PhD Research Proposal Form
China Scholarship Council (CSC) - ENS Group

FIELD: Biology – Immuno-virology

Thesis subject title:
Uncovering how the plasmacytoid dendritic cells sense and control SARS-CoV-2 infection.

Name of the French doctoral school: Integrative Molecular and Cell Biology Doctoral School BMIC
https://edbmic.universite-lyon.fr

Name of the Research team:
CIRI, INSERM U111, École Normale Supérieure de Lyon.
Team VIV (Vesicular trafficking, Innate response & Viruses), led by M Dreux
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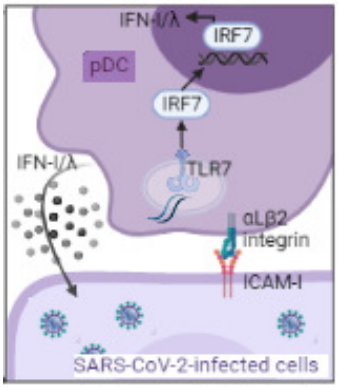
Lab Language:
English - international PhD students and post doc, e.g., Portugal, India, China, etc..

Research Proposal Abstract:
Keywords: innate response, interferon, SARS-CoV-2, COVID-19, dendritic cells, antiviral signaling.

All cells can detect infection by viruses and trigger an innate immune response. This first line of defense is initiated by the recognition of viral elements by host receptors (*e.g.,* Toll-like receptors; TLRs). This detection leads to the production of antiviral molecules, including type I and III interferons (herein referred to as IFN-I/III). IFN-I/III are essential response to initiate the immunity against viral infections.

The plasmacytoid dendritic cell (pDC) is a unique immune cell type specialized for rapid and massive production of IFN-I/III. The main viral-sensors responsible for pDC activation are the Toll-like receptors (TLR)-7 and -9, which recognize viral RNA and DNA, respectively. Studies have demonstrated that pDCs migrate into infected tissues, *e.g.,* the lungs during pulmonary infection, such as SARS-CoV-2. The rapid production of IFN-I/III by pDCs is essential to control various viral infections, as we previously uncovered[1].
Importantly, we have demonstrated that pDCs establish cell contact with infected cells that sustains their robust antiviral responses[2]. This is mediated by synapse formed at the contact site between pDCs and infected cells (shown for genetically-distinct viruses) by the molecular reorganization, including a specific polarization of actin-network and the endocytosis machinery[2]. This is a specialized platform for PAMP transfer from infected cell to the TLR7 sensor in pDCs, leading to IFN-I/III production. We thus named it: *interferogenic synapse (**iSYN**)*. The signaling cascade triggered by sending via ***iSYN*** leads to an antiviral response. This signaling also acts as positive feed-forward loop culminating in a robust IFN-I/III secretion at the contact site with infected cells[2]. This **specialized ability of pDCs** to sense infected cells brings key questions on how pDC achieve **an optimal antiviral response against viral infection**, while minimizing the deleterious impact of hyperactive inflammation (*e.g.,* TNFa and IL6).

Our recent results further revealed that ***iSYN*** is also a structural platform for polarization of effector functions leading to targeted IFN-I/II secretion directly toward the eliciting infected cells. An important remaining question is how the **dynamics of *iSYN*** (duration, strength and renewal) **impact the amplitude, nature and optimum of the IFN-I/III response.** Therefore, this project is designed to **functionally dissect the molecular basis of the targeted antiviral response by *iSYN***. Additionally, our studies shall provide mechanistic insights on the largely still-unknown secretion process of cytokines, including IFN-I/III and other pro-inflammatory cytokines.



Model of the innate responses against SARS-CoV-2. The short-range sensing of SARS-CoV-2-infected cells (at the bottom) by pDCs (at the top) requires cell contact mediated by adhesion complexes, identified as $\alpha_L\beta_2$ integrin and ICAM-1. This triggers a TLR7-induced signaling leading to an IFN-I/λ-prioritized response, w hile leaving inactive the NF-κB-mediated signaling.

Objective: Define the dynamics and intersect of the molecular events at iSYN leading to pDC antiviral response.

1. How is the antiviral secretion of pDCs targeted towards the infected cells?
2. What are the dynamics and cross-regulation of the sensing and effector functions at play at the *iSYN*?
3. Defining how to directly and temporally control the dynamics of *iSYN* to reach an optimal IFN-I/III response.

To address these questions, we have already established a methodology to monitor at the single-cell level the impact of pDC response on SARS-CoV-2-infected cells by live-imaging analysis using our BSL3-based spinning-disk confocal and using an infectious reporter-SARS-CoV-2. Our results uncovered that SARS-CoV-2 replication greatly decreases only after physical contact of infected cells with pDCs. SARS-CoV-2 can inhibit IFN-I/III signaling within infected cells. Thus, the observed locally concentrated antiviral response by pDCs can overcome this viral inhibition.

Methodology: We plan to tackle these questions using approaches mastered in our laboratory (Dr Dreux)[1-6] and the complementary expertise of our collaborator, Dr Olivier Destaing for optogenetic approaches[7-10]. This includes cutting-edge imaging technology (*i.e.,* ImageStream X, live-imaging using spinning disk confocal microscopy, super-resolution microscopy analysis), novel functional model and optogenetic approaches to study the dynamics and regulations of pDC antiviral response.
Novelty of the project: The strength of this project is to answer questions on the host/pathogen interactions by combining approaches from disparate fields: cellular biology (live-imaging using cutting-edge methods of confocal microscopy), molecular biology and immunology (antiviral response: RT-qPCR, ELISA, Western blot) and virology (viral replication: immunofluorescence, RT-qPCR). So far, optogenetic methods are scarcely-employed to define antiviral responses. Nonetheless, this type of approach is essential to go deeper in the molecular understanding of the cascade events.
The quantification at **single-cell level of the dynamics of the molecular cascade and probing for an optimal response is a new angle**, emerging from the single-cell analysis era. Our 3D and 4D (with time) studies will be critical to understand: *i/* the molecular regulations at the single-cell level, and *ii/* how the cell environment and coordination with surrounding cells (and in future studies other immune cell types) impact the functionality of individual cells.

References:

1 Webster, B. *et al.* Plasmacytoid dendritic cells control dengue and Chikungunya virus infections via IRF7-regulated interferon responses. *Elife* 2018, **7**.
2 Assil, S. *et al*. Plasmacytoid Dendritic Cells and Infected Cells Form an Interferogenic Synapse Required for Antiviral Responses. *Cell Host Microbe* 2019, **25**, 730-745 e736.
3 Dreux, M. *et al*. Short-range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity. *Cell Host Microbe* 2012, **12**, 558-570.
4 Decembre, E. *et al.* Sensing of immature particles produced by dengue virus infected cells induces an antiviral response by plasmacytoid dendritic cells. *PLoS Pathog* 2014, **10**, e1004434.
5 Coleon, S., Assil, S. & Dreux, M. Monitoring of Interferon Response Triggered by Cells Infected by Hepatitis C Virus or Other Viruses Upon Cell-Cell Contact. *Methods Mol Biol* 2019, **1911**, 319-335.
6 Assil, S. *et al*. Sensing of cell-associated HTLV by plasmacytoid dendritic cells is regulated by dense beta-galactoside glycosylation. *PLoS Pathog* 2019, **15**, e1007589.
7 Kerjouan, A. *et al.* Control of SRC molecular dynamics encodes distinct cytoskeletal responses by specifying signaling pathway usage. *J Cell Sci* 2021, **134**.
8 Moitrier, S. *et al.* Collective stresses drive competition between monolayers of normal and Ras-transformed cells. *Soft Matter* 2019, **15**, 537-545.
9 Vellino, S. *et al.* Cross-talk between the calcium channel TRPV4 and reactive oxygen species interlocks adhesive and degradative functions of invadosomes. *J Cell Biol* 2021, **220**.
10 Destaing, O. *et al*. beta1A integrin is a master regulator of invadosome organization and function. *Mol Biol Cell* 2010, **21**, 4108-4119.

Selected recent publications of the team on this topic

Venet, M., Ribeiro, M.S., Décembre, E., Bellomo, A., Joshi, G., Villard, M., Cluet, D., Perret, M., Pescamona, R., Paidassi, H., et al. (2021). SARS-CoV-2 infected cells trigger an acute antiviral response mediated by Plasmacytoid dendritic cells in mild but not severe COVID-19 patients. medRxiv, **2021**. https://doi.org/10.1101/2021.09.01.21262969

Sa Ribero M, Jouvenet N, Dreux M*, Nisole S*. Interplay between SARS-CoV-2 and the type I interferon response **PLoS Pathog 2020.** 16(7): e1008737. https://doi.org/10.1371/journal.ppat.1008737 * co-last authors

Assil S, Coléon S, Décembre D, Sherry L, Allatif O, Webster B & Dreux M. Plasmacytoid Dendritic Cells and Infected Cells Form an Interferogenic Synapse Required for Antiviral Responses. **Cell Host and Microbe 2019** May 8;25(5):730-745.e6. https://doi.org/10.1016/j.chom.2019.03.005

Assil S., Futsch N., Décembre E., Alais S., Gessain A., Cosset F-L., Mahieux R., Dreux M* & Dutartre H*. Sensing of Cell-associated HTLV by Plasmacytoid Dendritic Cells is Regulated by Dense beta-Galactoside Glycosylation. **PLoS Pathogens**. **2019** Feb 28;15(2):e1007589. https://doi.org/10.1371/journal.ppat.1007589 * co-last authors

Webster B, Werneke SW, Zafirova B, This S, Coléon S, Décembre E, Paidassi H, Bouvier I, Joubert PE,Duffy D, Walzer T, Albert ML & Dreux M. Plasmacytoid dendritic cells control dengue and Chikungunya virus infections via IRF7-regulated interferon responses. **Elife. 2018** Jun 19;7. pii: e34273. https://elifesciences.org/articles/34273

Webster B, Assil S & Dreux M. Cell-Cell Sensing of Viral Infection by Plasmacytoid Dendritic Cells. **J Virol.** 2016 Oct 28;90(22):10050-10053. doi: 10.1128/JVI.01692-16.

Décembre E, Assil S, Hillaire MLB, Dejnirattisai W, Mongkolsapaya J, Screaton GR, Davidson AD, Dreux M. Sensing of Dengue virus infected cells producing immature particles induces an antiviral response by plasmacytoid dendritic cells. **PLoS Pathogens**. 2014 Oct 23;10(10):e1004434. doi: 10.1371/journal.ppat.1004434.

Type of PhD:

1.Full PhD

- Joint PhD/cotutelle (leading to a double diploma): **NO**
- Regular PhD (leading to a single French diploma): **YES**

2. Visiting PhD (for students enrolled at a Chinese institution who will be invited to a French institution to carry out a mobility period) : NO