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Cloning and Expression of Low Molecule Protein Antigens from *Mycobacterium tuberculosis* $H_{37}Rv$

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The secreted proteins of *Mycobacterium tuberculosis* have attracted considerable attention both in vaccine research and as potential tools for diagnostic skin tests and serological diagnosis of tubeculosis (TB). In this study, we searched out T cell stimulating antigens from *M. tuberculosis* culture filtrates (CF) after 10~60% ammonium sulfate precipitation and removed Ag85 complex by hydroxylapatite. The CF was separated into 12 fractions on DEAE-Sepharose CL-6B column using a linear gradient of 10 to 300 mM NaCl in 20 mM Tris-HCl (pH 7.4). Aliquots of different fractions were further screened for their abilities to induce interferon (IFN)-γ production in peripheral blood mononuclear cells (PBMCs) from purified protein derivatives (PPD) (+) donors. The second fractionation was performed using non-reducing SDS-polyacrylamide gel electrophorsis (PAGE) and mini whole gel eluter. Among fourteen fractions, the protein bands induced the strong IFN-γ production in PPD (+) donors and IFN-γ content was assayed by ensyme-linked immunosorbent assay (ELISA). These were subjected for N-terminal protein sequencing. Each of the seven N-terminal sequences obtained was used for a homology search in the Sanger *M. tuberculosis* database with the Blast program and in GenBank. Of the identified proteins, none of the three proteins with the molecular mass of 17 kDa, 14 kDa, and 11 kDa were identical to previously described proteins from *M. tuberculosis*. These three proteins were expressed in *Escherichia coli* and purified by affinity chromatography. T cell stimulating activites by these recombinant proteins are now being studied. These results will provide an infromation for good candidates of diagnostic antigens and vaccines in human TB.