



**Karolinska
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Department of Microbiology, Tumor and Cell Biology

Immune regulation during pulmonary TB and during M. tuberculosis/HIV-1 co-infection

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Chaniya Leepiyasakulchai, M.Sc.

Huvudhandledare:

Markus Sköld, PhD
Karolinska Institutet
Department of Microbiology, Tumor and Cell
Biology

Bihandledare:

Prof. Markus Maeurer, MD, PhD
Karolinska Institutet
Therapeutic Immunology (TIM)
Department of Laboratory Medicine

Prof. Hans Wigzell, MD, PhD
Karolinska Institutet
Department of Microbiology, Tumor and Cell
Biology

Fakultetsopponent:

Prof. T. Mark Doherty, PhD
University of Bergen, Norway
Institute for International Health
Faculty of Medicine

Betygsnämnd:

Prof. Anders Lindén, MD, PhD
Karolinska Institutet
The Institute of Environmental Medicine

Docent Benedict Chambers, PhD
Karolinska Institutet
Center of Infectious Medicine (CIM)
Department of Medicine

Docent Helena Aro, PhD
Stockholm University,
Department of Genetics, Microbiology and
Toxicology

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ABSTRACT

Individually, tuberculosis (TB) and acquired immunodeficiency syndrome (AIDS) pose major global health problems and together, they form a deadly liaison. Preventive vaccines for any of the diseases are not yet available. Therefore, better understanding of protective immunity to each pathogen that cause the diseases and how co-infection influences host immune responses are urgently needed. The overall aim of this thesis was to increase our understanding of immune regulation and protective immune responses during pulmonary TB and during *Mycobacterium tuberculosis* (Mtb)/ human immunodeficiency virus-1 (HIV-1) co-infection in the mouse model.

In **study I**, we explored CD103⁺ dendritic cell (α E-DC) and CD4⁺Foxp3⁺ regulatory T (Treg) cell function during pulmonary TB. We showed that in mice resistant to Mtb infection the number of α E-DCs increased dramatically in response to Mtb infection. In contrast, highly susceptible mice failed to recruit α E-DCs even during chronic infection. Instead of producing TNF α , α E-DCs preferentially produced TGF β . In contrast to resistant mice, the Treg cell population was diminished in the lungs, but not in the draining pulmonary lymph node (PLN) of highly susceptible DBA/2 mice during chronic infection. Further, we showed that Treg cells produced IFN γ in response to infection with a virulent clinical Mtb isolate. The reduced number of lung α E-DCs and Treg cells in susceptible mice coincided with severe lung inflammation and increased bacterial burden. Our results indicated that α E-DCs and Treg cells may play a role in regulating the host immune response during pulmonary TB.

In **study II**, we further investigated the origin, tissue localization, infection rate and cytokine profile of α E-DCs during pulmonary TB. We showed that alveolar epithelial cells support monocyte survival and differentiation *in vitro*. We demonstrated that bone marrow-derived monocytes were precursors of α E-DCs in the lungs and PLN during pulmonary TB. We confirmed the localization of α E-DCs beneath the bronchial epithelial cell layer and near the vascular wall during steady state conditions, and showed that α E-DCs had a similar localization in the lungs during pulmonary TB. In addition, α E-DCs were detected in the bronchoalveolar lavage during the infection. In contrast to other DC subsets, we found that only a minor fraction of lung α E-DCs was infected with the bacterium. We also showed that virulent Mtb did not significantly alter the cell surface expression level of MHC II on infected cells *in vivo* and that α E-DCs contain the highest frequency of IL-12p40⁺ cells among the myeloid cell subsets in infected lungs. Our results support a model in which inflammatory monocytes are recruited into the Mtb-infected lung tissue and, depending on which non-hematopoietic cells they interact with, differentiate along different paths to give rise to multiple monocyte-derived cells, including DC with a distinctive α E-DCs phenotype.

In **study III**, we determined the impact of chronic Mtb infection on the immunogenicity of a HIV vaccine candidate. We found that, depending on the vaccination route, Mtb-infected mice displayed impairment in both the magnitude and in the quality of both antibody- and T cell responses to the vaccine components p24Gag and gp160Env. Mtb-infected and HIV-vaccinated mice exhibited reduced p24Gag-specific serum IgG and IgA titers, and suppressed gp160Env-specific serum IgG titers compared to uninfected HIV-1-vaccinated controls. Importantly, the virus neutralizing activity in serum of intramuscular HIV-vaccinated Mtb-infected mice was significantly decreased relative to the uninfected controls. In addition mice concurrently infected with Mtb had fewer p24Gag-specific IFN γ -expressing T cells and multifunctional T cells in the spleen. These results suggested that Mtb infection may interfere with the effectiveness of HIV vaccines in humans.

In **study IV**, we established a mouse model for Mtb/HIV-1 co-infection by utilizing the chimeric EcoNDK virus. During the time-course of the experiment, we did not detect signs of immunodeficiency. However, we confirmed that the virus was present in Mtb/EcoNDK co-infected mice at least 14 days after a single injection of the virus. In fact, the viral load was significantly higher in the lungs and in the spleen of Mtb/EcoNDK co-infected mice compared to animals infected with the virus alone. We showed that EcoNDK influence the adaptive T cell response directed towards the bacterium. We observed that the number of Mtb-specific CD8⁺ T cells was significantly increased in the spleen compared to Mtb-infected animals. Furthermore, we characterized the cell surface expression profile of T cell immunoglobulin and mucin domain-3 (Tim-3) and Program Death 1 (PD-1) on T cell subsets during TB and during Mtb/EcoNDK co-infection. Even though we did not detect any significant difference between Mtb-infected and co-infected mice, we did find that Tim-3 and PD-1 were utilized differently by CD4⁺ T cells and CD8⁺ T cell subsets. Finally, we showed that TB10.4-specific CD8⁺Tim-3⁺ T cells were enriched for both TNF α - and IFN γ -producing cells. Our murine co-infection model may be a useful tool to elucidate why pulmonary TB is such a problem in patients with HIV-1/AIDS.