vendredi, août 26, 2022

INTERNATIONAL PhD PROPOSAL

PhD Supervisor full name **Mickael Menager**

PhD PROPOSAL IDENTIFICATION

PhD Project title	Comprehensive Links between immune sensing Of hiv and ClocK
Project Acronym	CLOCK
Project Keyword	HIV-1 innate sensing, Circadian rhythm, CLOCK, cGAS, type I IFN production, Dendritic cell maturation, Actin, Virology, Immunology, Cellular biology, Confocal microscopy, Single-cell OMICs

LABORATORY PRESENTATION

Laboratory Team Name	Inflammatory Responses And Transcriptomic Networks in Diseases
Department IP	Immuno/infectio
Doctoral school	BiosPC
University	University Paris Cité
Laboratory website	https:/ www.institutimagine.org/en/mickael-menager-187

PhD PROPOSAL

PhD Supervisor full name	Mickael Menager
PhD Supervisor position	Group Leader and head of the Lab
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PhD Proposal abstract (1000 characters maximum)

We previously used the power of machine learning algorithms combined with the generation of whole transcriptomic and chromatin accessibility changes to better understand how HIV-1 is sensed by human DCs and how this sensing leads to type I IFN production and DC maturation. Among the top Transcription Factors (TFs) inferred to be linked with DC responses to HIV-1, we identified CLOCK, the master regulator of the circadian rhythm, as predicted to have a differential activity during HIV-1 innate sensing. Using shRNA knockdown in monocyte-derived dendritic cells (MDDCs), we observed several phenotypes upon CLOCK decrease of expression: (1) a more mature phenotype at steady state, (2) a decrease in cGAS expression, the HIV-1 innate sensor, combined with a (3) change of its localization, alongside a (4) loss of actin polymerization. This PhD project will now aim to understand at the molecular level the links between, CLOCK, DCs maturation, HIV-1 innate sensing and actin polymerization.

PhD Proposal (4000 characters maximum)

Background

Type I interferons (IFNs) help limit HIV-1 spread through induction of interferon-stimulated genes (ISGs) that restrict HIV-1 replication and stimulate the immune response. IFNs are also involved in hyperactivation of the immune system during the chronic phase of infection and participate in the establishment of HIV-1 cellular reservoirs. DCs are relatively resistant to viral replication due to the expression of restriction factors such as SAMHD1. The protein Vpx (encoded by HIV-2 or introduced experimentally in DCs by using lentiviral particles) can promote SAMHD1 degradation and enforce reverse transcription. DCs can then sense HIV-1 via the innate sensor cGAS and produce type I IFNs through the STING/TBK1/IRF3 signaling pathway. However, it remains unclear how multiple transcription factors (TFs) downstream of innate immune sensing regulate the differential expression of hundreds of innate immune genes, production of IFNs and DC maturation. We previously combined the power of machine learning algorithms with the generation of whole transcriptomic (RNA-seq) and chromatin accessibility data to better understand how HIV-1 is sensed by human DCs and how this sensing by the innate immune system leads to IFNs and DC maturation (PMID: 31968263). Among the top Transcription Factors (TFs) inferred to be linked with DC responses to HIV-1, we identified CLOCK as predicted to have a differential activity during DC maturation following HIV-1 innate sensing. This protein has been well described as the master regulator of the circadian rhythm and plays a key role in the regulation of a variety of biological pathways (fatty acids uptake, lipogenesis, glycolytic metabolism, etc…).
Using shRNA knockdown in monocyte-derived dendritic cells (MDDCs), we have already observed several phenotypes in the absence of CLOCK: (1) a more mature phenotype at steady state, (2) a decrease in cGAS expression, the HIV-1 innate sensor, combined with a (3) change of its localization, alongside a (4) loss of type I IFN production and (5) effects on actin polymerization. Taken together, our results highlight a yet undescribed role for CLOCK in the control of DC response to HIV infection.

Proposed project

With this PhD project, we would like now to better understand, at the molecular level,

how CLOCK expression is linked to DCs maturation, how it can control cGAS expression at the protein level and how it can affect cGAS localization and if this effect is linked with actin polymerization. The PhD project will be divided into 4 different but interdependent parts/questions:
How CLOCK can control DCs maturation status at steady state?
How CLOCK can control cGAS (HIV-1) sensor expression, et the protein level?
How CLOCK controls cGAS perinuclear localization? Is it through interconnections with molecular pathways involving actin and TSPAN7, a protein identified by the team as a positive regulator of actin nucleation?
Are the newly described roles of CLOCK, dependent on its activities as a master regulator of circadian rhythm?

Technics and material that will be used

Flow cytometry, Cytokines detection by bioplex and digital ELISA (SIMOA), shRNA knock down in human primary cells, cell culture of human blood cells from healthy and HIV seropositive patients, Viral production and infection of cells in a BSL3 facility, Confocal microscopy, Single-cell OMICS experiments, Possibility to learn OMICs analyses at the computational level
During this project the candidate will work with already well-established technics in the lab covering molecular, cell biology and microscopy coupled with viral production and cell infections in a BSL3 facility. Most of the work will be done on human cells from healthy blood donors, infected with replicative competent and non-replicative competent viruses.

Expected profile of the candidate

We are looking for a highly motivated research scientist, who will be embarking with us for a PhD on this very exciting journey, where we are intending to characterize the molecular links between CLOCK, master regulator of circadian rhythm and innate sensing of HIV-1 in human dendritic cells. The candidate must have first-class skills in immunology, virology, molecular and cellular biology. Candidates with interests and background in autoimmunity/inflammation are encouraged to apply. Knowledge of open-source packages for single-cell analyses such as SEURAT, combined with programming languages such as R, would be plus but is not necessary.

The PhD student will be expected to participate to the conception, generation and the analyses of the different molecular and cellular experiments. To this purpose, he will collaborate with a senior postdoc an engineer and with several computational biologists, all members of Mickael Menager's team. The PhD student will also benefit from the several core facilities present at Imagine Institute (https:/ www.institutimagine.org/en/), such as the LabTech Single-cell@Imagine the genomics and bioinformatics core facilities and the confocal core facility.

The Imagine Institute represents a unique setting in which to develop such collaborative project based on human disease related studies, with ample opportunity for collaborations with national and international top-level scientists and clinicians from Imagine, Curie, Pasteur, CEA, INRA institutes and industrial from Sanofi and Ariana Pharma.