Proposal for the 2020 Imagine International PhD program

Laboratory: *Immunogenetics of pediatric autoimmune diseases*
Head of laboratory: Frédéric Rieux-Laucat, PhD
Project and student supervisor: Aude Magerus (HDR Y~~/N~~)
Number of HDR in the lab: 5
Field of research: Immunology, Genetic and molecular basis of cellular mechanisms involved in autoimmunity
Number and names of PhD students currently in the lab in 2020:
2nd Y: 2: Laure Delage and Caroline Besnard
3rd y: 1: Jerome Hadjadj
Number and names of PhD students under the Imagine program: 1-Jérome Hadjadj
Project Title: Autoimmunity Through multi-OMICS analysis (ATOMICS)

Project description (Max 2 pages including abstract and publications)

In this project, we aim to determine the mechanisms involved in the control of self-tolerance human by studying the ALPS-FAS model[1]. Autoimmune lymphoproliferative syndrome (ALPS) is a human genetic disorder with childhood onset usually caused by defective lymphocyte homeostasis. The ALPS-FAS is a non-infectious non-malignant lymphoproliferative disease along with autoimmune cytopenia. It is associated with heterozygous dominant mutations of the *FAS* gene. By discovering the first germline heterozygous mutations of *FAS*[2] and the first somatic mutations in sporadic cases[3], our group has shown that the ALPS is a consequence of lymphocyte apoptosis defect. We then discovered that some *FAS* mutations exhibiting a partial clinical penetrance (healthy relatives carrying the same *FAS* mutation, HC-FAS) are accompanied by somatic mutations of the second allele of *FAS* in patients but not in healthy relatives[4]. However, a second event remains to be elucidated in 18% of the ALPS-FAS patients. Using a whole exome sequencing approach in 16 ALPS-FAS families carrying mutations associated with low penetrance, we already identified 4 candidate genes in 8 families (analysis still in progress in other families). The first candidate gene encodes a protein regulating the FAS-Ligand (FASLG) expression. The homozygous variation identified in the patients is associated with an *in vitro* apoptosis defect related to a decreased FASLG expression. The second gene, located on the X chromosome, is inherited from the mother in 3 different families, fitting with the sex ratio imbalance observed in these ALPS-FAS families. The third gene is involved in the recycling of the endosomal vesicles including the FAS receptor, and the fourth could induce cell death after engagement of the membrane attack complex. These preliminary results indicate that in some instances the ALPS can exhibit a digenic inheritance. Together with the cases of combined germline and somatic *FAS* mutations previously described, these observations account for the lack of clinical prognosis (development or not of the disease) in front of a newborn diagnosed with a *FAS* mutation in the context of a familial ALPS.

The goals of this project will be:

1-To study the consequences of the identified genetic defects associated with a germline *FAS* mutation with regards to the immunological mechanisms underlying the self-tolerance checkpoint defects.

We will first determine whether the identified mutations are gain or loss function by analyzing the expression at the protein level and the function of the corresponding genes. To analyze their consequences with regards to the ALPS-onset, these genes will be cloned in a lentiviral vector in order to express the mutants in HC-FAS lymphocytes (hypothesis of GOF mutations), or the wild-type gene in the FAS-patients lymphocytes (hypothesis of LOF). We will assess the apoptosis functions and the proliferation of the transduced lymphocytes. All cellular models are available and animal models can be developed when necessary (i.e characterization of the 4th gene).

2- To analyze the transcriptional landscape at the bulk and single cell level in patients (before and after the ALPS onset) and healthy carriers as well as in patients before and under treatment.

Using the power of multi-omics analyses such as Whole exome/genome sequencing (WES/WGS), Single-cell CITEseq (RNA-seq coupled with bar-coded antibodies) combined with more classical cell biology analyses of

lymphocytes, we will decipher the mechanisms leading to lymphoproliferation and/or autoimmunity occurrence in the patients exhibiting a *FAS* mutation. Our first goal is to identify biomarkers and molecular targets to accelerate the diagnosis and improve the treatments of such patients. The second goal will be the development of biomarker-based diagnostic and prognostic tools to identify patients eligible for pathway-specific treatments. We will compare the transcriptomic profile of ALPS-FAS patients carrying a dominant FAS mutations with complete clinical penetrance (n=5) and patients with haplo-insufficient mutations and incomplete clinical penetrance for whom a digenic cause was identified (3 patients and 2 HC-FAS) or not (5 patients and 5 HC-FAS). For the later, the availability of the WGS data will allow the search for possible variants in the deregulated genes. Lastly, we have selected 2 families for which we obtained blood samples from patients' relatives who were asymptomatic at the time of genetic diagnosis (and supposed to be HC-FAS) but who developed ALPS symptoms later in life. This will provide a unique opportunity to analyze the transcriptomic profiles at the single cell level before and after the disease onset. Since these patients were not initially treated at disease onset, we will have the opportunity to analyze them untreated and under treatment (mTOR inhibitors).
This project will benefit from a unique cohort of >200 biobanked ALPS-FAS patients' samples as well from the genomics, Single cell RNAseq and bioinformatics core facilities of the Imagine Institute

This proposal should lead to the identification of the molecular mechanisms leading to autoimmunity in children and of molecular signatures that will help the clinicians in the prognosis and treatment monitoring of the ALPS-FAS patients.

Lab members

Staff scientists: Frédéric Rieux-Laucat (Head), Aude Magérus-Chatinet and Fabienne Mazerolles (CR-Inserm) Sonia Meynier and Laura Barnabei (Post-Doc)
Research Assistants: Marie-Claude Stolzenberg (IR) and Sajedeh MIRSHAHVALAD (AI)
PhD Students: Jérome Hadjadj, Laure Delage and Caroline Besnard
Master Students: Claire Mayer and Victor Haas
Pediatricians: Bénédicte Neven, Brigitte Bader-Meunier, Pierre Quartier-Dit-Maire, Florence Aeschlimann

Major Publications

1. Rieux-Laucat F, Magerus-Chatinet A, Neven B. The Autoimmune Lymphoproliferative Syndrome with Defective FAS or FAS-Ligand Functions. J Clin Immunol. 2018 Jul;38(5):558–568.

2. Rieux-Laucat F, Le Deist F, Hivroz C, Roberts I, Debatin K, Fischer A, de Villartay JP. Mutations in *FAS* associated with human lymphoproliferative syndrome and autoimmunity. Science. 1995;268:1347–1349.

3. Holzelova E, Vonarbourg C, Stolzenberg MC, Arkwright PD, Selz F, Prieur AM, Blanche S, Bartunkova J, Vilmer E, Fischer A, Le Deist F, Rieux-Laucat F. Autoimmune lymphoproliferative syndrome with somatic *FAS* mutations. N Engl J Med. 2004 Sep 30;351(14):1409–18.

4. Magerus-Chatinet A, Neven B, Stolzenberg MC, Daussy C, Arkwright PD, Lanzarotti N, Schaffner C, Cluet-Dennetiere S, Haerynck F, Michel G, Bole-Feysot C, Zarhrate M, Radford-Weiss I, Romana SP, Picard C, Fischer A, Rieux-Laucat F. Onset of autoimmune lymphoproliferative syndrome (ALPS) in humans as a consequence of genetic defect accumulation. J Clin Invest. 2011 Jan;121(1):106–12. PMCID: PMC3007148