Austin Clinical Microbiology

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Review Article

Immunogenicity of ESX Family Antigens of Mycobacterium Tuberculosis: An Overview

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Received: August 09, 2016; **Accepted:** September 22, 2016; **Published:** September 23, 2016

Abstract

Using of different ESX family antigens as subunit vaccine is one of the most promising strategies to develop a novel and improved TB vaccine. The aim of this study was to review the immunogenicity of various ESX family antigens for induction of host immune responses and their application as TB subunit vaccine. Here, by using gene name/synonym name and their accession number, only published articles with English language were obtained from PubMed, Google Scholar and Scopus and reviewed to identify relevant studies. According to various studies, among 23 members of *Mycobacterium tuberculosis* ESX family antigens, EsxA, EsxB, EsxG, EsxH, EsxN, EsxO, EsxQ, EsxR, EsxS, EsxV, and EsxW antigens were the most immunogenic antigens. Unlike *esxA* and *esxB* genes which are encoded by RD1, *esxO, esxP, esxV*, and *esxW* genes are encoded by RD7 and RD9 regions and due to high sequence homology of these genes with their subfamily members proteins which are present in vaccine strains of *M. bovis* BCG, these proteins could not be used for distinguish between TB infection and vaccination with BCG. However, they are potent candidates as subunit vaccine. Based on this review, no study has evaluated the immune profile of EsxM, EsxJ, EsxK, and EsxP antigens as vaccine candidates. Therefore, further preclinical and clinical studies are needed.

Keywords: Mycobacterium tuberculosis; ESX family antigens; Immunogenicity

Introduction

Mycobacterium Tuberculosis (MTB) that is the causative agent of Tuberculosis (TB) infection remains as one of the leading causes of human mortality worldwide [1]. Unlike newborns and children, protective efficacy of the only approved and available vaccine against TB infection, i. e. BCG or *Mycobacterium bovis* bacillus Calmette-Guérin, in adults is low (0-80 %) [1,2]. Therefore, developing a novel TB vaccine is an important issue. Several studies in preclinical animal models have demonstrated that various *Mycobacterium tuberculosis (M. tuberculosis)* antigens could induce robust immune responses (compared to the BCG) against TB infection [3]. Therefore, the second-generation of effective immunization strategy against TB infection have been developed to improve BCG-primed immunity based on subunit vaccines [4]. Subunit vaccines are based on different antigens of bacteria which are expressed in different phases of TB pathogenesis in order to adapt to different environments [5]. During different stages of TB infection, *M. tuberculosis* produces dormancy or latent antigens which are expressed by dormant or non-growing *M. tuberculosis* located in stress condition like nutrient deprivation, hypoxia and host immune responses, as well as early expressed antigens which expressed by growing or replicating and metabolically active bacteria [6,7]. The choice of suitable MTB antigens as a possible vaccine candidate in order to develop effective subunit TB vaccines is necessary. Most TB subunit vaccines have been designed based on antigens expressed by replicating bacteria [8]. Among the most promising TB antigens, members of the *esx* gene family or ESAT-6 like proteins or ESX family antigens, which are expressed in replicating stage, are attractive immune targets in order to induce the cell-mediated immunity against TB infection [9,10]. The ESX family antigens are the low-molecular weight and short-length proteins, approximately 100 amino acids in length, which are consist of 23 members (EsxA to EsxW) and except for EsxQ, all genes localized as pairs [9,10]. These antigens, except for EsxQ, secreted as heterodimers by the type-VII secretion system (T7SS) which known as ESX secretion system (the ESAT-6 secretion system) (ESX-1 to ESX-5) [9,10]. Based on high amino acid sequence homology, the ESX family antigens divided into subfamilies including, the QILSS subfamily (EsxM, EsxJ, EsxK, EsxP and EsxW) with >98% homology, the TB10.4 subfamily (EsxH, EsxR and EsxQ) with 67%-84%homology, the MTB9.9 subfamily (EsxN, EsxI, EsxL, EsxO and EsxV) with 93-98% homology, and the TB9.8 subfamily (EsxS and EsxG) with~96% protein homology [9].

The present study focused on the potential of ESX family of MTB antigens as immunodominant molecules for induction of host immune responses and their application as TB subunit vaccines.

EsxA

MTB *esxA* gene encodes a 6 kDa-early secretory antigenic target EsxA (ESAT-6) protein. ESAT-6 protein plays a direct role in granuloma formation and facilitating bacterial escape from the phagosome to the cytosol [11]. The role of ESAT-6 protein as a candidate vaccine has been established in several studies. According to the conducted studies, ESAT-6 protein as fusion or single protein or in an adjuvant combination provides promising results in term of efficient long-term memory immunity, and protective immunity from TB infection [12-16]. Villarreal and colleagues reported that

Citation: Khademi F, Yousefi-Avarvand A, Derakhshan M and Tafaghodi M. Immunogenicity of ESX Family Antigens of Mycobacterium Tuberculosis: An Overview. Austin Clin Microbiol. 2016; 1(1): 1006.

Table 1: Characteristics of the ESX family antigens of *M. tuberculosis.* (http://tuberculist.epfl.ch) [11-30].

Gene/Synonym	Accession number	Protein function	Profile of immune responses	Potential candidate vaccine
esxA/esat-6	Rv3875	cell wall and cell processes	Improving the effectiveness of BCG vaccination Induce a strong humoral and cellular immune response in animal models	Yes
esxB/lhp/cfp10	Rv3874	cell wall and cell processes	Conferring protection in animals challenged model	No
esxC/ES6_11	Rv3890c	cell wall and cell processes	As fusion protein (EsxD-EsxC) gave protection at the level of BCG	No
esxD/-	Rv3891c	cell wall and cell processes	As fusion protein (EsxD-EsxC) gave protection at the level of BCG	No
esxE/ES6_12	Rv3904c	cell wall and cell processes	Modest but significant stimulation of CD4 and CD8 T cells	No
esxF/ES6_13	Rv3905c	cell wall and cell processes	Modest but significant stimulation of CD4 and CD8 T cells	No
esxG/TB9.8	Rv0287	cell wall and cell processes	Immunogenic antigen	No
esxH/cfp7/TB10.4	Rv0288	cell wall and cell processes	Strongly immunogenic and induces protection against TB	Yes
esxI/ES6_1/Mtb9.9D	Rv1037c	cell wall and cell processes	Immunogenic antigen	No
esxJ/ES6_2/TB11.0	Rv1038c	cell wall and cell processes	NA	No
esxK/ES6_3/TB11.0	Rv1197	cell wall and cell processes	NA	No
esxL/ES6_4/Mtb9.9C	Rv1198	cell wall and cell processes	Immunogenic antigen	No
esxM/TB11.0/QILSS	Rv1792	cell wall and cell processes	NA	No
esxN/ES6_5/Mtb9.9A	Rv1793	cell wall and cell processes	Immunogenic antigen	Yes
esxO/ES6_6/Mtb9.9E	Rv2346c	cell wall and cell processes	Immunogenic antigen	No
esxP/ES6_7/QILSS	Rv2347c	cell wall and cell processes	NA	No
esxQ/TB12.9/ES6_8	Rv3017c	cell wall and cell processes	Immunogenic antigen	No
esxR/ES6_9/TB10.3	Rv3019c	cell wall and cell processes	Immunogenic antigen	No
esxS/PE28	Rv3020c	cell wall and cell processes	Immunogenic antigen	No
esxT/-	Rv3444c	cell wall and cell processes	Modest but significant stimulation of CD4 and CD8 T cells	No
esxU/-	Rv3445c	cell wall and cell processes	Modest but significant stimulation of CD4 and CD8 T cells	No
esxV/ES6_1/Mtb9.9D	Rv3619c	cell wall and cell processes	Induce a strong humoral and cellular immune response in animal models	No
esxW/ES6_10/QILSS	Rv3620c	cell wall and cell processes	Induce a strong Th1 response immune response in animal models	No

NA: Not Available, potential candidate vaccine is based on http://tuberculist.epfl.ch.

immunization with EsxA DNA construct, as fusion or alone antigens, elicits strong Th1 responses in mice model, as compared with BCG [9]. As discussed in Chen et al. study, the ESAT-6protein in combination with CFP-10 as fusion protein and mixed with aluminum hydroxide and CpG DNA adjuvants, was able to induce high levels of antibody and IFN-γ against TB antigens [15]. Lin and colleagues showed that intradermal administration of ESAT-6 protein along with other TB antigens and mixed with IC31 adjuvant as multistage vaccine (H56) was able to boost BCG-primed immunity and control the late-stage of TB infection in cynomolgus macaques. This subunit vaccine has been entered into clinical studies [17]. Xin and colleagues reported that subunit vaccine consists of ESAT-6 antigen as multistage fusion protein and mixed with an adjuvant composed from DDA (dimethyldioctadecylammonium bromide), poly (I:C) (polyinosinic acid: polycytidylic acid) and gelatin has high protective efficacy against TB infection in C57BL/6 mice [7]. Characteristics of the ESX family antigens of *M. tuberculosis* presented in this Table 1.

EsxB

The *esxB* gene encodes a10-kDa MTB protein named culture filtrate antigen EsxB (LHP, CFP10) protein and belongs to the ESAT-6 family. Like ESAT-6, EsxB protein encoded in the region of difference 1 (RD-1) and secreted by ESX-1, a T7SS which is encoded in RD1 [3,12]. Both proteins have low sequence homology with other ESX family proteins and used in the diagnosis of latent TB infection by using the IFN-γ release assay (IGRA). They can distinguish between TB infection and vaccination with BCG and are present in virulent MTB and *M. bovis*, but deleted in the attenuated or a virulent strains of *M. bovis* BCG vaccine [3,12]. The immunogenicity characteristics of EsxB antigen was evaluated and proved in other studies [8,12,15]. They have reported that EsxB protein, like ESAT-6, could induce strong immunity against MTB infection in animal models and may act as a good subunit vaccine candidate. Therefore, EsxB antigen has been suggested for TB vaccine development.

EsxC and EsxD

The esxC and the esxD genes express two ESAT-6 like proteins with unknown functions which secreted by the type-ESX-2 as heterodimer [18]. Proteins length is 95 and 107 amino acids and molecular mass is 9.9 and 11 kDa, respectively. Villarreal and colleagues study showed that EsxC and EsxD antigens, compared with the other ESX antigens such as EsxH, EsxR, and EsxS, elicit lower levels of IFN-γ and TNF-α [9]. However, Knudsen et al. reported that dimer form of EsxD and EsxC antigens (EsxD-EsxC) are promising TB vaccine candidates and protected against MTB infection in B6C3F1 mice model, comparable to BCG-vaccinated group [18].

EsxE and EsxF

The proteins expressed by these genes have 90 and 103 amino acids in length and molecular mass of 9.5 and 10 kDa, respectively. Their function is unknown and their ESX secretion system has not been identified [18]. The immunogenicity profile of EsxE and EsxF antigens has been investigated by Villarreal and colleagues [9]. They observed that like EsxC and EsxD antigens,EsxE and EsxF antigens showed low IFN-γ and TNF-α responses [9].

The MTB9.9 subfamily

The MTB9.9 subfamily antigens with 94 amino acids in length and molecular mass of 9.9 kDa contains five members as EsxN, EsxI, EsxL, EsxO andEsxV [9,18]. The function of MTB9.9 subfamily antigens is unknown. They are secreted by type-ESX-5 as a heterodimer pair along with the QILSS subfamily antigens, e.g., EsxN-EsxM, EsxI-EsxJ, EsxL-EsxK, EsxO-EsxP, and EsxV-EsxW [18]. Peng and colleagues studied on the MTB9.9 subfamily proteins and showed that they are immunogenic in C57BL/6 mice and induce humoral and Th1-type cellular memory responses. It represents them as potent subunit vaccine candidates [19]. The *esxO* and *esxV* genes, like *esxP* and *esxW* genes, located in RD7 and RD9 regions and are absent in BCG. As discussed in study by Villarreal and colleagues, among the MTB9.9 subfamily antigens, the strongest CD4 T cells immunity responses was observed for EsxO antigen as well as EsxV was the most prominent antigen to induce CD8 T cells immunity [9]. Mahmood et al. and Ansari et al. reported that EsxV antigen induced strong Th1 immune response and reduced bacterial load in the lungs of infected mice with TB infection and increased the IFN-γ, IL-12 and IgG2 a levels, and can acts as a sub unit vaccine candidate [20,21]. Based on comprehensive literature data and bioinformatics analyses, Zvi and colleagues showed thatEsxA, EsxN and EsxH antigens are located among top-ranking antigens to induce immune protective responses against TB infection and predicted as possible vaccine candidates [22].

The QILSS subfamily

The QILSS subfamily has 5 members consisting of EsxM, EsxJ, EsxK, EsxP and EsxW, which secreted along with the MTB9.9 subfamily proteins by the type-ESX-5 [9,18]. Members of the QILSS subfamily have 98 amino acids in length and molecular mass of ~11 kDa with an unclear function (http://tuberculist.epfl.ch). Among the QILSS subfamily genes, the genome regions containing *esxP* and *esxW* genes are located in RD7 and RD9 regions. These are deleted during the attenuation of *M. bovis* and these genes are absent in BCG vaccine [23]. Therefore, using of these immunogenic antigens could be useful for designing more effective vaccines against TB infection. In order to evaluate immunization potential of EsxW antigen, several immunization studies have been performed in animal models. Baldwin et al. reported that a vaccine called ID83 containing *esxW* gene plus Rv1813and Rv2608 and synthetic TLR4 or TLR9 agonists, could induce Th1 immune responses and protect against TB infection in mice [24]. Knudsen et al. and Bertholet et al. showed that EsxW and EsxV antigens (with each other, or in combination with PE/PPE family antigens and latency antigen, as ID93), were immunogenic in animal model. These are promising TB vaccine candidates and protects against MDR-TB (multidrug-resistant) [18,25]. However, no study was found about the immune profile of EsxM, EsxJ, EsxK, and EsxP antigens as vaccine candidate.

The TB10.4 subfamily

The TB10.4 subfamilyhas 3 members consisting of EsxH, EsxR and EsxQ, which secreted by an export system known as ESX-3 [9,18]. Except for EsxQ protein, EsxH and EsxR proteins secreted as a heterodimer pair along with the TB9.8 subfamily antigens, EsxH-EsxG and EsxR-EsxS [18]. Also, among the TB10.4 subfamily antigens, except for EsxH protein, their function is unclear. EsxH protein may be involved in virulence (http://tuberculist.epfl.ch). Protein length of EsxH and EsxR proteins is 96 amino acids and for EsxQ protein are 120 amino acids (http://tuberculist.epfl.ch). The esxH gene encoded hypothetical protein called the TB10.4 antigen with molecular mass of ~10 kDa which secreted as heterodimers with EsxG (EsxG-EsxH). This molecule presumably plays the main role in zinc ion acquisition [11]. Hoang and colleagues assessed the post-exposure vaccine activity of TB10.4 antigen and observed a very modest reduction of bacterial load compared to the ESAT-6 antigen [11]. However, Dietrich et al. demonstrated TB10.4 antigen is strongly immunogenic and as fusion protein strongly induced protection against TB infection at the same level of BCG vaccine. Therefore, this is a promising candidate for exchanging with ESAT-6 protein [26]. Other groups were also evaluated the effectiveness of novel TB vaccines expressing TB10.4 protein as fusion with other mycobacterial antigens to improve the BCG protection against TB and observed a strong humoral and cell-mediated immune responses [7,18,27-30]. Villarreal and colleagues demonstrated that members of the TB10.4 subfamily antigens are the most immunogenic ESX antigens [9].

The TB9.8 subfamily

The TB9.8 subfamily has 2 members consisting of EsxS and EsxG, secreted by the type-ESX-3 [9,18]. Members of the TB9.8 subfamily antigens with 97 amino acids in length and molecular mass of ~9.8kDa belong to the ESAT6 family antigens and are a distant member of the *M. tuberculosis* PE family (http://tuberculist.epfl.ch). According to the conducted study by Villarreal and colleagues, among 23 ESX family members, EsxH, EsxR, EsxS, and EsxQ were the most immunogenic antigens, respectively [9]. As a multi-antigenic recombinant adenoviral booster TB vaccine, the effectiveness of the EsxG protein in improving the protective efficacy of BCG against TB infection was studied. In animals challenged, this antigen in combination with additional potential immunogenic antigens boosted the protection conferred by BCG vaccine [28]. According to the Knudsen et al report, EsxG antigen as dimer form (EsxG-EsxH) is immunogenic antigen and the promising TB vaccine candidate [18].

EsxT and EsxU

EsxT antigen is a putative ESAT-6 like protein with 100 amino acids length and molecular mass of ~11 kDa. This TB antigen secreted by the type-ESX-4, along with EsxU protein which is ESAT-6 like protein with protein length of 105 and molecular mass of ~11 kDa (http://tuberculist.epfl.ch). The performance of both proteins is still remained unclear. The immunogenicity profile of EsxT and EsxU antigens has been investigated by Villarreal and colleagues [9]. In terms of inducing CD4 and CD8 T cells responses, EsxT and EsxU antigens induced lower immune responses compared to EsxR, EsxH, EsxQ and EsxS antigens, respectively.

Conclusion

Among various MTB virulence factors, the ESX family antigens are important TB vaccine candidates. They could induce strong humoral and cellular immune responses in preclinical and clinical studies. Among 23 members of *M. tuberculosis* ESX family antigens, EsxA, EsxB, EsxG, EsxH, EsxN, EsxO,EsxQ, EsxR, EsxS, EsxV and EsxW were the most immunogenic antigens. However, in order to induce excellent immune responses, these early-expressed antigens could be used in combination with latency-associated antigens as multistage fusion protein. Adjuvants and delivery systems are also required to establish an effective vaccine. Some of these genes were located in the RD1, RD7 and RD9 regions and missed from the genome of *M. bovis* BCG strains. Unlike the *esxA* and *esxB* genes (encoded by RD1), *esxO*, *esxP*, *esxV*, and *esxW* genes (encoded by RD7 and RD9) could not distinguish between TB infection and vaccination with BCG. This is because of their high sequence homology with their subfamily member's proteins present in vaccine strains of *M. bovis* BCG and virulent MTB. However, using of these immunogenic antigens could be useful for designing more effective vaccines against TB infection. No study was found to evaluate the immune profile of EsxM, EsxJ, EsxK and EsxP antigens as vaccine candidate.

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Citation: Khademi F, Yousefi-Avarvand A, Derakhshan M and Tafaghodi M. Immunogenicity of ESX Family Antigens of Mycobacterium Tuberculosis: An Overview. Austin Clin Microbiol. 2016; 1(1): 1006.