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ESID medium term fellowship October 2016-March 2017- FINAL REPORT

Dear ESID Board,
In October 2016 I started my internship at the Department of Rheumatology and Clinical Immunology at the University of Freiburg in Dr Marta Rizzi's research group.

In these six months I had the possibility to learn several techniques related to human B cell research:
- Freezing and thawing of primary human B cells and hematopoietic stem cells
- CD34+ hematopoietic stem cells and B lymphocyte isolation by magnetic beads
- Flow cytometry: surface and intracellular staining, acquisition (FACS Canto II BD), and analysis by FlowJo software; identification of B cell subpopulations (naïve B cells, transitional B cells, marginal zone B cells, switched memory cells, plasmablasts, CD21 low B cells); identification of B cell precursors (myeloid and lymphoid progenitors, Pro B cells, Pre B cells, Immature B cells). Intracellular calcium flux by ratiometric analysis of Fura Red; intracellular phosphoprotein staining.
- Human primary B cells culture; in vitro human B cell activation with cytokines or other stimuli (CD40L,IL-21, CpG, anti-IgM); effect of small molecules on B cell development in vitro; activation, proliferation and survival assays. Human early B cell development in vitro from cord blood or bone marrow derived CD34+ cells.

I used these techniques to characterize the B cell compartment of patients affected by Common Variable Immunodeficiency and other PID and to study the in vitro B cell development from BM derived CD34+ cells from patients affected by primary antibody deficiencies (PAD) as well as healthy donors.

Primary antibody deficiencies are a heterogeneous group of diseases characterized by antibody production failure. They can present with a wide spectrum of clinical phenotypes, complications and disease severity. Molecular defects have been identified only in about 15-25% of patients affected by PAD. Analysis of patients' bone marrow showed that about 20% of patients carry a block in early B cell development, but the physiopathology behind this block is still unknown. To study the mechanisms of this developmental block, an in vitro model to study early human B cell development was set up and recently published by Marta Rizzi's research group. This is a three-stage cultivation system, independent of feeder cells. In the first stage CD34+ cells isolated from bone marrow are expanded for 7 days in the presence of SCF, Flt3-L and IL-6. Then cells are collected and plated again in medium containing SCF, Flt3-L and IL-7 in order to favor the development of lymphocyte precursors. From day 14 to day 49 (third stage) cells are cultivated in cytokine-free medium and analyzed once a week by flow cytometry. In healthy donors this leads to the development of lymphocyte progenitors until the stage of IgM+ immature B cells.

I tested BM derived CD34+ cells from PAD patients *in vitro* by feeder free differentiation system for their capability to develop into Immature B cells. CD34+ cells from patients showing a normal B cell development *in vivo* (normal presence of B cell precursors in BM aspirates

analyzed by flow cytometry) developed lymphocyte progenitors until the stage of IgM+ Immature B cells, while hematopoietic stem cells isolated from PAD patients presenting a block in the early B cell development could not reach the immature B cell stage. Therefore, this system is suitable to model in vitro the block in early B cell development observed *in vivo*.

During my internship I could also participate to the seminars organized by the CCI (Center for Chronic Immunodeficiency), to the BBI (Bed and Bench Immunology) course, the Translational Immunology School of the German Society of Immunology and to the B cell forum, where I could present the preliminary results of my project and get in touch with the main experts in the field of immunology and PID. This will contribute to build a network for collaboration on PID projects when I will be back to Prof D'Elios´ research group at University of Florence.

The friendly and stimulating atmosphere in Marta Rizzi's lab, as well as her strong expertise in the field, allowed me to make the most of my experience.

I would like to warmly thank ESID for this great opportunity that permitted me to strongly improve both my laboratory skills and my knowledge in PID.

Best regards

Dr. Arianna Troilo