Project NL-1

Complement resistance mechanisms by multi-drug resistant Klebsiella pneumonia

(Supervisors: **Prof. Suzan Rooijakkers, Prof. Anna Blom**)

The aim of this project is to characterize complement evasion molecules in the opportunistic bacterium *Klebsiella pneumoniae* (*K. pneumoniae*) where carbapenem-resistance grew from 1.6% to 10.4% in 10 years. In order to survive in the human body, bacteria have evolved mechanisms to avoid clearance by the immune system. For instance, our lab discovered that the prominent Gram-positive pathogen *Staphylococcus aureus* secretes a number of proteins that block critical steps in the complement cascade [Rooijakkers, Nat. Immunol. 2015, 2019]. Only little is known about the mechanisms that *K. pneumoniae* uses to withstand killing by complement attack.

This project has two main objectives:

1) Identify *Klebsiella* proteins (either secreted or expressed in the outer membrane) that interfere with complement activation. To this end, we will employ an efficient phage display library (running in the lab) for identification of bacterial secreted virulence factors that can be screened against various complement targets. Also, we already have a list of potential 'immune evasion' candidates. All protein candidates will be produced recombinantly and subsequently tested in a variety of functional assays (bacterial killing in serum, flow cytometric-based viability assays, neutrophil phagocytosis, complement deposition assays etc). For membrane protein candidates, we will generate deletion mutants or knock-ins of the relevant genes in bacterial strains to assess their relevance in context of the whole organism.

2) Decipher how complement-resistant Klebsiella strains withstand direct killing by the complement system. The complement system can directly kill Gram-negative bacteria by formation of a large, ring-structured, multi-protein Membrane Attack Complex (MAC). The MAC inserts into the bacterial outer membrane and, through an unknown mechanism, causes permeation of the bacterial inner membrane [Heesterbeek, EMBO J, 2019]. Unpublished data from our lab suggest that some Klebsiella strains specifically resist the lethal inner membrane damage. We will use transposon library mutagenesis and RNA sequencing approaches to study how these bacteria protect from MAC-mediated inner membrane damage.

General description of your individual PhD-schedule:

- Your main university will be Utrecht University/ University Medical Center Utrecht (Netherlands) with Prof. Rooijakkers as supervisor.
- You will have a 6-months research secondment at Lund University (Sweden) with Prof. Blom as supervisor, where you continue to scientifically work on your thesis project.
- You will have a 1-month clinical training at Tirol Kliniken Innsbruck (Austria).
- You will have a 1-month entrepreneur training at Statens Serum Institut (Copenhagen, Denmark).
- You will finally receive a PhD issued by Utrecht University and Lund University if you fulfil the respective requirements.

Application

The position is advertised from 10.09.2019 – 10.11.2019 on **www.corvos.eu**. Please apply via this homepage during that time.