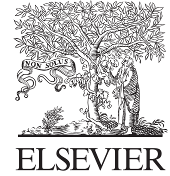
Osong Public Health Res Perspect 2015 6(1), 34–38
http://dx.doi.org/10.1016/j.phrp.2014.12.002
pISSN 2210-9099 eISSN 2233-6052



ORIGINAL ARTICLE

Evaluation of *Mycobacterium tuberculosis* Early Secreted Antigenic Target 6 Recombinant Protein as a Diagnostic Marker in Skin Test

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Received: October 8, 2014
Revised: November 15, 2014
Accepted: December 3, 2014

KEYWORDS:
early secretory antigenic target 6,
Mycobacterium tuberculosis,
pQE60 vector,
purified protein derivative,
recombinant early secretory antigenic target 6

Abstract

Objectives: Tuberculosis (TB) is the leading infectious disease in the developing world. Delayed-type hypersensitivity skin test diagnoses TB using tuberculin purified protein derivative (PPD), but this test is incapable of distinguishing *Mycobacterium tuberculosis* (MTB) infection from bacillus Calmette–Guérin (BCG) vaccination or an infection caused by nontuberculous mycobacteria (NTM). This study was performed to evaluate the use of recombinant early secretory antigenic target 6 (rESAT-6), a secretory protein found only in MTB, *Mycobacterium bovis*, and few other mycobacterial species, as a skin marker for MTB in guinea pigs.
Methods: We prepared recombinant MTB ESAT-6 and evaluated its use as a specific antigen for MTB in guinea pigs.
Results: Our results show that the purified MTB rESAT-6 antigen is capable of inducing a positive reaction only in guinea pigs sensitized to MTB. No such reaction was observed in the animals sensitized to *M. bovis*, BCG vaccination, or NTM (*Mycobacterium avium*).
Conclusion: Our study results confirm that the ESAT-6 antigen is more specific to MTB infection than PPD and could be used in more specific skin tests for detection of MTB in large animals and in humans.

1. Introduction

In 2012, estimates indicated 8.6 million new TB cases and 1.3 million TB-related deaths, of which there were 1 million human immunodeficiency virus (HIV)-negative patients, with the remaining 0.3 million being HIV-positive patients. These data indicate that TB is one of the important health problems [1]. Furthermore, a pandemic of tuberculosis (TB) is influenced by increases in the HIV/acquired immunodeficiency syndrome cases and emergence of multidrug-resistant and extensively drug-resistant strains, all of which aggravate the

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problem. Therefore, there is a necessity to identify strategies for controlling TB infection [2].

TB-controlling strategies are based on accurate diagnosis, but this is not possible with the classical common technique that is used to screen patients for TB [3]. Tuberculin purified protein derivative (PPD) test is a delayed-type hypersensitivity reaction that is widely used for screening patients with exposure to *Mycobacterium tuberculosis* (MTB) for several decades [4]. PPD is a mixture of large number of MTB antigens that are present in nontuberculous mycobacteria (NTM) and *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) [5]. Therefore, the PPD skin test can produce a false-positive reaction in patients who have received the BCG vaccination or have had exposure to NTM [5]. The *early secretory antigenic target 6* (*ESAT-6*) gene is located in the RD1 region and is present in the pathogenic strains of *Mycobacterium* such as MTB, *M. bovis*, *Mycobacterium africanum*, *Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium szulgai*, *Mycobacterium flavescens*; however, this gene is absent in all strains of *M. bovis* BCG and in a large number of NTM [6]. The gene activates one of the most important virulence factors in MTB and is responsible for inducing secretion of interleukin-8 (neutrophils) and T-lymphocyte chemotactic cytokine [7,8]. In recent years, this gene was evaluated as a diagnostic tool for detection of MTB infection in enzyme-linked ImmunoSpot assay technique and as a candidate for vaccination [9]. We have successfully cloned and expressed ESAT-6 protein and evaluated its sensitivity and specificity as a skin test antigen and compared recombinant ESAT-6 (rESAT-6) skin test reaction with locally prepared PPD in guinea pigs.

2. Materials and methods

2.1. Bacterial strains and product

MTB H37Rv genome, *Mycobacterium avium*, and *M. bovis* BCG were obtained from the Tuberculosis Research Laboratory of Razi Vaccine and Serum Research Institute (Karaj, Iran). The vector pQE60 was obtained from the Iranian Recombinant Gene Bank (Institut Pasteur, Tehran, Iran). PPD produced from MTB (50 IU/mL) was obtained from Razi Vaccine and Serum Research Institute. *Escherichia coli* strains M15 and XL1-blue were grown in Luria-Bertani liquid media.

2.2. Cloning, expression, and purification

The *ESAT-6* gene from H37Rv strains of MTB was amplified by polymerase chain reaction (PCR). Forward and reverse primers have sites for *Bgl*II and *Bam*HI. After digesting PCR products with appropriate enzymes, the fragments were ran on 1% agarose gel and purified. The *ESAT-6* gene was ligated to the pQE60 vector using T4 DNA ligase and transformed into *E. coli* XL1-blue cells. Restriction enzyme analysis was used to screen the transformants using *Eco*RI and *Hind*III and these were confirmed by sequencing. The pQE60-E6 was purified from the culture of recombinant *E. coli* XL1-blue and transformed into the competent *E. coli* M15 cells. The transformants were placed on lysogeny broth plates containing 50 μg/mL ampicillin and 30 μg/mL

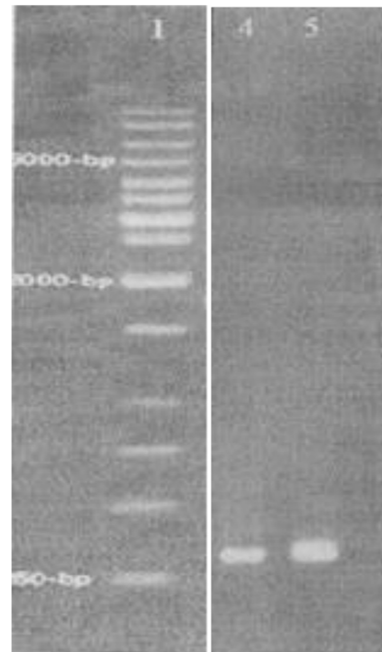

Figure 1. Evaluation of recombinant early secretory antigenic target 6 (*rESAT-6*) gene as polymerase chain reaction product. Lanes 4 and 5 = *rESAT-6* gene.

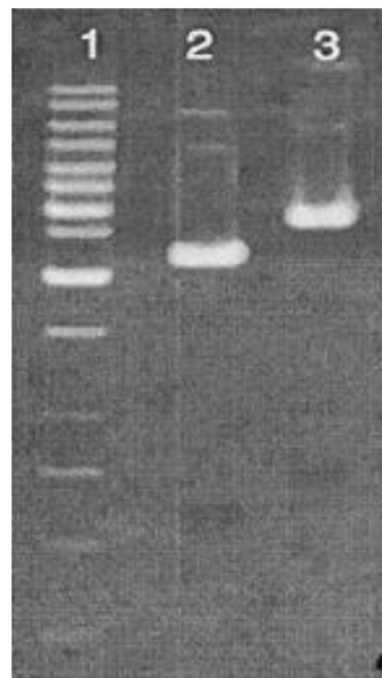

Figure 2. Electrophoresis of recombinant plasmid after digestion by restriction endonucleases.

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