jeudi, août 18, 2022

INTERNATIONAL PhD PROPOSAL

PhD Supervisor full name **Anne Puel**

PhD PROPOSAL IDENTIFICATION

PhD Project title	Inborn errors of immunity and chronic mucocutaneous candidiasis
Project Acronym	IEI – CMC
Project Keyword	Genetic predisposition; human; fungal diseases; IL-17; whole exome sequencing

LABORATORY PRESENTATION

Laboratory Team Name	Human Genetics of Infectious Diseases
Department IP	Imagine Institute
Doctoral school	BioSPC
University	Paris Cité
Laboratory website	https://www.institutimagine.org/fr/jean-laurent-casanova-177

PhD PROPOSAL

PhD Supervisor full name	Anne Puel
PhD Supervisor position	Research Director (DR1) Inserm
PhD Supervisor email	anne.puel@inserm.fr

PhD Proposal abstract (1000 characters maximum)

Chronic mucocutaneous candidiasis (CMC) is characterized by severe lesions of the nails, skin, and mucosae by the fungus Candida albicans. We have discovered 14 inborn errors of IL-17 immunity underlying isolated or syndromic CMC. The mechanisms that disrupt IL-17 immunity and underlie CMC remain incompletely understood, while half of the 800 patients of our cohort with idiopathic CMC lack a genetic etiology. Using powerful genetics/genomics, computational, molecular and cellular approaches, we aim to pursue the molecular, cellular, immunological, and clinical study of known and novel inborn errors of IL-17A/F immunity in patients with isolated or syndromic CMC. This proposal will provide new insights into the mechanisms of mucocutaneous immunity to fungi, while dissecting the molecular and cellular control of human IL-17, and also provide new molecular diagnoses for patients and genetic counseling for families, while paving the way for new cytokine-based approaches in these patients.

PhD Proposal (4000 characters maximum)

lesions
of the nails, skin, and mucosae by the fungus *Candida albicans*. From 2008 onwards, we have discovered 14 inborn
errors of IL-17 immunity underlying 'isolated' (autosomal recessive (AR)
IL-17RA, IL-17RC, and ACT1 deficiencies, and autosomal dominant (AD) IL-17F
deficiency) or 'syndromic' (AR CARD9, IL12p40, IL-12Rβ1, ROR-g/gT,
ZNF341, c-Rel deficiencies, AD STAT3, IL6ST/GP130, and JNK1 deficiencies, and
AD STAT1 gain-of-function (GOF)) CMC. Cells with IL-17RA, IL-17RC, ACT1, or
JNK1 deficiency respond poorly to IL-17A/F. Patients with CARD9, IL12p40,
IL-12Rβ1, ROR-g/gT, ZNF341, c-Rel, STAT3, IL6ST, or JNK1 deficiency, or
STAT1 GOF, display low proportions of IL-17A/F-producing T cells. However, the mechanisms
that disrupt IL-17 immunity and underlie CMC remain incompletely understood.
Moreover, half of the 800 patients of our cohort with idiopathic CMC lack a
genetic etiology. We
aim to pursue the molecular, cellular, immunological, and clinical study of novel
inborn errors of immunity in patients with isolated or syndromic CMC. We
propose here to discover novel CMC-causing genes using genome-wide (GW)
approaches, including whole-exome or whole-genome sequencing (WES/WGS), and to characterize
their molecular, cellular, immunological, and clinical impact by comprehensive functional
studies. Briefly, using bioinformatics
tools developed in the lab, the student will analyze WES/WGS data,
applying various filters up to the selection of rare or private candidate variants
prioritized based on several criteria (e.g. the number of patients bearing rare
variants in the identified gene, the degree of purifying selection, the
potential damaging effect of the variants, the known expression pattern and
function of the gene in host immune pathways). The candidate variants will be
studied by assessing the intra-familial segregation and their frequency in the
general population and the corresponding ethnic group.

The
most promising variants will be studied experimentally, at the molecular,
cellular, and immunological levels using overexpression systems (e.g. HEK293T,
Jurkat cells) and patients' cells (whole blood, PBMCs, immortalized T or B cells,
fibroblasts, keratinocytes). The expression of the mutant allele will be
assessed, at the RNA and protein levels by RT-qPCR, flow cytometry and/or Western
blotting, by overexpression in the appropriate cell populations. At the
molecular level, the functional consequences of the variants will be tested
with relevant techniques (e.g. phosphorylation assessed by Western-blot/flow
cytometry, gene induction by RT-qPCR or luciferase assays) depending on the
gene and the pathway involved. At the cellular level, cytokine/chemokine

induction will be assessed by intracellular flow cytometry or multiplex ELISA. A comprehensive cellular immunophenotyping using a 40-color flow cytometry panel or CyTOF will be performed to test the impact of the variants on the development and/or differentiation of leucocyte subsets. Leukocyte subset function will be analyzed using transcriptomic analyses with RNAseq of specific cell types or sc-RNAseq of PBMCs, as the basal state and upon appropriate stimulations. For selected cellular phenotypes, rescue experiments will be performed with the expression of an exogenous wild type copy of the candidate gene. CRISPR/Cas9 technology will be used to create the patients' variant in control cells (e.g. T cells) or cell lines (e.g. Jurkat cells), and to correct the variant in the patients' cell line or primary cells. In all experiments, appropriate controls cells will be included. From a biological standpoint, this research will provide new insights into the mechanisms of mucocutaneous immunity to *C. albicans*. The clinical implications
will help the patients and their families in terms of molecular diagnosis, genetic counseling, treatment and clinical outcome.

Expected profile of the candidate

A highly motivated and talented candidate with, if possible, skills in genetics, molecular and cellular biology.