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Institutet**

Department of Microbiology, Tumor and Cell Biology (MTC)

Studies on the host immune response during pulmonary TB and during M. tuberculosis/HIV-1 co-infection

AKADEMISK AVHANDLING

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av

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ABSTRACT

Population studies have shown that HIV-infected individuals co-infected with *Mycobacterium tuberculosis* (MTB) are at up to 10-fold higher risk of developing active tuberculosis (TB) than their seronegative counterparts. Co-infected individuals also progress faster to AIDS than patients infected only with the virus. Therefore, understanding the relation between these two diseases is of a crucial importance. The overall aim of this thesis was to investigate the interaction between HIV and MTB infection from the perspective of host immunity. Using *in vitro* and *in vivo* models, we investigated innate and adaptive immune responses to better understand the mechanisms behind the increased susceptibility to MTB and HIV during co-infection.

In the first study we examined regulation of the host immune response during pulmonary TB mediated by CD103⁺ dendritic cells (α E-DC) and CD4⁺ Foxp3⁺ regulatory T (Treg) cells. We found that in resistant mice, the number of lung α E-DCs increased dramatically during MTB infection. In contrast, susceptible DBA/2 mice which failed to recruit α E-DCs even during chronic infection. α E-DCs remained essentially TNF α negative but contained a high number of TGF β -producing cells in the lungs of infected mice. Further, we show that Treg cells in resistant and susceptible mice induced interferon IFN γ during pulmonary TB. We also showed that the Treg cell population was diminished in the lungs, but not in the draining pulmonary lymph nodes, of susceptible mice. The reduced number of lung Treg cells in susceptible mice coincided with an increased bacterial load and exacerbated lung pathology compared to resistant strains. These results demonstrate that immune-regulatory mechanisms may play an important role in protective immune responses during pulmonary TB.

In study II we analyzed the impact of MTB-infected macrophages on DC functionality, including HIV transfecting ability. An *in vitro* system was used in which human monocyte-derived DCs were exposed to soluble factors released by MTB- or BCG-infected macrophages. Soluble factors secreted from infected macrophages resulted in the production of proinflammatory cytokines and partial upregulation of maturation markers on DCs. Interestingly, the HIV transfecting ability of DCs was enhanced. In summary, this study shows that DCs respond to soluble factors released by mycobacteria-infected macrophages. These findings highlight the important role of bystander effects mediated by MTB-infected macrophages, and suggest it contributes to DC-mediated HIV spread and amplification in co-infected individuals.

In study III we assessed the impact of MTB infection on the immunogenicity of a HIV vaccine. We found that, depending on the vaccination route, mice infected with MTB before the administration of the HIV vaccine showed impairment in both the magnitude and the quality of antibody and T cell responses directed towards the vaccine. Mice infected with MTB prior to HIV vaccination exhibited reduced IgG and IgA antibody levels and neutralizing activity of serum against the virus. In addition mice infected with MTB had significantly lower HIV-specific multifunctional T cells. These results suggest that chronic MTB infection might interfere with the outcome of prospective HIV vaccination in humans.

In study IV we developed a new murine model of MTB/HIV co-infection using conventional, immunocompetent mouse strains. We utilized the chimeric EcoNDK virus together with a low dose MTB aerosol infection. To date, we have observed that the viral burden increased in the spleen and in the lungs of animals infected with MTB prior to virus inoculation. We have also shown that co-infection did not affect control of bacterial growth. During the early stage of co-infection, EcoNDK induced a significantly higher frequency of MTB antigen-specific CD8⁺ T-cells in the spleen. Also, MTB-specific CD8⁺ T-cells in both MTB-infected and MTB/EcoNDK co-infected animals were enriched for Tim3⁻ and PD1 expressing cells.

In conclusion, this small animal model may provide a useful tool to increase our understanding of how MTB and HIV influence the host immune response during co-infection *in vivo*.