ImmunoTools IT-Box-139 Award 2012

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The role of macrophages and dendritic cells during dengue virus infection in humans.

Dengue virus (DENV) is a mosquito-borne virus, causing the most common viral infection in humans transmitted by mosquitoes, with ~50 million cases annually [1]. DENV has four serotypes, 1 – 4. Upon primary infection, humans develop antibodies which can neutralize homotypic re-infection. Yet, during secondary, heterotypic re-infection, antibodies can enhance infection, a phenomenon called antibody-dependent enhancement (ADE) of infection [2-4]. ADE infection shows higher number of infected cells, higher virus production and altered immune responses. Taken together, the resulting viraemia gives higher mortality-risks during secondary infection. Currently, there is no effective, clinical dengue-vaccine available [1].

In humans, dengue virus infects monocytes, macrophages, and DCs [5,6]. Based on total virus particle production, previous research identified DCs as the major dengue host cell, thus much research focuses on this cell type [7-9]. However, my preliminary results indicate that macrophages actually are more important than previously appraised *(to be published)*. Furthermore, macrophages show antibody-mediated ADE, whereas dendritic cells do not show ADE [10].

This sub-project of my PhD aims to dissect the importance of macrophages and DCs in dengue disease pathogenesis in humans. I will investigate the role of macrophages in human dengue infection relative to DCs by quantifying the virus production by these cells and the activation state of the infected cells and their bystander, non-infected, cells. This project will advance the field and medical understanding of dengue pathogenesis by characterizing the role of macrophages and DCs during human infection in terms of virus production and immune activation state.

Within this project, we will investigate the role of macrophages during human dengue infection, relative to DCs by looking at the following questions;

I) Are macrophages more efficient in producing infectious virus than DCs?
II) Does DENV-infection induce higher "pro-inflammatory" state in infected macrophages or DCs?
III) For macrophages; Does ADE suppress antiviral responses or apoptosis relative to infection in the absence of antibodies?
IV) (long term): Capacity for T cell proliferation by DENV-infected macrophages or DCs.

We already have optimized protocols to grow monocultures of Macrophages and DCs from human buffy coats. The IT-139 box will be used to continuously confirm the right phenotype

of macrophages and dendritic cells with e.g. CD11c, CD14, CD40, CD80, CD86 and the HLA antibodies.

I) Using the phenotype markers together with our own DENV-antibodies, we can determine the fraction of infected cells after DENV-infection. At the same time, the supernatant can be assayed by plaque assay to determine the virus production per cell, as to quantify how much DENV is produced by macrophages or DCs.

II) Additionally, to study whether DENV suppresses immune-activation of host cells, we will use our own DENV-antibodies in conjunction with the activation markers, like CD16, CD25, CD36, CD83 etc, from the IT-139 box.

III) In macrophages, we will also investigate whether ADE may (further) suppress cell death or pro-inflammatory responses. Annexin V will be used to quantify cell death, and IL6, CD25 and CD45RO can be used for pro-inflammatory responses and activation of macrophages. We will further strengthen these observations by performing "protection-assays" with vesicular stomatitis virus. This assay has been set-up and optimized within our lab. With this assay we can confirm whether activation-differences in macrophage cultures are reflected by antiviral cytokines in the supernatant.

IV) A potential, long term, experiment would be to study the proliferation of T cells after co-culture with DENV-infected macrophages and DCs, using e.g. CD3, CD4, CD8, CD25, CD44, and CD69 in conjunction with CFSE-labelling of naïve T cells. This observation may give insight in whether macrophages or DCs induce a more massive T cell response after DENV infection.

References:

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