**PROJECT 1**

Title: **Unraveling the molecular mechanisms controlling dendritic cell (DC)-mediated immune tolerance**

Curriculum: BAIO

Link to OSR/UniSR personal page:
https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/mechanisms-of-peripheral-tolerance/silvia-gregori.html

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

Peripheral immune tolerance is an active process that prevents immune response to self/non-dangerous antigens, thus controlling the development of chronic inflammation and autoimmune diseases. The balance between the activity of effector and regulatory cells plays a pivotal role in this system. Still, the molecular mechanisms regulating identity and function of regulatory cells are partially obscure. In the recent years several lines of evidence have shown the relevance of epigenetic control combined with the activity of specific transcription factors in ruling immune cell differentiation and function. Highlighting this role, several studies have linked autoimmune manifestations to defects of epigenetic regulation.
We identified and characterized DC-10, an inducible subset of human DC endowed with the ability of spontaneously and upon activation releasing high levels of IL-10, and of inducing Tr1 cells (1). DC-10 can be identified in the peripheral blood and in tissues thanks to the discovery of CD141, CD163, CD14, and CD16 (2). More recently, by investigating the epigenome and transcriptome of in vitro differentiated and ex vivo isolated DC-10 from healthy subjects, we have identified a set of IL-10-induced enhancer-gene nodes that exploit specific transcription factors, which might be involved in tolerogenic activity of human DC.
Aim of the project is to dissect the role of the identified transcription factors in controlling the tolerogenic functions of DC, and specifically of DC-10, and to assess their relevance in pathological conditions. The project involves high throughput analyses of chromatin status and transcriptome in tolerogenic and inflammatory cells ex vivo isolated from healthy donors and autoimmune patients, reverse genetic/gene editing approaches to study the role of selected candidate gene(s) and state of the art immunological assays to study functionality of human immune cells.
This approach will allow to identify novel molecular mechanisms that control the activity of regulatory and effector immune cells, which are aberrant in pathological conditions due to breaking of immune tolerance. The results of this project will help better understanding the mechanisms controlling immune tolerance and immune response, and might help designing new therapies to re-establish immune tolerance in autoimmune disease.

***Skills to be acquired by the student:***

- Multi-parametric flow cytometric analysis
- In vitro immunological assays, such as proliferation, suppression and cytokine production assays
- FACS-sorting of rare populations of cells
- Nucleic acid extraction and analyses by low- and high-throughput techniques (ddPCR, NGS)
- Genetic modification of primary cells and cell lines

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**References** (max. 3)

1. Gregori S., Tomasoni D., Pacciani V., Scirpoli M., Battaglia M., Magnani CF., Hauben E., Roncarolo MG. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. Blood. 2010 Aug 12;116(6):935-44. doi: 10.1182/blood-2009-07-234872.
2. Comi M., Avancini D., Santoni de Sio F., Villa M., Uyeda M.J., Floris M., Tomasoni D., Bulfone A., Roncarolo M.G., Gregori S. Co-expression of CD163 and CD141 identifies human circulating IL-10-producing dendritic cells (DC-10). Cell Mol Immunol. 2019 Mar 6. doi: 10.1038/s41423-019-0218-0.