Internship Proposition
(one page max)
Master 2 GP Immunology & ImmunoIntervention (I^3)
2024-2025

Lab: ***Laura Hulea lab***
Team: ***Regulation of cellular metabolism and mRNA translation***
Name and position of the supervisor: ***Laura Hulea***
Email of the supervisor: ***laura.hulea@umontreal.ca***
Candidate (if internship filled):
Title of the internship: ***Metabolic and translational dependencies of activated T cells***
Summary of the internship proposal:

Rationale and hypothesis. Upon T cell receptor (TCR) stimulation by antigens, naïve quiescent T cells undergo extensive proliferation. This is associated with profound metabolic reprogramming, which sustains an increase in energetic requirements, as well as the generation of precursors of growth promoting biomolecules. Aspects of metabolic remodeling accompanying T cell activation depend upon mTOR-mediated translational regulation. Yet, a more comprehensive understanding of the orchestrated regulation of proliferation, mRNA translation and metabolic adaptive programs during T cell activation is lacking. We propose a systematic temporal characterization of large-scale changes occurring at different levels of gene expression (transcription, translation) and metabolic regulation in T cells upon TCR activation. We expect such an approach will uncover novel mechanisms underpinning the extremely dynamic adaptive processes that allow the transition from quiescent naïve T cells to the highly proliferative state of activated T cells. Moreover, we postulate that mTOR and the translational machinery will play an important role in at least some of these molecular aspects of T cell activation, and that pharmacological/genetic modulation of translation would allow the regulation of T cell activation.

Aims. We will use cutting edge technologies we are experienced with to measure metabolism (CG/MS, LC/MS for metabolite measurement; Seahorse for cell bioenergetics assessment) and protein synthesis (polysome profiling) in naïve and activated T cells in vitro. This will be performed at short (2h, 6h, 12h) and long (24h, 48h) time points, as both translation and metabolism are extremely dynamic processes. We will also investigate how these two processes talk to each other in order to better coordinate the required cellular adaptations for rapid proliferation and T cell activation. Our research aims to document extensively the molecular changes in metabolite, bioenergetic capacity and protein synthesis, and to uncover metabolic pathways that are needed for T cell activation. The novelty of our research resides into an unbiased approach and extensive analysis of all possible changes that take place during T cell activation.

We will employ genetic approaches (CRISPR/Cas9, shRNA) and classical molecular and cellular biology techniques to functionally validate top translationally regulated genes and the corresponding metabolites and/or metabolic pathways identified. To further investigate how these validated genes/pathways impact on T cell metabolism during activation, we will perform metabolic tracing experiments, whereby the incorporation of ^{13}C carbons from ^{13}C-glucose or ^{13}C-glutamine into metabolites of various pathways (glycolysis, TCA cycle, one carbon metabolism) is measured over time. This powerful technique allows the dissection of changes in direction/flow and speed of metabolic pathways and how it impacts T cell biology during activation.

Since mTOR is a critical node linking integration of extracellular cues, mRNA translation and metabolism, we will assess the effects of modulating mTOR and the translational machinery on the translational and metabolic changes occurring during T cell activation. We will use mTOR inhibitors, knock-down of downstream translational inhibitors eIF4E-binding proteins (4E-BPs) or inhibitors of eIF4A, a key member of the translation initiation factor eIF4F. Experiments will be performed *in vitro* and *in vivo* models of T cell activation and immune response. We expect that at least part of the genes/pathways regulating T cell activation validated before will be affected by such manipulations.

Impact and feasibility. We expect that our work will elucidate fundamental aspects of T cell biology and the roles played by metabolism and translational adaptations in T cell activation and the immune response.

Option(s) linked to the project:

☐ Clinical Research Profile (Recherche Clinique)
☐ Data Analyst Profile (Recherche et Analyse de Données Biologiques)
☒ Experimental Biology Profile (Recherche Expérimentale)

Form to be sent by email to : gpi3@univ-nantes.fr