Sorbonne Université/China Scholarship Council program 2021

Thesis proposal

Title of the research project: **Identification of Protective Immune Mechanisms in Diabetes Complications**

Keywords: Diabetes, Immunity, Monocytes, Transcriptomics, Epigenetics

Joint supervision: YES (RONAN ROUSSEL)

Joint PhD (cotutelle): no

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Institution: Sorbonne Univiersité

Doctoral school (N°+name): ED 394_ Physiologie, physiopathologie et thérapeutique

Research laboratory: CORDELIERS RESEARCH CENTRE (U1138), IMMUNITY AND METABOLISM OF DIABETES-IMMEDIAB

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Subject description (2 pages max):

1) Study context

Diabetic complications are a major fear of patients with diabetes. Chronic complications of diabetes affect many tissues and organs, causing retinopathy, nephropathy, neuropathy, and cardiovascular diseases **[1]**. If diabetes duration and glycaemic control are the main driving forces for developing diabetic complications, other factors could mediate this risk. Indeed, for the same glycaemic exposure (duration and level of hyperglycaemia), risk of complications could vary between individual with diabetes. For decades, there have been efforts to identify these factors with limited success so far. Immunological features have been shown to play a role in the onset of both micro and macrovascular complications **[2]**. However, the mechanisms resulting in the differential activation of the immune system which could modulate the susceptibility to chronic diabetes complications are still unresolved. Many studies have focused on deciphering the immune mechanisms involved in the complications of both types of diabetes, 1 and 2, as well as the role of age and diabetes duration **[1]**. Although these disorders occur in the majority of individuals with diabetes, a proportion of diabetics (10-15%) do not develop any (referred to in the proposal as "uncomplicated diabetes"). The phenotypic characterisation and immune mechanistic insights related to "uncomplicated diabetes" are currently lacking. Thus, an extensive phenotypic characterisation at the clinical, cellular and molecular levels is of interest to understand the protective mechanisms linked to diabetes complications. **The PhD proposal aims at integrating the clinical phenotype, immune activation profile and molecular characterisation of monocytes in diabetes subjects classified as "uncomplicated" and "complicated". The project may provide systematic evidence that an unmet immune response may limit diabetes complications.**

2) Details of the proposal

Our project is organized in 3 complementary tasks, combining human studies and computational skills.

Study populations: Type 1 and Type 2 Diabetes: In the PhD proposal, we will have a the great opportinoty to have acces to human samples (blood) from ongoing clinical studies funded by ANR-DGOS (2015_Angiosage (NCT02671864) and 2019_Glutadiab (NCT04353869) including type 1 (T1D) and type 2 (T2D) diabetes patients. We will select two groups of patients with opposite phenotype regarding the presence of complications or not: **1/** A group of **uncomplicated patients** (without any diabetic complication and 2/ a group of **complicated patients** defined by a previous history of lower limb amputation for diabetic foot ulcer (DFU) (this complication is chosen since it is one of the most severe among diabetic complications and involves both micro and macrovascular injuries). **Feasibility :** Blood samples from the present analyses are available from the Angiosafe and Glutadiab studies which are coordinated by PIs of Immediab.

Task 1: Characterisation of the blood immune response in "uncomplicated" diabetes
The immune-inflammatory activation extends to circulating cells and 2) the dynamics of the immune population may reflect the susceptibility to severe diabetes complications. In line with this, our laboratory has developed a highly sensitive cytometric method characterising the human immunophenotyped **[3, 4]**. Our approach quantifies 15 major innate and adaptive immune populations and subpopulations through lineage (CD45, CD3, HLA-DR, CD14, CD56 and CD20) and phenotypic markers (CD8, CD4, CD16, CD123, CD11c) in a single assay. In the PhD project, this sensitive method will be used to define the immune profile

of diabetic participants. Analyses will allow a discrete quantification of cell frequencies and absolute counts as well as rare population discovery or validation through unsupervised analyses and dimensional reduction algorithms. Additionally, we will characterise the serum and intracellular metabolome associated with immune cell profiling (with a focus on monocytes) in the diabetics' populations. This investigation will allow the discovery of novel metabolite pathways that may per se modulate the protective immune response associated with no or delayed diabetes complications.

Task 2: Applying transcriptomics and epigenomics to characterise monocytes activation status

In recent years, it has become clear that on the genome scale, DNA sequences called enhancers, more so than promoters, orchestrate the majority of cell-type-specific patterns of gene expression, particularly in phenotypically plastic cells such as monocytes. Enhancers are discrete regions of the genome that function to increase transcription from nearby promoters **[5, 6]**. In a given cell, enhancer elements can be broadly categorized as inactive, primed, poised, or active **[7]**. Defining the repertoire of such enhancers' categories of blood monocytes in diabetes is crucial to better understand the susceptibility to diabetic complications. The main challenge of this Task will be to identify enhancers with different characteristics (based on chromatin accessibility, histone modification and gene expression) between groups of diabetic participants (uncomplicated versus uncomplicated) (description in task 1).To address this matter, we will combine transcriptomic (RNA-Seq) and epigenomic approaches including identification of open-chromatin regions (ATAC-seq), DNA-methylation status (Arrays) and mapping repressive and active histone marks (H3K27ac and H3K27me3) by ChIP-sequencing.

Task 3: Identification of the molecular/Immune score through data integration

The aim of this task is to identify molecular and immune pathways in diabetes with no or late onset complications. An immune score will be established through bio-analyses that integrate data of clinical phenotype, immune profile, metabolomics and genomics. The PhD candicate will be involved in data integration in close collaboration with the experts in bio-analyses of IMMEDIAB team (Gilberto Velho, Louis Potier, Claire Vandiedonck and Marc Diedisheim).

3) References

1-Forbes JM, DOI: 10.1152/physrev.00045.2011
2-Grossman V, DOI: 10.2337/dc14-3008
3-Julla JB, DOI: 10.1007/978-1-4939-9130-3_3
4-Alzaid F, DOI: 10.15252/emmm.202013038
5-Bulger M, DOI: 10.1016/j.ydbio.2009.11.035
6-Ong CT, DOI: 10.1038/nrg2957
7-Diedisheim M, DOI: 10.1016/j.molmet.2020.101041

4°) Profile of the Applicant (skills/diploma…)

The successful candidate should:

1) Have a Master degree in molecular biology, biomedicine, medicine, biochemistry, genetics or related fields.

2) Have solid knowledge of molecular biology and extensive wet lab experience in molecular biology techniques.
3) Have experience/ knowledge in Flow Cytometry Analysis (FACS) and immunology
4) Already have or keen to learn bioinformatics skills to analyze genome-wide sequencing data, in addition to above wet lab skills.
5) Have proficiency in English

Above that, we also expect the successful candidate to have excellent communication and organizational skills, and work both independently and as a team player.

Contacts:

Thesis supervisor

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