

Abstract

Mycobacterial infections are a health problem worldwide with *Mycobacterium tuberculosis*, the causative agent of tuberculosis, being responsible for over one million deaths every year. The opportunistic pathogen *Mycobacterium avium* is another member of the mycobacteria family, mainly causing disease in immunocompromised patients. The treatment of mycobacterial infections is extensive, and the emergence of mycobacterial strains resistant to the current treatments presents an urgent need for development of new treatments and effective vaccines.

KEAP1 is an oxidative stress sensor with several binding partners in the cell. Research in our group has shown that KEAP1 negatively regulates the induction of pro-inflammatory cytokines and autophagy in response to *M. avium* infection in primary human macrophages. The mechanism of how this regulation occurs is not understood, and has been under investigation in this thesis. HEK293 cells were tested out as a model for our investigation and were proved to be unsuitable for our purpose. The investigation of how KEAP1 negatively regulates inflammatory responses following *M. avium* infection was therefore carried out in primary human macrophages. Findings obtained throughout this study show that the E3 ubiquitin ligase complex proteins Cul3 and Rbx1 associated with KEAP1 might have an important role in the negative regulation of inflammation seen following *M. avium* infection.

Mycobacterial infections and innate immune receptor signalling can affect antigen-presentation. Since KEAP1 appears to be involved in anti-mycobacterial responses in primary human macrophages, we investigated the role of KEAP1 in regulation of antigen presentation and shaping of the following adaptive immune response. Using siRNA knockdown of KEAP1 in primary human macrophages, we obtained evidence suggesting that KEAP1 positively influences macrophage expression of the molecules CD80 and HLA-DR, both important for the activation of the adaptive CD4+ T cell response. How this alteration changes the following adaptive immune response is not known. We therefore wanted to establish an assay with expanded human mycobacteria-specific T cells in order to analyse whether or not KEAP1 affects the activation of mycobacteria-specific T_H1 cells upon stimulation with *M. avium* infected autologous macrophages. T cells survived the expansion and produced cytokines upon stimulation with a positive control (stimulation cocktail). However, preliminary experiments did not allow us to establish a solid protocol to analyse activation of mycobacteria-specific T cells upon stimulation with primary human macrophages. More experiments are hence necessary to optimise this assay.

This thesis has increased our knowledge of how KEAP1 is involved in the innate immune response in primary human macrophages. Future experiments should aim to verify and further elaborate on the findings done in this thesis, as well as continue the work on investigating the links to adaptive immunity.