PROJECT 1

DoS: ALESSANDRA MORTELLARO

Title: Study of disease pathogenesis and development of gene therapy for adenosine deaminase 2 deficiency

Curriculum: Gene and Cell Therapy

Link to OSR/UniSR personal page:

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

The deficiency of adenosine deaminase 2 (DADA2) is a rare genetic disease caused by autosomal recessive mutations in the ADA2 gene. The most common presentation of DADA2 is cutaneous and cerebral vasculopathy, stroke, systemic inflammation, hematological and immunological manifestations. Disease onset is usually in childhood, and a significant proportion of patients are deceased before the age of 30. The clinical benefit of the existing therapies is variable, and sustained disease remission is difficult to achieve. Therefore, there is a high unmet medical need for the development of targeted therapies for DADA2. However, there is a complete lack of knowledge of the immune mechanisms underlying DADA2. A better understanding of how ADA2 regulates immune responses will significantly contribute to identifying new therapeutic strategies for the treatment of patients with this puzzling syndrome.
The goals of this Ph.D. project are:
1) To elucidate the mechanisms regulated by ADA2 during the innate immune response.
Preliminary results suggest that loss of ADA2 in patients' macrophages causes increased production of pro-inflammatory cytokines (IL-1, TNF, IFN) and poor differentiation of anti-inflammatory M2 macrophages. The role played by ADA2 in controlling macrophage homeostasis remains to be identified.
2) To evaluate the efficacy of a lentiviral vector-mediated gene therapy approach to correct DADA2 cellular defects in patients' cells.
An ADA2-encoding lentiviral vector already generated in the laboratory will be used to restore ADA2 expression in patients' hematopoietic stem cells and macrophages and determine whether ADA2 correction restores a more physiological immune response in DADA2 syndrome.
Collectively, these studies will lead to a better understanding of the molecular basis of DADA2 in terms of hematopoietic and innate immune compartments and contribute to establishing a clinically applicable gene therapy protocol that will be exploited for future clinical translation.

Skills to be acquired by the student:

Molecular biology techniques: cDNA cloning in a plasmid, gene delivery, DNA and RNA sequencing, RNA isolation and real-time qPCR, gel electrophoresis.

Cellular biology techniques: ELISA, Western blot, immunoprecipitation of proteins, immunofluorescence microscopy, flow cytometry and cell sorting.
Cell culture techniques: isolation of immune cells from blood, mouse bone marrow and spleen, maintenance of immune and non-immune cell lines.
Basic and advanced in vivo experimentation techniques: mouse handling, generation and maintenance of colonies, genotyping, administering injections, bone marrow transplant, isolation of immune organs.
The student will be prepared to integrate the scientific method and critical thinking, and report project results clearly and effectively to a small/medium audience. The student will also be empowered with effective writing skills.

References

Zhou Q, et al. Early-onset stroke and vasculopathy associated with mutations in ADA2. N Engl J Med. 2014 Mar 6;370(10):911-20.

Pui Y. Lee, et al. Genotype and functional correlates of disease phenotype in deficiency of adenosine deaminase 2 (DADA2). J Allergy Clin Immunol. 2020 Jun;145(6):1664-1672.e10.

Barzaghi F, et al. ALPS-Like Phenotype Caused by ADA2 Deficiency Rescued by Allogeneic Hematopoietic Stem Cell Transplantation. Front Immunol. 2019 Jan 14;9:2767.