PASTEUR PARIS UNIVERSITE (PPU) INTERNATIONAL DOCTORAL PROGRAM 2020

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PROJECT

- **FILE #13**
- **ACRONYM:** ANCIENT
- **TITLE:** Actin cytoskeleton in cell migration in 3D-environments

LABORATORY

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LABORATORY PRESENTATION AND RESEARCH TOPICS

- **SUPERVISOR HDR:** SEPULVEDA, Fernando, fernando.sepulveda@inserm.fr

- **SPECIFY THE TEAM NAME:** Molecular basis of altered immune homeostasis laboratory

- **WEBSITE OF THE TEAM:** team : https://www.institutimagine.org/fr/188

DESCRIPTION OF THE PROPOSED PROJECT

- **KEYWORDS:** actin, cell migration, leukocytes, primary immunodeficiencies, microdevices

- **ABSTRACT**

Patients affected by primary immunodeficiencies (PIDs) caused by deleterious mutations altering actin cytoskeleton dynamics, present impairments in several immune processes, highlighting the critical role of actin cytoskeleton in the regulation of immune homeostasis. Indeed, several immune cell functions (*e.g.* migration, cell-cell interactions, phagocytosis and cell division) heavily relay in the dynamic regulation of actin polymerization. In particular, during leukocytes' migration it has been shown that the coordinated polymerization of actin generates the force required for cells to move inside the tissues.
Leukocytes of these patients present impairments in almost every aspect of the immune response, emphasizing the need to better characterize the intricate molecular networks that regulate the reorganization of the actin cytoskeleton in healthy and diseased states. In this project, we aim to determine the impact that deleterious mutations in actin regulatory genes have (i) in the migratory capacity of leukocytes under confinement, (ii) to better characterize the molecular mechanisms involved, and (iii) to evaluate the putative contribution of alterations in cell migration in clinical manifestations of patients.

To successfully address these questions, the candidate will implement a multi-disciplinary approach mixing microscopy, cell biology, immunology and microfluidics. The candidate will leverage (i) state-of-the-art microfabricated devices (which allow to study complex cellular events at the single-cell level), and (ii) a large cohort of human samples from several PID patients. The successful accomplishment of this project will provide fundamental and clinical insights, and will set the ground for future studies aiming to the development of better therapeutic approaches for these severe disorders.

- **DESCRIPTION OF THE PROJECT**

The compartmentalization of the immune system is one of the main regulatory mechanisms maintaining immune homeostasis. This phenomena implies the spatial separation between the place where leukocytes' activation occurs (*i.e.* lymph nodes), and the compartments where effector responses take place (*i.e.* extra-lymphoid tissues). For this compartmentalization to happen, leukocytes move between the different tissues involved in the immune response, by recognizing various tissue-specific chemotactic signals. *In vivo*, at the cellular level leukocytes migration relies on the use of confinement to generate forces that allows cells to circulate between tissues. This type of migration is known as amoeboid migration, which requires cell contractility in a process depending on the dynamic regulation of the actin cytoskeleton, and it is independent of cell adhesion or degradation of extracellular matrix.

The actin cytoskeleton comprises the mechanical support for the cells, and is fundamental to fulfill specific functions (*e.g.* antigen internalization, immune synapse or cell migration). Alterations in any regulatory protein involved in these processes can have broad consequences, leading to severe alterations in immune homeostasis.
Over the last years, next generation sequencing analysis has allowed the identification of several primary immunodeficiencies (PID) caused by deleterious mutations altering actin cytoskeleton dynamics. Leukocytes of these patients present impairments in almost every aspect of the immune response, highlighting the critical role of actin cytoskeleton in the regulation of several immune cell functions, emphasizing the need to better characterize the intricate molecular networks that regulate the reorganization of the actin cytoskeleton in healthy and diseased states.

Even if the study and characterization of PID caused by alterations in actin cytoskeleton dynamics has improve our understanding of the individual role of different actin regulators in immune regulation, the impact of these mutations in amoeboid cell migration has not been addressed in detail. In this PhD project, we aim to determine how defects in these genes affect the migratory capacity of leukocytes. In particular, we aim:

1.- To evaluate the impact that deleterious mutations in actin regulatory genes cause in leukocyte migration using micro-fabricated devices
The study of cell migration in microfabricated devices has allowed researchers to mimic *in vitro* the constrains encountered by leukocytes when migrating in physiological situations. This approach enables the spatiotemporal analysis of cell motility by the imaging of migrating cells at high-resolution microscopy, obtaining important kinetic parameters (cell size, cell positioning, cell velocity and persistence, response to chemo-attractants, etc). In collaboration with researchers of the Curie Institute, we have implemented the use of microfabricated devices which allow us to gain insights of the role of different actin regulators in: (i) the spontaneous and chemokine induced cell migration in 1D and 2D environments (microchannels and 2D-confined devices), (ii) the migration capacity of leukocytes in complex environments, and their ability to deform nuclear envelop during migration and to respond to chemotactic cues (collagen gels and constricted-microchannels), and (iii) to assess the dynamics of the actin cytoskeleton during cell migration.

2.- To better characterize the molecular networks by which these actin regulators control cell migration
In this part of the project we shall leverage microdevices and biochemistry, cell biology and live cell imaging approaches to better characterize the molecular machinery by which these actin regulators control cell migration. Besides, we aim to determine the impact of these mutations in the activity of other important actin regulators known to control different steps of cell migration (i.e. mDIA1, ARP2/3, etc).

3.- To evaluate the putative contribution that alterations in leukocytes' migration can have in the clinical manifestations of the patients.
In this part of the project we shall determine the impact that cell migration in complex environments can have in immune effector functions and gene expression profile in patients cells. This will indicate whether alterations in cell migration under confinement translates in functional changes in tissues and contribute to clinical phenotype of patients.

To successfully address these questions, the candidate will implement a multi-disciplinary approach mixing microscopy, cell biology, immunology and microfluidics. The candidate will leverage state-of-the-art approaches and a large cohort of human samples from several PID patients. The successful accomplishment of this project will provide fundamental and clinical insights, and will set the ground for future studies aiming to the development of better therapeutic approaches for these severe disorders.

- **REFERENCES**

EXPECTED PROFILE OF THE CANDIDATE

- **EXPERIENCE REQUIRED**
The successful candidate should have a background in immunology, cell biology or similar.