

Therapeutic Monoclonal Antibodies and their Engineered Antibody Fragments Specific to *LipL32* for Passive Immunotherapy of Leptospirosis

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Abstract

Leptospirosis is a globally re-emerging neglected zoonotic disease that continues to be a significant human and veterinary public health concerns, 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 zoonotic 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, infertility, 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- or 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]. Aminoglycosides 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 administering antibiotics [18,19]. The JHR associated leptospirosis was reported to be 80% in a

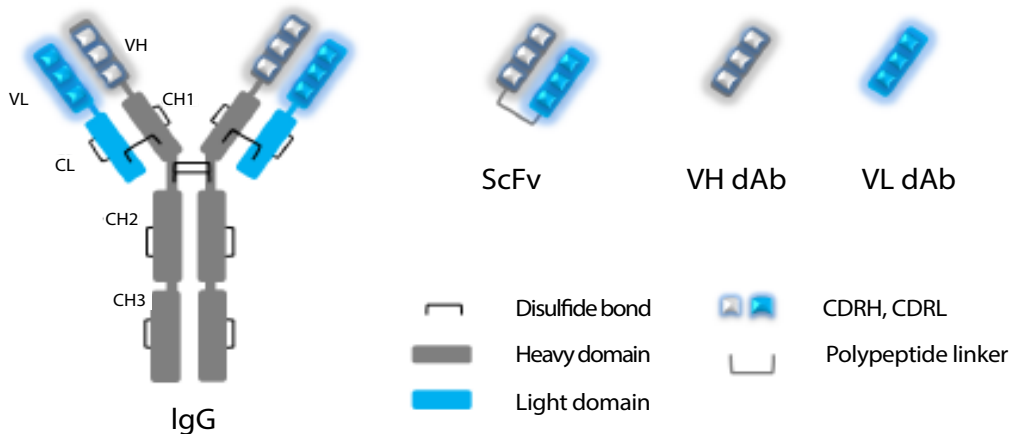


Figure 1: Immunoglobulin G (IgG), single-chain variable fragment (ScFv) and single-variable domain (dAb) antibodies.

IgG antibody consists of two identical heavy chains (grey) and two identical light chains (blue). Heavy chain consists of one variable domain (VH) and three constant CH1, CH2, and CH3 domains, Light chain consists of one variable (VL) and one constant (CL) domain. Complementarity determining regions (CDRH, CDRL) in variable domains are indicated. Full-length IgG contains two antigen-binding regions responsible for binding to specific antigen and constant Fc region (CH2, CH3) responsible for interaction with Fc receptors. Single-chain variable fragment antibody (ScFv) consists of variable domains of heavy (VH) and light (VL) chains joined by a peptide linker.

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 mAbLPP1 and mAbLPP2, 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 mAbLPP1. 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 LPP1 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 mAbLPP2 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.

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References

- Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, et al. (2003) Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* 3: 757-771.
- Adler B, de la Pena-Moctezuma A (2010) *Leptospira* and leptospirosis. *Vet Microbiol* 140: 287-296.
- Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, et al. (2015) Global morbidity and mortality of leptospirosis: A systematic review. *PLoS Negl Trop Dis* 9: e0003898.
- Baranton G, Postic D (2006) Trends in leptospirosis epidemiology in France. Sixty-six years of passive serological surveillance from 1920 to 2003. *Int J Infect Dis* 10: 162-170.
- Ricaldi JN, Vinetz JM (2006) Leptospirosis in the tropics and travelers. *Curr Infect Dis Rep* 8: 51-58.
- Bandara M, Ananda M, Wickramage K, Berger E, Agampodi S (2014) Globalization of leptospirosis through travel and migration. *Global Health* 10: 61.
- Abela-Ridder B, Sikkema R, Hartskeerl RA (2010) Estimating the burden of human leptospirosis. *Int J Antimicrob Agents* 36: S5-7.
- Hinjoy S (2014) Epidemiology of leptospirosis from Thai national disease surveillance system, 2003-2012. *OSIR* 7: 1-5.
- Thipmontree W, Suputtamongkol Y, Tantibhedhyangkul W, Suttinont C, Wongswat E, et al. (2014) Human leptospirosis trends: Northeast Thailand, 2001-2012. *Int J Environ Res Public Health* 11: 8542-8551.
- Levett PN (2001) Leptospirosis. *Clin Microbiol Rev* 14: 296-326.
- Segura E, Ganoza C, Campos K, Ricaldi JN, Torres S, et al. (2005) Clinical spectrum of pulmonary involvement in leptospirosis in an endemic region, with quantification of leptospiral burden. *Clin Infect Dis* 40: 343-351.
- Matthias MA, Ricaldi JN, Cespedes M, Diaz MM, Galloway RL, et al. (2008) Human leptospirosis caused by a new, antigenically unique *Leptospira* associated with a *Rattus* species reservoir in the Peruvian Amazon. *PLoS Negl Trop Dis* 2: e213.
- Vijayachari P, Sugunan AP, Shriram AN (2008) Leptospirosis: an emerging global public health problem. *J Biosci* 33: 557-569.
- Marchiori E, Lourenco S, Setubal S, Zanetti G, Davaus T, et al. (2011) Clinical and imaging manifestations of hemorrhagic pulmonary leptospirosis: a state-of-the-art review. *Lung* 189: 1-9.
- Plank R, Dean D (2000) Overview of the epidemiology, microbiology, and pathogenesis of *Leptospira* spp. in humans. *Microbes Infect* 2: 1265-1276.
- Anselmo M, De Leo P, Calcagno G, Donelli E, Tirota A, et al. (2000) Ceftazidime in the treatment of Weil's disease. *Infez Med* 8: 106-109.
- Kobayashi Y (2005) Human leptospirosis: management and prognosis. *J Postgrad Med* 51: 201-204.
- Pound MW, May DB (2005) Proposed mechanisms and preventive options of jarisch-herxheimer reactions. *J Clin Pharm and Therapeutics* 30: 291-295.
- Watt G, Warrell DA (1995) Leptospirosis and the Jarisch-Herxheimer reaction. *Clin Infect Dis* 20: 1437-1438.
- Haake DA, Chao G, Zuerner RL, Barnett JK, Barnett D, et al. (2000) The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. *Infect Immun* 68: 2276-2285.
- Guerreiro H, Croda J, Flannery B, Mazel M, Matsunaga J, et al. (2001) Leptospiral proteins recognized during the humoral immune response to leptospirosis in humans. *Infect Immun* 69: 4958-4968.
- Murray GL (2013) The lipoprotein LipL32, an enigma of leptospiral biology. *Vet Microbiol* 162: 305-314.
- Sakolvaree Y, Maneewatch S, Jiemsu S, Klaysing B, Tongtawe P, et al. (2007) Proteome and immunome of pathogenic *Leptospira* spp. revealed by 2DE and 2DE-immunoblotting with immune serum. *Asian Pac J Allergy Immunol* 25: 53-73.
- Chalayan P, Chanket P, Boonchawalit T, Chattanadee S, Srimanote P, et al. (2011) Leptospirosis serodiagnosis by ELISA based on recombinant outer membrane protein. *Trans R Soc Trop Med Hyg* 105: 289-297.
- Bomfim MR, Ko A, Koury MC (2005) Evaluation of the recombinant LipL32 in enzyme-linked immunosorbent assay for the serodiagnosis of bovine leptospirosis. *Vet Microbiol* 109: 89-94.
- Toyokawa T, Ohnishi M, Koizumi N (2011) Diagnosis of acute leptospirosis. *Expert Rev Anti Infect Ther* 9: 111-121.
- Aviat F, Rochereau-Roulet S, Branger C, Estavoyer JM, Chatrenet B, et al. (2010) Synthetic peptide issued from Hap1/LipL32 for new early serodiagnosis of human leptospirosis. *Comp Immunol Microbiol Infect Dis* 33: 375-387.
- Branger C, Blanchard B, Fillonneau C, Suard I, Aviat F, et al. (2005) Polymerase chain reaction assay specific for pathogenic *Leptospira* based on the gene hap1 encoding the hemolysis-associated protein-1. *FEMS Microbiol Lett* 243: 437-445.
- Seixas FK, da Silva EF, Hartwig DD, Cergueira GM, Amaral M, et al. (2007) Recombinant *Mycobacterium bovis* BCG expressing the LipL32 antigen of *Leptospira interrogans* protects hamsters from challenge. *Vaccine* 26: 88-95.
- Lee SH, Kim KA, Park YK, Seong IW, Kim MJ, et al. (2000) Identification and partial characterization of a novel hemolysin from *Leptospira interrogans* serovar lai. *Gene* 254: 19-28.
- Hauk P, Negrotto S, Romero EC, Vasconcellos SA, Genovez ME, et al. (2005) Expression and characterization of HlyX hemolysin from *Leptospira interrogans* serovar Copenhageni: potentiation of hemolytic activity by LipL32. *Biochem Biophys Res Commun* 333: 1341-1347.
- Hauk P, Macedo F, Romeo EC, Vasconcellos SA, de Moraes ZM, et al. (2008) In LipL32, the major leptospiral lipoprotein, the C terminus is the primary immunogenic domain and mediates interaction with collagen IV and plasma fibronectin. *Infect Immun* 76: 2642-2650.
- Hoke DE, Egan S, Cullen PA, Adler B (2008) LipL32 is an extracellular matrix-interacting protein of *Leptospira* spp. and *Pseudoalteromonas tunicata*. *Infect Immun* 76: 2063-2069.
- Vieira ML, de Moraes ZM, Gonçalves AP, Romero EC, Vasconcellos SA, et al. (2010) Lsa63, a newly identified surface protein of *Leptospira interrogans* binds laminin and collagen IV. *J Infect* 60: 52-64.
- Breiner DD, Fahey M, Salvador R, Novakova J, Coburn J (2009) *Leptospira interrogans* binds to human cell surface receptors including proteoglycans. *Infect Immun* 77: 5528-5536.
- Maneewatch S, Adisakwattana P, Saengjaruk P, Srimanote P, Chairisri U, et al. (2014) Therapeutic epitopes of *Leptospira* LipL32 protein and their characteristics. *Protein Eng Des Sel* 27: 135-144.
- Wang B, Sullivan J, Sullivan GW, Mandell GL (1984) Interaction of leptospire with human polymorphonuclear neutrophils. *Infect Immun* 44: 459-464.
- Maneewatch S, Sakolvaree Y, Saengjaruk P, Srimanote P, Tapchaisri P, et al. (2008) Monoclonal antibodies to LipL32 protect against heterologous *Leptospira* spp. challenge. *Hybridoma (Larchmt)* 27: 453-65.

39. Maneewatch S, Sakolvaree Y, Tapchaisri P, Saengjaruk P, Songserm T, et al. (2009) Humanized-monoclonal antibody against heterologous *Leptospira* infection. *Protein Eng Des Sel* 22: 305-312.
40. Casadevall A, Dadachova E, Pirofski LA (2004) Passive antibody therapy for infectious disease. *Nat Rev Microbiol* 2: 695-703.
41. Weiner GJ. (2015) Building better monoclonal antibody-based therapeutics. *Nature Reviews Cancer* 15: 361-370.
42. Huston JS, Levinson D, Mudgett-Hunter M, Tai MS, Novotný J, et al. (1988) Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*. *Proc Natl Acad Sci USA* 85: 5879-5883.
43. Adler B, Faine S (1978) The antibodies involved in the human immune response to leptospiral infection. *J Med Microbiol* 11: 387-400.
44. Jost BH, Adler B, Vinh T, Faine S (1986) A monoclonal antibody reacting with a determinant on leptospiral lipopolysaccharide protects guinea pigs against leptospirosis. *J Med Microbiol* 22: 269-275.
45. Schoone GJ, Everard CO, Korver H, Carrington DG, Inniss VA, et al. (1989) An immunoprotective monoclonal antibody directed against *Leptospira interrogans* serovar *copenhageni*. *J Gen Microbiol* 135: 73-78.