Production of Mycobacterium Tuberculosis ESAT-6 Recombinant Protein and Use of This in Skin Test

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Background & Objectives: Tuberculosis (TB) is the leading infectious disease in the developing world. In the 1882, Robert Koch has identified Mycobacterium tuberculosis and then in 1920 a delayed-type hypersensitivity skin test reaction has introduced based on tuberculin purified protein derivative (PPD). Unfortunately, this test is incapable of distinguishing Mycobacterium tuberculosis (MTB) infection from bacille Calmette-Guérin (BCG) vaccination or infection with non-tuberculous mycobacteria. Thus, there is an urgent need to develop a perfect and sensitive test for detection of tuberculosis. For introducing a more specific diagnostic tool for TB detection, this study was performed for cloning and expression and skin test reaction of early secreted antigen target 6 (rESAT6), a secretory protein found only in MTB, M. bovis, and few other mycobacterial species.

Methods: After amplification of esat-6 gene from M. tuberculosis H37Rv genome, it was cloned in expression vector (PQE60) and followed for expression in E.coli M15 and purified with Ni-NTA agarose affinity chromatography. The expressed protein was confirmed with electrophoresis and western blotting. For skin test, different groups of guinea pigs were sensitized with M. tuberculosis, M. avium and BCG vaccine and two months later skin test was performed with ESAT-6 and PPD.

Results: Our results showed that recombinant protein of ESAT-6 (rESAT-6) was successfully expressed and purified in prokaryotic system. Skin test data show that, unlike PPD skin tests, purified rESAT6 antigen elicited a positive skin response in animals exposed only to MTB and no skin responses were observed in the guinea pigs sensitized with BCG vaccine, or with M. avium. In compare of PPD, The sensitivity of rESAT-6 was reported as 114 in potency test.

Keywords: Mycobacterium tuberculosis; Skin Test; PPD; ESAT-6, rESAT-6; PQE60 Vector