Stuck in the throat: Dissecting the role of the Leishmania biofilm in parasite development and transmission

Supervisory team

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Project in summary

Leishmaniasis is a neglected tropical disease caused by the unicellular parasite Leishmania that disproportionately affects socio-economically disadvantaged communities and is challenging to control. It is transmitted by the bite of a female sand fly in which the parasite has multiple developmental stages and adapts its environment by the generation of a biofilm. The parasites secrete a large amount of proteophophoglycans (PPGs) that condense in the sand fly midgut to generate a biofilm in which the parasites are embedded. The presence of the biofilm modifies the blood feeding behaviour of the vector and forces them to regurgitate parasites.

We will investigate the cellular impact of biofilm biogenesis for Leishmania and sand flies, with an overarching aim to define its key features using molecular microbiology, cell biology, biochemistry and a variety of light and electron microscopy techniques of parasites within the biofilm to better understand Leishmania infection of the sand fly vector, its transmission and pathogenesis.

Project Key Words
parasitology, biofilm, insect vector, pathogenesis, transmission

MRC LID Themes
Global Health = Yes
Health Data Science = No
Infectious Disease = Yes
Translational and Implementation Research = No

Skills
MRC Core Skills
Quantitative skills = Yes
Interdisciplinary skills = Yes
Whole organism physiology = Yes

Skills we expect a student to develop/acquire whilst pursuing this project

- Molecular biology – Creation of defined isogenic mutants and complemented strains. These PPG mutants will be used for sand fly infections and downstream in vitro and in vivo assays.
- Parasitology – We will use a range of in vitro and in vivo host-pathogen models, including sand fly infection to investigate gel biogenesis and function.
- Microscopy – We will use a variety of microscopic techniques (light, confocal, electron) to track the behaviour of Leishmania, assess their distribution in situ and investigate the ultrastructure of the interaction with the biofilm.
- Biochemistry – We will use a variety of techniques such as Western blotting, ELISA, and surface plasmon resonance to assess the role of CRP in biofilm and parasite distribution.

Routes

Which route/s is this project available for?
1+4 = Yes
+4 = Yes

Possible Master's programme options identified by supervisory team for 1+4 applicants:

- LSHTM – MSc Medical Parasitology

Full-time/Part-time Study

Is this project available for full-time study? Yes
Is this project available for part-time study? No

Eligibility/Requirements

Particular prior educational requirements for a student undertaking this project
LSHTM's standard institutional eligibility criteria for doctoral study.
The doctoral candidate should have completed an undergraduate or postgraduate degree related to microbiology or parasitology with an element of molecular biology.

Other useful information

Potential CASE conversion? = No

Project in more detail

Scientific description of this research project

Project Objectives:
Leishmania are parasitic protozoa that infect sand flies for transmission to vertebrates and use surface and secreted glycans to accomplish this. Leishmania block the midgut of the vector with a glycan-rich biofilm forcing it to regurgitate infective metacyclic forms into the skin. This is analogous to plague transmission by fleas blocked with Yersinia pestis bacteria. However, unlike plague which glue themselves to cuticular spines in the flea's proventriculus, Leishmania use proteophophoglycans (PPGs) to form a biofilm inside the insect – filling the midgut lumen. Inside this biofilm, Leishmania complete their development to become infectious and biofilm accumulation can influence the proportion of the infective metacyclics transmitted. Recently, we found that C-reactive protein P (CRP), a component of vertebrate serum, has very high affinity for PPGs. Collectively, these observations raise important developmental, biophysical and biochemical questions about the biogenesis of the biofilm, how Leishmania interacts with it and how this might change when the sand fly takes another bloodmeal. To investigate these, we seek to observe the behaviour of Leishmania in

the biofilm and how this is modified when specific PPGs are not present. Leishmania have an array of five large genes responsible for the production of distinct PPGs, which have roles for different parasite developmental stages. We will interrogate their role in the formation and function of the biofilm through Cre-Lox mediated deletion and use our pooled expertise in cell biology, microscopy (including serial block face electron microscopy), biochemistry, sand fly infection and transmission to address how biofilm integrity affects Leishmania:
Development in sand flies: by comparing the establishment of mutant infection in sand flies and their developmental progression with reference to position in the sand fly gut. Key aspects of the vector-parasite interaction will be focused on, namely: midgut attachment, migration and movement in the biofilm, valve attachment and metacyclogenesis.
Transmission from sand flies: by assessing the relationship between the composition of the infectious dose (parasite number, proportion of different promastigote forms, and quantity of co-regurgitated biofilm) with biofilm biogenesis, its biophysical properties and its impact on the blood feeding behaviour of the vector.
Distribution and ultrastructural arrangement in the biofilm: by tracking fluorescent parasite distribution in the gel and serial block face electron microscopy to explore the relationship between Leishmania and the biofilm in situ.
Investigate how CRP modifies these features when the infected sand fly takes a second bloodmeal. Specifically, explore the possibility that CRP can alter the local distribution of biofilm and parasites within and how it influences transmission.

Techniques to be used:

- Molecular biology – Creation of defined isogenic mutants and complemented strains. These PPG mutants will be used for sand fly infections and downstream in vitro and in vivo assays.
- Parasitology – We will use a range of in vitro and in vivo host-pathogen models, including sand fly infection to investigate gel biogenesis and function.
- Microscopy – We will use a variety of microscopic techniques (light, confocal, electron) to track the behaviour of Leishmania, assess their distribution in situ and investigate the ultrastructure of the interaction with the biofilm.
- Biochemistry – We will use a variety of techniques such as Western blotting, ELISA, and surface plasmon resonance to assess the role of CRP in biofilm and parasite distribution.

Confirmed availability of any required databases or specialist materials:
N/A

Potential risks to the project and plans for their mitigation:
If the PPG knockout is unsuccessful the rest of the project remains viable with analysis of wild type parasites in their biofilm and expand the study to other Leishmania species.

Further reading

(Relevant preprints and/or open access articles)

Additional information from the supervisory team

The supervisory team has provided a recording for prospective applicants who are interested in their project. This recording should be watched before any discussions begin with the supervisory team.
Rogers-Sunter-Raynes Recording

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