrum of clinical manifestations ranging from mild to severe and fatal infections. Additionally, most of the severe infections are often caused by serovars belonging to *L. noguchii*, *L. interrogans*, and *L. kirschneri*, which are considered as evolutionarily related species (Brenner et al. 1999; Slack et al. 2009). Meanwhile, group II comprises of five intermediate pathogenic species including *L. broomii*, *L. fainei*, *L. wolffii*, *L. licerasiae*, and *L. inadai*. Infection caused by these species are usually mild, self-resolving, and without fatal complications (Schmid et al. 1986; Brenner et al. 1999; Petersen et al. 2001; Levett et al. 2006; Matthias et al. 2008; Slack et al. 2008). On the other hand, six saprophytic *Leptospira* species have been reported thus far, including *L. terpstrae*, *L. yanagawae*, *L. meyeri*, *L. wobachii*, *L. vanthielii*, and *L. biflexa*. These saprophytic species are harmless to human and reside freely in the environment (Brenner et al. 1999).

The establishment of infection by pathogenic *Leptospira* during human leptospirosis often results in invasion, colonization, dissemination, and extraction of host nutrients. In addition, the outcome of the leptospiral infection are also largely dependent on a few determinants such as inoculum size (Ganoza et al. 2006), host immunity (Chin et al. 2018), and the virulence factors expressed by pathogenic *Leptospira* (Thaipadungpanit et al. 2007). A myriad of studies have documented *Leptospira* virulence factors including leptospiral immunoglobulin-like (Lig) proteins, hemolysins, OmpA-like Loa22, lipopolysaccharides (LPS), outer membrane protein (OMPs)–like lipoprotein, phingomyelinases, and adhesion molecules (Ko et al. 2009; Adler et al. 2011; Wang et al. 2012; Narayanavari et al. 2012).

In the context of organs pathology during human leptospirosis, liver is one of the organs affected during leptospiral infection with the severity varying from mild to severe hepatic dysfunction (Gancheva 2009). Grossly, the liver is often enlarged and tender; however, deaths rarely occur due to hepatic failure ((Vijayachari et al. 2008). Meanwhile, the histopathological changes in liver autopsy of leptospirosis fatal cases vary from mild interstitial edema and vascular congestion to advanced degenerative changes (Arean 1962). In addition, distention of the space of Disse by clear fluid with mixtures of cells including mononuclear cells, lymphocytes, and councilman bodies were observed (Arean 1962). These findings could be explained by a recent animal study which documented the infiltration of leptospires in the Disse's space and preferential attachment and invasion of leptospires in the perijunctional region between hepatocytes in a hamster model of Weil's disease infected with *L. interrogans* strain K37 (Miyahara et al. 2014).

Jaundice, marked by hepatocytes dystrophy and necrotic changes, intrahepatic cholestasis, and elevations of bilirubin and transaminase, is a common feature during leptospirosis. Matiash (1999) reported that the incidence of jaundice was observed in a lesser extent in the early stage of leptospiral infection due to the absence of marked disturbances in the protein-synthetizing function of the liver and low enzymatic activity. However, at later stage of infection (2 to 3 weeks after exposure to leptospiral infection), jaundice with high bilirubinemia was observed. This could be due to toxic affliction of hepatocytes and advancing centrilobular cholestasis (Matiash 1999).

On the other hand, histological analysis of the liver infected with *L. interrogans* strain K37 in hamster model of Weil's disease revealed that hyperpermeability of the liver vascular wall, dyscomplexation of the liver crosspieces together with inflow of bile to the sinusoidal capillaries, and interstitial edema were reported at different time intervals during a leptospiral infection. The authors suggested that major contributing factor towards the early damage in the liver could be due to toxic vascular affection of the microcirculatory bed by leptospires (Miyahara et al. 2014). The authors also postulate a novel pathogenicity of leptospires where pathogenic leptospires tend to invade and disrupt the intercellular junctions

of host hepatocytes, resulting in leakage of the bile from bile canaliculi and jaundice formation (Miyahara et al. 2014).

Besides the liver, the lungs are extremely affected during leptospiral infection. Histologically, pulmonary congestion accompanied with hemorrhage is commonly seen in patients with leptospirosis. A study of fatal leptospirosis by Arean (1962) revealed that all 33 fatal cases showed pulmonary petechiae on the plural surfaces with 60% of patients having gross hemorrhage on the cut surfaces of the lung with hemorrhage found in intra-alveolar spaces and alveolar septa (Arean 1962). Another study by Nicodemo et al. (1997) on the lung tissue of 12 fatal cases revealed that pulmonary hemorrhage alone or the combination of pulmonary hemorrhage with gastrointestinal bleeding was the main cause of death seen in eight patients. On the other hand, the authors also reported that edema was observed in the intra-alveolar septa, accompanied with mild-to-moderate inflammatory infiltrates, predominant by lymphocytes, plasma cells and macrophages. Meanwhile, immunohistochemistry studies on pulmonary tissues showed the deposition of leptospires within macrophages and endothelial cells in septa and alveoli, where leptospires were found attached to capillary endothelial cells (Nicodemo et al. 1997; Silva et al. 2002). Besides that, hyaline membrane formation in the lung also provide an indicative of diffuse alveolar damage caused by leptospires (O'Neil et al. 1991; Kiatboonsri et al. 1995).

The underlying mechanism of lung pathogenesis is still poorly understood. At present, pathogenesis in lung could be due to a toxin-mediated mechanism and/or the host immune responses. Hemorrhage seen in lung tissues during leptospiral infection could be due to toxin secreted by leptospires which resulted in capillary vasculitis (Luks et al. 2003). On the other hand, the numbers of leptospires present in lung tissues are much lower than the liver and blood counts, indicating that pulmonary abnormalities could be due to the exposure of circulating toxin produced by leptospires from distant sites such as the liver (Bharti et al. 2003).

In the lung, Na^+/K^+ pump is crucial for its role in removing sodium from alveolar fluid for edema clearance and homeostatic regulation in order to maintain lung integrity (Sznajder et al. 1994; Sznajder 2001). Inhibition of the Na^+/K^+ pump may result in lung failure in severe leptospirosis cases (Vadász et al. 2005). The evidence of alteration of Na-K-2Cl (NKCC) co-transporter and epithelial sodium channel (ENac) in the lungs during leptospirosis was demonstrated in an animal model. This alteration resulted in the impairment of pulmonary function and consequently pulmonary damage. In the study, ENac expression was found to be reduced while the NKCC expression increased in pulmonary cells of hamsters infected with *Leptospira* (Andrade et al. 2007). Furthermore, numerous sources reported the involvement of cytokines, such as tumor necrosis factor-alpha (TNF-α) in decreasing ENac and interleukin 1 (IL-1) in increasing Na-K-2Cl co-transporter during leptospiral infection (Dagenais et al. 2004; Choi et al. 2007; Yamagata et al. 2009). Taken together, these findings suggest that cytokines could be involved in the modification of ion channels resulting in pulmonary damage.

Apart from that, Bernardi et al. (2012) investigated the involvement of immune receptors and intercellular and vascular cell adhesion molecules in the lungs of patients with pulmonary involvement. The authors reported the increase in the expression of the VCAM-1, ICAM-1, C3a receptor, and Toll-like receptor 2 on alveolar septa of patients who died from leptospirosis. The authors speculated that adhesion molecules and immune receptors participate in the phenomena leading to pulmonary hemorrhage in fatal leptospirosis. The expression of these complement receptors and adhesion molecules could promote leukocyte recruitments to infected tissues. On the other hand, mild inflammation on lung tissues was observed in most of the fatal cases. The authors postulated that besides classical inflammatory responses, thrombocytopenia could be another pathological mechanistic contributor towards hemorrhagic phenomena in severe pulmonary hemorrhagic syndrome (Bernardi et al. 2012).

The kidney is also a preference target during human leptospirosis, which could be due to the intrinsic renal-tropic homing ability of leptospires on the host (Haake and Levett 2015). Renal involvement during leptospirosis can vary from mild non-oliguric renal impairment to complete renal failure, a typical presentation of Weil's syndrome. The major histological findings often involve tubular necrosis and interstitial nephritis. Arean (1962) reported that tubular damage includes necrosis and thinning of tubular epithelium, with tubular lumen distended with cellular debris and hyaline casts (Arean 1962). In the reservoir host, leptospires enter host via penetration of the capillary lumen and interstitial tissue which causes edema and cell infiltration. On the other hand, *Leptospira* (*Leptospira canicola*) can adhere to renal tubule and tubular lumen. Immunohistochemistry analysis showed that *Leptospira canicola* antigens are attached in the proximal tubule epithelium cells and appear as big extracellular clusters in the interstitium (Morrison and Wright 1976).

Two major factors contributing towards the pathogenesis of acute kidney injury (AKI) in leptospirosis are toxin-induced immune response and direct nephrotoxic action of pathogenic *Leptospira* through toxin productions (Barnett et al. 1999). Previous study has shown that acute interstitial nephritis (AIN) is the main factor leading to AKI in leptospirosis (Cerqueira et al. 2008). Acute interstitial nephritis (AIN) is triggered by the presence of leptospires in renal tissues and usually occurs after tubular damage. Arean (1962) documented that patients who died within the first week of illness displayed acute tubular necrosis and cellular swelling while those patients who died between 2 and 3 weeks of illness exhibited acute tubular necrosis (ATN) and interstitial edema. Patients who died after 3 weeks of illness presented with

diffuse and severe interstitial nephritis (Arean 1962). Another study also reported on the incidence of ATN in 13/15 patients from Moldova (Covic et al. 2003). These findings suggested that ATN could be due to direct toxic effect of leptospiral components on tubular epithelial cells or indirectly due to inability to concentrate urine and ionic wasting defects resulting from hypovolemia, dehydration, and ischemia.

On the other hand, the outer membrane of *Leptospira* contain antigenic components including endotoxins, lipoproteins, peptidoglycans, and lipopolysaccharides which can contribute towards kidney injury, inflammation, and tubular dysfunction. Several *Leptospira* outer membrane proteins (OMPs) including lipopolysaccharide (LPS), lipoprotein (LipL41), and a porin (OmpL1) have been identified to be located in the interstitium and proximal tubules of infected hamsters challenged with host-derived *Leptospira kirschneri* (Barnett et al. 1999). The authors suggest that OMPs could have crucial roles in inducing and causing persistent leptospiral interstitial nephritis (Barnett et al. 1999). Meanwhile, Yang et al. (2002) demonstrated the role of *Leptospira* outer membrane protein, LipL32 in the pathogenesis of tubulointerstitial nephritis. In this study, the authors showed that LipL32 from pathogenic *Leptospira shermani* affects mouse proximal tubular cells directly and leads to the increase in gene expression of T cells (RANTES), monocyte chemotactic protein-1 (CCL2/MCP-1), tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS). Further, induction of these genes could be responsible for cellular damage in renal tissue (Yang et al. 2002). Similarly, a recent study by Chang et al. (2016) also demonstrated the role of LipL32 in inducing inflammation and causing renal damage in zebrafish larvae infected with *Leptospira santarosai* serovar Shermani (Chang et al. 2016). On the other hand, Humphryes et al. (2014) reported that vaccination with LipL32 had improved kidney invasion in hamsters infected with *Leptospira interrogans* Serovar Canicola (Humphryes et al. 2014), indicating the undisputable role of LipL32 in renal pathology during leptospirosis. Besides LipL32, Abreu et al. (2017) reported that Lp25 protein expressed by pathogenic *Leptospira* spp. is associated with rhabdomyolysis-induced AKI in a guinea pig model of leptospirosis (Abreu et al. 2017).

Host Immune Evasion by Pathogenic *Leptospira*

Polymorphonuclear Neutrophils

Innate immunity is the frontline of host defense during leptospirosis. Under normal circumstances, leptospires can reach up to 10^6–10^7 organisms per gram (g) in the tissues or per milliliter (mL) of blood of infected hosts (Truccolo et al. 2001) where phagocytosis or complement system is involved in the killing of leptospires. However, there is a high possibility where leptospires can evade from host innate immune through complement killing or clearance by phagocytosis.

Neutrophils and macrophages are important effectors during phagocytosis. Earlier studies have reported that human polymorphonuclear neutrophils (PMN) are effective in killing non-pathogenic leptospires (*L. biflexa* sp.), but not to pathogenic leptospires (*L. interrogans* sp.) in non-immune cells (Cinco and Banfi 1983a, b; Wang et al. 1984). Meanwhile, Murgia et al. (2002) demonstrated that pathogenic strain (*Leptospira interrogans* strain Hardjoprajitno) and non-pathogenic strain (*Leptospira biflexa* strain Patoc1 (serovar Patoc)) can be destroyed through oxygen-dependent and oxygen-independent reactions aided by hydrogen peroxide (H_2O_2), with or without the presence of myeloperoxidase (MPO) (Murgia et al. 2002). Further, the authors also confirmed the leptospiracidal activity of H_2O_2 alone in other pathogenic strains including Wijmberg (serovar Copenhageni), 142 (serovar Icterohaemorrhagiae), and Ballico (serovar Australis). Moreover, in this study, the authors reported that non-pathogenic *Leptospira biflexa* strain Patoc1 is more prone to killing by H_2O_2 than pathogenic *Leptospira* strains (Murgia et al. 2002). Besides killing by H_2O_2, leptospires are susceptible to the cationic peptides of neutrophils in oxygen-independent killing. Cathelicidin-derived peptides from neutrophils displayed anti-leptospiral activity among different *Leptospira* strains (*L. interrogans* serovars and *Leptospira biflexa)* (Scocchi et al. 1993; Sambri et al. 2002). On the other hand, a recent study showed that neutrophil extracellular traps (NETs) is involved in host innate immunity towards *Leptospira*. The authors reported that *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130 (LIC) triggers the release of DNA extracellular traps (NETs) and kills the leptospires through NETosis, a process where extrusion of the neutrophil DNA with bactericidal proteins results in trapping and/or killing of the pathogens. The authors suggest that these DNA traps are crucial in preventing the early leptospiral dissemination (Scharrig et al. 2015).

Taken together, neutrophils are playing crucial role in killing leptospires. However, current evidences showed that leptospires are scarcely phagocytosed and killing occurs only in the presence of specific antibodies. Furthermore, these findings as discussed above demonstrated that neutrophils are mostly effective against saprophytic leptospires rather than pathogenic leptospires, thus increasing the chances of pathogenic leptospires to evade from the host immune system and establish infection in infected hosts. The actual mechanism on how pathogenic *Leptospira* escaped from neutrophil killings remains largely unknown. Indeed, a recent study documented that pathogenic leptospires (*L. interrogans* serovar Copenhageni) are able to inhibit myeloperoxidase (MPO) activities (peroxidase and chlorination), without meddling with neutrophil degranulation. The authors further identified a

putative virulence factor, LipL21 as a potent MPO inhibitor, and postulated a novel innate immune evasion mechanism for the survival of leptospires in the host (Vieira et al. 2018).

Complement System

Apart from phagocytosis, the invasivity of leptospires is more closely related to complement system. Complement system comprises of more than 50 receptors and plasma proteins and is crucial in innate immune defense. Complement system is imperative in conferring protection against foreign microbes due to its inflammatory, lytic, and opsonic activities (Bloom 2002). The functions of complement effectors arise from the activation of classical (CP), alternative (AP), and/or lectin (LP) pathways. The lectin (LP) and alternate (AP) pathways are involved in the host innate immunity while classical pathway (CP) is triggered by the presence of IgM or IgG specifically bound to antigens. Meanwhile, the alternate pathway is activated when the intra-chain thioester bond located in the C3 molecule is hydrolyzed. C3 molecule, being the main element of the complement cascade, will generate C3a and C3b (Zipfel et al. 2007a, b) where C3b attaches to microbial surfaces and behaves like an opsonin to enhance the recognition of microbes and phagocytosis by host immune phagocytic cells (van Lookeren Campagne et al. 2007). On the other hand, lectin pathway is triggered when lectins, including ficolins or mannose-binding lectin, attach to carbohydrate moieties found on the microbial surfaces (Fraga et al. 2016).

Host Complement Evasion by Pathogenic *Leptospira*

Johnson and his colleague first reported on the complement-mediated anti-leptospiral activity against different *Leptospira* serotypes by using serum from different mammals. In their studies, they found that non-pathogenic *Leptospira* are more susceptible to complement killing as compared to pathogenic *Leptospira* (Johnson and Muschel 1966). Similarly, Cinco and his co-worker also demonstrated the complement resistance mechanism within the *Leptospira* genus (Cinco and Banfi 1983a, b). The authors reported that pathogenic *Leptospira* strains (*Leptospira interrogans*) showed resistance towards complement killing, but non-pathogenic leptospires (*Leptospira biflexa* strains) were killed to various degrees, probably via direct activation of complement alternative pathway. These studies indicate that pathogenic *Leptospira* strains are resistant to complement killing. However, the underlying mechanism on how the pathogenic *Leptospira* escape from complement attack remains elusive.

It is not surprising that pathogenic microorganisms employ various strategies to avoid attack by antibody or complement in the host, including *Leptospira*. Apparently, pathogenic *Leptospira* strains are more resistant towards complement-mediated killing, while saprophyte *Leptospira* strains are more prone to serum killing (Meri et al. 2005a, b; Barbosa et al. 2009). However, there is lack of current evidence on the efficacy of complement killing on intermediate pathogenic leptospires, for example, *Leptospira licerasiae*. Figure 1 depicts an overview on the complement evasion strategies recruited by pathogenic *Leptospira*. These strategies include (i) capturing or mimicking host complement regulators, (ii) cleavage of complement proteins on the *Leptospira* surface via acquisition of host proteases, and (iii) inactivation of complement through secretion of proteases (Fraga et al. 2016). Additionally, these diverse mechanisms are also employed by other pathogens such as fungi, bacteria, and viruses to avoid complement attack (Zipfel et al. 2007a, b; Rooijakkers and van Strijp 2007; Lambris et al. 2008; Blom et al. 2009).

Capturing or Mimicking Host Complement Regulators

Meri et al. (2005a, b) had first reported the mechanism undertaken by pathogenic *Leptospira* in avoiding complement killing. In this study, the authors surmised that the resistance of *Leptospira* serum-intermediate and serum-resistant strains towards complement-mediated killing was associated with the binding capacity of *Leptospira* towards factor H and factor H–related protein 1 (FHR-1a and FHR-1b) in human serum. Factor H and factor H–related protein 1 (FHR-1) are alternative complement pathway regulators. These proteins have multiple functions where they (i) can be the cofactor for the cleavage of C3b by factor I, (ii) accelerate the degeneration of the C3-convertase C3bBb, and (iii) prevent the binding of factor B to C3b (Meri et al. 2005a, b).

The association of *Leptospira* surface proteins with human factor H have been elucidated, including outer membrane protein LenA (leptospiral endostatin–like protein A) and LenB (leptospiral endostatin–like protein B) (Barbosa et al. 2006; Verma et al. 2006). It is documented that LenA binds to both factor H (FH) and factor H–related protein 1 (FHR-1), whereas LenB is only associated with factor H (Verma et al. 2006; Stevenson et al. 2007). Further, genetic analysis on *L. interrogans* revealed five more *lenA* paralogs, which are *lenB*, *len*C, *lenD*, *lenE*, and *lenF*. These Len proteins showed their capacities to bind with laminin and fibronectin (with the exception on LenA, which could only interact with laminin), where these bindings facilitate host colonization and invasion by pathogenic *Leptospira* (Stevenson et al. 2007). Meanwhile, in a review by Zipfel et al. (2007a, b), the authors surmised that pathogens can escape from complement attack by binding to other host molecules, such as fibrinogen, plasminogen, IgA, IgG, extracellular matrix components, and thrombin, which eventually contribute to tissue adhesion and degradation of host cells (Zipfel et al. 2007a, b). In this context, several *Leptospira* proteins are also shown to interact with host complement regulators, including Lsa23 (binds to FH and C4BP)

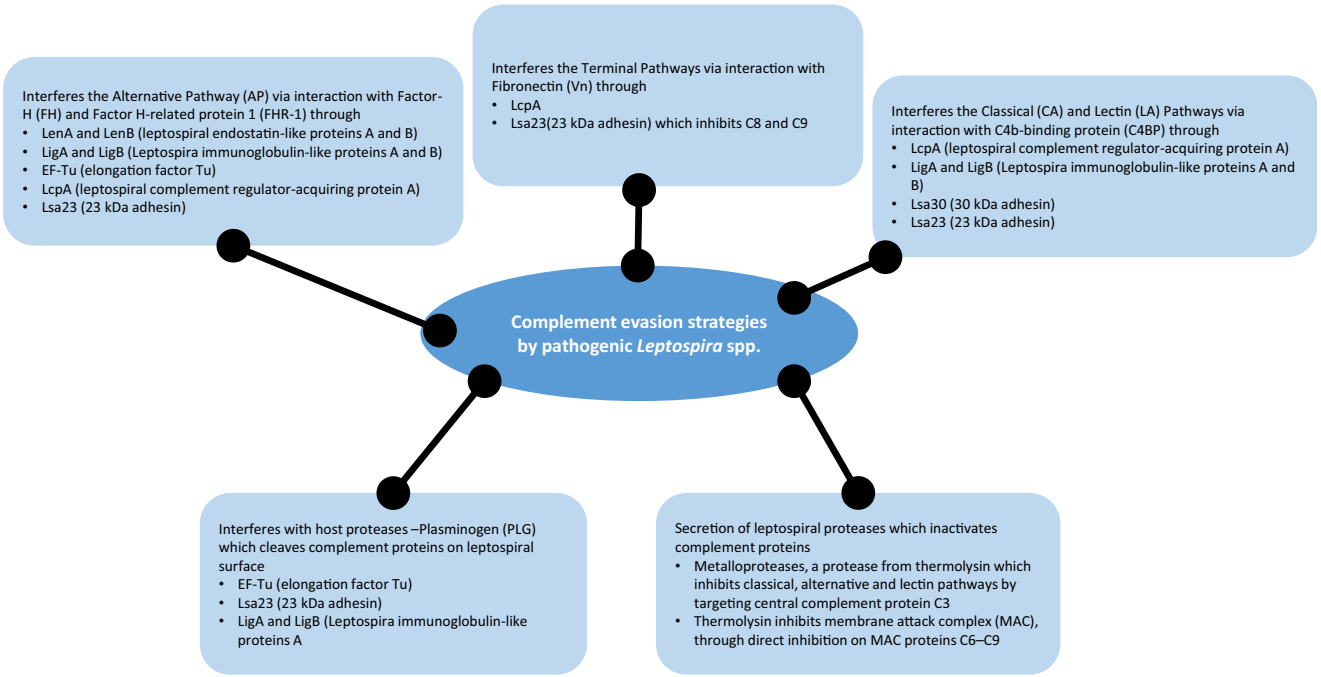

Fig. 1 An overview depicting the various strategies employed by pathogenic *Leptospira* spp. to evade from complement attack. These strategies involved (i) interference with complement pathways (classical, alternative, lectin, and terminal) by capturing or mimicking host complement regulators, (ii) cleavage of complement proteins on the *Leptospira* surface via acquisition of host proteases (plasminogen), and (iii) inactivation of complement proteins through secretion of leptospiral proteases (metalloproteases and thermolysin)

(Siqueira et al. 2016), Lsa30 (binds to C4BP) (Souza et al. 2012), and elongation factor Tu (EF-TU) (binds to FH) (Wolff et al. 2013).

Meanwhile, Barbosa et al. (2009) reported that both serum-resistant and serum-intermediate pathogenic leptospires have the ability to bind to human C4BP, whereas serum-sensitive strain Patoc1 binds in negligible amounts (Barbosa et al. 2009). Human C4BP is the fluid phase inhibitor involved in the lectin and classical pathways. The surface-bound C4BP promotes the factor I–mediated cleavage of C4b, which could contribute towards complement resistance by leptospires. In a later year, the authors identified that leptospiral complement regulator-acquiring protein A (LcpA) is expressed by leptospiral strains which showed partially resistant towards complement-mediated killing. LcpA is surface-exposed and can bind to both soluble and purified C4BP from human serum. It remains functionally active when bound to LcpA and facilitates the cleavage of C4b by factor 1 (Barbosa et al. 2010). Recent study also showed that LcpA of pathogenic *Leptospira* spp. can bind to factor H (FH), vitronectin (Vn), and complement terminal pathway. Competitive binding assays revealed that interaction of LcpA with C4BP, Vn, and FH occurs through distinct sites. Vitronectin (Vn) is a glycoprotein involved in the regulation of complement terminal pathway through inhibition of C5b7 complex formation and C9 polymerization. Hence, binding of Vn to leptospiral surface can eventually assist *Leptospira* from complement attack. It also helps to protect *Leptospira* from lysis by impairing membrane attack complex (MAC) formation (Preissner and Seiffert 1998; da Silva et al. 2015).

On the other hand, leptospiral immunoglobulin (Ig)–like proteins (Lig proteins): LigA and LigB, are multiple proteins involved in the interaction with cell lines, extracellular matrix (ECM), and complement regulators in vitro. These proteins may have role in bacterial attachment to host tissues and colonization. As demonstrated by Castiblanco-Valencia et al. (2012), binding of recombinant LigA and LigB of pathogenic *L. interrogans* serovar Pomona strain Fromm to C4BP, FH, FHR-1, and FHL-1 can interfere and control all complement pathways. This cleavage is believed to circumvent complement attack by pathogenic *Leptospira*. Furthermore, the authors demonstrated that C4BP and FH do not compete each other to bind to Lig proteins, suggesting that C4BP and FH have different binding sites on these molecules but interacting with their targets simultaneously (Castiblanco-Valencia et al. 2012). A study by Choy (2012) revealed that multiple activities of LigB enhances the virulence of *L. interrogans*. The author also demonstrated that both classical and alternative pathways are inhibited by LigB in hemolytic assays with erythrocytes. Further, the author reported that expression of LigB confers protection in saprophyte *Leptospira biflexa* and deduced that resistance of *ligB*-transformed *L. biflexa* towards complement killing could be due to the acquisition of FH and C3b by these bacteria (Choy 2012). These findings are further consolidated through a study by Castiblanco-Valencia et al. (2016) where resistance of saprophyte *L. biflexa* towards serum killing is greatly enhanced by

the expression of both *ligA* and *ligB* genes, with a reduction in the membrane attack complex (MAC) deposition on *lig*-transformed *L. biflexa* as compared to the wild-type strain (Castiblanco-Valencia et al. 2016).

Pathogenic *Leptospira* spp. are also shown to block terminal pathway of the complement system to evade innate immunity. A study by Siqueira et al. (2017) demonstrated the ability of pathogenic, virulent strain *L. interrogans* L1-130 in binding to the immobilized human C8. Additionally, the authors also showed that virulent strain of *L. biflexa* was more competently interacting with C8 and C9 than the saprophyte *L. biflexa* strains, at physiological concentration (50 μg/mL). The authors reported that a novel leptospiral adhesion, Lsa23, could be responsible for the interaction between pathogenic *Leptospira* and C8 and C9 terminal complement components and further suggest that inhibiting the complement terminal pathway could be one of the strategy employed by pathogenic leptospires to evade host innate immunity (Siqueira et al. 2017).

Cleavage of Complement Proteins on the *Leptospira* Surface via Acquisition of Host Proteases

Vieira et al. (2009) discovered that *Leptospira* species could bind to human plasminogen (PLG) and generate plasmin (PLA) on its outer surface in the presence of urokinase-type plasminogen activator (uPA) (Vieira et al. 2009; Verma et al. 2010). The authors reported that PLG binding on the pathogenic *Leptospira* outer surface followed by activation of PLA leads to fibronectin degradation, which could explain the leptospiral invasiveness in hosts. Previous studies also reported that plasmin could degrade important biological substrates such as fibrinogen, ECM proteins, and human IgG and cleave immobilized IgG in physiological conditions (Harpel et al. 1989; Barthel et al. 2012). In addition, plasmin can interfere with C3b and lead to cleavage of this central complement protein. Hence, the ability of *Leptospira* to bind to human plasmin is a typical example on how pathogenic *Leptospira* can evade from complement attack through enzymatic degradation. Moreover, Vieira and his co-workers (Vieira et al. 2010) also found that pathogenic *Leptopsira* express multiple PLG-binding proteins. Eight proteins from PLG-binding receptors for *Leptospira interrogans* were identified and characterized, including the major outer protein, LipL32 (one of the key virulence factors of *Leptospira*). The binding/activation of PLG on the pathogenic *Leptopsira* surface proteins helps the bacteria to overcome tissue barriers, which in turn facilitate invasion and colonization of mammalian tissues. Furthermore, since PLG formation is occurring on *Leptospira* surface, it does increase the pathogenic *Leptospira* survival upon infection and creates an opportunity to modulate innate host immunity through protease-associated activity (Vieira et al. 2010).

Meanwhile, Vieira and his co-workers (Vieira et al. 2011) demonstrated that the association between plasmin (PLA) activity and *Leptospira* outer surface in bracket (pathogenic Leptospira interrogans) could result in host immune evasion and increase the survival of *Leptospira*. The authors demonstrated that bacteria-associated PLA can reduce C3b and IgG depositions on leptospiral surface, probably through degradation which diminishes the opsonization process. The authors speculated that the decrease in the opsonization process through PLA generation could be one of the crucial strategies for leptospires to escape and survive from host immune attack. These findings greatly enhance our understanding on leptospiral pathogenesis as well as leptospiral-host interaction. Furthermore, the survival of *L. interrogans* serovar Pomona in human serum was enhanced when bound to plasmin, which indicates the prominent role of plasmin in complement resistance (Vieira et al. 2011). Besides that, a numbers of *Leptospira* membrane proteins have also been reported as PLG ligands, including LigA and LigB (Castiblanco-Valencia et al. 2016), EF-Tu (Wolff et al. 2013), and Lsa23 (Siqueira et al. 2016), where interaction of these proteins with PLG causes the cleavage of C3b, C4b, and/or C5.

Inactivation of Complement Proteins Through Secretion of Leptospiral Proteases

Secretion of proteases by pathogenic *Leptospira* spp. will directly inactivate complement. Fraga et al. (2013) demonstrated that culture of pathogenic *Leptospira* can directly inhibit all three complement pathways, classical, alternative, and lectin pathways, through leptospiral proteases secretion. The authors postulated that inhibition of all three complement pathways were contributed by metalloproteases, a protease from thermolysin, which is found exclusively in *Leptospira* pathogenic species. Metalloproteases targets central complement protein C3, a key factor involved in the amplification of the complement cascade. Hence, degradation and cleavage of C3 will result in the functional inactivation of complement which consequently attenuated the host immune response towards *Leptospira* (Fraga et al. 2013). Furthermore, the inhibition of complement activation by thermolysin is further substantiated by findings from Amamura et al. (2017). The authors demonstrated the capability of thermolysin to inhibit membrane attack complex (MAC), through direct inhibition on MAC proteins C6–C9 (Amamura et al. 2017). Thus, it is plausible that secretion of proteases by pathogenic *Leptospira* helps in the evasion from complement system. Understanding the interlink between *Leptospira* proteases and inhibition of complement system activation can aid us in attaining more holistic views and for better designation of therapeutic regimens in the future to control this zoonotic disease.

Conclusion

Human leptospirosis remains one of the zoonotic diseases that causes high mortality and morbidity worldwide. Despite much

effort have been put in, the underlying mechanism of the pathogenesis remains poorly understood. Much effort are needed to unravel the mechanisms of pathogenesis in the target organs during infection. On the other hand, *Leptospira*, being a highly invasive bacterium, employs diverse strategies to subvert host immunity to establish infection and invade target organs in the host. Identification of *Leptospira* putative virulence factors that interfere with host immune evasion could be potential therapeutic or vaccine candidates for better management of human leptospirosis in the future.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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