MOI Project 16

The impact of Tspan2 on differentiation and function of neutrophils in anti-infectious immune responses

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Tetraspanin proteins play an important role in regulating cellular processes critical for immune defence against pathogens. Either through lateral association within membrane domains or direct binding to interaction partners, these proteins are involved in pathogen recognition, proliferation and trafficking of immune cells. While the tetraspanin Tspan2 was originally identified in oligodendrocytes of the central nervous system, among immune cells we have shown that Tspan2 is highly expressed in neutrophils. New insights into neutrophils, their different stages of differentiation, special cell migration patterns such as swarming, and functions in the first phase of infection defence are bringing this cell type to the forefront of immunological research. It is becoming increasingly clear that neutrophils are not a homogeneous cell population. Rather, these cells exist in different stages of differentiation associated with distinct effector functions that are genetically (re)programmed dynamically and adapted to the course of the infection. The expression and functional role of Tspan2 in different neutrophils has not yet been elucidated.

Using Tspan2/DTR:EGFP reporter mice, we were able to show that Tspan2 is expressed in the majority of neutrophils in the immune system. Functionally, neutrophils from Tspan2-deficient mice show increased antibacterial activity in vitro. Overexpression of Tspan2 leads to altered cell morphology with increased formation of long membrane extensions within which Tspan2 is accumulated. In an exploratory approach to characterise the molecular interaction partners of Tspan2, mass spectrometry analysis of GFP-Tspan2 expressing MEFs compared to GFP-expressing control cells revealed that Tspan2 may interact with proteins associated with processes of endocytosis and cytoskeletal reorganisation.

The aim of this project is to characterise the functions of Tspan2 in neutrophils, with particular focus on their specific differentiation stages. The influence of Tspan2 in anti-infectious immune responses will be investigated in a chlamydial infection model, where neutrophils are critically involved in the local inflammatory response and associated immunopathology.

Our studies will determine how Tspan2 influences neutrophil differentiation and function using single cell RNA sequencing of wild-type vs. Tspan2-deficient neutrophils at steady state or after stimulation. Specifically, the involvement of Tspan2 in neutrophil effector functions such as myeloperoxidase and NET production will be analysed in the context of infection with the intracellular bacterium *Chlamydia trachomatis*.

Extending our exploratory mass spectrometry screen, molecular interaction partners of Tspan2 will be defined using APEX2 / BioID proximity labelling. Truncated versions of Tspan2 will be generated to define the specific interaction sites on the intra- or extracellular loops of the membrane protein. Candidate interaction partners will be verified by co-immunoprecipitation and co-localisation visualised by confocal microscopy.