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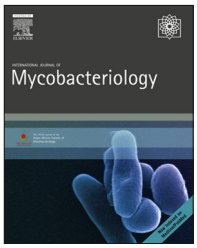


# T cell cytokine responses in peripheral blood mononuclear cells from patients with multidrug-resistant tuberculosis following stimulation with proteins purified from *Mycobacterium tuberculosis* MDR clinical isolates

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ABSTRACT

*Objective:* Tuberculosis (TB) is a devastating disease that remains a major health threat worldwide. The appearance of *Mycobacterium tuberculosis* strains resistance to current antibiotics is a growing problem, both in the third world and in developed countries. Completion of genomic sequencing of *M. tuberculosis* provides a strong foundation for subsequent identification of proteins to aid the understanding of protein function and the discovery of new drug targets or a TB vaccine. This study employed a proteomics approach to identify proteins from antibiotic resistant *M. tuberculosis* isolates and compare them to drug-sensitive isolates to determine the role of T cells in multidrug-resistant (MDR)-TB patients against *M. tuberculosis*-purified proteins (Rv0147) as compared with healthy subjects.

*Methods:* Proteins were extracted by Triton X-114 detergent-phase separation and precipitated by adding saturated ammonium sulfate to the supernatant. Following isoelectric focusing, proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Mass spectrometry was performed, and protein sequences were determined. Peripheral bloom mononuclear cells (PBMCs) were cultured, and autologous T cells were isolated from PBMCs by negative selection. Cells were subsequently cultured at 37°C in 5% $CO_2$, followed by stimulation with 10 μg/mL of the protein candidate (Rv0147) for 72 h. Culture supernatants were assayed for interleukin (IL)-10 and interferon (IFN)-γ by enzyme-linked immunosorbent assay.

*Results:* The identified proteins included Rv3057c, Rv0009, Rv3161c, Rv3614c, Rv0685, Rv2986c, Rv0443, Rv2114, Rv3311, Rv0831, Rv3804, and Rv3614c, and our results showed that the majority of upregulated or overexpressed proteins belonged to pathways associated with cellular metabolism, cell wall integrity, respiration, or cell membrane construction.

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Additionally, Rv1876 from MDR-TB isolates was predicted to be involved in the expression of bacterioferritin exclusively in MDR-TB-related resistance to first-line TB drugs. Furthermore, Rv2031c (HspX) was induced under oxygen-deficient conditions, and hypothetical protein (Rv2744c) and two membrane- and cell-wall-fraction proteins (Rv0379 and Rv1886c) were also identified. Analysis revealed increased percentages of INF-$\gamma$ and decreased IL-10 levels in MDR-TB patients as compared with those observed in normal subjects.

*Conclusion:* Four identified membrane or membrane-associated proteins, including bacterioferritin, GroEs, HspX, and Ef-Tu, may be potential targets for the development of novel prophylactic diagnostics and therapeutic strategies against TB. Our results suggested that T cells stimulated by the protein candidate Rv0147 may be shifted to T helper 1 status in MDR-TB patients.

## Conflicts of interest

The authors declare no conflicts of interest.