ImmunoTools *special* Award 2014

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Human dendritic cell subsets in severe dengue disease

Dengue is a mosquito-transmitted disease caused by one of the four strains of dengue virus (DENV), which infects an estimated 400 million people annually. Currently there are no approved vaccines or specific treatments available for dengue. Primary infection with one strain is usually associated with mild symptoms, but secondary infections with a different strain can result in dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Both are life-threatening diseases which result from the massive induction of proinflammatory cytokines, the so called 'cytokine storm'. It is believed that he C-type lectin CLEC5A plays a role in the production of inflammatory cytokines in response to DENV infection. However, little is known about the expression and function of CLEC5A at the side of infection, e.a. the skin.

The aim of my PhD project is to investigate the expression and function of CLEC5A in human skin. We have identified that all CLEC5A-expressing cells in human skin are dendritic cells (DCs). These cells are key players in orchestrating primary immune responses, by capturing and processing antigen in the periphery and subsequent activation of T and B cells in lymphoid organs. Immune activation by DCs depends on the antigen, but also on the type of DC subset. DCs are a diverse population of cells and the capacity to activate lymphocytes differs greatly between subsets. Identification of DCs in human tissue is achieved by using the expression of cell surface molecules and are typically characterized as $CD3^{-}CD19^{-}CD20^{-}CD56^{-}$ and $HLA\text{-}DR^{+}$. Further characterization requires CD1a, CD1c, CD14, CD141 and CD304 to identify the different subsets. The anti-human antibodies of ImmunoTools would be of great value to identify these subsets.

C-ype lectins are a class of pattern recognition receptors (PRRs), which are able to recognize conserved structures on a wide range of pathogens, including fungi and viruses. We have previously shown that the C-type lectin Dectin-1 is crucial in orchestrating anti-fungal T helper 17 responses. This shows that C-type lectins are powerful PRRs, which are capable to activate and skew adaptive immune responses. The ability of DCs to activate adaptive immunity depends, in part, on the expression of co-stimulatory molecules, such as CD80 and CD86. We will make use of an activating antibody for CLEC5A to investigate if CLEC5A is able to induce the cell surface expression of co-stimulatory molecules on DCs. The anti-human antibodies

of ImmunoTools would provide us with the ability to asses the expression of these molecules on DCs.

In addition to co-stimulatory molecules, we are very interested in the ability of CLEC5A to induce the production of proinflammatory cytokines by DCs. The cytokines which are believed to be key players in the cytokine storm are IL-1β, IL-6 and TNF-α. We will therefore investigate if CLEC5A triggering leads to the secretion of these molecules by DCs using enzyme-linked immunosorbent assays (ELISA).

One of the challenges of using primary human material is the limited number of cells than can be isolated from tissue. We have therefore developed a technique to differentiate hematopoietic precursor cells, isolated from human cord blood, into different DC subsets. For this, several recombinant proteins such as Flt-3, SCF, GM-SCF and TNF-α are crucial to obtain the preferred subsets. These *in vitro* generated DC subsets share many functional similarities with their *ex vivo* counterparts.

With this research, we aim to elucidate the mechanisms that underlie the cytokine storm, which is thought to be responsible for the severe symptoms of dengue fever. Elucidating these mechanisms may lead to the identification of targets to develop a specific treatment for this disease. The anti-human antibodies, ELISA's and recombinant proteins of ImmunoTools would be of great value to achieve this.

ImmunoTools *special* AWARD for **Joris K. Sprokholt** includes 25 reagents

FITC - conjugated anti-human CD1a, CD3, CD11a, CD11b, CD14, CD16, CD19, CD20, CD40, CD45, CD56, HLA-ABC, HLA-DR,

PE - conjugated anti-human CD14, CD45,

PerCP - conjugated anti-human CD45,

APC -conjugated anti-human CD11c, CD14, CD16,

recombinant human cytokines rh IL-5, rh IL-13, rh TNFα,,

human IL-6 ELISA-set, human IL-8 ELISA-set, human TNF-alpha ELISA-set,

DETAILS