Impact of vitamin D on the epigenetic programming of CD34$^+$ hematopoietic progenitor cells derived from human cord blood

Our immune system protects us against various types of microbes that may cause infectious diseases. However, in order to fulfill this function perfectly, the cells forming the immune system need to be trained as good as possible. This training takes place during hematopoiesis, which is a differentiation process happing inside our large bones. In this proposal, we aim to understand how hematopoiesis is influenced by vitamin D and its receptor VDR. The transcription factor VDR works together with other transcription factors like PU.1 and CEBPα in shaping the epigenome of hematopoietic stem and progenitor cells, *i.e.*, VDR epigenetically programs these cells. This determines what type of immune cells are produced and how well these cells are prepared for their function in the defense against microbial infections. Thus, we aim to understand how vitamin D and its receptor prepare our immune system, in particular monocytes of innate immunity, for their on optimal function.

The easiest and less harming way to isolate hematopoietic stem and progenitor cells is from cord blood of newborns, *i.e.*, using blood that remains after birth in the placenta. We will use cord blood of 40 newborns (after cutting the cord, *i.e.*, the blood remaining in the placenta) as well as blood samples of their mothers before giving birth. From both type of blood samples we will isolate mononuclear immune cells and from these mature monocytes. Based on the immunophenotype and the DNA methylation pattern, we will select 20 mother-newborn pairs for further analysis. From these we will prepare CD34$^+$ cells (*i.e.*, hematopoietic stem and progenitor cells), multiply them *in vitro* for 4-6 days and differentiate them then within 3 weeks into monocytes. We will perform his differentiation process in the absence and presence of the vitamin D metabolite 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and take samples every week. As reference, also mature monocytes from mothers and cord blood will be tested for their response to $1,25(OH)_2D_3$. All samples will be analyzed for changes in their epigenome, such as chromatin accessibility, active chromatin (H3H27ac marks) and the binding of VDR, PU.1 and CEBPα, as well as in their transcriptome. These genome-wide data will allow us to build for each individual a computer model of the regulatory processes, each of which involve thousands of genomic regions and hundreds of genes. These models will be compared with data from a vitamin D intervention study, which we performed recently with 47 healthy adults.

We expect that our study will allow us to

- describe the effect of vitamin D on the training (epigenetic programming) of immune cells during hematopoiesis
- discover interindividual differences in this process that allow to understand individual-specific responses to vitamin D
- discover the mechanistic understanding on how vitamin D modulates the functionality of the innate immune system

In conclusion, this project will provide mechanistic insight into interindividual differences in vitamin D-triggered immune responses. This will allow a better understanding why vitamin D supplementation, in particular in the summer months is essential for our health.