mardi, août 2, 2022

INTERNATIONAL PhD PROPOSAL

PhD Supervisor full name **Ménasché Gaël**

PhD PROPOSAL IDENTIFICATION

PhD Project title	New molecular effectors and therapeutic targets regulating mast cell degranulation and anaphylaxis
Project Acronym	Mastarget
Project Keyword	Mast cells, allergies, anaphylaxis, secretory granules, mouse models, Rab44, FceRI

LABORATORY PRESENTATION

Laboratory Team Name	Molecular basis of altered immune homeostasis
Department IP	Immunology
Doctoral school	BioSCP
University	Université Paris Cité
Laboratory website	https://www.institutimagine.org/fr/gael-menasche-et-fernando-sepulveda-188

PhD PROPOSAL

PhD Supervisor full name	Ménasché Gaël
PhD Supervisor position	Permanent researcher (INSERM, HDR)
PhD Supervisor email	gael.menasche@inserm.fr

PhD Proposal abstract (1000 characters maximum)

Mast cells (MCs) are key effectors of allergies triggered by crosslinking IgE bound to high-affinity IgE receptors (FceRI) by specific allergens. Triggering initiates MC degranulation and the release of inflammatory mediators stored in secretory granules (SGs). Previous work from the team used a conditional KO mouse model where MCs lack Kif5b to demonstrate that Kif5b regulates microtubule-dependent SG transport upon FceRI activation through PI3K-dependent recruitment to the granular Rab27b/Slp3 complex. More recently, we demonstrated that Rab44 interacts and functions with kinesin-1 to regulate SG translocation to the plasma membrane upon FcεRI activation. The goal of this PhD project is i) to continue to better understand the molecular mechanisms regulating the late signaling events of vesicular trafficking leading to SG release by MC and ii) to assess whether Rab44 can be considered as a potential target for modulating MC degranulation and inhibiting IgE-mediated allergic reactions.

PhD Proposal (4000 characters maximum)

Allergic diseases are driven by activation of mast cells (MCs) and release inflammatory mediators in response to IgE-directed allergens. These mediators are stored in cytoplasmic secretory granules (SGs). Activation of the high-affinity IgE receptor (FcεRI) by allergen-IgE complexes results in the translocation of SGs and their fusion with the plasma membrane also known as MC degranulation. Previous work from the team demonstrated that Rab44 interacts and functions with kinesin-1 to regulate SG translocation to the plasma membrane upon FcεRI activation. This PhD project aims i) to continue to better understand the molecular mechanisms regulating the late signaling events of vesicular trafficking leading to SG release by MC and ii) to assess whether Rab44 can be considered as a potential target for modulating MC degranulation and inhibiting allergic reactions.

The PhD project is organized around three axes:

1) To decode the molecular network supervised by Rab44 and modulating MC degranulation

Our work has shown that that Rab44 interacts and functions with kinesin-1 to regulate SG translocation upon stimulation, however we have not yet been able to co-immunoprecipitate Rab44 and members of the kinesin-1-dependent transport machinery. For this reason, we now aim to focus our effort on identifying the molecular network supervised by Rab44 on MC. We will first delineate the Rab44 interactome in MCs by combining immunoprecipitation or tandem affinity purification strategy to high-throughput mass-spectrometry to identify associated unknown candidate effectors. Then, identified candidates will be validated through the use of

functional assays for their role in MCs. We will also define the Rab44-dependent molecular network, by establishing the bulk RNA-sequencing transcriptome profiling of bone marrow-derived MCs of Rab44-deficient and WT adult mice at the steady-state. By conducting a bioinformatic analysis, we will address whether key molecular pathways affected by Rab44 disruption directly related to MC degranulation.

2) Therapeutic antisense oligonucleotides targeting Rab44 for inhibiting MC degranulation to treat allergy

The antisense oligonucleotide (ASO) strategy is a novel innovative therapeutic approach that aims to selectively modulate gene expression. ASOs are short (12-24-mers) chemically modified oligonucleotides that can base-pair RNA and modulate gene expression through the activation of RNase H1. An antisense oligonucleotide approach targeting Rab44 is here proposed to reduce the expression of Rab44 in murine and human MCs leading to an inhibition of MC degranulation upon activation. The validation of this strategy will be performed in vivo on anaphylaxis mouse models.

3) Identification of new molecular signaling pathways regulating late events of MC degranulation

To identify unknown, specific effectors of MC degranulation, we performed in silico proteomic analysis of primary human and mouse MCs purified from skin and fat. By focusing on proteins of unknown function that are strongly and specifically expressed in MCs, we have selected 20 possible effectors/targets. In order to characterize their function a siRNA screen will be performed assaying degranulation as a read-out. A selected panel of identified effectors/targets exhibiting strong effects on degranulation will be further characterized to validate their implication in exocytosis and to decipher their mechanism of regulation in MC function in vitro and in vivo.

Impact: Altogether, this project will i) provide new insights into the molecular mechanisms regulating the late signaling events of vesicular trafficking leading to SG release by MC and ii) propose new therapeutic strategy to specifically inhibit MC degranulation using antisense oligonucleotides targeting Rab44.

Expected profile of the candidate

We are looking for a highly motivated candidate holding a Master degree. The candidate requires previous laboratory experience in basic molecular and cellular biology techniques and in cell culture. Prior lab experience with mice is a plus.