ImmunoTools *special* Award 2024

Jeanne Le Cléac'h, PhD-student

Supervisor: Jonathan D. Turner

Department of Infection and Immunity, Luxembourg Institute of Health, 29, rue Henri Koch, 4354 Esch-sur-Alzette, LUXEMBOURG

Long-term immunological consequences in adversity-divergent twins

The early life development period, from conception until late adolescence, represents an opportunity for environmental factors to disturb the maturation of the individual. These factors are present in various forms such as infections, pollutants, psycho-socioeconomic status and are known as Early Life Adversities (ELA). ELA is associated with diseases such as type-2 diabetes, coronary heart diseases or neuro-psychologic disorders. Epidemiologic studies have shown that psycho-socioeconomic status in early life can predict these health outcomes in adults. Although recognized as decisive drivers of health inequalities, the mechanisms are still poorly understood.

Low grade inflammation is normally observed in ELA exposed individuals and it correlates with poor health. Literature shows that the functionality of immune cells is often impacted by ELA leading to inflammeageing and immunosenescence later on. As the immune system is a major operator of health and seems to be a proxy in the apparition of health inequalities, we are investigating its role and the mechanisms by which it could participate in this major public health challenge.

It has not been possible, so far, to exclude genetic effects in human environmental studies. As monozygotic twins share the same genetic background, they provide a robust approach to identify the influence of the environment on biological features. The German cohort "TwinLife" has followed over 4,000 twin pairs since 2014 to assess genetic predisposition and environmental influence on health and social outcomes. We screened this cohort for divergence in adverse experience in early life and identified over 150 adversity-divergent pairs of monozygotic twins between 18 to 30 years old.

The collection of fresh blood and saliva samples as well as the clinical psychological screening of the participants is still ongoing. To investigate our main hypothesis, we will use immune cells from the whole blood. We are cell sorting immune population on fresh blood but also isolating and storing peripheral blood mononuclear cells (PBMCs), plasma, DNA, RNA. First, using flow and mass cytometry we are currently assessing the immunophenotype and seeking differences with a twin-twin comparison. Then, we will take a closer look at cells functionality

and metabolism with in vitro essays using frozen PBMCs and plasma. Finally, underlying molecular mechanisms will be explored with epigenetic modification such as differentially methylated regions and DNA expression with RNA specimen. This method will allow us to understand the impact of early life psycho-socioeconomic factors on biological features at every levels.

We previously showed in rats that Natural Killer (NK) cells were particularly impacted by the social stress of maternal separation with a decrease of cytotoxicity, increase of inflammaging and senescence markers. As it has not yet been shown in humans, we will conduct functional tests on NK cells in our cohort. NK cells are derived from common lymphoid progenitors, represent up to 16.87% of PBMCs in healthy adults but are also resident in some extra medullar sites such as lymph nodes, thymus, liver, uterus etc. These innate lymphoid cells are the first necessary line of defence for our organism. They are characterized by their cytotoxicity, degranulation (perforine, granzyme B) capacities to eliminate the threat and cytokine secretion capacities (IFNg, TNFa) for immune cell recruitment.

NK cells can be subdivided in many categories according to their location, role or maturation states. These populations will be deeply investigated during the single cell analysis of our cohort. As everything is already set up for the immunophenotyping, we want to move on to the next step: **assessment of functional differences in NK cells of monozygotic divergent twins**.

In order to estimate these capacities, we will optimize a cytotoxicity and degranulation essay for NK cells after sorting the PBMCs.

To evaluate the cytotoxicity we will use target cells (K562) marked with a cell tracer and cultivate them with NK cells to assess the deaths ratio of the target cells. As a positive control for NK cell activation (with and without target cells) we will use **IL-2**. For the degranulation assay, we will activate NK cells with **IL-12** and **IL-15** to mimic physiological activation without target cells and expand them. After incubation, we will collect the supernatant to measure the levels of **IFNg** and **Granzyme B** produced.

The ImmunoTools products selected will allow us to characterize the early life psycho-socioeconomic impact on these NK cells functioning and lead to new leads on further investigation about the characteristics of NK cells metabolism.