Pathogenesis insights from an ancient and ubiquitous spirochete

Jenifer Coburn, Mathieu Picardeau, Christopher W. Woods, Timothy Veldman, David A. Haake

1 Medical College of Wisconsin, Milwaukee, Wisconsin, United States of America, 2 Pasteur Institute, Paris, France, 3 Duke University Medical Center, Durham, North Carolina, United States of America, 4 Durham VA Medical Center, Durham, North Carolina, United States of America, 5 VA Greater Los Angeles Healthcare System, Los Angeles, California, United States of America, 6 The David Geffen School of Medicine at the University of California, Los Angeles, California, United States of America

* dhaake@ucla.edu

Introduction

Leptospirosis is a ubiquitous zoonotic infection caused by bacterial spirochetes that are equally adapted to life in the aqueous environment as they are to infection of their eucaryotic hosts. Leptospires owe their ubiquity to having evolved from free-living saprophytes to become non-pathogenic commensals of a wide range of mammals and, although not as well documented, birds, amphibians, and reptiles [1,2]. They colonize the proximal renal tubules of the host, in which they proliferate in the nutrient-rich glomerular filtrate, and from which they are shed into the environment by host urination. Most infections are mild or asymptomatic, but others result in organ failure and death (Fig 1). Significant impacts on human well-being have been documented, with an estimated 1 million cases and approximately 59,000 deaths per year, many of which occur in tropical, medically underserved regions of the world [3]. Leptospirosis affects not only human health but also livestock farming, causing great economic or subsistence resources losses. Despite the fact that leptospirosis has been much less investigated than other illnesses with comparable or even lower burden [4], a number of remarkable discoveries have recently emerged about these organisms and the infections they cause.

Massive species diversity

With the description of many new leptospiral genomes, a striking pattern of massive species diversity has emerged: Leptospira species belonging to the P1 clade, which includes human pathogens, have an open pan-genome with a relatively high number of genes found only in a single species (Fig 2) [5]. The open pan-genome reflects the leptospiral life cycle, which includes the ability of leptospiral pathogens to form biofilms to withstand environmental stress and survive for prolonged periods in milieux such as soil and aqueous habitats [6]. Such settings contain complex microbial and chemical compositions, facilitating a high rate of horizontal gene transfer that enables reworking of cellular functions to allow rapid adaptation to new environmental conditions and hosts. Leptospiral genes and gene fragments are derived both from unrelated bacteria and other leptospires, resulting in mosaic genes with diverse phylogenetic ancestry [7,8]. Clues to which pathogen-specific genes are required for virulence have been obtained from RNAseq studies examining differential expression during adaptation to the mammalian host [9]. Determining the impact of specific genes on in vivo fitness has also been determined by high-throughput sequencing of tissues from animals challenged with transposon mutant pools [10]. Targeted mutagenesis in leptospiral pathogens has been
challenging and has only recently become more reliable through the development of the CRISPR dCas9 and transcription activator-like effectors (TALEs) as gene silencing approaches [11,12]. These novel tools made it possible to show that the pathogen-specific, multifunctional Leptospiral immunoglobulin-like domain (Lig) proteins were required for virulence [11,12].

**Rapid dissemination**

Rapid and widespread dissemination to all organs, including the eye and brain, is a hallmark of leptospirosis. Remarkably, *Leptospira interrogans* is detectable in all organs of perfused animals within 1 hour after intraperitoneal inoculation of hamsters [13]. Consistently, flagellar mutants with decreased motility are attenuated for virulence in animal models [14,15]. Leptospires are uniquely equipped for dissemination through their corkscrew morphology, 200 nM diameter (in Greek, leptos means “thin”), and powerful propulsion by their endoflagella. As in other spirochetes, the organs of motility are called “endoflagella” because they are not surface exposed; instead, they are entirely within the periplasm. Leptospires are unique in that they have a single supercoiled endoflagellum extending axially toward the middle of the cell from a flagellar motor embedded in the inner membrane at each pole, without overlap. Endoflagellar rotation imparts a swimming motility that enables leptospires to be particularly invasive at liquid–gel borders, such as the interface between the vascular lumen and the endothelium, with 30 times the swimming force of *Escherichia coli* [16]. Recent elegant high-resolution cryo-electronic tomography studies have shown that the function of leptospiral endoflagellar filaments relies on an asymmetric sheath that imparts their supercoiled structure [17].

**Host tissue barrier invasion**

Another key to efficient transmigration across host tissue barriers appears to be targeting of E- and VE-cadherins, which are integral to the intercellular adherens junctions of epithelium and
Virulent *L. interrogans*, but not the saprophytic *Leptospira biflexa*, disrupt adherens junctions of endothelial and epithelial cells in vitro, resulting in loss of VE- and E-cadherins and disruption of the associated catenins, which link cadherins to the intracellular actin cytoskeleton. A clue to the molecular mechanisms for endothelial barrier disruption emerged from a study examining the crystal structures of 4 *L. interrogans* leucine-rich repeat (LRR) proteins [20]. As has been observed with other LRR proteins, the 23-residue repeating leucine-rich motifs formed a characteristic curved solenoid structure. One of these LRR proteins, LIC10831, has a structure and binding pocket similar to that of the *Listeria monocytogenes* internalin InlA. InlA mediates the first step of listerial invasion of the intestinal epithelium by binding to unpaired E-cadherin proteins as they become accessible through the normal sloughing of intestinal epithelial cells at the tip of the brush border. LIC10831 is actively secreted by *L. interrogans* and specifically binds E- and VE-cadherins at a binding coefficient 10-fold lower than InlA [21]. Importantly, LIC10831, but not an LRR protein (LIC12234) that bound other host factors, bound specifically to the cell–cell junctions of endothelial cells. Two other VE-cadherin adhesins were identified using phage display [22].
suggesting that targeting of endothelial barrier integrity may be a key feature of pathogenic *Leptospira* species.

**Evasion of innate immune recognition**

Reflecting their ancient history of coevolution with eukaryotes, pathogenic leptospires have evolved an array of novel strategies to evade and/or alter the innate immune response. These strategies may well be important in extending the time of persistence in the renal tubules of reservoir hosts for shedding in the urine. Leptospires are stealth pathogens that evade recognition with altered microbial-associated molecular patterns (MAMPs) structures [23]. For example, *Leptospira* spp. escape murine TLR5 recognition through the peculiar subsurface localization and stability of the FlaB flagellar subunits, combined with specific down-regulation of FlaB transcription during mammalian infection [23]. An additional immune evasion strategy involves leptospiral lipoproteins that bind MAMPs and block their recognition by host pattern recognition receptors (PRRs) of the Toll- and NOD-like families. In some cases, these PRR-blocking lipoproteins are some of the most abundant proteins in pathogenic leptospires. This strategy applies to leptospiral lipid A, which is similar to gram-negative endotoxin but differs structurally in key ways such that, although it is recognized by mouse TLR4, it is not recognized by human TLR4 [24]. In fact, C3H/HeJ mice lacking TLR4 are more susceptible to leptospiral infection than C3H mice with an intact TLR4 [25]. However, even in mice with intact TLR4, leptospiral lipopolysaccharide (LPS) O-antigen and multiple LPS-binding lipoproteins reduce TLR4-mediated uptake by macrophages and their TRIF-dependent activation [26]. Similarly, LipL21, a major leptospiral lipoprotein has now been recognized as a peptidoglycan-binding lipoprotein that enables escape from NOD1 and NOD2 recognition [27].

**Evasion of innate killing mechanisms**

Leptospires have evolved a variety of strategies for evasion of host innate killing mechanisms. These include escape from complement by surface presentation of LigA, LigB, and other proteins that bind host complement regulators [28,29]. Pathogenic leptospires also express catalase, encoded by *katE*, which is required for resistance to reactive oxygen species (ROS) and for virulence in the hamster model [30]. Expression of *katE* is transcriptionally controlled by the peroxide stress regulator PerR, a novel H$_2$O$_2$ sensor [31–33]. Recently, a second potential PerR was identified, called PerRB, which is present only in pathogenic *Leptospira* species. Inactivating *perRA* or *perRB* led to an increased tolerance to 2 different components of the phagocytic oxidative burst, H$_2$O$_2$ and superoxide, respectively, indicating that the 2 regulators do not have a redundant functions [34]. While single *perRA* and *perRB* mutants were virulent for hamsters, the double mutant was avirulent for hamsters [34]. These results indicate that, although the double mutant has the metabolic pathways required for infection, it lacks expression of specific virulence-related gene products. Interestingly, RNAseq and protein expression studies involving *perRA* and *perRB* single and double mutants revealed complex regulons, including many coding and noncoding RNAs, some of which are likely unrelated to resistance to oxidative stress [34].

**Rapid diagnostics**

Leptospirosis is a common cause of acute febrile illness in areas where dengue, malaria, and rickettsial infections are also common. Though the majority of cases are mild, life-threatening leptospirosis is common in many tropical countries with a case fatality rate exceeding that of other common febrile illnesses. Our ability to identify the etiology of febrile illness and provide
targeted therapy is limited by the nonspecific clinical presentations, low sensitivity of molecular methods, and low specificity and delayed utility of serological methods. Standard biomarkers such as C-reactive protein (CRP) and procalcitonin have relatively low specificity for leptospirosis [35]. In contrast, the transcriptional response to leptospirosis and scrub typhus is distinguishable from that of patients with viral etiologies [35]. Likewise, proteomic approaches utilizing multiple inflammatory biomarkers such as serum amyloid A and leucine-rich alpha-2 glycoprotein provide diagnostic utility greatly superior to CRP or PCT alone and are more readily translated to rapid diagnostic platforms suitable for use in low resource healthcare settings. Host response signatures and biomarkers such as decreased cathelicidin may also enable prediction of disease severity among patients with early infection [36] and identify patients who would benefit from antibiotics and other interventions.

Conclusions

While much more work is needed to validate and expand these discoveries, leptospirosis research is providing unprecedented insights regarding these ancient pathogens and their host interactions. Further elucidation of leptospiral genetic diversity and its global health impacts are needed, both in humans and in both domestic and wild animals using a One Health perspective. The coevolution of leptospires with their eukaryotic hosts is reflected in their novel mechanisms for immune evasion and escape from innate killing strategies. A better understanding of the remarkably rapid and efficient dissemination process will provide opportunities for improved prevention strategies including vaccine development. Finally, host response and biomarker approaches to diagnosis are needed to rapidly diagnose suspected cases and expedite their management to improve patient outcomes. A better disease burden estimate of causes of fever, including leptospirosis, in developing countries would also be most useful for policy and decision makers.

References

1. Picardeau M, Bulach DM, Bouchier C, Zuerner RL, Zidane N, Wilson PJ, et al. Genome sequence of the saprophyte Leptospira biflexa provides insights into the evolution of Leptospira and the pathogenesis of leptospirosis. PLoS ONE. 2008; 3(2):e1607. https://doi.org/10.1371/journal.pone.0001607 PMID: 18270594.
2. Xu Y, Zhu Y, Wang Y, Chang YF, Zhang Y, Jiang X, et al. Whole genome sequencing revealed host adaptation-focused genomic plasticity of pathogenic Leptospira. Sci Rep. 2016; 6:20020. Epub 2016/02/03. https://doi.org/10.1038/srep20020 PMID: 26833181; PubMed Central PMCID: PMC4735792.
3. Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, et al. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. PLoS Negl Trop Dis. 2015; 9(9):e0003898. Epub 2015/09/18. https://doi.org/10.1371/journal.pntd.0003898 PMID: 26379143; PubMed Central PMCID: PMC4574773.
4. Goarant C, Picardeau M, Morand S, McIntyre KM. Leptospirosis under the bibliometrics radar: evidence for a vicious circle of neglect. J Glob Health. 2019; 9(1):010302. Epub 2019/01/04. https://doi.org/10.7189/joghl.09.010302 PMID: 30603077; PubMed Central PMCID: PMC6304173 form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no conflict of interest.
5. Vincent AT, Schiettekatte O, Goarant C, Neela VK, Bernet E, Thibeaux R, et al. Revisiting the taxonomy and evolution of pathogenicity of the genus Leptospira through the prism of genomics. PLoS Negl Trop Dis. 2019; 13(5):e0007270. Epub 2019/05/24. https://doi.org/10.1371/journal.pntd.0007270 PMID: 31120895; PubMed Central PMCID: PMC6532842.
6. Thibeaux R, Soupe-Gilbert ME, Kainiu M, Girault D, Bierque E, Fernandes J, et al. The zoonotic pathogen Leptospira interrogans mitigates environmental stress through cyclic-di-GMP-controlled biofilm production. NPJ Biofilms Microbiomes. 2020; 6(1):24. Epub 2020/06/14. https://doi.org/10.1038/s41522-020-0134-1 PMID: 32632986; PubMed Central PMCID: PMC7293261.
7. Haake DA, Suchard MA, Kelley MM, Dundoo M, Alt DP, Zuerner RL. Molecular evolution and mosaicism of leptospiral outer membrane proteins involves horizontal DNA transfer. J Bacteriol. 2004; 186
21. Eshghi A, Gaultney RA, England P, Brule S, Miras I, Sato H, et al. An extracellular Leptospira interrogans leucine-rich repeat protein binds human E- and VE-cadherins. Cell Microbiol. 2019; 21(2):e12949. Epub 2021/04/18. https://doi.org/10.1111/cmi.12949 PMID: 33469138; PubMed Central PMCID: PMC7815788.

22. Lambert A, Picardeau M, Haake DA, Sermswan RW, Srikram A, Adler B, et al. FlaA proteins in Leptospira spp. and Pseudoalteromonas tunicata. Infect Immun. 2015; 83(1):186–92. Epub 2015/01/10. https://doi.org/10.1128/IAI.01643-07 PMID: 18285490.

23. Evangelista KV, Hahn B, Wunder EA Jr., Ko AI, Haake DA, Coburn J. Identification of cell-binding adhesins of Leptospira interrogans. PLoS Negl Trop Dis. 2014; 8(10):e3215. Epub 2014/10/03. https://doi.org/10.1371/journal.pntd.0003215 PMID: 25275630; PubMed Central PMCID: PMC4183468.
24. Werts C, Tapping RI, Mathison JC, Chuang TH, Kravchenko V, Saint Girons I, et al. Leptospiral lipo-polysaccharide activates cells through a TLR2-dependent mechanism. Nat Immunol. 2001; 2(4):346–52. Epub 2001/03/29. https://doi.org/10.1038/86354 PMID: 11276206.

25. Viriyakosol S, Matthias MA, Swancutt MA, Kirkland TN, Vinetz JM. Toll-like receptor 4 protects against lethal Leptospira interrogans serovar icterohaemorrhagiae infection and contributes to in vivo control of leptospiral burden. Infect Immun. 2006; 74(2):887–95. Epub 2006/01/24. https://doi.org/10.1128/IAI.74.2.887-895.2006 PMID: 16428731; PubMed Central PMCID: PMC1360355.

26. Bonhomme D, Scontecchia I, Vernel-Pauillac F, Caroff M, Germon P, Murray G, et al. Leptospiral LPS escapes mouse TLR4 internalization and TRIF-associated antimicrobial responses through O antigen and associated lipoproteins. PLoS Pathog. 2020; 16(8):e1008639. Epub 2020/08/14. https://doi.org/10.1371/journal.ppat.1008639 PMID: 32790743; PubMed Central PMCID: PMC7447051.

27. Ratet G, Scontecchia I, Fanton d’Andon M, Vernel-Pauillac F, Wheeler R, Lenormand P, et al. LipL21 lipoprotein binding to peptidoglycan enables Leptospira interrogans to escape NOD1 and NOD2 recognition. PLoS Pathog. 2017; 13(12):e1006725. Epub 2017/12/07. https://doi.org/10.1371/journal.ppat.1006725 PMID: 29211798; PubMed Central PMCID: PMC5764436.

28. Breda LC, Hsieh CL, Castiblanco Valencia MM, da Silva LB, Barbosa AS, Blom AM, et al. Fine Mapping of the Interaction between C4b-Binding Protein and Outer Membrane Proteins LigA and LigB of Pathogenic Leptospira interrogans. PLoS Negl Trop Dis. 2015; 9(10):e0004192. Epub 2015/10/31. https://doi.org/10.1371/journal.pntd.0004192 PMID: 26517116; PubMed Central PMCID: PMC4627802.

29. Barbosa AS, Isaac L. Strategies used by Leptospira spirochetes to evade the host complement system. FEBS Lett. 2020; 594(16):2633–44. Epub 2020/03/11. https://doi.org/10.1002/1873-3468.13768 PMID: 32153015.

30. Eshghi A, Lourdault K, Murray GL, Bartpho T, Sermawan RW, Picardeau M, et al. Leptospira interrogans catalase is required for resistance to H2O2 and for virulence. Infect Immun. 2012; 80:3892–9. https://doi.org/10.1128/IAI.00466-12 PMID: 22927050.

31. Zavala-Alvarado C, Sismeiro O, Legendre R, Varell H, Bussotti G, Bayram J, et al. The transcriptional response of pathogenic Leptospira to peroxide reveals new defenses against infection-related oxidative stress. PLoS Pathog. 2020; 16(10):e1008904. Epub 2020/10/07. https://doi.org/10.1371/journal.ppat.1008904 PMID: 33021995; PubMed Central PMCID: PMC7567364.

32. Keboouchi M, Saul F, Taher R, Landier A, Beaudeau B, Dubrac S, et al. Structure and function of the Leptospira interrogans peroxide stress regulator (PerR), an atypical PerR devoid of a structural metal-binding site. J Biol Chem. 2018; 293(2):497–509. Epub 2017/11/18. https://doi.org/10.1074/jbc.M117.804443 PMID: 29146596; PubMed Central PMCID: PMC5767856.

33. Lo M, Murray GL, Khoo CA, Haake DA, Zuerer RL, Adler B. Transcriptional response of Leptospira interrogans to iron limitation and characterization of a putative fur mutant. [Submitted for Publication]. 2010.

34. Zavala-Alvarado C, Samuel Garcia Huete S, Vincent AT, Sismeiro S, Legendre R, Varell H, et al. The oxidative stress response of pathogenic Leptospira is controlled by two peroxide stress regulators which putatively cooperate in controlling virulence. bioRxiv. 2021; 2021.11.06.371039v2. https://doi.org/10.1101/2021.11.06.371039

35. Tillekeratne LG, Suchindran S, Ko ER, Petzold EA, Bodinayake CK, Nagahawatte A, et al. Previously Derived Host Gene Expression Classifiers Identify Bacterial and Viral Etiologies of Acute Febrile Respiratory Illness in a South Asian Population. Open Forum Infect Dis. 2020; 7(6):ofaa194. Epub 2020/07/04. https://doi.org/10.1093/ofid/ofaa194 PMID: 32617371; PubMed Central PMCID: PMC7314590.

36. Lindow JC, Wunder EA Jr., Popper SJ, Min JN, Mannam P, Srivastava A, et al. Cathelicidin Insufficiency in Patients with Fatal Leptospirosis. PLoS Pathog. 2016; 12(11):e1005943. Epub 2016/11/05. https://doi.org/10.1371/journal.ppat.1005943 PMID: 27812211; PubMed Central PMCID: PMC5094754.