HLA-A*0201-restricted CTL epitopes in Rv0350 and Rv0351 of latent Mycobacterium tuberculosis

Y. Sun¹, Y. Wang¹, H. Wang¹, S. Wang², G. Ping¹, L. Zhang¹

¹ Capital Medical University, Beijing, China
² Beijing Research Institute for Tuberculosis Control, Beijing, China

**Background:** Mycobacterium tuberculosis (Mtb) infects approximately one third of the world’s population, 10% of which develop disease during lifetime. Beijing genotype strains are the most predominant strains in China. The aim of this study was to screen and validate the possible HLA-A*0201 restricted specific T cell epitopes of latent phase Mtb strains H37Rv and Beijing genotype.

**Methods & Materials:** MTB DNA microarray gene expression analysis was performed to screen the Mtb genes which expressed up-regulated under hypoxia. SYFPEITHI and NetCTLpan databases were used to predict the HLA-A*0201 restricted cytotoxic T lymphocyte (CTL) epitopes on Mtb, followed by peptide/HLA-A*0201 affinity and complex stability assays using the T2 cells. IFN-gamma-producing T cells were detected by enzyme-linked immunospot assay (ELISPOT) and a LDH release assay were performed to detect peptide-specific CTL activity using PBMC derived from HLA-A*0201-positive human donors latently infected with Mtb (LTBI).

**Results:** Using a whole genome microarray, we identified 130 genes of Mtb strain H37Rv and Beijing genotype whose expression was up-regulated under hypoxic conditions, 37 genes were responsible for encoding membrane protein, cell wall proteins or exported proteins. Of selected four proteins coded by up-regulated genes, Six potential epitopes on Rv0350, Rv0351 and Rv0440 were selected as candidate HLA-A*0201 restricted CTL epitopes by SYFPEITHI and NetCTLpan prediction. Four of these 6 study epitopes (Rv0440 p416-424, Rv0440 p362-370, Rv0351 p122-130 and Rv0350 p363-371) were showed high binding affinity and stabilization to HLA-A*0201 molecules by T2 binding studies. Synthesis of multi-epitope peptides Rv0351-A-T (containing Rv0351 p122-130, APDRE and Trojan peptide) and Rv0350-A-T (containing Rv0350 p363-371, APDRE and Trojan peptide) via ufrin-sensitive linker VRKR. Cytotoxic activity showed that significant lysis of T2 cells pulsed with Rv0351-A-T or Rv0350-A-T was induced by peptide-specific T cells derived from HLA-A*0201-positive LTBI. IFN-γ was released by PBMC in vitro from HLA-A*0201-positive LTBI when challenged with the peptides by an ELISPOT assay.

**Conclusion:** Our data suggest that Rv0351 p122-130 and Rv0350 p363-371 are HLA-A*0201-restricted CTL epitopes and could be useful in the design of an Mtb vaccine for LTBI.

**Acknowledgments:** This investigation was funded by a grant from Key Projects in the National Science & Technology Program (2009ZX10004-305).

http://dx.doi.org/10.1016/j.ijid.2014.03.1055