Title: Immune cell migration in congenital disorders of glycosylation.
Department: Cell Biology
Supervision: Prof. Dr. Alessandra Cambi (alessandra.cambi@radboudumc.nl)
Dr. K. van den Dries (koen.vandendries@radboudumc.nl)
Paola de Haas (paola.dehaas@radboudumc.nl)
Duration: 6 months

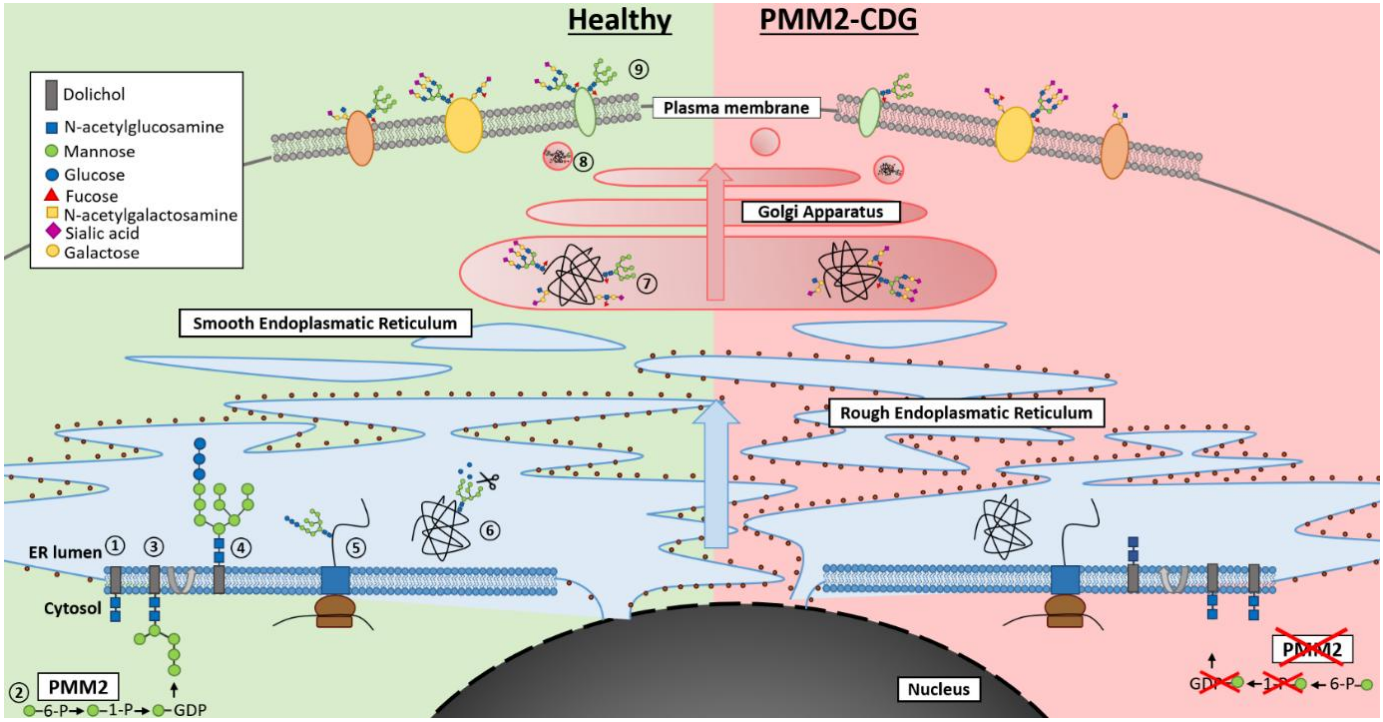
Main aim:
The aim of this internship project is to investigate the effect of a glycosylation defect on immune cell migration in an *in vitro* PMM2-CDG model.

Background:
PMM2-CDG is the most prevalent type of Congenital Disorders of Glycosylation I (CDG-I). CDG-I is a group of diseases affecting the assembly of sugars (glycans) in the process of protein glycosylation. PMM2-CDG is caused by mutations in the gene phosphomannomutase 2 (PMM2), which occur in about 50% of the CDG-I patients. PMM2 is an enzyme that converts mannose 6-phophate to mannose 1-phosphate. Mannose 1-phosphate is converted to GDP-mannose, which is a crucial step for subsequent assembly of the glycan chain. Addition of glycans to proteins is known as glycosylation. Mutations in PMM2 result in loss of complete N-glycans in almost every cell of the body.

PMM2-CDG patients therefore present a large spectrum of symptoms including muscular hypotonia, delayed psychomotor development, hepathopathy, and skeletal abnormalities. In addition, approximately 20 % of the patients does not survive the first year of life, and the main cause of this early mortality is microbial infection. So far, research on this disease has mainly focused on the neurological aspects, however, the high infection driven mortality in the first year strongly indicates that hampered immunological processes could play an important role in the clinical presentation and disease progression.

Several studies indicate that glycosylation indeed plays important roles in the immune system. For example, glycan-binding proteins such as selectins and galectins are important for leukocyte trafficking and cell-extracellular matrix (ECM) interaction, respectively. Furthermore, glycosylation plays a role in the immunological synapse formation and influences the threshold of T cell receptor and B cell receptor activation.

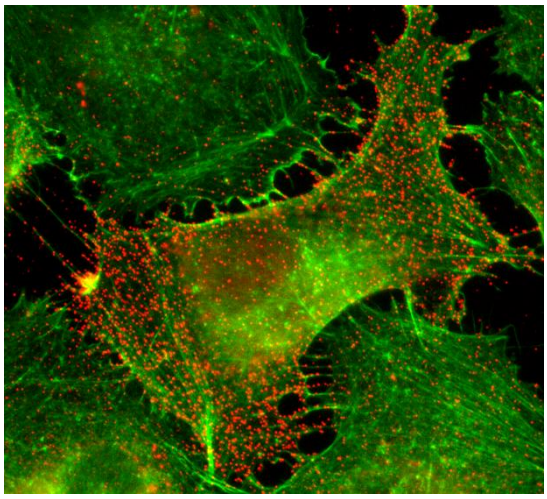
Despite this information, the molecular mechanisms and the impact of this PMM2 genetic defect on the immune system have not been clarified yet, and key immune cell types such as T cells or antigen-presenting cells have not been thoroughly studied in this pathology.



Plan of investigation:

This internship project will focus on immune cell function in PMM2-CDG. In specific, the migratory properties of antigen-presenting cells, such as dendritic cells and macrophages will be analyzed. moDCs and a THP1 cell line will be used and treated with tunicamycin. Tunicamycin is an antibiotic that inhibits the enzyme N-acetylglucosamine transferase, responsible for the addition of the first monosaccharide to the dolichol anchor in N-glycosylation. Tunicamycin therefore inhibits the N-glycan assembly one step upstream of the PMM2 mutation and provides a good *in vitro* model for PMM2-CDG.

The migratory properties of antigen-presenting cells in PMM2-CDG will be analyzed by using a diversity of techniques. A 2D random migration assay and a transwell migration assay will be performed to study the random and directed movement of the immune cells. Flow cytometry will give insight into the expression of differentiation markers and integrins. The binding capacity of the integrins will be assessed by measuring the binding to fluorescent labeled ligand coated beads.



Heparan sulphate (red) and actin (green) on endothelial cells

Techniques used:

- Generation of moDCs
- 2D random migration
- Fluorescence microscopy
- Transwell migration assay
- Flow cytometry

References:

1. Barone et al. Semin Neurol. 2014 Jul;34(3):357-66
2. Blank et al. J Inherit Metab Dis. 2006 Aug;29(4):592
3. He et al. Glycobiology. 2014 Apr;24(4):392-8
4. Hauser et al. J Leukoc Biol. 2016 Jun;99(6):993-1007