Therapeutic Monoclonal Antibodies and their Engineered Antibody Fragments Specific to Lipl32 for Passive Immunotherapy of Leptospirosis

Santi Maneewatchararangsri*

Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400, Thailand

*Corresponding Author: Santi Maneewatchararangsri, Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400, Thailand. Tel: +66 2306 9100 Ext. 2035; Fax: +66 2306 9139; E-mail: santi.man@mahidol.ac.th

Abstract

Leptospirosis is a globally re-emerging neglected zoonotic disease that continues to be a significant human and veterinary public health concern, with 0.1-1/100,000 population and estimated 350,000-500,000 severe cases annually (International Leptospirosis Society surveys). The disease is caused by infections of pathogenic spirochetes of the genus *Leptospira* which are classified into 20 genomospecies and placed more than 250 serovars/strains. Treatment by antibiotics such as doxycycline, ceftriaxone, azithromycin is predominant. Antibiotics provide therapeutic activity when initiated early of illness, and might be less effective at late and severe of human leptospirosis such as Weil’s disease and severe pulmonary hemorrhagic syndrome and also in animal reservoirs. Besides, antibiotics might cause adverse effects, i.e., Jarisch-Herxheimer reaction, due to massive release of the bacterial toxic substances. An immunomodulation or passive immunotherapy by using therapeutic antibodies against the *Leptospira* virulent factors might be the optimal therapeutic approach for the late and severe leptospirosis. *LipL32*, an immunodominant outer membrane protein of pathogenic *Leptospira* spp. has been used as diagnostic biomarker, vaccine candidate for a broad spectrum vaccine development, and therapeutic target for passive immunotherapy of leptospirosis. Passive immunotherapy of experimental leptospirosis by using therapeutic mouse monoclonal antibodies and their single-chain variable fragment antibodies specific to LipL32 have been demonstrated.

Keywords: Leptospirosis; Re-emerging zoonosis; Passive immunotherapy; Therapeutic monoclonal antibody; *LipL32*

Leptospirosis remains one of the most common zooonopathogenic infections found throughout the world, particularly in subtropical and tropical areas, such as central and tropical areas of America, Oceania, and Southeast Asia [1-3]. In Southeast Asian countries, the disease is endemic in Thailand, Lao PDR, Philippines, Indonesia, Malaysia, and Vietnam [2-7]. For industrialized countries, leptospirosis cases tend to be imported subsequent to travel to the disease endemic areas [4-6]. World health organization has estimated incidence of leptospirosis is 0.1-1 case per 100,000 populations annually. The incidence of leptospirosis in Thailand is 10 per 100,000 populations with a 19.7% fatality rate in 2011-2012 [8,9].

The disease is caused by infections of pathogenic spirochetes in the genus *Leptospira*. Within the genus, more than 250 serovars/strains of the spirochetes are classified into 20 genomospecies based on 16S rRNA relatedness [2]. According to pathogenicity in human, *Leptospira* spp. are placed into three clades: pathogenic (cause disease), non-pathogenic (free-living saprophytic, do not infect), and intermediate (infect and may cause disease). Pathogenic *Leptospira* spp. infects both humans and animals such as ruminants, i.e., cattle, buffalo, sheep, goat, horse, and swine, domestic animals, rodents, and wildlife. Animal infections may lead to a variety of clinical outcomes including jaundice, infertiltity, abortion in the late pregnancy, still-birth, failure to thrive, decreased milk production, and death. *Leptospira* spp. are highly adapted to a broad range species, including environments, mammalian reservoirs to human [10]. Animal reservoirs shed *Leptospira* in urine to soil, water and environments and the spirochete infects human at skin and mucosae such as conjunctival, oral, and genital surfaces [11,12]. Risk associated leptospirosis are occupational health and safety regulations such as veterinarians, abattoir workers, animal caretakers, gardeners or farmers [1,10]. In recent years, outbreak of human leptospirosis is found in fresh water sports, such as caving, canoeing, kayaking, rafting, and triathlons. The disease is also a travel-associated bacterial zoonosis [6].

Clinical symptoms of leptospirosis ranges from asymptomatic, non-specific and self limiting febrile illness including chill, headache, fever, which may be confused with other acute febrile illness such as influenza, malaria and dengue fever to life-threatening illness. Muscle pain, myalgias, and ocular suffusion are also present. Weil’s disease, severe pulmonary hemorrhagic syndrome and meningoencephalitis are severe forms of leptospirosis which characterized by renal, hepatic, cardiac and neurologic complications leading to jaundice, acute renal failure, hemorrhage, meningitis and septic shock, and multi-organ failure. Leptospirosis-associated severe pulmonary hemorrhagic syndrome has been reported sporadically or in the disease outbreaks with 5-40% fatality rate [13,14]. Antibiotics such as doxycycline, ceftriaxone, azithromycin, penicillin G, ampicillin have been used for treatment of leptospirosis. Doxycycline has been used in pre- and post-exposure prophylaxis. Severe leptospirosis requires both antibiotics and supportive therapy for improving mortality rate. Treatment with antibiotics may prevent disease severity from mild to severe forms if antimicrobial therapy initiated early and preferably before the fifth day after the onset of the illness. For mild symptom, beta-lactam class antibiotics such as penicillin and amoxicillin are useful. Third-generation cephalosporin in treatment of leptospirosis is exists [15,16]. Amino-glycosides have been considered in the past [17]. Most of the antibiotics treatment may cause adverse effects, i.e., Jarisch-Herxheimer reaction (JHR), a ‘cytokine storm’ caused by the massive release of bacterial toxic substances after administrating antibiotics [18,19]. The JHR associated leptospirosis was reported to be 80% in a
Malayan study. Doxycycline-associated phototoxicity and gastrointestinal side effects have been reported.

Outer membrane of pathogenic Leptospira displays various components play roles in bacterial pathogenesis by acting as adhesins, porins and receptors. LipL32 is regarded as a dominant lipoprotein located at the outer membrane. The LipL32 protein is restricted to pathogenic and intermediate clades of Leptospira serovars. Sequence homology of LipL32 of pathogenic serovars showed more than 94% and decreased to 67% homology in intermediate clade of Leptospira spp. [20-22]. Pathogenic Leptospira expresses LipL32 constitutively in both in vitro culture and during infection in mammalian hosts [20,23]. The protein is highly immunogenic, i.e., LipL32-specific IgG can be detected in acute and convalescing leptospirosis patient's sera [21,23-24]. Thus, LipL32 is a molecular target for leptospirosis diagnosis [24-26]. It is also a potential immunogen for developing universal leptospirosis vaccines [27-29]. LipL32 exhibits hemolytic activity and enhance the hemolytic activity of sphingomyelinase-H (SphH), hence its synonym, hemolysis-associated protein-1(Hap-1) [30-31]. LipL32 was also identified as a member of the Leptospira adhesive matrices (MSCRAMMs), responsible for binding to extracellular matrix (ECM) molecules, including matrigel, laminin, collagens (I and IV) and both intact to 30 and 45-kDa proteolytic fragments of fibronectin (FN) [32-33]. LipL32 also binds to the zymogen plasminogen to generate plasmin [34] which adheres to the proteoglycan of human cell surface receptors [35], to cultured mammalian cells [36] and to neutrophils [37]. Passive immunotherapy of experimental leptospirosis by using LipL32-specific monoclonal antibodies (mAbs) and recombinant antibody fragments have been demonstrated. [38-39]. Thus, LipL32, an immunodominant outer membrane protein of pathogenic Leptospira spp. has been used as diagnostic biomarker, vaccine candidate for a broad spectrum vaccine, and therapeutic target in passive immunotherapy for leptospirosis.

Passive immunotherapy by using therapeutic monoclonal antibodies, conjugated antibodies, bispecific antibodies, antibody fragments such as Fab, F(ab')2, single-chain variable fragment (ScFv), single-variable (dAb) domain have been developed and used as non-drug therapeutic agents for treatment and intervention of infectious diseases, cancer, inflammatory and autoimmune diseases, intoxications, and envenomations [40-42]. Antibody therapy of experimental leptospirosis by monoclonal antibodies (mAbs) directed against agglutinating serovar-specific lipopolysaccharide have been demonstrated in animal model [43-45]. Two murine hybridoma clones secreting monoclonal antibodies, namely mABLPF1 and mABLPF2, specific to the Leptospira LipL32 outer membrane protein have been produced [37]. Both mAbs neutralized Leptospira-mediated hemolysis in vitro, and exhibited therapeutic activity when passively given to experimental hamsters infected with Leptospira spp. [37].

Single-chain variable fragment antibody (ScFv; VH-linker-VL) molecule [42] is an effective therapeutic small molecule with an expected lower (or lack of) immunogenicity and better target epitope accessibility. The molecular mass of ScFv is about 30 kDa compared to the 150 kDa of intact IgG lacks of functional domain (Fc) of immunoglobulin. Murine single chain antibody fragments, as well as humanized-ScFv have been produced from the original mouse mABLPF1. The scFv exhibited therapeutic activity when passively given to experimentally hamsters infected with heterologous Leptospira [39]. Therapeutic LipL32 epitopes and membrane binding inhibitory activity of mAb to MDCK monolayer cells were also investigated [36]. The epitope peptide of mAB LPF1 was mapped to a non-contiguous carboxy-terminal β-turn and amphipathic α-helix of LipL32 structure contributing to phospholipid/host cell adhesion and membrane insertion. We found that the mABLPF2 epitope was located on the interacting loop of peptide binding groove of the LipL32 molecule responsible for interactions with host constituents. Epitope sequences are highly conserved among Leptospira spp. and are absent from the LipL32 super family of other microorganisms. Both epitopes are surface-exposed, readily accessible by mAbs, and immunogenic. However, they are less dominant when revealed by LipL32-specific immunoglobulins [36]. Therapeutic antibodies, particularly the humanized-ScFv, have potential for further development as a non-drug therapeutic agent for human leptospirosis, especially in subjects allergic to antibiotics.

Acknowledgement
Scholarship for academic research presentations aboard (2015) was supported from ICTM grant, Faculty of Tropical Medicine, Mahidol University and from Faculty of Graduate Studies, Mahidol University.

References


