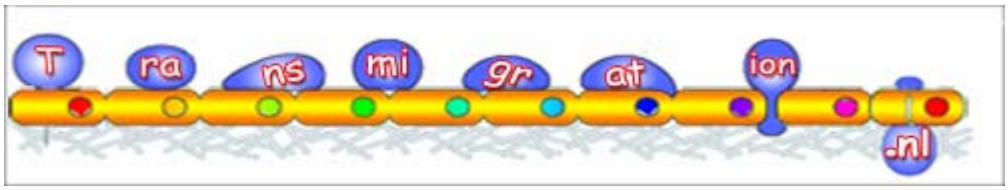
MCB Internship project 1:



Signals that regulate the passage of leukocytes across the vessel wall.

Introduction: Our group studies the mechanisms leukocytes use to find their way across the vessel wall. The main goal is to unravel the signalling routes that are induced in the endothelium by adherent leukocytes. This signalling induces formation of docking structures and is required for proper diapedesis. The signalling induced by clustering of adhesion molecules such as ICAM-1 and VCAM-1 is analyzed using real-time fluorescence microscopy in combination with physiological flow as well as by biochemical and cell biological assays.

Aim: For this project, the student will learn to study protein-protein interactions, including biochemical approaches such as immuno-precipitations, Western blotting. In addition, focus will be on measuring the activity of small GTPases and GEFs using specific GST-pull down assays. In addition, transmigration of leukocytes across endothelial monolayers in real-time using flow-based migration set up will be used. Moreover, to study the role of some specific proteins, RNAi techniques to silence proteins of interest will be learned.
The project will be finished with an oral presentation in front of the department including a small institute committee (still to assemble), together with a written report.

Techniques: To produce GST-fusion proteins, E.coli bacteria will be transformed, grown and protein of interest will be isolated. Using cells such as primary endothelial cells or HeLa cells, activity of GTPases such as Rac1, RhoG and RhoA will be performed. Also, with nucleotide-free mutants of specific GTPases, the activity of GEFs will be measured. Samples will be analyzed by Western blotting.
Transmigration assays will be carried out using confocal and wide-field microscopy in time. Using a 37°C incubator chamber, together with a controlled infusion pump, leukocytes, e.g. monocytes and neutrophils, will be perfused over the endothelial cells. Silencing of specific proteins in the endothelial cells will answer the contribution of these proteins to this process. If sufficient time, we will attempt to image the transmigration process in 4D, i.e. 3 dimensional in time using fluorescently-tagged proteins and confocal laser scanning microscopy.

Duration: at least 6 months. Students from the University or HLO who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the group leader, Jaap van Buul, either by e-mail: j.vanbuul@sanquin.nl or phone: 020-5121219.