PhD PROJECT PROPOSAL

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Dynamics of parasite-immune cell interactions at the mucus-epithelium interface

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Abstract*
Coccidian parasites are known to infect the epithelial tissue that covers the digestive tract and airways. To successfully infect the tissues, parasites have to overcome immune response and cross a layer of a viscoelastic and sticky fluid called mucus present at the surface of both tissues. Yet, the cascade of events that lead to the infection of epithelia by coccidian parasites remains unknown. This PhD project aims at understanding the dynamics of infection by using two in-vitro model systems i) artificial mucus whose rheological properties can be tuned and ii) a bronchial epithelium reconstituted from human primary cells. We will combine cell biology techniques, video-microscopy, confocal microscopy, single-cell force measurement techniques and advanced image processing to image and quantify interactions and fate of the parasites in the model epithelia. This project could be decisive in deciphering the complex interactions between pathogens and host tissues and open prospects for new therapeutic avenues.

Keywords*
Mucus; Pathogens; Epithelium; Immune cells; Active matter physics; Image processing; Videomicroscopy.

Scientific question and Objectives*
The overall objective is to study the interactions between coccidia, a large group of multilayered-wall protected parasites, immune cells and mucus, a viscoelastic and sticky fluid that covers the surface of several epithelia to protect them against external pathogens. The mucus forms the first physical barrier that is believed to prevent parasite infection. Yet, in some cases, parasites manage to infect the underlying epithelium and replicate within. The cascade of events that leads to a successful infection remains largely unknown. The scientific questions addressed in this project are: (i) how do parasites interact with mucus? And what is the role of rheological properties of mucus in these interactions? (ii) do immune cells such as phagocytes patrolling close to the mucus barrier target parasites? (iii) are interactions between the mucus, parasites and immune cells detrimental or beneficial to the parasites in the infection of the epithelium?

Proposed approach (experimental / theoretical / computational) and research plan*
We will use two type of model systems infected by *Cryptosporidium*, a coccidian parasite that can multiply in the bronchial and the intestinal epithelia: (i) Artificial mucus, whose rheological properties can be tuned to mimic the mucus barrier in small intestine, which is a primary site of infection by many digestive coccidia *in vivo*. This system will allow to finely characterize and quantify the adhesion and fate of the parasites facing the mucus as a function of the parasite surface biochemistry, mechanics and adhesion, and of the mucus composition and rheology (Task 1 on the Year 1 of the project).
(ii) A bronchial mucosal epithelium, reconstituted *in vitro* at the air/liquid interface from human primary cells, in which the mucus is propelled via the continuous beating of active cilia exposed by the epithelial ciliated cells. This system will enable to study to what extend muco-ciliary clearance is efficient to fight against parasite infection or whether specific mucus flow patterns (e.g. vortices), previously observed in air/liquid interface

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cultures (Loiseau et al., 2020), lead to the trapping and a local increase of parasite concentration thus enhancing the probability of infection of the epithelium and/or contact with the patrolling immune cells (see below) (Task 2 on Year 2). In a second step, phagocytic cells (macrophages, neutrophiles) will be introduced into each of these systems to investigate whether these cells target and eliminate the parasites or serve as Trojan horses to allow the parasite infecting the epithelium as shown previously with the coccidian *T. gondii* (Freppel et al. 2016; Ndao et al., 2020) (Task 3 on Year 3). We will combine cell biology techniques, video-microscopy, confocal microscopy, single-cell force measurement techniques, i.e. AFM, optical tweezers (OT), micropipette (MP) aspiration-based techniques, and advanced image processing to image and quantify interactions and fate of the parasites in these systems.

Interdisciplinarity and Implication of the two labs*

This is a new project that combines complementary expertise of a microbiologist (Aurélien Dumètre) and a physicist (Etienne Loiseau). In this collaboration, LAI lab (Biology) will bring AD's expertise on the biology and mechanics of the parasite and immune cells, especially on parasite-phagocyte interactions. CINaM lab (Physics of living systems) will bring expertise on artificial mucus, mucus rheology and on *in vitro* reconstituted bronchial epithelium. At LAI, the PhD student will quantify the mechanical and adhesion properties of the parasites and immune cells at the single-cell level, characterize their surface biochemistry and tune the parasite-immune cell co-culture systems. At CINaM, he/she will develop quantitative tools (microrheology, particle image velocimetry…) and image processing techniques that will be used to analyse experiments performed in both laboratories. Overall, the LAI/CINaM implication in this project will be 50/50, segmented as follows:

- Task 1, Year 1: the PhD student will be at LAI 60% for quantification of the structure, mechanics, and adhesion of the parasites and immune cells and of the dynamics of their interactions by using AFM, MP, OT, and flow cytometry. He/She will be at CINaM 40% to prepare artificial mucus with controlled rheological properties and study to what extent a mucus layer forms a physical protective barrier against parasites.
- Task 2, Year 2: the repartition will be CINaM 60% to investigate the fate of the parasites facing the bronchial mucosal epithelial system the CINaM developed recently. The PhD student will study the competition between mucociliary clearance and parasite motility within the mucus layer to reach the underlying tissue. At LAI (40%), the student will investigate whether modifications of the surface biochemistry and ultrastructure of the parasite wall affect its interactions and fate with the mucosal epithelium.
- Task 3, Year 3: the repartition will be LAI 50% / CINaM 50% as the student will need the equal scientific and technical expertise of the two labs on the epithelial systems (CINaM) in which we will add immunes cells (LAI) and study the intricate parasite-immune cell interactions at the mucus-epithelium interface.

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Specify with whom the person recruited will collaborate and on what aspects *

At LAI, the PhD student will be supervised by A. Dumètre. He/She will collaborate for this project with permanent researchers (mainly Pierre-Henri Puech, Laurent Limozin), ITAs (Martine Pélicot-Biarnes, Thi Thien Nguyen) and, during Year 1, a 3rd year ANR PhD student (Jana ElHusseiny) for the manipulation of the parasites/immune cells and quantification of their mechanics, structure and surface biochemistry. At CINaM the PhD student will be supervised by E. Loiseau. He/She will interact with a postdoc (Alice Briole) for the quantification of mucus microrheology and with a PhD student (Camille Darthenay-Kinnemeann) who studies the dynamics of ciliated epithelia. Preparation of artificial mucus will be done in collaboration with Hugues Bodiguel (laboratory of rheology, Grenoble). In addition, LAI actively collaborates with local and external laboratories that could benefit from the project: (i) the Mattie Pawlovic's lab at Dundee, Scotland, that could bring her expertise on the cell biology and genetics of the *Cryptosporidium* parasites, (ii) the Electron Microscopy Platform at IMM, Marseille, for imaging the ultrastructure of the parasites and immune cells in the mucosal epithelial systems.

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PhD student's expected profile*

Biologist or physicist, with an interest in quantitative biology and biophysical experiments. Some basic knowledges in programming (Python…) would be a plus. Curiosity, independence and tenacity are some of the "must have" for such an interdisciplinary project.

This project is an entirely new one.

Two to five references related to the project*

- Ndao O et al. Dynamics of *Toxoplasma gondii* oocyst phagocytosis by macrophages. Front Cell Infect Microbiol. 2020. doi:10.3389/fcimb.2020.00207
- Loiseau E et al., Active mucus–cilia hydrodynamic coupling drives self-organization of human bronchial epithelium. Nat. Phys., 2020. https://doi.org/10.1038/s41567-020-0980-z
- Freppel W et al. Structure, composition, and roles of the *Toxoplasma gondii* oocyst and sporocyst walls. The Cell Surface 2019. doi.org/10.1016/j.tcsw.2018.100016
- Freppel W et al. Macrophages facilitate the excystation and differentiation of *Toxoplasma gondii* sporozoites intotachyzoites following oocyst internalization. Sci Rep 2019. doi.org/10.1038/srep33654
- Dumètre A et al. Mechanics of the *Toxoplasma gondii* oocyst wall. PNAS 2013. doi:10.1073/pnas.1308425110

Two main publications from each PI over the last 5 years*

A. DUMETRE

- Ndao O et al. Dynamics of *Toxoplasma gondii* oocyst phagocytosis by macrophages. Front Cell Infect Microbiol. 2020;10: 207. doi:10.3389/fcimb.2020.00207
- Freppel W et al. Structure, composition, and roles of the *Toxoplasma gondii* oocyst and sporocyst walls. The Cell Surface 2019, 5:100016. doi.org/10.1016/j.tcsw.2018.100016

E. LOISEAU

- Mesdjian O., et al. Longitudinal to transverse metachronal wave transitions in an in vitro model of ciliated bronchial epithelium. *Physical Review Letters* 129.3 2022. https://doi.org/10.1103/PhysRevLett.129.038101
- Loiseau E., et al. Active mucus–cilia hydrodynamic coupling drives self-organization of human bronchial epithelium. *Nature Physics* 2020. https://doi.org/10.1038/s41567-020-0980-z

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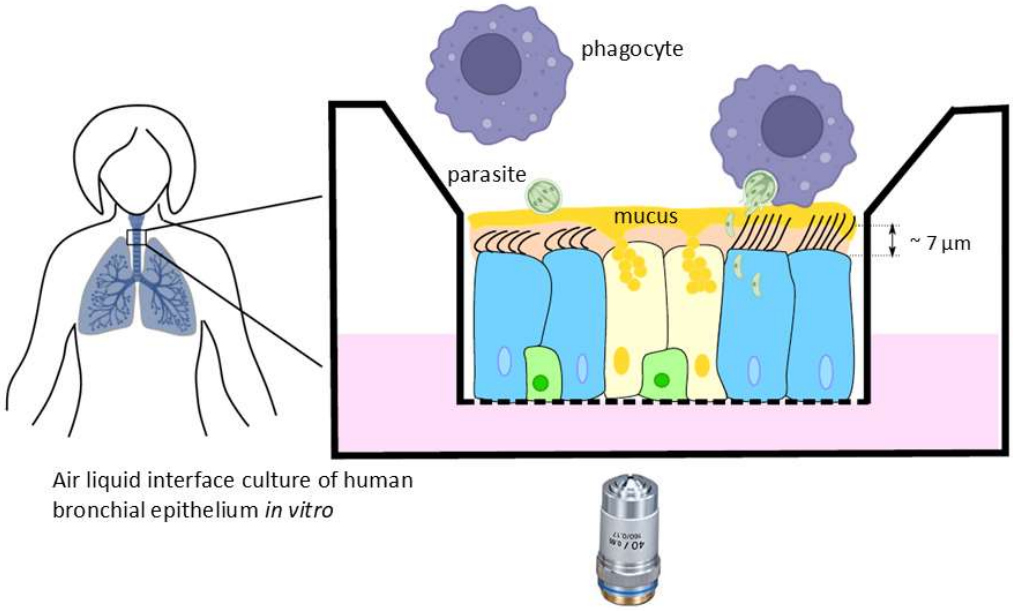
Project's illustrating image



Air liquid interface culture of human bronchial epithelium *in vitro*

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