Monday, August 14, 2023

International PhD PPU-IMAGINE Proposal

Call 2023/2024

PhD PROPOSAL IDENTIFICATION

PhD Project title	Exploring interferon-mediated lung inflammation at the cellular spatial level
Project Acronym	SC-LUNG
Project Keywords	spatial single-cell transcriptomic, interferonopathy,lung, inflammation

LABORATORY PRESENTATION

Laboratory Team Name	Inflammatory Responses and Transcriptomic Networks in diseases
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PhD PROPOSAL

PhD Supervisor full name	Mickaël Ménager
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Number of ongoing PhD students supervised by the Supervisor	1

PhD Proposal abstract (1000 characters maximum)

As emphasized by the SARS-CoV-2 pandemic, understanding the pathogenesis of inflammatory lung disease is of high relevance to human health, with persistent pulmonary inflammation leading to fibrosis and respiratory failure. The potential of type I interferon (IFN) to mediate lung inflammation has been recently highlighted. However, the precise mechanism remains unknown. We propose to study the type I interferonopathies, a group of inborn errors of immunity with chronically enhanced type I IFN signaling, to explore how IFN-mediated lung inflammation is precisely triggered and induces fibrosis, employing state-of-the-art technologies (including single-cell transcriptomics and induced pluripotent stem cells) on never

assessed rare human samples. Importantly, this proposal will not only improve the management of rare monogenic disorders, it will also be relevant for more common diseases (e.g. systemic lupus erythematosus, idiopathic pulmonary fibrosis, virally-induced lung inflammation).

PhD Proposal (10 000 characters maximum)

State of the art/Context: Lung inflammation is central to the pathology observed in a variety of disease settings, including infection, allergy, cancer and toxin exposure. In addition, a persistent inflammatory state can lead to fibrosis and end-stage respiratory failure. Attention has been drawn to the potential of type I interferon (IFN) to mediate lung inflammation after self-derived nucleic acid recognition[1]. However, the precise underlying mechanism remains poorly understood. We propose to unravel how IFN-mediated lung inflammation is triggered and induces fibrosis through the study of the type I interferonopathies (TI1s), a group of inborn errors of immunity in which enhanced type I IFN signalling is considered central to pathology. Of importance, life-threatening pulmonary disease is observed in two TI1s, for which Dr Frémond is a renowned expert, i.e. STING-associated vasculopathy in infancy (SAVI) and COPA syndrome[1]. The underlying pathogenesis of lung inflammation and progression to pulmonary fibrosis in SAVI and COPA syndrome remains to be deciphered.

In mammalian cells, immune responses to cytoplasmic double-stranded DNA involve the sensor cGAS (cyclic GMP-AMP synthase) that activates STING to trigger IFN production. Gain-of-function mutations in STING1, encoding STING, were reported by us and others to explain a severe T1I referred to as SAVI, encompassing systemic vasculopathy with interstitial lung disease. A year later, heterozygous mutations in COPA, encoding coatomer protein subunit alpha (COPA), were described to cause COPA syndrome, a hereditary inflammatory syndrome mainly affecting the lungs. Striking pulmonary clinical and histopathological overlap between SAVI and COPA syndrome, suggested a common molecular pathogenesis and we defined a role for COPA in STING intracellular trafficking[2]. In addition, anti-MDA5-positive dermatomyositis[3] and systemic lupus erythematosus (SLE), both polyfactorial diseases associated with high type I IFN signalling, can manifest severe inflammatory lung disease. Of further interest, STING pathway has been recently involved in COVID-19 immunopathology related to lung and skin features[4].

These data highlight the potential of studying rare monogenic disorders characterised by IFN signalling and lung inflammation (designated here as IFN-LIs), combined with state-of-the-art single-cell transcriptomic experiments, to gain unique insight into lung inflammation-induced fibrosis to decipher pathological mechanisms, also relevant to a larger group of disorders, including SLE, and idiopathic lung fibrosis. Importantly, with this clinically-orientated research program, we will be able to provide translational leads for novel therapeutic strategies in all these disease settings.

Scientific objectives of the proposal: Under expert supervision and using state-of-the-art technologies, including single-cell transcriptomics (both from cell suspension and at the spatial level on lung biopsies), the objectives of your PhD proposal are:

(i) WP1: to explore the cellular and molecular pathogenesis of monogenic disorders characterized by IFN signaling and lung inflammation (IFN-Lis)

(ii) WP2: to pave the way to design an integrated disease model to study pathogenic pathway in IFN-LIs.

WP1: Deciphering the cellular and molecular pathology of IFN-mediated lung inflammation (IFN-LI)

The relative contribution of intrinsic lung cells and cells derived from the hematopoietic system to the pulmonary inflammation and fibrosis seen in SAVI and COPA syndrome is unknown. We will derive transcriptomic data using lung tissue and peripheral blood mononuclear cells (PBMCs) from patients with SAVI, COPA syndrome and controls, with samples already biobanked. Specifically, we will analyze lung biopsies at the single cell scale with scRNAseq, comparing these data with expression profiles recorded concomitantly in PBMCs. In doing so, we will identify the major cell types responsible for IFN signalling and pro-fibrotic molecule expression, deriving a fibrotic signature curated from the literature and relevant to our dataset. We will analyze pathways enriched in differentially expressed genes, with algorithms, such as GSEA (Gene Set Enrichment Analysis) and IPA (Ingenuity Pathway Analysis), to define novel molecular pathways relevant to IFN-LI pathology. In an already-established collaboration between the team of Mickael Menager and Dr Marie-Louise Frémond, we generated preliminary transcriptomic data from PBMCs of SAVI patients (N=5), COPA patients (N=2) and healthy controls (N=8), compared to patients with Aicardi-Goutières syndrome (AGS N=4) which does not present lung involvement. Our results indicate an IFN pathway activation, mainly in myeloid cells, higher in SAVI patients, but markedly lower in COPA than in AGS patients. This suggests distinct pathophysiologies and highlights the power of comparative

transcriptomic studies in rare monogenic diseases to discover pathology-relevant pathways[5,6]
Using then the genes of interest selected through scRNAseq, we will analyse lung biopsies spatially at the lung tissue scale, using different single-cell spatial transcriptomic approaches already implemented in the LabTech Single-Cell@Imagine, also headed by Mickael Menager.
Molecules central to signaling pathways identified through transcriptomic analyses will be compared in the blood versus lung biopsies and then validated experimentally in primary patient cells material and using induced pluripotent stem cells (iPSCs)

WP2: Disease modeling and experimental validation of molecular pathways identified in WP1

To specifically decipher the cellular interplay involved in IFN-driven lung inflammation and identified through single-cell experiments performed in WP1, we will generate iPSCs from selected IFN-LI patient (SAVI, COPA) and healthy control material with the iPSC platform of the Imagine Institute, with the final aim to generate lung organoids, so to provide an integrated disease model to study pathogenic pathways. We will use a combined approach to the generation of iPSC-derived cellular models based on (i) clinical observation and study of the available literature, and (ii) the results of our single-cell analyses (from WP1). One can predict that (at least) three cell types will be of particular relevance to these studies: (i) type II alveolar epithelial cells; (ii) endothelial cells; (iii) alveolar macrophages. We will focus on establishing the differentiation of type II alveolar epithelial cells (iATIIs), considering their described capacity to induce a rapid and broad inflammatory response to SARS-Cov-2, and their role in inducing lung fibrosis. iPS-derived cells will be analysed at baseline and after stimulation of DNA sensing pathways to acutely challenge our models of STING-mediated IFN-LIs (COPA, SAVI). Thus, we will evaluate IFN production and signalling, the expression of pro-fibrotic markers (e.g. TGFβ1), and key molecules involved in previously unknown signalling pathways identified in our scRNAseq data.

Teams involved, scientific resources and synergies. For this scientific proposal, the PhD student will benefit from the existing expertise of two experimental groups at Imagine Institute (Mickael Menager's team and Dr Marie Louise Frémond) and several Imagine platforms (LabTech Single-cell@Imagine, iPSC, genomics and bioinformatics) and the involvement with clinicians at Necker Hospital. The PhD student will then be in a unique environment, combining knowledge of *in vitro* systems, IFN biology, clinical expertise, single-cell skills at both wet and computational level, and technical skills of the different platforms. This will provide a rich source of cross-fertilisation converging on a single theme on lung inflammation leading to the optimization of innovative and integrated study models providing benefit to project partners and the wider institute. Notably, our unprecedented access to rare patient material, and already established expert collaborations, including with the team of Pr Sermet-Gaudelus (INEM, Paris), place us in a unique position to develop this exciting new area of translational clinical medicine, with also the goal to set up and foster the development of single-cell multi-OMICs technologies directly in tissue biopsies to compare biomarkers/pathophysiology discovered in the blood versus inflamed tissues in rare monogenic diseases but also in more common disorders.

References.

1.Frémond, M.-L. & Crow, Y. J. STING-Mediated Lung Inflammation and Beyond. *J. Clin. Immunol.* **41**, 501–514 (2021).
2.Lepelley, A. *et al.* Mutations in COPA lead to abnormal trafficking of STING to the Golgi and interferon signaling. *J. Exp. Med.* **217**, e20200600 (2020).
3.Ye, Y. *et al.* Single-cell profiling reveals distinct adaptive immune hallmarks in MDA5+ dermatomyositis with therapeutic implications. *Nat. Commun.* **13**, 6458 (2022).
4. Domizio, J. D. *et al.* The cGAS-STING pathway drives type I IFN immunopathology in COVID-19. *Nature* **603**, 145–151 (2022).
5.Cevins, C. de *et al.* Single-cell RNA-sequencing of PBMCs from SAVI patients reveals disease-associated monocytes with elevated integrated stress response. 2023.04.25.23288913 Preprint at https://doi.org/10.1101/2023.04.25.23288913 (2023).
6.Maxime Batignes *et al.* Enhanced inflammatory signaling driven by metabolic switch in Aicardi-Goutières syndrome. *bioRxiv* 2023.02.23.529707 (2023) doi:10.1101/2023.02.23.529707

Laboratory's best 5 publications

1. Camille de Cevins, Laure Delage, Maxime Batignes, Quentin Riller, Marine Luka, Anne Remaury, Boris Sorin, Tinhinane Fali, Cécile Masson, Bénédicte Hoareau, Catherine Meunier, Mélanie Parisot, Mohammed Zarhrate, Brieuc P. Pérot, Víctor García-Paredes, Francesco Carbone, Luc Canard, Charlotte Boussard, Etienne Crickx, Jean-Claude Guillemot, Marie-Louise Frémond, Bénédicte Neven, Galina Boldina, Franck

Augé, Fischer Alain, Michel Didier, Frédéric Rieux-Laucat, Mickael M. Ménager. Single-cell RNA-sequencing of PBMCs from SAVI patients reveals disease-associated monocytes with elevated integrated stress response. medRxiv 2023.04.25.23288913; doi: https://doi.org/10.1101/2023.04.25.23288913 2. Maxime Batignes, Marine Luka, Surabhi Jagtap, Camille de Cevins, Ivan Nemazanyy, Tinhinane Fali, Víctor García-Paredes, Francesco Carbone, Brieuc P. Pérot, Bénédicte Neven, Brigitte Bader-Meunier, Pierre Quartier dit Maire, Marie Hully, Alexandre Belot, Alice Lepelley, Marie-Louise Frémond, Yanick J. Crow, Alain Fischer, Mickaël M. Ménager. Enhanced inflammatory signaling driven by metabolic switch in Aicardi-Goutières syndrome. bioRxiv 2023.02.23.529707; doi: https://doi.org/10.1101/2023.02.23.529707 3. Quentin Riller, Jacques Fourgeaud, Julie Bruneau, Suk See De Ravin, Grace Smith, Mathieu Fusaro, Samy Meriem, Aude Magerus, Marine Luka, Ghaith Abdessalem, Ludovic Lhermitte, Anne Jamet, Emmanuelle Six, Alessandra Magnani, Martin Castelle, Romain Lévy, Mathilde M. Lecuit, Benjamin Fournier, Sarah Winter, Michaela Semeraro, Graziella Pinto, Hanène Abid, Nizar Mahlaoui, Nathalie Cheikh, Benoit Florkin, Pierre Frange, Eric Jeziorski, Felipe Suarez, Françoise Sarrot-Reynauld, Dalila Nouar, Dominique Debray, Florence Lacaille, Capucine Picard, Philippe Pérot, Béatrice Regnault, Nicolas Da Rocha, Camille de Cevins, Laure Delage, Brieuc P. Pérot, Angélique Vinit, Francesco Carbone, Camille Brunaud, Manon Marchais, Marie-Claude Stolzenberg, Vahid Asnafi, Thierry Molina, Frédéric Rieux-Laucat, Luigi D. Notarangelo, Stefania Pittaluga, Jean Philippe Jais, Despina Moshous, Stephane Blanche, Harry Malech, Marc Eloit(*), Marina Cavazzana(*), Alain Fischer(*), Mickaël M. Ménager(*), Bénédicte Neven(*)(* co-last authors). Late-onset enteric virus infection associated with hepatitis (EVAH) in transplanted SCID patients, Journal of Allergy and Clinical Immunology, 2023, ISSN 0091-6749, https://doi.org/10.1016/j.jaci.2022.12.822. 4. C de Cevins, M Luka, N Smith, S Meynier, A Magerus, F Carbone,..., Darragh Duffy, Frederic Rieux-Laucat, Julie Toubiana, Mickael M Menager. A monocyte/dendritic cell molecular signature of SARS-CoV2-related multisystem inflammatory syndrome in children (MIS-C) with severe myocarditis. Med. Cell Press August 2021. https://doi.org/10.1016/j.medj.2021.08.0022
5. Lepelley A, Martin-Niclós MJ, Le Bihan M, Marsh JA, Uggenti C, Rice GI, Bondet V, Duffy D, Hertzog J, Rehwinkel J, Amselem S, Boulisfane-El Khalifi S, Brennan M, Carter E, Chatenoud L, Chhun S, Coulomb l'Hermine A, Depp M, Legendre M, Mackenzie KJ, Marey J, McDougall C, McKenzie KJ, Molina TJ, Neven B, Seabra L, Thumerelle C, Wislez M, Nathan N, Manel N, Crow YJ, Frémond ML. Mutations in COPA lead to abnormal trafficking of STING to the Golgi and interferon signaling. J Exp Med. 2020 Nov 2;217(11):e20200600. doi: 10.1084/jem.20200600. PMID: 32725128; PMCID: PMC7596811.

Expected profile of the candidate

We are looking to recruit a dedicated and hard-working student who is motivated to explore deep molecular and cellular mechanisms of lung inflammation-induced fibrosis in type I interferonopathies. The student must be engaged to his/her work, committed, thoughtful and eager to learn. The PhD Student will participate in setting up and analyze single-cell experiments in collaboration with engineers and computational biologists, and how to work with iPSCs and establish cellular models to validate experimentally pathways identified through single-cell experiments. The PhD student will also have a unique opportunity to embark with us on the fascinating journey of analyzing human biopsises at the spatial and single-cell resolution, a fast growing and revolutionary field. Previous experience in bioinformatics and innate immunity signalling would be a plus. Interested students are encouraged to contact us at: mickael.menager@institutimagine.org and marie-louise.fremond@institutimagine.org.