ESAT-6 AS AN EFFECTIVE MYCOBACTERIUM TUBERCULOSIS SUBUNIT VACCINE

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ABSTRACT: Tuberculosis is still one of the important infectious diseases and a major cause for public health problems, particularly in low- and middle-income countries. Prevention of this disease is a priority of WHO. Many antigens have been candidate for vaccination. Evaluating the safety and efficacy of these antigens is the concern of many researchers. This review is about ESAT-6 as an effective Mycobacterium tuberculosis Subunit vaccine.

Keywords: ESAT-6, Mycobacterium Tuberculosis, Vaccine.

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), was isolated and identified by Robert Koch in 1882 (1). Since then, tuberculosis continues to be one of the prominent infectious diseases and a major cause for public health problems, particularly in low- and middle-income countries (2). Although its incidence rate is slowly decreasing, the absolute number of cases is still increasing. Nearly one third of the world population are involved with mycobacterium tuberculosis (3). Estimation of world health organization (WHO) for the global burden of disease in 2009 was 9.4 million new cases of TB and 14 million prevalent cases. WHO estimated that about 1.3 million deaths will occur in HIV-negative patients and approximately 0.4 million deaths will occur in HIV-positive ones, during 2009. Most of TB cases are in South-east Asia (35%), Africa (30%) and Western Pacific regions (20%). Though, the only available tuberculosis vaccine is attenuated strain of M. tuberculosis, M. bovis bacillus Calmette-Guerin (BCG). Many trials have shown the protective efficacy of this vaccine between 0% to 85% (4). Despite the beneficial effects of BCG, it cannot be fully protective in adults(5). As a result a need for a more efficient vaccine is sensible.

Various investigations have been performed for making new vaccines including DNA and subunit protein vaccine (6-7). In 1908, after serial passages of Mycobacterium bovis, Albert Calmette and Camile Guérin succeeded to generate a vaccine against tuberculosis, which is known as Bacille Calmette Guérin (BCG). The only available vaccine against tuberculosis is BCG vaccine (8).

Although BCG induces high levels of protection in animal models of tuberculosis, its efficacy in humans is proven highly variable by human trials. In addition, despite widespread use of BCG in neonates, it does not provide effective prevention for adult pulmonary disease. Therefore, it has not decreased the global burden of tuberculosis and designing a beneficial vaccine against TB has been a research priority in many countries (4). HIV appearance in recent decades has led to re-emergence of tuberculosis. Therefore, many efforts have been put into the research and development of new effective vaccines against tuberculosis and better diagnostic methods for the disease (9). Present candidates for TB vaccination immunologically contain the remaining M. tuberculosis organisms and transform them into a dormant form with low replication and metabolism. This can
lead to disease reactivation at later stages and post-exposure vaccines are needed to prevent reactivation of dormant bacteria and re-infection (10).

Among current candidates are live vaccines. Live mycobacterial vaccines are genetically improved BCG vaccines (recombinant BCG or rBCG) or M. tuberculosis isolates that are attenuated by deletion of virulence genes. Other candidates are killed whole bacteria and bacterial fragments (11).

In recent years, increased emergency of multi-drug-resistant (MDR) strains of M.TB, co-infection with HIV and limitation of effective available vaccine against TB complicated the situation (12). So, the attempts have been done to identify M. tuberculosis antigens and epitopes as candidates for new protective vaccines and specific diagnostic reagents against TB. The studies led to the identification and characterization of specific diagnostic reagent, ESAT-6, for TB (13).

Recent sequencing of the M. tuberculosis genome has introduced new approaches to antigen discovery. An immunodominant gene family is the early secretory antigen target 6 (ESAT-6) gene families, which encodes immunodominant molecules and strongly recognized by the immune system of animals and humans whose T cells are exposed to M. tuberculosis.

Various investigations have assessed the influence of 6-kDa early secretory antigen target (ESAT-6) on TB (14-15). Culture filtrate from Mycobacterium tuberculosis contains protective antigens of relevance for the generation of a new anti-tuberculosis vaccine (The ESAT-6 family of low mass protein) (16). It is expected that an ideal anti-TB vaccine should provide consistent and long-term protection in humans, irrespective of the geographical location of use (13). One of the most extensively considered antigens to improve new vaccines against TB is ESAT-6 (13). Antigen epitope of ESAT-6 can be detected by B and T cell in active TB (17-21), further studies showed an increase antibody level in M.tuberculosis-infected patients (19-21).

M. tuberculosis is an intracellular pathogen for which the defense relies on cell-mediated immunity, and T-cell effector mechanisms rather than antibody are needed to control or eliminate the bacteria. Virus vectors carrying genes for the proper antigens can not only deliver them within a recipient cell but also many of them have the added advantage of generating strong cell-mediated immune responses themselves. Based on findings, CD4+ T cells present among the most protective response against Mycobacterium tuberculosis, as in HIV infection loss of CD4+ T cells greatly raise susceptibility to both severe and reactivation tuberculosis. Both interferon-γ and several cytokines including interleukin-2 of play important role in fighting against tuberculosis which are produced by CD4+ T cells (22).

The family of Fc receptors (FcRs) for IgG (FcγR) delivers a leading instance of how simultaneous triggering of activating and inhibitory signaling pathways sets thresholds for cell activation and thus generates a well-balanced immune response. In addition to their role in binding antigen, antibodies can regulate immune responses through interacting with Fc receptors (FcRs) (23). Fcγ receptors bind to M. tuberculosis that are opsonized with immunoglobulin G (IgG) and lead to phagocytosis of bacteria, activation of reactive oxygen species and pro-inflammatory reactions. This pathway might be favored by the immune system to induce macrophage responses before the activation of adaptive immunity (24-25). Also, Immunity to M. tuberculosis is characterized by some basic features; specifically sensitized T lymphocytes mediate protection, and the most important mediator molecule seems to be interferon gamma (IFN-γ) (26).

In this study, we aim to review the literature regarding the development of vaccines against TB and focus on ESAT-6 subunit vaccine with Fcγ IgG as a delivery system, which is a good replacement for previous ineffective vaccines.

**ESAT-6 AS NEW ANTI-TB VACCINE CANDIDATE**

ESAT6 protein is one of the secreted antigens of Mycobacterium tuberclusis and is expressed only in virulent Mycobacterium bovis strains and M. tuberculosis (12). ESAT-6 reactive has a large proportion of memory T cells in M.TB-infected. Hence, ESAT6 is a promising candidate antigen for vaccine goals (12). Immunization with peptides along with appropriate adjuvants induced high level of cellular immune response and the epitope 51–70 of ESAT-6 induced a level of protection, which was equivalent to that achieved after immunization with ESAT-6 and BCG. These results are highly encouraging with respect to the potential of subunit and peptide-based vaccines in providing protection against TB (13).

Early secreted antigenic target protein 6 (esat6) is one of the genes stand by region of difference 1 (RD1) of Mycobacterium tuberculosis (M. tb) genome. This RD1 is a characteristic of virulent strains of M. tb and Mycobacterium bovis and this is one of the major differences between the disease causing strains and Bacillus-Calmat Guerin (BCG) vaccine strains. Studies have proved the presence of large number of memory T cells in...
M. tb infected individuals and these memory cells are reactive towards Esat6 antigen, which highlighted the importance of this gene especially in early infections stage (16). Initial studies demonstrated that ESAT-6 protein is a dominant T-cell determinant in M. tuberculosis infection of human and animals (26). The ESAT-6 antigen was known in the low-molecular-mass fraction of culture filtrate due to a strong T-cell response with high levels of gamma interferon (IFN-γ) released. According to some studies, this antigen has been shown to own acceptable stimulatory antigenic features and is recognized highly by a great number of TB patients (27).

Knowledge of biochemical structure of ESAT-6 is unperfected yet. Being a secreted protein, no signal peptide has been recognized on ESAT-6, and it is still a mystery how the ESAT-6 protein is translocated into the surrounding milieu. Bioinformatics analysis has suggested that the genes flanking ESAT-6 encode a novel secretion pathway of M. tuberculosis and the indispensability of this functional unit in the secretion of ESAT-6 has recently been demonstrated by several research groups. As yet, the molecular mechanisms underlying this type of transport still continue to be elucidated (26).

Newly and recently, secreted protein antigens present in the culture filtrate (CF) of M. tuberculosis have been the focus of studies because these antigens are considered immune dominant and involved in inducing protective immunity (13). The crucial factor of protective immunity against TB is a T-cell mediated response characterized by the secretion of IFN-γ and other cytokines (12).

**SUBUNIT EAST-6 AND DNA ESAT-6 VACCINE**

Evaluating the immunogenicity and protective efficacy of secreted proteins encoded by RD1 is important for development of subunit and DNA anti-tuberculosis vaccines (26). Identification of appropriate adjuvant and delivery systems has shown the promise to overcome the problem of poor immunogenicity associated with subunit and peptide based vaccines (13). These are non-live, or in the case of viral vectors, non-replicating vaccines, which can be delivered safely into the human host regardless of immune competence. Subunit vaccines in TB are mostly based on recombinant proteins admixed with proper adjuvants, or the use of attenuated viral vectors. Although subunit vaccines theoretically could be used as priming vaccines, current views are that they may be mostly used as booster vaccines on top of BCG-, recombinant BCG-, or attenuated Mtb-priming vaccines (26). The subunit vaccines ESAT-6 are then expected to boost strong, long-lived immune responses in already primed individuals, such that these will persist to levels high enough to protect the 3%–10% vulnerable individuals against TB disease. Subunit vaccine ESAT-6 candidates are based on antigens that are recognized by T cells from patients with latent infection or whose tuberculosis has been cured. The great obstacle to the development of protein subunit vaccines is the restricted availability of new adjuvants capable of eliciting a suitable pattern of immune response (28).

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<th>Adjuvant</th>
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<td>ESAT-6</td>
<td>DDA</td>
<td>Predominant T cell antigenicity but less inherent immunogenicity</td>
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<tr>
<td>ESAT-6</td>
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* Adjuvants: LANAC, liposome-Ag-nucleic acid complex; DDA, dimethyl dioctadecylammonium bromide; MPL, monophosphoryl lipid A; SAF, syntax adjuvant formulation (pluronic L121, squalene, Tween-80 and soybean muramyldipeptide); TDR, trehalose dihydrate; K31, a vehicle based on the cationic peptide KUL5/UL6 and the immunostimulatory oligodeoxynucleotide ODN 1811 signaling through the TLR9 receptor; PLG, poly(lactic-co-glycolic acid microparticles; IFA, incomplete Freund's adjuvant; A020A, a cationic lipophilic vehicle containing 3-decenedial monophosphoryl lipid A and a purified fraction of O-antigen assoiciated with T cell responses. 

**Table 1. Conflict of antigenicity and immunogenicity and protective efficacies of protein subunit vaccines against experimental TB (29)**
Humoral and cellular immune responses, as well as confers protection against some viral, bacterial and parasitic pathogens are come off by DNA vaccine (12). T helper cells play an important role in eliciting both humoral and cellular immune responses via expansion of antigen-stimulated B cells and expansion of CD8 +T cells respectively. Hence, it is the key to measure proliferation of T cells after immunization with vaccines when stimulated in vitro with a specific antigen. The immunogenicity of DNA vaccine encoding ESAT-6 was increased by DNA priming and protein subunit boosting. The convincing example in this regard can be ESAT-6, the epitome of T cell antigenicity. The molecule is documented by about 25–35% of the total M. tuberculosis reactive T cell selection recruited during recall response of mouse model of memory immunity (16).

It has been reported that DNA vaccines specially encouraged Th1 -dominant immune response. Caring immunity against tuberculosis mainly depends on cellular immune responses and some cytokines of Th1 -type, such as IFN-γ. ESAT6 DNA vaccine elicited a Th1 -type immune response. Similarly, the lymphocyte proliferation response and IFN-γ secretion in DNA vaccination mice were significantly increased by the boost of ESAT6 protein. Those suggested that the ESAT6 DNA vaccine primed the correct pathway of the immune response (Th1 -type) and the ESAT6 protein boost enhanced the DNA–primed memory responses (28).

While DNA immunization induced Th1 -polarized immune response, protein–in-adjuvant vaccination elicited a Th2-dominant response. When animals were primed with DNA and boost with protein, both antibodies and Th-cell proliferative response were significantly enhanced. Moreover, production of Th1 -type cytokine (IFN-γ ) was increased significantly by DNA priming-protein boosting. This protocol also resulted in an increased relative ratio of IgG2a to IgG1 and the cytotoxicity of T cells. Thus, this study demonstrated that the formation of ESAT6 DNA prime-protein boost inoculation could improve antigen-specific cellular immune responses, which are important for protection against TB infection (12). Priming by the DNA vaccine was critical for the type of protection immune response, since the ESAT6 protein on its own induced a different pathway (Th2 type) of the immune response.

Efficacy of Available Vaccines

Evaluating the safety and efficacy of new vaccines requires accurate analyses with assays and trials that are well harmonized. The countries in urgent need for TB vaccine are those with low income and have the least facilities. Therefore, analysis of beneficence remains essential to persuading the governments and international communities in order to support adult TB vaccination programs. Given that, the necessity of clinical trials in this field is trivial.

Ag85B-ESAT-6 (H1) vaccine, accompanied by a strong Th1 adjuvant (IC31), has been evaluated through two clinical trials. The trials suggest that this vaccine has no serious side effects and reactions and has a strong immunogenic effect on human immune system. Besides, it is well tolerated and leads to better patient compliance. It leads to strong Th1 responses to the Ag85B protein (30-32). These findings are also supported by van Dissel et al. the results revealed besides a minor local or systemic adverse effect and a tenderness in the site of infection, H1 vaccine with IC31 has strong T-cell immune response, which lasts during 32 weeks follow up, suggesting the a persistent memory response in vaccinated individuals (33).

Another fusion protein, which came later, was Ag85B-TB-10.4. It was designed as an alternative for Ag85B-ESAT-6. In this vaccine, ESAT-6 was replaced by TB-10.4 to decrease the risk of interference with diagnostic assays based on ESAT-6 (34). However, Hoang et al also concluded that ESAT-6 containing vaccines has a notable protective effect against previously established TB infection (35).

TB-10.4 and CFP10 are members of ESAT-6 gene family. Skjøt et al. compared the features of CFP10 and TB10.4. They found that both of them were strongly recognized by 70% of the TB patients. In addition, with increase in the concentration of these antigens, high levels of IFN-γ were induced. IFN-γ level in response to these antigens was even higher compared to ESAT-6, in many cases (27).

The role of genetic diversity of M. tuberculosis in Ag85B-TB-10.4 and Ag85B-ESAT-6 has also been established. it is shown that the efficacy of these two vaccine cannot affected by Tuberculosis genetic diversity (36).

In their later study in 2002, Skjøt et al. assessed the differences in T cell response between healthy BCG-vaccinated patients, and non-vaccinated control group. The subjects underwent in vitro stimulation with recombinant TB10.4 (rTB10.4). They confirmed TB10.4 to be the immunodominant member of ESAT-6 family.
gene (37). Rindi et al. also worked on the virulent and avirulent isolates of M. tuberculosis and found that TB10.4 was produced only in virulent isolates. This observation confirms that TB10.4 gene and the consequent protein are involved in the virulence of M. tuberculosis (38).

CFP-10 is the other member of ESAT-10 family group. More recent studies have shed light on impact of specific and sensitive assays measuring IFN-γ production by CD4+ T lymphocytes against defined mycobacterium tuberculosis antigens, especially CFP-10 and ESAT-6. These so-called IFN-γ release assays (IGRA) can detect mycobacterium tuberculosis -infected not only from uninfected individuals but also from BCG-vaccinated individuals, because CFP-10 and ESAT-6 are produced by mycobacterium tuberculosis but not by BCG (28).

In conclusion, this review suggests that these antigens may be effective diagnostic reagent against TB, a mixture of synthetic peptides of ESAT-6 and other M. tuberculosis-specific antigens. In addition, this data suggested that DNA prime-protein boost mainly augmented Th1 -type cell mediated immune response. As it drive cytotoxic T-cell response, production of IgG2a as well as IFN-γ were all enhanced. IFN-γ cytokines should also play a role in the initial control of TB infection. Taken together, our results demonstrated that DNA prime-protein boost protocol could be as a new strategy to improve the efficacy of TB DNA vaccine. However, this study was consisted on the role of the some ESAT-6 family members, TB10.4 and CFP-10 in the immunization against tuberculosis, there were a number of such antigens have been characterized and some, for example, the proteins from the antigens-85 complex and ESAT-6 have proven to be highly immunogenic. Further investigations have needed to prove the possible advantages and disadvantages of widely utilization of such these TB-vaccines.

REFERENCES


