

# Genome and HLA-wide scanning and validation of cytotoxic CD8+ T cell responses against *Mycobacterium tuberculosis*

Sheila T. Tang<sup>1\*</sup>, Krista E. van Meijgaarden<sup>2\*</sup>, Michèl R. Klein<sup>2</sup>, Pascale van Weeren<sup>2</sup>, Fatima Kazi<sup>2</sup>, Søren Buus<sup>3</sup>, Alexander Zaigler<sup>4</sup>, Ugur Sahin<sup>4</sup>, Ole Lund<sup>1#</sup>, Tom H. M. Ottenhoff<sup>2#</sup>

<sup>1</sup>Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Building 208, 2800 Lyngby, Denmark. <sup>2</sup>Departments of Immunohematology & Blood Transfusion and of Infectious Diseases, Leiden University Medical Center, 2300 RC Leiden, The Netherlands. <sup>3</sup>Division of Experimental Immunology, Institute of Medical Microbiology and Immunology, The Panum Institute 18.3.12, University of Copenhagen, Blegdamsvej 2, 2200 Copenhagen N, Denmark. <sup>4</sup>Ganymed Pharmaceuticals AG, Germany, Freiligrathstr. 12, DE-55131 Mainz, Germany. \* shared 1<sup>st</sup> authorship. # shared 1<sup>st</sup> authorship

## Abstract

One-third of the world's population is latently infected with *Mycobacterium tuberculosis* (*Mtb*), and each year 2-3 million people die of tuberculosis. The attenuated *Mycobacterium bovis* Calmette-Guérin (BCG) vaccine against TB does not provide consistent protection. Currently, the TB problem is greatly aggravated by human immunodeficiency virus (HIV) co-infection, and the emergence of multi drug resistant *Mtb* strains. New and better vaccines against tuberculosis are therefore urgently needed.

It is well-established that CD4 T cell responses play a major role in the generation of acquired immunity against *Mtb*. There is, however, also mounting evidence from animal studies that CD8 T cells are involved in the control of *Mtb* infection, and it is increasingly recognized that CD8 cytotoxic T cells (CTL) also contribute to optimal host defense against mycobacteria. *Mtb* genome contains 3985 coding open reading frames (ORFs) but *Mtb* specific epitopes have only been defined for 270 of the ORFs (7%) and 30 ORFs (consisting of primarily proteins identified from culture filtrate) account for 65% of the defined epitopes. This indicates that our view of the TB epitome is probably very limited and incomplete, and that more epitopes remain to be discovered. This necessitates, however, new search strategies, including investigating a much broader selection of *Mtb* proteins.

In the present study we have used novel bioinformatics approaches in combination with medium throughput functional immunological screening strategies to select new *Mtb* proteins, which are likely to contain CTL epitopes. The predicted epitopes were pre-validated by measuring binding to relevant HLA molecules, and finally validated by CTL response from TB patients and immune donors.

**Results:** We have identified 70 *Mtb* specific CD8+ T cell epitopes restricted by 3 of the major HLA class Ia supertypes (A2 (44), A3 (9) and B7(17)) in healthy PPD+ individuals. The selected supertypes cover more than 80% of individuals from different ethnic groups. Sixty-one of these epitopes were novel and have not previously been described by others. This is a considerable expansion of the existing list of known *Mtb* CD8+ T cell epitopes in IEDB (as of 22<sup>nd</sup> Nov 2008 a total of 80 MHC-I known epitopes for *Mtb* (<http://www.immuneepitope.org/>)).

Some pathogenic proteins such as secreted, immunomodulatory proteins or conserved proteins may be targeted more frequently by the immune system than others. Eight different selection schemes were therefore compared in this study in order to examine if some selection schemes work better than others. Here we found that the more functional selection criteria, such as that a protein is secreted or predicted to be secreted lead to a higher hit frequency than the broader selection schemes (all proteins and conserved proteins). Furthermore, we found that the more immunological relevant selections had a higher frequency of reactive CD8+ T cell epitopes.

Our study showed a significant correlation between the binding affinity of a peptide to an MHC molecule and a positive CD8+ T cell response in immune donors.

Some of these epitopes were tested and preliminary results from proliferation assay and tetramer analysis showed that the epitopes were recognized in TB patients. This suggests that the epitopes are processed and presented to CD8+ T cells via the MHC-I pathway during a natural TB infection.