DARC complex arrangements in maturing reticulocytes

Introduction: Erythropoiesis occurs in the bone marrow and is the process that leads to the production of erythrocytes (red blood cell) from hematopoietic stem cell (HSC). The resulting enucleated reticulocyte exits the bone marrow and into the circulation where it matures into erythrocyte within 48-72 hours.

During reticulocyte maturation in the peripheral blood, the plasma membrane and the cytoskeleton are subjected to an intense remodelling, in part due to the fact that the cell has to gain its typical biconcave shape, which will allow getting through the narrow capillaries, but also due to the active vesicle formation process to get rid of unwanted proteins.
We are in particular interested in DARC (Duffy antigen receptor for chemokines), which is a membrane protein and besides being the basis of the FY blood group system, is the main receptor recognized by the malaria parasite Plasmodium Vivax. This parasite invades only reticulocytes and causes the majority of malaria cases outside Africa. What we have seen before is that DARC is expressed in all erythroid cells from early erythropoiesis to erythrocyte stage. Although its protein expression is stable, different epitopes within DARC molecule are exposed in immature reticulocytes and masked in erythrocytes, which could partly explain why Plasmodium Vivax invades only reticulocytes. Why there is a differential exposure of certain epitope is still to be elucidated. A possible reason could be that DARC belongs to a certain protein complex, or is in a specific conformation. Such a knowledge will be helpful to identify the major candidate to a vaccine against Plasmodium vivax.

Aim: This project aims to elucidate whether DARC belongs to the cytoskeleton or to a certain protein complex that could be responsible of the differential epitope exposure in maturing reticulocytes. We will initially optimize the isolation of reticulocyte from peripheral blood in order to have the highest purity; we will perform detergent extractability assays to determine whether DARC is in a soluble or cytoskeleton fraction. Finally, we will perform immunoprecipitation on DARC with the endpoint of performing a proteomic analysis to identify binding partners both in reticulocytes as well as erythrocytes. For all the experiments we will compare reticulocytes and erythrocytes isolated from peripheral blood to in vitro cultured reticulocytes.

Techniques: This project involves isolation of membrane proteins and red cell membrane preparation, immunoprecipitation, cell culture, Western Blotting, flow cytometry.

Duration: 9 months. Students from HLO or University who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the group leader, Emile van den Akker, by e-mail: e.vandenakker@sanquin.nl