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

Jale Moradi*; Nader Mosavari; Majid Tebyanian; Mahmood Ebrahimi

Razi Vaccine and Serume Research Institute, Iran

jale_moradi@yahoo.com

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 prefect 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. tuberclusis*, *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