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Development of MHC class I African alleles and *ex vivo* detection of *M.tuberculosis*-reactive CD8+ T-cells

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ABSTRACT

The T-cell mediated adaptive immune response is important in controlling infection with *Mycobacterium tuberculosis* (*M.tb*). *S*everal types of T-cells participate in the anti-*M.tb* defense, including CD4+ and CD8+ cells. CD8+ T-cells recognize small parts, so-called epitopes, of foreign antigens as well as self-derived antigens in association with MHC class I molecules. Identification of T-cell epitopes might therefore aid in the development of diagnostic markers and vaccine candidates. They may also guide to monitor CD8+ T-cell responses in disease settings where CD8+ T-cells play a role in biologically and clinically relevant immune responses.

In this thesis, we evaluated the previously identified *M.tb*-derived T-cell epitopes (*Paper V*), as well as identified novel *M.tb*-derived CD8+ T-cell epitopes from several proteins (TB10.4, Ag85B, ESAT-6, glycosyl transferase I, glycosyl transferase 2 and cyclopropane fatty acid synthase) (*Papers I–III*). The epitopes were restricted by a wide range of MHC class I allotypes, including some of the most common alleles in Caucasian, Asian and African population groups. Most of the MHC class I alleles common in the African groups were not commercially available. Therefore, they were cloned and subsequently expressed as recombinant proteins in order to be used in peptide binding detection and to construct peptide-MHC class I multimeric complexes for the first time (*Papers II–III*).

We studied peptide-MHC interactions to 13 different allotypes by using overlapping peptide libraries. A variable broadness of peptide binding patterns was identified. Some alleles showed a diverse pattern, allowing binding of many epitopes, while others displayed a more restricted peptide binding pattern. Another interesting feature was the very frequent occurrence of promiscuous binding epitopes. Subsequent evaluation of the binding characteristics of a majority of the 672 identified binding epitopes showed a wide range of affinities and dissociation rates with both inter- and intra-allelic differences (*Papers I–III*).

An extensive panel of 62 MHC class I multimers was constructed in order to validate some of the previously identified binding epitopes as being CD8+ T-cell epitopes. We also used these reagents to characterize *M.tb*-specific CD8+ T-cell responses in patients with pulmonary tuberculosis (TB) with diverse ethnic background (Caucasian, Asian and African). Generally, a low CD8+ T-cell response reflecting a diverse *M.tb*-specific reactivity could be detected, with only a few immunodominant epitopes. The majority of the *M.tb*-specific CD8+ T-cells had a precursor-like phenotype (CD45RA+CCR7+), despite expressing high frequencies of the degranulation marker CD107a, indicating that antigen-experienced effector cells reside in this population (*Papers II–IV*). One explanation for the high number of specific ‘naïve-like’ T-cells might be that they belong to a compartment of memory cells with ‘stem-cell like’ features, including expression of c-kit (CD117) and CD95 (*Paper IV*).

This thesis shows that both MHC class I allotypes and epitope-derived proteins might influence immune recognition on several levels including peptide-MHC binding, T-cell receptor (TCR) engagement as well as T-cell effector functionality and phenotype of the antigen-specific T-cells (*Paper III*); The T-cell phenotype and *M.tb*-specific T-cell frequency appear to be determined by both the restricting allele *and* the antigen.

In conclusion, we identified and validated many novel CD8+ T-cell targets from *M.tb*-derived proteins restricted via a broad range of MHC class I molecules, with the hope that these tools will aid future diagnostics and prevention strategies in different disease settings.

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