Iranian Journal of Veterinary Science and Technology

Received: 2020- Mar- 04
Accepted after revision: 2020- Dec- 27
Published online: 2021- Feb- 27

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2020.64256.0

Purification and biological analysis of specific antigens (ESAT6/CFP10) from Mycobacterium tuberculosis

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ABSTRACT

The pathogenesis of *Mycobacterium tuberculosis* (Mtb) is related to its low molecular weight proteins mainly ESAT6 and CFP10 that are highly specific and potentially useful for the diagnosis of tuberculosis. This research focused on isolation, purification, and characterization of low molecular weight proteins from Mtb. Cultures of Mtb were inactivated by heating at 68 °C for 90 min and 100 °C for 3 hrs, respectively. Inactivated cultures were filtered and the proteins in the supernatant fluid precipitated with two rounds of ammonium sulfate, at 4 °C. The collected precipitates were dialyzed and subjected to gel chromatography (G-50) and the obtained fractions were analyzed for protein concentrations and molecular weight. ESAT6 and CFP10 protein complex in the purified fraction was confirmed by Western blotting. Guinea pig sensitization assay was used for estimating the potency of the purified fraction compared to the standard PPD. The maximum amount of low molecular weight proteins were precipitated by 20% ammonium sulfate. SDS-PAGE analysis revealed protein bands of approximately 10-15 kDa. The purity of the proteins was ≥95%, as confirmed by SDS-PAGE. The presence of the ESAT-6/CFP10 complex was confirmed by Western blot analysis. The purified fractions showed no cross-reaction with BCG or *M. avium* strain. ESAT-6/CFP-10 purified by the ammonium sulfate method appeared to be suitable for the development of a diagnostic kit for the detection of Mtb.

Keywords

Mycobacterium tuberculosis, ESAT-6/CFP10, Ammonium sulphate, Chromatography, Western blotting

Number of Figures : 6
Number of Tables : 3
Number of References : 35
Pages : 59-67

Abbreviations

AA: The first step of precipitation with ammonium sulfate
AT: The first step of precipitation with TCA
BA: The second step of precipitation with ammonium sulfate
BCG: Bacille Calmette-Guérin
BT: The second step of precipitation with TCA
CFP10: Culture filtrate protein
ECL: Enhanced chemiluminescence
ESAT6: Early secretory antigenic target
GMP: Good manufacturing practices
HIF: heat-inactivated filtrate
HRP: horseradish peroxidase
IgG: Immunoglobulin G
Mtb: Mycobacterium tuberculosis
PBST: Phosphate buffer saline with tween
PPD: purified protein derivative
RD1: Region of difference 1
SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TB: Tuberculosis
TST: Tuberculin skin test
TCA: Trichloroacetic acid
WHO: World health organization

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Introduction

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (Mtb). TB is a major public health problem. Each year about eight million new cases of TB are notified, of which 2 to 3 million prove fatal [1]. To control the disease, one of the most important requirements is the early and specific diagnosis of TB.

Mantoux test or the tuberculin skin test (TST) is one of the most widely used diagnostic procedures that is based on the detection of the cell-mediated immune response [2]. Tuberculin is the crude protein extracts of thermally treated cultures of Mtb. These proteins "referred to as purified protein derivative (PPD)" is used for *in vivo* skin diagnostic tests for detecting sensitization to mycobacterial antigens that might have resulted from infection, vaccination, or environmental exposure [3].

The primary concern with TST diagnostic procedure is the high number of false-positive results, as the test is unable to distinguish between Mtb infection and either exposure to non-tuberculosis mycobacteria or vaccination with *M. bovis* Bacille Calmette-Guérin (BCG) [4, 5]. False-negative results are also reported especially in children and immune-compromised individuals [6, 7].

The low specificity of the TST is attributed to the undefined nature of PPD antigens. Therefore, the presence of cross-reactive antigenic epitopes existing in Mtb and other mycobacterial and non-mycobacterial strains has hampered the development of specific immunodiagnostic test methods [8, 9].

Efforts have been made to identify and characterize immunologically active antigens from Mtb that could react specifically with antibodies from TB patient's sera. Several secretory antigens that are secreted into the extracellular environment by Mtb are known as the target of the immune response in the infected host. Up to date a number of these antigens have been purified, characterized, and evaluated. Among these antigens, the low molecular weight proteins called early secretory antigenic target (ESAT6) and culture filtrate protein (CFP10) are known to be involved in the virulence of Mtb [10]. These complex protein antigens are known to be specific targets for the diagnosis of TB infections. The genes for culture filtrate protein (CFP10) and early secretory antigenic target (ESAT6) are located within the 9455 bp region of difference 1 (RD1 region). This region is known to be highly specific for Mtb complex and is absent in M. bovis BCG strain [11]. Both ESAT6 and CFP10 mediate a specific Th-1 host immune response and have a strong diagnostic potential for both the virulent and latent form of Mtb [12-15].

Different purification procedures have been used for the purification of secretory antigens from the culture filtrate of Mtb. Seiber et al. (1934) used trichloroacetic acid (TCA) for the precipitation of proteins from heat-killed Mtb cultures [26]. The obtained product designated as PPD is a complex mixture of several protein antigens and their aggregated and degraded products. Hence, poor sensitivity and low specificity of the PPD in a diagnostic test might be due to the mixture of protein antigens present in the TCA purified fractions [14].

Thus, the development of new improved test procedures that could specifically diagnose Mtb and have higher sensitivity and specificity might be an effective tool for accurate diagnosis of TB and the control of the disease. In this study, we precipitated and purified low molecular weight secretory proteins (ESAT6 and CFP10 complex) from heat-inactivated Mtb. The purified proteins were then analyzed by Western blotting and finally, animal studies were performed.

Results

Protein/antigen purification

The Mtb C strain was grown on Lowenstein-Jensen solid medium and Dorset Henley liquid medium. As seen in Figure 1, 20% w/v of the mentioned salt allowed maximum precipitation of low molecular proteins, compared to other methods. Figure 1 clearly shows the protein bands more pronounced in $(NH_4)_2SO_4$ precipitated filtrates inactivated at 68 °C, for 90 min. (NH4)2SO4 precipitation yielded proteins with a molecular weight of about 10-50 kDa. While by the TCA method, higher molecular weight proteins ranging from 18 to 90 KDa was apparent. Fraction BT and the standard PPD appeared as a smear on the gel showing no precise protein bands that might be due to the presence of complex protein mixtures.

The results showed that the precipitation of tuberculin solution with 40% TCA at 100°C (BT) had the highest protein concentration. However, the precipitation with 20% ammonium sulfate at 68 °C (AA) had the lowest protein concentration (Table 1).

The amount of protein in fraction AA increased from 0.58 to 1.48 mg/ml after PEG 6000 concentrations. Figure 2 indicates the protein profile of concentrated AA fractions. After subjecting the fraction AA to Sephadex G-50 column chromatography four peaks (F1 to F4) were obtained (Figure 3). Fractions 1 to 3 showed the presence of protein while F4 showed no protein.

All fractions were further analyzed by SDS-PAGE to estimate their protein profile. Fraction 3 showed a single protein band of approximately 12 KDa which corresponds to the ESAT6/CFP10 complex (Figure 4). Fractions 1 and 2 (F1 and F2) had several pro-

tein bands, while no protein bands were observed in fractions 4 (F4). Table 2 represents the purification steps with its recovery during this study. So that by performing chromatography in the last stage, the recovery was about 3.25% and 38.65 mg/ml yield was observed at the F3.

The presence of purified ESAT-6/CFP10 complex protein in F3 fraction in the range of approximately 12 kDa was confirmed, as anti ESAT6/CFP10 monoclonal antibodies blotted with the protein in the mentioned fraction (Figure 5).

Animal study

Thirty-six male guinea pigs were divided equally into four groups and sensitized with Mtb, BCG vaccine, *M. avium*, and PBS, respectively. The animals were later injected with the PPD produced at Razi Institute of Iran as the positive control, purified ESAT6/CFP10 complex (F3 fraction), and PBS as the negative control, respectively (Figure 6).

The results were read after 24 hours and indurations occurring on the skin of the animals were measured (Table 3). The animals sensitized with *M. avium* reacted to PPD fractions, however, the level was approximately 5 mm, while this reaction was enhanced in BCG sensitized animals. The purified ESAT6/CFP10 complex protein showed positive results in Mtb sensitized animals only, while the non-sensitized animals (PBS control) showed no skin reaction to any of the tested treatments.

Table 1.
The concentrations of protein in the four fractions as determined by the Lowry method

Precipitation Method	TCA precipitation		$(NH_4)_2SO_4$ precipitation	
	AT	BT	AA	BA
Inactivation method	68 °C 90 min	100 °C 3 h	68 °C 90 min	100 °C 3 h
Protein concentration (mg/ml)	1.6 ± 0.22 to 2.2 ± 0.31	3.3 ± 0.45 to 5.1 ± 0.28	0.3 ± 0.02 to 0.6 ± 0.08	1.1 ± 0.2 to 1.8 ± 0.33

The results are expressed as mean (± SD) of three individual experiments

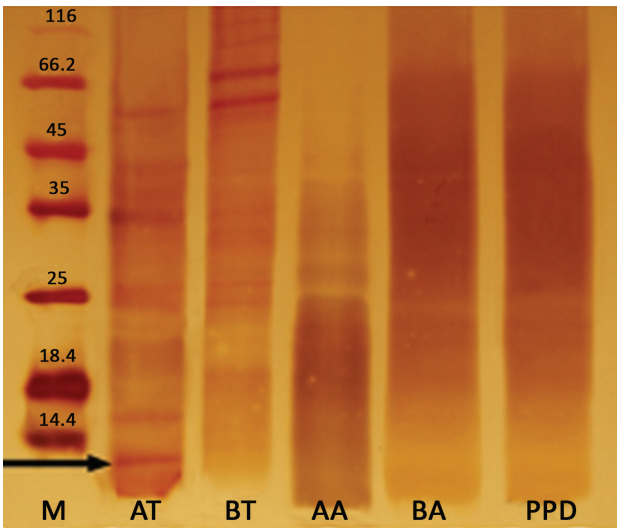

Figure 1.
The protein profile of the ammonium sulphate and TCA precipitated fractions on 15% SDS-PAGE. AT: Cell free filtrate of cultures inactivated at 68 °C for 90 min and precipitated with 40% TCA; BT: Cell free filtrate of cultures inactivated at 100 °C for 3 hrs and precipitated with 40% TCA; AA: Cell free filtrate of cultures inactivated at 68 °C for 90 min and precipitated with 20% ammonium sulphate; BA: Cell free filtrate of cultures inactivated at 100 °C for 3 hrs and precipitated with 20% ammonium sulphate ; PPD: Standard tuberculin produced at Razi Vaccine and Serum research Institute, Iran; M: Standard protein molecular weight marker 10-120 KDa.

Discussion

The worldwide burden of TB occurs in low- and middle-income countries where the diagnosis of this disease still relies on sputum smear microscopy and clinical findings including chest radiography. There is a great need for rapid diagnostic tests at all levels of the health system especially in developing counties [22].

Secreted proteins of Mtb have been reported as a rich source of immunogens. In the last decades, these proteins have gained specific attention as virulence factors, vaccine, and diagnostic candidates [22]. ESAT-6 and CFP-10 have been described as dominant antigens recognized by T-cells and considered as virulence factors in Mtb. These two major proteins (as a complex 1:1) facilitate translocation of Mtb from the phagosome into the host cell cytoplasm at later stages of infection. They do induce a strong T-cell response and, presumably, are involved in the lysis of the host cell membrane or the overall host cell. [35]. In this regard, ESAT6/CFP10 complex proteins are recognized by over 70% of TB patients and are considered promising candidates for the accurate diagnosis of TB in man and animal [15, 23]. The ESAT6/CFP10 has been demonstrated recently to be one of the major targets for memory effector cells during the recall of memory

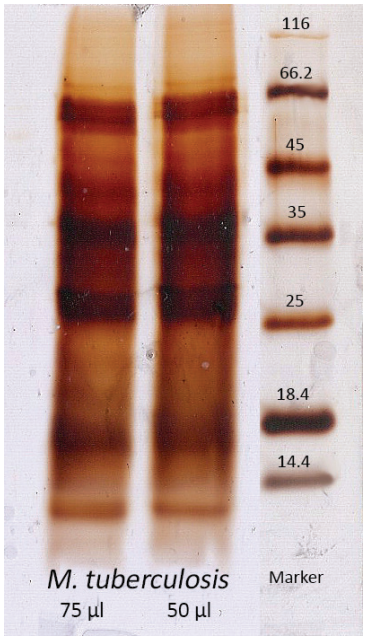

Figure 2.
SDS-PAGE analysis of two different concentrations of fraction AA after concentrating it one-to-tenth volume by PEG.

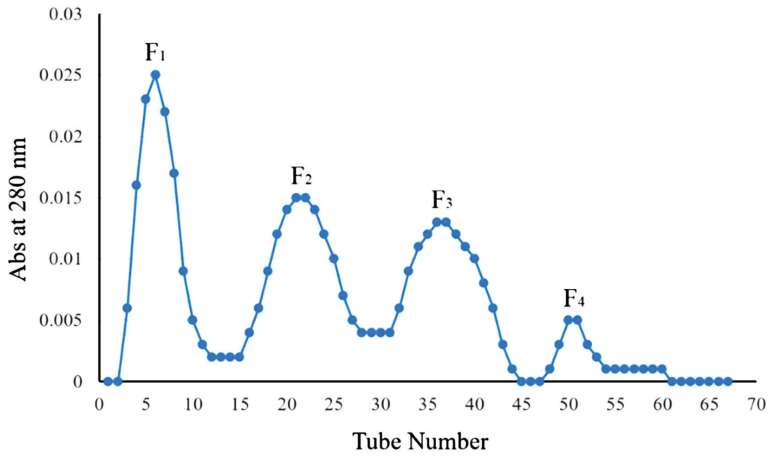

Figure 3.
Sephadex G-50 chromatography of fraction AA of *Mycobacterium tuberculosis* C strain

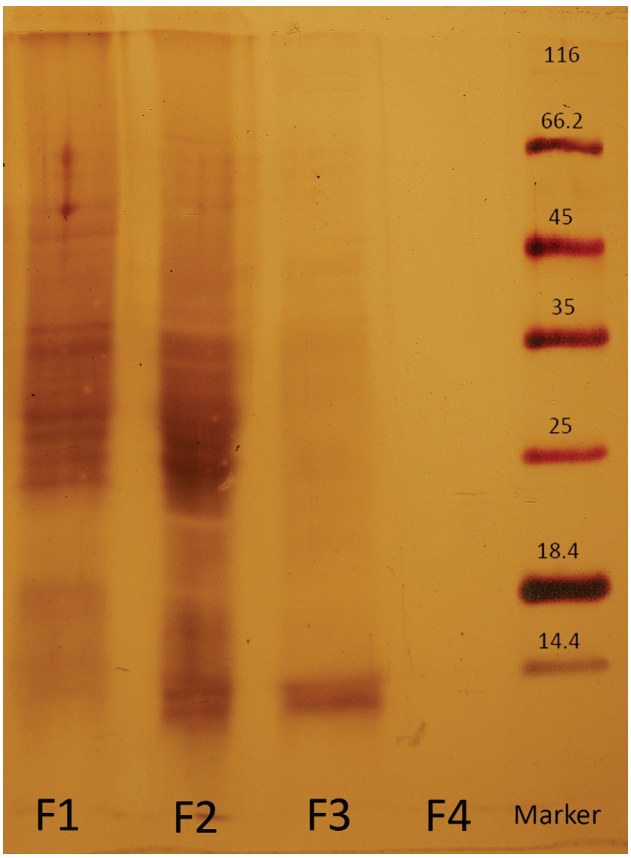

Figure 4.
SDS-PAGE analysis of four fractions obtained after gel filtration in Sephadex G-50 columns; F1, F2, F3 and F4 are the fractions collected. Lane M is molecular weight marker (10-180 kD).

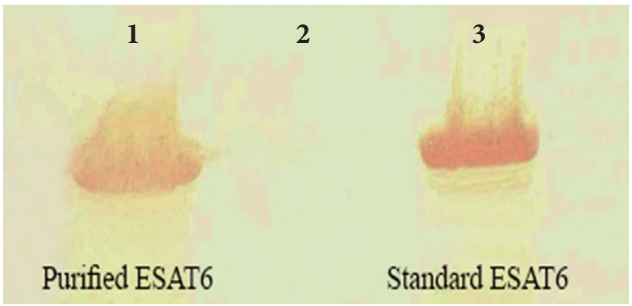

Figure 5.
Western blotting of ammonium sulfate-purified low molecular weight protein fractions using ESAT6 specific antibody. lane 1, purified ESAT6 protein; lane 2, negative control; lane 3, positive control.

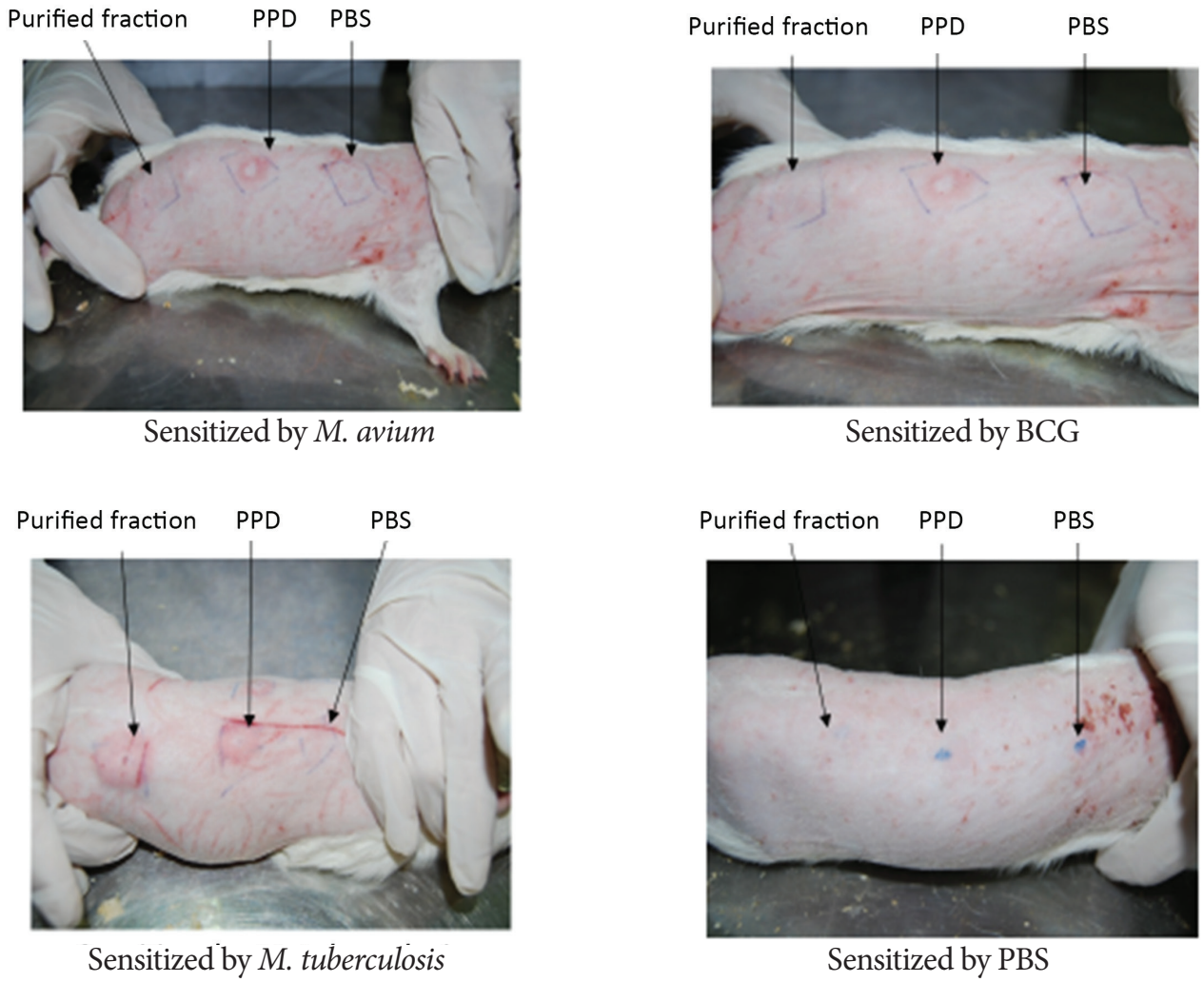

Figure 6.
Skin reactivity after injecting PPD, purified ESAT6/CFP10, and PBS in guinea pig models sensitized with *M. avium*, *M. tuberculosis*, BCG, and PBS.

Table 2.
Purification stages adopted for purification of low molecular weight secretory proteins form Mtb.

No.	Purification stages	Initial Volume (ml)	Final volume (ml)	Protein (mg)	Total Protein mg/ml	pH	Recovery %	Yield mg
1	Heat inactivated (HI)	350	-	12.68	4438.0	6.7	100	-
2	HI filtered (0.2 µm)	350	170	12.63	2147.1	6.6	48	-
3	NH_4SO_4 ppt (20%)	170	70	-	-	-	-	-
4	NH_4SO_4 2nd (20%)	70	38	0.28	10.64	6.2	0.64	533
5	Dialysis	30	58	0.24	13.92	-	-	-
6	PEG concentration	58	35	1.31	45.85	6.9	2.13	578
7	Gel Chromatography	F1	21	1.42	29.82	-	1.38	3.25	386
		F2	18	0.45	8.1		0.18	
		F3	75	0.47	35.25		1.64	
		F4	15	0.067	1.005		0.05	

Table 3.
Skin indurations measured after 24 h in sensitized guinea pigs injected intradermally with PPD, purified ESAT6/CFP10, and PBS

Treatments	Dose	Skin reaction (SD ± mean diameter in mm)			
		M. tuberculosis	BCG	*M. avium*	PBS
PPD	5 IU	12.64 ± 0.92	6.19 ± 1.14	4.21 ± 0.61	0
F3	0.001 mg/ml	16.91 ± 2.76	0	0	0
PBS	0.1 ml	0	0	0	0

PPD: purified protein derivative
F3: purified ESAT6/CFP10 protein obtained during gel chromatography
PBS: Phosphate buffer saline

immunity in a mouse model of TB [24].

Previous studies have studied the specificity of recombinant ESAT6, CFP10 antigens for the diagnosis [31-33], but in this study, we chose to improve our existing PPD product by replacing TCA with ammonium sulfate and autoclaving of culture filtrate with heat inactivation at lower temperatures (68 °C, 90 min).

Several impurities in the tuberculin PPD has been reported, including polysaccharides, nucleic acis, ash, and metals such as iron, sodium, zinc, copper, etc [25, 26]. The aim was to purify low molecular weight proteins mainly ESAT6 and CFP 10 that could have enhanced specificity and sensitivity for skin testing. In contrast to TCA, ammonium sulfate is predicted to precipitate tubercle proteins with lower nucleic acid contaminations and additionally maintain the protein integrity in its natural state [3, 27].

As mentioned earlier, these proteins are usually obtained by cloning methods and gene expression as they are expressed in small quantities under *in vitro* conditions [22]. However, some methods such as cloning might not have desirable outcomes as the recombinant or synthetic proteins might not possess the same biological functions as the natural proteins [14].

In this study, we used the ammonium sulfate precipitation technique to isolate and purify the target proteins while maintaining their natural integrity. The method appeared to be especially suitable for the purification of low molecular weight proteins from the culture filtrate of the bacterium. Although we observed lower yields for the ammonium sulfate method compared with the TCA method, the homogeneous protein precipitates achieved during two rounds of 20% ammonium sulfate constituted mainly of low molecular weight proteins including ESAT6 and CFP10. Hence, it was predicted that this purified fraction would be significantly more specific for the diagnosis of TB compared to the currently available Razi institute PPD.

A skin test was performed in guinea pig models that were sensitized previously with Mtb, BCG vaccine, and *M. avium*. Guinea pig models are considered the most suitable animal models for studying vaccine response and additionally for the development and standardization of PPD [28].

Previously, a group of researchers approved the use of guinea pig models for evaluating the specificity and sensitivity of combinations of ESAT6/CFP10 antigens. According to their reports, a skin test reagent containing these two molecules together could be considered highly specific and sensitive reagent for the detection of TB infection [23, 29].

Similarly, our purified fraction which had both ESAT6/CFP10 protein complex demonstrated greater specificity compared to the standard PPD in these animal models. The purified fraction injected in sensitized guinea pigs was able to distinguish between Mtb from BCG and *M. avium*. While PPD showed cross-reactive immunity to BCG and *M. avium* strain. Hence we could differentiate the exact agent responsible for TB infections. These results are consistent with previously reported studies and showed that ESAT6 protein can elicit skin test reaction in guinea pigs immunized with Mtb but not in the BCG vaccinated guinea pigs [30-33].

Since PPD is a mixture of a large number of proteins, its production in uniformity and compliance with good manufacturing practices (GMP) is very difficult, as a batch to batch variations are often recorded. We here were able to show that our purified fraction had greater specificity to TB than our manufactured PPD at the Razi Institute. This also offers a greater advantage to develop a skin test that utilizes the lower molecular weight proteins particularly ESAT6 and CFP10 rather than a whole mixture of proteins. The use of these antigens is likely to produce consistent and easily interpretable responses when used. However, as might be expected in a genetically diverse population, the sensitivity of a diagnosis based on a single antigen is lower than that with a complex

antigen mixture [34].

In summary, our results confirm that the ESAT6/CFP10 antigen is more specific than PPD to Mtb infection and could be used in more specific skin tests for detection of Mtb in animals and humans. However, further studies need to be performed to identify additional low molecular weight proteins of Mtb strain C. Additionally, alternate separation and detection methods must be employed to resolve and identify extremely basic proteins. Based on the immune response to these proteins in suspected cases, it seems that these proteins might be a suitable candidate for effective vaccination against TB.

Materials & Methods

Bacterial strain and Cultural conditions

The Mtb strain C (ATCC 35808) employed in this study was obtained from the Tuberculosis Reference Laboratory at Razi Vaccine and Serum Research Institute, Karaj, Iran. The bacterial strain was cultivated on Lowenstein-Jensen medium at 37 °C for 40 days and later inoculated into 15 Liters of Dorset Henley liquid medium (with a final pH of 6.8). Confluent growth of Mtb was observed after 6 weeks on the liquid medium.

Inactivation of Mtb strain C

To inactivate the tuberculosis bacterial cells, the freshly grown cultures (15 L) were divided into two equal parts and placed in a hot water bath set at 68 °C for approximately 90 min (Fraction A), and 100 °C for 3 h (Fraction B), respectively. To assure complete inactivation of the bacterial cells, drops of heat-treated cultures were placed on Lowenstein-Jensen solid medium and incubated at 37 °C for 40 days. No growth after 40 days indicated complete inactivation of the bacterium.

Filtration and collection of cell free extracts

The inactivated cultures (Fraction A and B) were passed through Buchner funnel to strain the cell debris and then filtered under pressure using K7 and 0.22 µm filters (Millipore, USA). The collected cell-free extracts were stored at 4 °C for further use. To assure complete inactivation, few drops of the filtered liquid were transferred to Lowenstein-Jensen medium and incubated for approximately 8 weeks. The work proceeded after no growth was observed after the incubation period (at 37 °C for 40 days). The obtained heat-inactivated filtrate (HIF) were further assessed for sterility according to in-house protocols.

Protein Precipitation and concentration

The HIF (fractions A & B) were further divided into two parts (AT, BT and AA, BA). AT and AA fractions were TCA and ammonium sulfate precipitated fractions of the filtrates inactivated at 68 °C for 90 min, respectively. While BT and BA were the culture filtrates inactivated at 100 °C for 3 h and inactivated by TCA and ammonium sulfate, respectively. For $(NH_4)_2SO_4$ precipitation, solid ammonium sulfate was added with stirring, in a 40-min period to reach 70% salt saturation at 4 °C. Based on gradient analysis, two rounds of 20% $(NH_4)_2SO_4$ was selected for further studies. The required amount of the salt was dissolved slowly in the appropriate fractions and kept on a stirrer for 4-8 h. Later, the precipitates were collected by centrifugation at 7000 rpm for 30 min. After dissolving the precipitates in phosphate buffer (pH 6.8), the trace amount of the salts were removed by dialysis against sterile distilled water, overnight at 4 °C. Finally, the dialyzed fractions were concentrated to one-tenth of the original volume by polyethylene glycol 6000. For the TCA method, the fractions (A and B) were subjected to the acid at 40% final concentrations. All purified fractions were stored at refrigerated temperatures until use.
Determination of protein concentrations and Molecular Weight. The protein concentrations were determined by the Lowry method using BSA as standard [17]. The approximate molecular weight of the obtained protein fractions was determined by 15% SDS-PAGE [16] and subsequent staining with Silver staining [18].

Gel Filtration Chromatography

The obtained concentrated filtrate was subjected to size exclusion chromatography with G-50 Sephadex (Sigma, USA) columns. The fractions were filter-sterilized (0.45 µm) and applied to Sephadex G-50 packed column (2×150 cm), at 4 °C. The column was equilibrated with 50 mM phosphate buffer (pH 6.8) and eluted with the same buffer. Fractions of 4 ml were collected at a flow rate of 6 mL/hour. Fractions were pooled and monitored at 280 nm [19].

Western blot analysis

The SDS-PAGE-fractionated antigen mixture was transferred to a nitrocellulose membrane, as described by Franco et al. [20]. Transfer efficiency was monitored by checking for the presence of pre-stained marker bands on the membrane. After transfer, the membrane was blocked with a blocking reagent (PBS/Tween-20 0.05%; PBST) containing 5% dried skim milk, with gentle agitation at room temperature. The membranes were incubated for 2 h at room temperature in 1:2000 diluted anti-ESAT6, anti-CFP10 (Abcam, UK) antibody, washed repeatedly and incubated with the secondary antibody i.e. horseradish peroxidase (HRP) conjugated antibody (goat anti-mouse IgG, Abcam). After thorough washes, the blots were developed by ECL (Enhanced chemiluminescence, Promega, USA) kit and visualized for immune-reactive bands.

Sensitization and Skin test in Guinea pigs

The purified fractions were tested for their potency based on a six-point assay, by sensitizing guinea pig models according to WHO recommendations [21]. 36 male outbred guinea pigs weighing 400-450 g, obtained from Razi Vaccine and Serum Research Institute, Animal care unit were divided into four groups of nine. Potency test was performed after 30 days of guinea pig sensitization with Mtb strain C (ATCC 35808), *M. aviam* strain D4 (ATCC 35713), BCG vaccine, and PBS, respectively. The purified fraction containing 2 mg/ml of the protein was diluted to obtain 0.001 mg/ml concentrations. The animals were injected intradermally with 5 IU PPD (positive control), 0.001 mg/ml of the purified fraction, and 0.1 ml of PBS (negative control) in the flank. The test results were read based on the size of erythema and edema at the injection site of the animals after 24 hrs. The diameters above 8 mm were regarded as a positive reaction.

Ethics committee approval

The present study was approved by the Ethics Committee of Razi vaccine and serum research institute, Karaj, Iran.

Acknowledgements

The Authors extend their appreciation to the Razi Vaccine and Serum Research Institute for funding the research group project No. 2-18-18-94127.

Authors' Contributions

The experiment was designed by N Mosavari. The experiments were conducted by MB, NS, MMT, and AG. The data were interpreted by N Mojgani and N Mosavari. The figures were prepared by MB. The manuscript was written by MB. The manuscript was reviewed by N. Mosavari, N. Mojgani, and KSB. The final manuscript was read and approved by all authors.

Conflict of interest

The authors declare no conflict of interest.

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How to cite this article
Mojgani N, Babaie M, Shakibamehr M , Mohammad Taheri M, Mosavari N, Ghaempanah A, Soleymani Babadi K (2020). Purification and biological analysis of specific antigens (ESAT6/CFP10) from Mycobacterium tuberculosis. Iran J Vet Sci Technol. 12(2):59-67.
DOI: https://doi.org/10.22067/ijvst.2020.64256.0
URL: https://ijvst.um.ac.ir/article_39739.html

Iranian Journal of Veterinary Science and Technology

Received: 2020- Mar- 04
Accepted after revision: 2020- Dec- 27
Published online: 2021- Feb- 27

Abstracts (in Persian)

خالص سازی و تجزیه و تحلیل بیولوژیکی آنتی ژن‌های خاص (ESAT6/CFP10) مایکوباکتریوم توبرکلوزیس

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چکیده

بیماری‌زایی مایکوباکتریوم توبرکلوزیس (Mtb) مربوط به پروتئین‌های با وزن مولکولی پایین آن به طور عمده ESAT-6 و CFP10 است که برای تشخیص سل بسیار خاص و بالقوه هستند. این تحقیق بر جدا سازی، خالص سازی و تعیین خصوصیات پروتئین‌های با وزن مولکولی پایین Mtb تمرکز دارد. کشت‌های Mtb به ترتیب با حرارت در ۶۸ °C به مدت ۹۰ دقیقه و ۱۰۰ °C به مدت ۳ ساعت غیرفعال گردیدند. کشت‌های غیرفعال، فیلتر شدند و پروتئین‌های مایع رویی در دو مرحله با آمونیوم سولفات در دمای ۴ °C رسوب داده شدند. رسوبات حاصله دیالیز شدند و ژل کروماتوگرافی (G-۵۰) انجام گرفت. فراکسیون‌های به دست آمده برای تعیین غلظت پروتئین و وزن مولکولی مورد تجزیه و تحلیل قرار گرفتند. کمپلکس پروتئینی ESAT-6 و CFP10 در فراکسیون خالص شده با وسترن بلات تایید شد. از روش سنجش حساسیت خوکچه هندی برای تخمین قدرت بیماری‌زایی فراکسیون خالص شده در مقایسه با PPD استاندارد استفاده شد. حداکثر مقدار پروتئین‌های با وزن مولکولی پایین آمونیوم سولفات ٪۲۰ رسوب داده شدند. تجزیه و تحلیل SDS-PAGE باندهای پروتئینی تقریباً ۱۵-۱۰ کیلو دالتونی را نشان داد. خلوص پروتئین حدود ≥۹۵، بود که توسط SDS-PAGE تایید گردید. حضور کمپلکس ESAT-6/CFP10 با تجزیه و تحلیل وسترن بلات تأیید شد. فراکسیون‌های خالص شده هیچ واکنش متقاطعی با سویه BCG یا مایکوباکتریوم اویوم نشان ندادند. به نظر می‌رسد ESAT-6/CFP10 خالص شده با روش آمونیوم سولفات برای توسعه کیت تشخیصی برای تشخیص Mtb مناسب می‌باشد.

واژگان کلیدی

مایکوباکتریوم توبرکلوزیس، ESAT-6/CFP10، آمونیوم سولفات، کروماتوگرافی، وسترن بلات