Microbiological Research 207 (2018) 108–115

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Leptospirosis in human: Biomarkers in host immune responses

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ARTICLE INFO

Keywords:
Leptospirosis
Host immune responses
Biomarkers
Cytokines
Immune mediators
Proteomic approach

ABSTRACT

Leptospirosis remains one of the most widespread zoonotic diseases caused by spirochetes of the genus *Leptospira*, which accounts for high morbidity and mortality globally. Leptospiral infections are often found in tropical and subtropical regions, with people exposed to contaminated environments or animal reservoirs are at high risk of getting the infection. Leptospirosis has a wide range of clinical manifestations with non-specific signs and symptoms and often misdiagnosed with other acute febrile illnesses at early stage of infection. Despite being one of the leading causes of zoonotic morbidity worldwide, there is still a gap between pathogenesis and human immune responses during leptospiral infection. It still remains obscure whether the severity of the infection is caused by the pathogenic properties of the *Leptospira* itself, or it is a consequence of imbalance host immune factors. Hence, in this review, we seek to summarize the past and present milestone findings on the biomarkers of host immune response aspects during human leptospiral infection, including cytokine and other immune mediators. A profound understanding of the interlink between virulence factors and host immune responses during human leptospirosis is imperative to identify potential biomarkers for diagnostic and prognostic applications as well as designing novel immunotherapeutic strategies in future.

1. Introduction

Leptospirosis is a globally widespread zoonotic disease caused by spirochetes *Leptospira* which has high impact on health consequences in human and domestic animals (Levett, 2001; Bharti et al., 2003). Human leptospirosis is transmitted through direct contact of wounds with urine and tissues of the infected animals or indirectly through the mucous membranes of the nose, eyes and mouth with contaminated water or soil (Bharti et al., 2003; Palaniappan et al., 2007). Pathogenic *Leptospira* species have their own affinity and special adaptations towards specific mammals and hence causes clinical manifestations in varying degrees (Levett, 2001). For example, rodent reservoirs are carriers for *Leptospira* species and do not show any diseases. Meanwhile, infected companion animals or livestock may suffer from abortion and multiple organs injuries and infected humans may present with a variety of clinical manifestations from asymptomatic infection to life threatening disease (Ashford et al., 2000; Ganoza et al., 2010). With mild infections, patients typically present with symptoms viz fever, headache, and myalgia, and less frequently by meningitis, conjunctival suffusion, rash, renal insufficiency and jaundice. Symptoms may be biphasic and some can resolve spontaneously. However, these symptoms and signs are non-specific and could be frequently misdiagnosed with other causes of acute febrile illness such as dengue fever, influenza or malaria (Bharti et al., 2003; Wuthiekanun et al., 2007).

The development of severe leptospirosis could be accompanied by symptoms which include jaundice, pulmonary haemorrhage, hepatic and kidney failure (Chirathaworn et al., 2016). Of the complications that arise from leptospiral infection, severe pulmonary haemorrhage syndrome (SPHS) and Weil's syndrome (combination of acute renal insufficiency, haemorrhage and jaundice), are among the well-known forms of severe leptospirosis. Notably, mortality recorded in Weil's disease and severe pulmonary haemorrhage syndrome (SPHS) are > 10% and 50%, respectively (McBride et al., 2005; Gouveia et al., 2008).

High incidences of leptospiral infection are often seen in tropical and subtropical regions. There are approximately 1.03 million cases and 59,000 deaths reported globally (Costa et al., 2015; Torgerson et al., 2015). Hence, the high frequency of leptospiral infection constitutes a serious clinical problem worldwide that needs to be addressed

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https://doi.org/10.1016/j.micres.2017.11.015
Received 8 June 2017; Received in revised form 23 November 2017; Accepted 25 November 2017
Available online 28 November 2017
0944-5013/

properly. Increased incidence of leptospiral infection indicates the difficulty of diagnosing and treating the disease and thus, a better understanding of leptospiral infection followed by the development of sensitive and specific diagnostic methods and appropriate strategies for leptospirosis treatment are beneficial.

Hitherto, the underlying pathogenesis of leptospirosis remains obscure. It is believed that both pathogen and host factors are responsible for the development and progression of severe infection. However, it is still poorly understood whether the final outcome of severe illness is a result from direct tissue damage caused by the *Leptospira*, or it is a consequence of an imbalance of host immune response to infection. Numerous studies have reported the involvement of immune mediators such as cytokines and chemokines and their potentials as promising biomarkers for disease severity prediction or therapeutic efficacy. However, there are still lots of unsolved questions pertinent to this disease that need to be addressed by further research. Hence, in this review, we focus on the leptospiral pathogenesis and host immune response during leptospirosis to gain insights and for better strategized planning in the future to combat this disease.

2. Leptospiral pathogenesis

Based on 16SrRNA phylogeny, *Leptospira* genus can be categorized in three large subgroups; Group I (pathogenic), Group II (intermediate pathogenic) and saprophytes (Lehmann et al., 2014). Hitherto, there are nine pathogenic *Leptospira* species in Group I including *L. kirschneri*, *L. interrogans*, *L. noguchii*, *L. borgpetersenii*, *L. alexendari*, *L. weilii*, *L. santarosai*, *L. kmetyi* and L. *alstoni*. Group I pathogens can be further classified into more than 250 distinct serotypes which produce a wide variety of clinical manifestations, ranging from subclinical infection to severe and fatal disease, with most of the severe disease arise from serovars belonging to evolutionarily-related species, *L. interrogans*, *L. noguchii* and *L. kirschneri* (Brenner et al., 1999; Slack et al., 2009). Meanwhile, Group II consists of five intermediately pathogenic *Leptospira* species including *L. licerasiae*, *L. wolffii*, *L. broomii*, *L. inadai* and *L. fainei*, where infection with these species will result in mild and 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). There are six saprophytic *Leptospira* species including *L. meyeri*, *L. terpstrae*, *L. biflexa*, *L. vanthielii*, *L. yanagawae* and *L. wobachii*, in which these species are free-living microorganisms in environment and do not cause any complications in human (Brenner et al., 1999). In addition, a new non-pathogenic saphroyte, *L. idonii* has been discovered and described recently (Saito et al., 2013).

Before leptospiral genome era, the underlying mechanisms on the pathogenesis of leptospira remains largely unknown (Ko et al., 2009). With the advancement of next generation sequencing (NGS), the first genome sequence of *Leptospira interrogans* was completed on 2003 (Ren et al., 2003). In-depth analysis on a series of genes responsible for chemotaxis and lipopolysaccharide (LPS) synthesis has revealed the unique physiological and pathogenic features of this species contributing towards pathogenesis in human. Furthermore, *Leptospira* genome project which was initiated on 2011 has enhanced our understanding on the pathogenesis mechanisms of *Leptospira* and aided us in distinguishing *Leptospira* species in terms of gene and pathway involvements (Ricaldi et al., 2012).

Establishment of human leptospirosis lies on the ability of pathogenic *Leptospira* to invade, colonise, disseminate and extract host nutrients for survival. The persistency in host invasion, colonisation and dissemination is assisted by the expression of various virulence factors by pathogenic *Leptospira*. The severity of leptospirosis can be determined by different virulence factors related to pathogenic *Leptospira* (Thaipadungpanit et al., 2007), inoculum size that modifies infecting pathogen burden (Ganoza et al., 2006) or differences in host immune response (Wagenaar et al., 2009a). Different *Leptospira* serotypes confer different virulence factors that play important roles in leptospiral pathogenesis to adapt to host's environment. To date, there are a lot of studies documented the *Leptospira* virulence factors (Ko et al., 2009; Adler et al., 2011; Narayanavari et al., 2012; Wang et al., 2012) including lipopolysaccharides (LPS) (outer membrane), outer membrane proteins (OMPs) like lipoprotein, hemolysins, OmpA-like Loa22, leptospiral immunoglobulin-like (Lig) proteins, sphingomyelinases and adhesion molecules.

Leptospiral lipopolysaccharides (LPS) is the major outer membrane of Leptospira, with varying LPS structure in different serovars and antigenically diverse (Bulach et al., 2000). Leptospiral LPS is a well-known virulence factor contributing towards the pathogenesis in human, due to its ability to adhere with host's extracellular matrix, including laminin, collagen and fibronectin (Hoke et al., 2008). Leptospiral LPS exhibits similarity to gram-negative-bacterial LPS, both structurally and immunologically. In human cells, leptospiral LPS activates macrophage through a Toll-like receptor 2 (TLR2) pathway, in the presence of CD14 (Werts et al., 2001). In contrast to mouse cells, TLR2 and TLR4 pathways were involved, suggesting host-specific activation (Nahori et al., 2005). Similarly, both pathogenic and intermediately pathogenic leptospiral LPS confers different carbohydrate and lipid compositions that undergo different leptospiral pathogenesis (Patra et al., 2015). Other leptospiral outer membrane proteins (OMPs), such as lipoproteins LipL32 is only conserved and expressed in pathogenic species during infection (Haake et al., 2000).

Hemolysins is a toxin made from protein and lipid that can lyse cell membrane of erythrocytes and other cells (Thompson and Manktelow, 1986). Pathogenic *Leptospira interrogans* can express leptospiral SphH that destroys mammalian cell membranes by pore formation, without the involvements of sphingomyelinase or phospholipase activities (Lee et al., 2002). However, leptospiral SphA demonstrated sphingomyelinase activity on cell membrane by increased membrane permeability, aggregation and fusion (Lee et al., 2002; Goni and Alonso, 2002). During *Leptospira interrogans* infection, hemolysin Sph1, Sph2, Sph3, HlpA and TlyA are secreted to stimulate proinflammatory cytokines via TLR2, TLR4, JNK and NFkB pathways (Wang et al., 2012). Meanwhile, Leptospiral immunoglobulin-like (Lig) proteins, such as LigA, LigB and LigC are expressed only in pathogenic *Leptospira* species, which they are anchored to the outer membrane to facilitate mammalian host cell invasion or attachment (Matsunaga et al., 2003). Exposure to physiological osmolarity leads to high expression of LigA and LigB that interacts with extracellular matrix proteins, such as collagen I and IV, laminin, fibronectin and fibrinogen that may be involved in the colonization and dissemination of leptospirosis (Choy et al., 2007).

A recent study on *Leptospira* genome revealed that approximately 900 genes in pathogenic *Leptospira* strains could be involved in the pathogenicity of leptospirosis. Moreover, the roles of most of the genes remain obscure and current known proteins failed to explain the virulence mechanisms of leptospira as a whole. Furthermore, mutation analysis has revealed that some of these genes have pronounced functions in leptospira pathogenesis where mutations in these genes reduce virulence of leptospira. These genes include Loa22, OmpA-family protein and several other proteins (Adler et al., 2011).

The latest breakthrough on pathogenesis of *Leptospira* was demonstrated by Fouts et al. (2016) where the authors performed comparative cross-species genomic analysis of the *Leptospira* genus to identify the virulence factors associated with the pathogenesis and mammalian host adaptation during leptospirosis. In the study, the authors have identified few metabolic mechanisms related to host adaptation by *Leptospira*, including pathogen-specific porphyrin metabolism, sialic acid biosynthesis and cobalamin (B12) autotrophy as a *Leptospira* virulence factor. Besides that, the authors also identified some novel *Leptospira* virulence proteins including pathogen-specific adhesins and Virulence Modifying (VM) proteins. The authors also suggested that CRISPR/Cas system, which is only present in pathogenic Leptospira, could be the underlying causes for *Leptospira* genus refractoriness to gene targeting (Fouts et al., 2016).