Institut Pasteur de Nouvelle-Calédonie

Leptospirosis Research and Expertise Unit

Master 2 Internship Proposal 2016-2017

(from January 2017 to June 2017)

Host laboratory:

Leptospirosis Research and Expertise unit
Institut Pasteur International Network, Institut Pasteur in New Caledonia,
9–11 Avenue Paul Doumer, BP 61, 98 845 Noumea, New Caledonia
- Head Dr Cyrille Goarant, cgoarant@pasteur.nc
Phone: +687 277531

Internship supervisor:

-Dr Roman Thibeaux, rthibeaux@pasteur.nc
Post-doctoral fellow in the "Leptospirosis Research and Expertise Unit"

Title: Role of *Leptospira* biofilm upon colonization of an *in vitro* model of proximal renal tubule

• Introduction, state-of-the-art and objectives of the internship

Leptospirosis is a widespread anthropozoonosis caused by multi-host pathogens within the genus *Leptospira*. Leptospirosis occurs as rural endemics, urban outbreaks related to floods, and emergent disease associated with water sports and recreational exposure in developed countries. The global burden of leptospirosis worldwide was recently reevaluated to over 1 million cases and almost 60,000 deaths annually.

Pathogenic leptospires are transmitted to humans by indirect contact with contaminated urine from infected mammals. Animals are most frequently divided into maintenance (or reservoir), and incidental hosts. Maintenance hosts are typically clinically asymptomatic, and *Leptospira* succeed in evading the immune response to colonize renal tubules from which they are shed in urine. Leptospiruria in maintenance hosts is of high intensity, constant, and of long duration. Rodents are known to be the main reservoir for *Leptospira* because they are persistent renal carriers, but rarely develop symptoms and are not impaired by the infection of their kidneys. In incidental hosts such as humans, tubulointerstitial nephritis is the most common lesion associated with acute infection, and may progress to fibrosis and subsequent renal failure.

Renal carriage is thus a key step in the persistence and epidemiology of leptospirosis. The kidney is a primary target of *Leptospira* during both acute and chronic infection, where conditions in the renal tubules favor *Leptospira* survival. However, details of the mechanisms of immune evasion by *Leptospira* during renal tubular colonization remain unclear, because leptospires survive and are shed from chronically infected hosts despite an initial specific host immune response.

As survival of planktonic leptospires in acidic urine is supposedly hampered, they might have evolved strategies for long-term colonization of renal tubules. *In vivo*, in reservoir mammals, long-term colonization of proximal renal tubules by pathogenic leptospires is believed to proceed via the formation of bacterial aggregates. Some reports suggest that virulent leptospires are able to form a biofilm in renal tubules, thought to help pathogenic strains escape the immune response and maintain long-term carriage in reservoir hosts. Biofilms are colonies of bacteria embedded within a protective matrix, which enables bacterial growth in hostile conditions. Genes thought to facilitate alginate biosynthesis involved in biofilm formation were found in the genomes of pathogenic *Leptospira*, corroborating their ability to form a biofilm *in vitro*. Interestingly, biofilm-associated microorganisms display increased resistance towards the host's immune response and several antibiotics and antimicrobial agents as compared to their free-living counterparts. Understanding

Leptospira biofilm unique organization and protective functions may yield important insights into *Leptospira* persistence in its host.

As yet, there is no evidence to support the hypothesis that biofilm formation is required for *Leptospira* renal tubule colonization and immune evasion during chronic infection. The current Internship proposal will aim at addressing the role of *Leptospira* biofilm as a mechanism of persistence for *Leptospira* in proximal renal tubules, allowing long-term survival in a nutrient-poor adverse environment.

The specific objective of this project will be to investigate the formation, the organization and the protective functions of *Leptospira* biofilm during chronic colonization of proximal renal tubules. As rats are consistently reported as asymptomatic carriers, they theoretically represent an ideal model to explore natural resistance to chronic *Leptospira* renal carriage. However, animal inoculations remain time-consuming and ethically-questionable. In order to avoid those pitfalls, we will develop an *in vitro*, tissue-based model as a replacement for animal models in testing chronic carriage of rat renal tubules.

• Research methodology and approach

a.) Set up the in vitro*, tissue-based model:*

Rat Renal Proximal Tubular Epithelial Cells (RRPTEpiC line) will be used as a cellular model to mimic the interaction of virulent bacteria with renal epithelial cells. In order to obtain a tissue like structure, RRPTEpiC will be grown in Epithelial Cell medium-A (EpiCM-a) on a fibrillar collagen layer. Proper differentiation of the *in vitro* model in a tissue-like structure will be assessed through expression (western-blot) and localization (immunofluorescence) analyses of standard markers of epithelium formation Villin, ZO-1, cytokeratin-18, α-SMA, E-cadherin). Cell polarization and morphology will be investigated by histology and scanning electron microscopy. Complexification of the model may be considered (i.e addition of macrophages within the collagen matrix) if previous goals are reached in a reasonable time.

b.) Biofilm formation and structural organization at the surface of kidney epithelium:

Biofilm formation will be challenged through co-culture of pathogenic leptospires with the *in vitro* Renal proximal tubular model. Following an initial set up under static co-cultures conditions, a transient flow mimicking urine discharge will be implemented to the model. The organization of *Leptospira* biofilm at the contact site with epithelial cells will be explored by histological analysis and scanning electron microscopy. The dynamic of matrix production during co-culture will be investigated by incorporating a fluorescent marker during synthesis of the extracellular polysaccharide matrix (Alexa fluor 647-labeled dextran) and visualized by fluorescent microscopy at different time points.

These experiments will considerably help to decipher and understand the establishment and the protective role of *Leptospira* biofilm within reservoir hosts.

A specific time slot dedicated to the writing of the report is planned at the end of the internship.

Practical aspects:

This study will take place in New Caledonia (NC, a French overseas collectivity) where Leptospirosis is endemic. Mean annual incidence is 45 cases per 100,000 inhabitants and highest incidence (500 cases/100,000 inhabitants) is reported in particular hot spots where leptospirosis is “hyper-endemic”. The Host Laboratory (UREL) belongs to the Institut Pasteur in New-Caledonia and has developed almost one decade of expertise in leptospirosis. This internship proposal takes place within the framework of a larger project granted by the AXA Research Fund, Postdoctoral Research Fellowship. Dr Thibeaux Roman (AXA post-doctoral grantee) commits to train the Master 2 trainee through daily support during the 6-month period of the internship. The granted will be paid in accordance with prevailing relevant legislation in New Caledonia. Neither the plane tickets nor housing commodities are taken in charge by the host laboratory.

Candidate requirements:

We are seeking outstanding candidates who have a passion for research. The selected candidate should be an enthusiastic, highly motivated and team-oriented scientist. Excellent scientific and organizational skills are requested with the ability to work in close collaboration (4 hands experiments will be performed). The successful candidate is expected to have good background in general microbiology, bacterial genetics and molecular biology. Good communication, French/English language skills and desire to work on interdisciplinary scientific questions will be appreciated. Working under sterile procedure is a plus.

Please send your application before end of November to rthibeaux@pasteur.nc with a CV, a motivation letter and the contact of two references.

References.

1. **Monahan, A., J. Callanan, and J. Nally, *Host-pathogen interactions in the kidney during chronic leptospirosis.* Vet Pathol, 2009. 46(5): p. 792-799.**
2. **Van der Hauwaert, C., et al., *Isolation and characterization of a primary proximal tubular epithelial cell model from human kidney by CD10/CD13 double labeling.* PLoS ONE, 2013. 8(6): p. e66750.**
3. **Baer, P.C., et al., *Differentiation status of human renal proximal and distal tubular epithelial cells in vitro: Differential expression of characteristic markers.* Cells Tissues Organs, 2006. 184(1): p. 16-22.**
4. **Tucunduva de Faria, M., et al., *Morphological alterations in the kidney of rats with natural and experimental Leptospira infection.* J Comp Pathol, 2007. 137(4): p. 231-8.**
5. **Ristow, P., et al., *Biofilm formation by saprophytic and pathogenic leptospires.* Microbiology, 2008. 154(Pt 5): p. 1309-17.**