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## Diagnostic potentials of *M. tuberculosis*-specific proteins and peptides

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**Introduction:** The genomic comparisons of *M. tuberculosis* with other mycobacteria have identified *M. tuberculosis*-specific regions of differences (RDs), which are absent in the vaccine strains of *M. bovis* BCG and many other mycobacteria. If found immunodominant, the proteins and peptides encoded by these RDs may be useful in the specific diagnosis of tuberculosis. The aim of this study was to identify the immunodominant proteins and peptides of *M. tuberculosis* RDs as reagents for serodiagnosis of tuberculosis.

**Methods:** A total of 775 peptides (25-mers overlapping by 10 residues) covering the sequence of 39 proteins predicted in RD1, RD4, RD5, RD6 and RD7 were synthesized using solid phase fmoc chemistry. The synthetic peptides were tested individually for sero-reactivity in 96-well microtitre plates using enzyme-linked immunosorbent assays (ELISA). The tested sera were obtained from 100 pulmonary tuberculosis patients and 100 *M. bovis* BCG-vaccinated healthy subjects. In addition, antibodies against immunodominant peptides/proteins were raised in rabbits by actively immunizing animals with pools of 11 peptides corresponding to each immunodominant protein. *M. tuberculosis* culture filtrate and whole cell lysates were probed in ELISA with the rabbit anti-sera to determine the natural expression of the proteins in *M. tuberculosis*. Bioinformatics analyses were performed to suggest the *M. tuberculosis*-specificity of the peptides and predict their immunodominance.

**Results:** Among the 775 peptides tested, 90 peptides, belonging to 28 proteins of RDs, reacted with antibodies present in sera of one or more TB patients. However, only four peptides could be considered immunodominant because they reacted with sera from >50% tuberculosis patients. Among these immunodominant peptides, three peptides (aa 346-370 of Rv3876, aa 241-265 of Rv1508c and aa 325-336 of Rv1516c) had significantly stronger antibody reactivity with sera from tuberculosis patients than healthy subjects ( $P<0.001$ ), and significantly higher positivity with tuberculosis patients' sera (% positives=66 to 93%) than healthy subjects' sera (% positives=10 to 28%). Bioinformatics analyses suggested *M. tuberculosis*-specificity of the peptides and predicted their immunodominance. Immunization of rabbits with pools of 11 peptides of Rv3876, Rv1508c and Rv1516c induced peptide-specific antibodies, and the peptides showing immunodominance in humans were immunodominant in rabbits as well. The testing of culture filtrate and whole cell lysates of *M. tuberculosis* with anti-peptide antibodies suggested the natural expression of Rv3876, Rv1508c and Rv1516c in whole cell lysates but absence in the culture filtrate of *M. tuberculosis*.

**Conclusion:** The immunodominant RD proteins and peptides are naturally expressed in *M. tuberculosis*, but not secreted, and may be useful in the serological of tuberculosis.

## Biography

Abu Salim Mustafa has completed PhD in 1979 from All India Institute of Medical Sciences and Postdoctoral studies from the National Institute of Cancer Research, Oslo, and Whitehead Institute for Biomedical Research, Cambridge. He is the Director of Research Core Facility at the Health Sciences Centre, Kuwait University. He has published >150 papers in reputed journals with >6100 citations and h-index 40. He has served as an editorial board member for 8 journals, invited speaker in 67 conferences and chairman of 23 scientific sessions, member of 16 academic and professional societies, and successfully completed 52 funded research projects.

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