ImmunoTools *special* Award 2023

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Expression profile and function of S1PR3 in primary human innate immune cells

Sepsis affects numerous patients in intensive care around the globe. The heterogeneity and complex pathophysiology of sepsis makes diagnosis complicated and treatment difficult. Standard methods of treatment involve support of organ functions, containment of infection and pathogen clearance, but new approaches focus on reducing the systemic inflammation. Promoting disease tolerance is one such way of modulating the immune response, where the body negates the negative impact of the infection on the host without eliminating the pathogen. The metabolism and signaling pathways of sphingosine-1-phosphate (S1P) are promising therapeutic targets for such strategies. Previous projects have shown that inhibition of S1P degradation and stimulation of S1P receptor type 3 (S1PR3) have huge beneficial effects on the health of septic mice and can significantly decrease the mortality rate. This was accompanied by a reduction in inflammation. Since the exact cells which contribute to this effect are unknown, deepened research has to be done.

The aim of this project is to investigate such potential cell types, primarily innate immune cells, and to see if the previous findings are translatable to humans. We mainly focus on neutrophils and macrophages, which are isolated from human blood. We already used anti-human antibody CD16 conjugated with FITC and anti-human antibody CD66b conjugated with PE from ImmunoTools to verify the efficiency of the isolation. The isolated neutrophils allow us to explore if S1PR3 stimulation can inhibit or reverse the activation of these cells. We would activate the neutrophils with LPS, your **recombinant human GM-CSF** and **recombinant human TNF-alpha**, respectively. Activation will be measured by Elastase release during NETosis as well as by flow cytometer using your **anti-human antibody CD63 conjugated with APC** in combination with anti-human antibody CD16 conjugated with FITC we already use.

We are also interested whether the activation of S1PR3 in macrophages has an inhibitory effect on their inflammatory potential. To generate the macrophages, monocytes would be cultured with your **recombinant human M-CSF** and **recombinant human IL-1beta**, leading to their differentiation. This *in vitro* differentiation would be confirmed by flow cytometry with

anti-human antibody CD86 conjugated with APC, **anti-human antibody CD14 conjugated with PerCP** and **anti-human antibody CD68 conjugated with PE** of ImmunoTools. Finally, activation of macrophages and potential inhibition by S1PR3 stimulation would be assessed with your **anti-human antibody CD40 conjugated with APC**, and **anti-human antibody HLA-DR conjugated with PerCP**.

ImmunoTools *special* AWARD for **Florian Anderfuhr** includes 10 reagents

PE - conjugated anti-human CD68, HLA-DR

PerCP - conjugated anti-human CD14

APC - conjugated anti-human CD40, CD63, CD86

recombinant human GM-CSF, IL-1beta, M-CSF, TNF-alpha

DETAILS more AWARDS