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Review

Optimizing antigen selection for the development of tuberculosis vaccines

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ABSTRACT

Tuberculosis (TB) remains a prevalent global infectious disease caused by genetically closely related tubercle bacilli in *Mycobacterium tuberculosis* complex (MTBC). For a century, the Bacillus Calmette-Guérin (BCG) vaccine has been the primary preventive measure against TB. While it effectively protects against extrapulmonary forms of pediatric TB, it lacks consistent efficacy in providing protection against pulmonary TB in adults. Consequently, the exploration and development of novel TB vaccines, capable of providing broad protection to populations, have consistently constituted a prominent area of interest in medical research. This article presents a concise overview of the novel TB vaccines currently undergoing clinical trials, discussing their classification, protective efficacy, immunogenicity, advantages, and limitations. In vaccine development, the careful selection of antigens that can induce strong and diverse specific immune responses is essential. Therefore, we have summarized the molecular characteristics, biological function, immunogenicity, and relevant studies associated with the chosen antigens for TB vaccines. These insights gained from vaccines and immunogenic proteins will inform the development of novel mycobacterial vaccines, particularly mRNA vaccines, for effective TB control.

1. Introduction

Tuberculosis (TB) is a contagious airborne disease caused by the *Mycobacterium tuberculosis* complex (MTBC) (Gagneux, 2012). Despite their highly similar nucleotide sequences (99.9% similarity), the strains in MTBC are classified into distinct species based on their host preference and molecular phylogenetics (Rodriguez-Campos et al., 2014). The most common cause of human TB worldwide is *Mycobacterium tuberculosis* (*M. tuberculosis* or *Mtb*), followed by *M. africanum*, which is responsible for roughly half of the cases in West Africa (de Jong et al., 2010). Additionally, several animal-adapted species, such as *M. bovis*, can infect a broad spectrum of hosts, leading to zoonotic tuberculosis, a significant challenge for both global human and veterinary public health (Olea-Popelka et al., 2017). Despite advancements in treatment, TB remains one of the deadliest infectious diseases. According to the World Health Organization (WHO), an estimated 10.6 million people worldwide contracted TB in 2021, leading to 1.6 million deaths (Bagcchi, 2023). Currently, around a quarter of the global population carries latent tuberculosis infection (LTBI), while most are asymptomatic, about 5%–10% will develop active TB in their lifetime (Sterling et al., 2020).

In 2015, the WHO launched the End TB Strategy, aiming to achieve an 80% reduction in TB incidence and a 90% decrease in TB-related deaths by 2030 (Uplekar et al., 2015). Nevertheless, the global dissemination of multidrug-resistant (MDR) strains, coupled with complications arising from human immunodeficiency virus (HIV) and type II diabetes contribute to an increased risk of infection (Pai et al., 2016; Zhou et al., 2023). Moreover, *Mtb* infection is the leading cause of mortality in individuals with HIV-1. TB and HIV-1 coinfection can disrupt the host immune system, especially by impairing $CD4^+$ T cells and macrophages (Guo et al., 2022). The development of innovative diagnostic methods, drugs, and vaccines is crucial in addressing these challenges effectively. Vaccination is considered one of the most cost-effective strategies for achieving annual global reductions in TB incidence and mortality. The Bacillus Calmette-Guérin (BCG) vaccine, currently the only licensed TB vaccine for human use, provides long-lasting and strong protection against miliary and meningeal TB in children. However, its effectiveness in preventing pulmonary TB is limited, especially in adults (Lange et al., 2022).

Due to the complex genome and structure of *Mtb*, the selection of effective and safe antigens has always been a primary consideration in

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vaccine design. As a result of the advances in immunology and molecular biology, TB vaccine development has made substantial progress. By 2022, sixteen vaccine candidates were at different phases of clinical trials, including whole-cell bacterial vaccines, subunit vaccines, and mRNA vaccines (Fletcher & Schrager, 2016). The antigens used in those vaccines involve multiple strains of *Mycobacterium* and over a dozen structural or secretory proteins of *Mtb*, which have yielded different evaluation results in laboratory and clinical experiments. This review provides an overview of both traditional and novel TB vaccines. Furthermore, it provides a comprehensive summary of the basic or clinical research findings on the antigens selected in TB vaccine development, offering valuable guidance and strategies for pioneering vaccine design.

2. Whole-cell bacterial vaccines

Whole-cell bacterial vaccines encompass attenuated, inactivated, and recombinant variations. The BCG vaccine, derived from attenuated *M. bovis*, is extensively utilized and has had a significant impact on global health. Compared to subunit vaccines, whole bacterial vaccines containing complex antigens may potentially induce a more diverse immune response. The BCG vaccine is administered to infants and young children, offering an 80% protective efficacy (Colditz et al., 1995; Rodrigues et al., 2011). However, the universal acceptance of BCG vaccination for preventing adult pulmonary tuberculosis is still a matter of debate (Gong et al., 2018a). This indicates that the BCG strain might have a distinct pathogenesis from *Mtb*, potentially resulting in an insufficient immune response. Actually, during several years of serial passage, the BCG strain underwent genetic changes and lost nine genes in the region of difference 1 (RD1), which are known to be important for virulence (Mahairas et al., 1996). The BCG vaccine has been found to confer protection against disseminated forms of tuberculosis and reduce all-cause mortality. Additionally, it can also provide some level of protection against infections caused by non-related pathogens like herpes and influenza viruses (Moorlag et al., 2019). This broad protection is thought to occur by triggering innate immune memory and stimulating heterologous lymphocyte activation, which results in heightened cytokine secretion, enhanced macrophage function, improved T-cell reactions, and elevated antibody levels (Netea et al., 2011). This non-specific "trained immunity" triggered by the BCG vaccine in infants may be more effective than in adults, who may have had previous exposure to nontuberculous mycobacteria (NTM) and other pathogens (Barreto et al., 2014).

To address the limitations of the BCG vaccine and enhance its specificity, several new whole-cell bacterial vaccines or vaccination strategies are currently being studied in clinical trials. These include the investigation of a BCG revaccination study and the evaluation of vaccines such as DAR-901 (heat-killed *M. obuense*) and RUTI (cell fragments of *Mtb*) in phase IIb, MIP (inactivated *M. indicus pranii*), MTBVAC (attenuated *M. tuberculosis*), and VPM1002 (recombinant BCG) in phase III (Sable et al., 2019).

DAR-901 is heat-killed *M. obuense*, an environmental pigmented mycobacterium of rapidly growing mycobacteria (RGM) (Luis et al., 2019). Preclinical studies have demonstrated that DAR-901 can induce cellular and humoral immunity, enhancing protection against *Mtb* (Lahey et al., 2016). However, a clinical trial in Tanzania indicated that the observed effect may not be significant in preventing TB infection in healthy adolescents (Wilson et al., 2023).

RUTI® is a therapeutic vaccine delivered in liposomes made from inactivated *Mtb* cell fragments (Cardona, 2006). It can induce cellular immunity and humoral immunity, notably triggering a Th1 response against a diverse array of *Mtb* antigens (Cardona et al., 2005). Clinical studies have shown that RUTI is safe and well-tolerated, with the most commonly reported adverse effects being mild to moderate injection site reactions (Nell et al., 2014).

MIP refers to inactivated M. *indicus pranii*, a nonpathogenic RGM. In a randomized, double-blind multicentric clinical trial, MIP has been shown to be safe with no reported adverse effects. Furthermore, a higher percentage of patients (67.1%) in the MIP group achieved sputum culture conversion at the fourth week compared to the placebo group (57%), suggesting a potential role of MIP in the clearance of *Mtb* (Sharma et al., 2017).

MTBVAC, designed with the deletion of *phoP* and *fadD26* genes in a strain of *Mtb*, has shown comparable safety and immunogenicity to BCG in a clinical trial conducted in Lausanne (Roy et al., 2019). MTBVAC is the only attenuated vaccine containing all the major immunodominant antigens of *Mtb*, such as ESAT6 and CFP10 (Gonzalo-Asensio et al., 2017).

VPM1002, a live attenuated vaccine based on recombinant BCG, has been tested in a clinical trial with newborn babies in South Africa, demonstrating safety and lower reactogenicity compared to BCG. Nevertheless, the immunogenicity of VPM1002 was found to be lower than that of the BCG vaccine (Cotton et al., 2022).

Vaccae™ is an inactivated vaccine derived from non-infectious *Mycobacterium vaccae*, which belongs to the same genus as *Mtb*. It is achieved through heat inactivation and high-pressure air shearing (Gong et al., 2018b). Vaccae™ has completed Phase III clinical trials as a preventive tuberculosis vaccine, the vaccination can induce cellular immunity and mitigate allergic inflammatory responses (Adams et al., 2004; Skinner et al., 1997). Furthermore, clinical studies have demonstrated that using Vaccae™ as an adjunctive immunotherapy, significantly enhances the sputum smear negative conversion rate (68%), facilitates lesion improvement, and cavity closure, shortens treatment duration, and mitigates chemotherapy-related damage (Bourinbaiar et al., 2020; Yang et al., 2011). As a prophylactic vaccine, Vaccae™ can effectively suppress the proliferation and activation of *Mtb*, hinder the progression of LTBI, and decrease the rate of infection (Liang et al., 2023; Sun ZhaoPing et al., 2013).

Given their comparable safety and efficacy to BCG, these novel whole-cell bacterial vaccines have the potential to become better alternatives. Particularly, it should be noted that attenuated vaccines may not be suitable for individuals with immune deficiency or HIV, as they have a higher risk of TB progression (Scriba et al., 2016). In such cases, the development of inactivated mycobacterial and subunit vaccines is more urgent and appropriate for this population.

3. Subunit vaccines

3.1. Viral-vectored vaccines

The subunit vaccines include viral-vectored vaccines and adjuvanted protein subunit vaccines. In contrast to traditional inactivated and attenuated vaccines that utilize whole pathogens as antigens, the subunit vaccines contain or express specific pathogen proteins, which reduces the likelihood of illness in vaccinated individuals (Condit et al., 2016). Currently, two viral-vectored vaccines (Ad5Ag85A and TB/FLU-04L) are in Phase I, while one (ChAdOx185A-MVA85A) is in Phase IIa (Bagcchi, 2023). The Ad5Ag85A and ChAdOx185A-MVA85A vaccines are based on adenovirus serotype 5 (Ad5) and simian adenovirus, respectively. While TB/FLU-04L is a live recombinant influenza A virus vaccine. These vaccines express the *Mtb* protein Ag85A, with TB/FLU-04L also expressing the antigen ESAT-6 (Li et al., 2020). The clinical trial results indicated that ChAdOx185A-MVA85A vaccine induced specific $CD4^+$ and $CD8^+$ T cell immune responses, generated IgG antibodies, and had no severe adverse reactions (Wilkie et al., 2020). Furthermore, TB/FLU-04L vaccine was found to be safe and capable of eliciting a *Mtb*-specific Th1 immune response (Shurygina et al., 2023). Another study demonstrated that Ad5Ag85A intramuscular vaccination was safe, immunogenic, and stimulated polyfunctional T cell responses, particularly in individuals who had previously received BCG vaccination (Smaill & Xing, 2014).

Viral vector vaccines have advantages in terms of production efficiency and cost-effectiveness. In contrast to inactivated vaccines, they have the capability to provide long-lasting immunity by inducing a strong

and persistent immune response (Travieso et al., 2022). However, it is crucial to consider certain limitations regarding the safety and effectiveness of viral-vectored vaccines. One concern is whether pre-existing immunity against the vector can impact its efficacy. Studies have demonstrated that previous exposure to influenza virus can influence the response to the targeted antigens (Gerlach et al., 2019). Similar challenges are seen with adenovirus vaccines, as elevated levels of neutralizing antibodies in vaccinated individuals against Ad5 have demonstrated the ability to suppress the immunogenicity of recombinant adenovirus vaccines, particularly in regions with high Ad5 antibody titers (Barouch et al., 2011). Furthermore, there is a risk of genetic reassortment with circulating wild-type viruses, potentially resulting in the loss of the antigen gene and restoration of virulence.

3.2. Adjuvant protein subunit vaccines

Apart from viral-vectored vaccines, five protein subunit vaccines are currently undergoing clinical trials. These include ID93 + GLA-SE (QTP101) and AEC/BC02 in Phase IIa, H56: IC31 and M72/AS01E in Phase IIb, and GamTBvac in Phase III.

ID93 + GLA-SE is a recombinant fusion protein comprising four *Mtb* antigens (Rv1813c, Rv2608, Rv3619c, and Rv3620c) combined with the TLR4L-containing adjuvant (GLA-SE) (Duthie et al., 2014). Phase I clinical trials demonstrated its safety and effectiveness in inducing strong antigen-specific serum antibodies and Th1-type cellular immune responses (Sagawa et al., 2023).

AEC/BC02 contains Ag85B antigen and the EC fusion protein (ESAT6-CFP10), along with the adjuvant BC02 derived from unmethylated cytosine-phosphate-guanine (CpG) DNA fragment and aluminum salt (Lu et al., 2022). While not effective as a pre-exposure prophylaxis vaccine, it shows potential as an immunotherapeutic against progressive disease in preclinical models of LTBI (Lu et al., 2015).

H56: IC31 combines three *Mtb* antigens (Ag85B, ESAT-6, and Rv2660c) with the IC31 adjuvant. A study conducted on animals demonstrated that H56: IC31 boosting has the potential to control late-stage *Mtb* infection and restrict latent tuberculosis (Lin et al., 2012). Although there were transient cardiovascular adverse events observed in some subjects in Phase I trials, the vaccine-induced antigen-specific IgG responses and Th1 cytokine-expressing $CD4^+$ T cells (Luabeya et al., 2015).

M72/AS01E is a recombinant fusion protein composed of two *Mtb* antigens Mtb32a (PepA) and Mtb39a (PPE18), with the liposome-based AS01 Adjuvant System (Montoya et al., 2013). In infected adults, it has shown 54.0% protection against active pulmonary TB disease without notable safety issues (Tait et al., 2019).

GamTBvac includes two mycobacterial fusion proteins, Ag85A and ESAT6-CFP10, which are immobilized on dextran. The vaccine also contains an adjuvant consisting of DEAE-dextran core as well as TLR9 agonists, the CpG oligodeoxynucleotides. It displays robust immunogenicity and a notable protective effect against *Mtb* strain H37Rv in both murine and guinea pig TB models (Tkachuk et al., 2017). Phase II studies have demonstrated its ability to induce antigen-specific IFN-γ release and Th1 cytokine-expressing $CD4^+$ T cells in healthy adults (Tkachuk et al., 2020).

Subunit vaccines for TB offer several advantageous features, including low cost, easy preparation, and safety. Some vaccines are very promising potential treatments for LTBI. It is important to gather more data and conduct studies to better understand the long-term effectiveness and safety profile of these TB subunit vaccines, especially in populations with comorbidities such as HIV. Ongoing studies will provide valuable insights into the potential use of these vaccines in clinical practice.

4. mRNA vaccine

Messenger RNA (mRNA) vaccines have emerged as a novel vaccine category that has gained significant attention and success in recent years, particularly with the development and authorization of COVID-19 mRNA vaccines (Chen et al., 2022). Due to their flexibility in vaccine design, short production time, cost-effectiveness, and capacity to elicit both cellular and humoral immune responses, mRNA vaccines have become one of the leading candidates to combat infectious diseases (Park et al., 2021). The design of mRNA vaccines hinges on careful antigen selection. An ideal target antigen should be immunogenic and capable of eliciting a protective immune response, ensuring the vaccine effectively generates the desired immune reaction against the specific pathogen. However, the thermostability of mRNA vaccines can present challenges, particularly in settings with limited access to reliable refrigeration systems. mRNA is fragile and prone to degradation if not stored and transported at appropriate temperatures, requiring cold chain storage and distribution. Overcoming this limitation by developing mRNA vaccines with improved thermostability would enhance their feasibility and accessibility in resource-constrained regions. Currently, the only one mRNA vaccine for TB, BNT164, is undergoing phase I clinical trials to determine a safe and well-tolerated dosage in a three-dose schedule (Matarazzo & Bettencourt, 2023). There are no public reports available regarding the protective effects, immunogenicity, and safety of BNT164 in preventing tuberculosis. Further research is needed to verify whether mRNA vaccines can induce a stronger and more comprehensive immune response against TB.

5. Antigen selection for TB vaccines

As mentioned earlier, antigens from mycobacteria are extensively used in the development of TB subunit and mRNA vaccines(Table 1). These antigens encompass structural proteins, virulence-associated proteins, and immune-regulatory factors present on the cell surface or secreted by the bacteria. This review section delves into specific genes and the cellular components they encode, providing valuable insights for future antigen selection and vaccine design.

5.1. fbpA (mpt44, Rv3804c; Ag85A)

The fibronectin-binding proteins (Fbps), also referred to as the antigen 85 complex comprising FbpA, FbpB, and FbpC, are situated in the cell wall of *Mtb* and possesses secretory capabilities (Nguyen et al., 2005). All the Fbps contained a carboxylesterase domain, and each protein functioned as a mycolyltransferase participating in the mycobacterial cell wall formation (Belisle et al., 1997). The antigen 85 complex plays a pivotal role in promoting the 6,6′-dimycolate (TDM) biosynthesis and maintaining the stability of the cell wall structure of *Mtb* (Elamin et al., 2011). Studies have indicated that the loss of FbpA expression inhibits *Mtb* replication in both human and mouse macrophage-like cell lines, suggesting that FbpA may have a role in the pathogenesis (Armitige et al., 2000). The secreted antigen 85 proteins are highly immunogenic and remain expressed continuously by the bacteria, and play a crucial role in stimulating both B cell and T cell responses (Khera et al., 2015). T cells, in particular, can activate the cellular and humoral immunity system by releasing cytokines and activating various immune cells such as macrophages, natural killer (NK) cells, dendritic cells (DC), and cytotoxic T (Tc) cells. This coordinated immune response helps in the destruction of intracellular *Mtb* infection. Among those currently in clinical trials, AEC/BC02, GamTBvac, Ad5Ag85A, TB/FLU-01/4L, and ChadOx185A use Ag85A as the antigen component.

5.2. fbpB (mpt59, Rv1886c; Ag85B)

FbpB is a secreted protein responsible for bacterial cell envelope biogenesis (Tucci et al., 2020). The Fbps share a high level of similarity, with 68–80% identity at the amino acid level. It suggested that all three enzymes are likely involved in cell wall mycoloylation, with FbpC playing a more critical role compared to FbpA and FbpB (Puech et al., 2002). Due to their enzymatic activities and status as secreted proteins, the Fbps are not only relevant as potential drug targets but also serve as diagnostic

Table 1
The immunogenic proteins of TB subunit vaccines.

Gene	Protein	Location	Function	Interaction protein	Vaccine	Immunogenicity	
						T cell response	B cell response
esxA	ESAT6, EsxA	Secretory	Hinders the antigen presentation process Inhibit the production of cytokines in host cells Modulates host cell death	EsxB, Rv2660	AEC/BC02, H56:1C31, GamTBvac, TB/FLU-01/4L	Yes (Brodin et al., 2004; Ravn et al., 1999)	Yes (Mohamud et al., 2013)
esxB	CFP-10, EsxB	Secretory	Regulates host cell death Inhibits the production of reactive oxidative species (ROS) Downregulates LPS-induced NF-κB activation Promote the recruitment and activation of neutrophils	EsxA	AEC/BC02, GamTBvac	Yes (Kamath et al., 2004)	Yes (Hanif & Mustafa, 2017)
esxV	EsxV	Secretory	Induce IFN-γ production and T cell response	EsxA, EsxB, EsxW, PPE18, PPE42	ID93+GLA-SE/QTP101	Yes (Safar et al., 2022)	Yes (Hanif & Mustafa, 2017)
esxW	EsxWEsxW	Secretory	Induce IFN-γ production and T cell response	EsxA, EsxB, EsxV, Rv1813c, PPE42	ID93+GLA-SE/QTP101	Yes (Safar et al., 2022)	No (Safar et al., 2020) [a]
Rv1813c	Rv1813c	Secretory	Modulates host energy metabolism Influences the metabolic and apoptotic in macrophages	PPE42	ID93+GLA-SE/QTP101	Yes (Fatma et al., 2021)	Yes (Liang et al., 2019)
Rv2660c	Rv2660c	Cell Wall	Induce increased production of IFN-γ Higher numbers of viable T cells		H56:1C31	Yes (Govender et al., 2010)	No (Liang et al., 2019)
pepA,	PepA	Cell Wall, Secretory	Stimulate the proliferation of peripheral blood mononuclear cells (PBMC) Induce the secretion of IFN-γ	PPE18, Rv1813c, Rv2660, EsxA, PPE42, EsxW	M72/AS01E	Yes (Al-Attiyah et al., 2004; Skeiky et al., 1999)	Yes (Ishida et al., 2024) [a]
PPE18	PPE18	Cell Wall, Secretory[a]	Suppresses the production of pro-inflammatory factors Suppress the production of proinflammatory cytokines in macrophages Hinders MHC class II antigen presentation and the B cell response	EsxA, EsxB, PepA, EsxV, EsxW	M72/AS01E	Yes (Dillon et al., 1999; Skeiky et al., 2004)	No (Dolasia et al., 2021)
PPE42	PPE42	Cell Wall	Serves as a potent B cell antigen	Rv2660c, Rv1813c, PepA, EsxA, EsxW	ID93+GLA-SE/QTP101	Yes (Chakhaiyar et al., 2004)	Yes (Chakhaiyar et al., 2004)
fbpA	Ag85A	Cell Wall, Secretory	Promoting the 6,6′-dimycolate (TDM) biosynthesis and maintaining the stability of the cell wall structure	EsxA	AEC/BC02, GamTBvac, Ad5Ag85A, TB/FLU-01/4L, ChadOx185A	Yes (Baldwin et al., 1999)	Yes (Baldwin et al., 1999)
fbpB,	Ag85B	Cell Wall, Secretory	Involved in cell wall mycoloylation	EsxA, EsxB	H56:1C31	Yes (Ahmad et al., 2017)	Yes (Wang et al., 2023)

[a] Uncertain.

antigens (Ernst et al., 2019). Quantifying the levels of secreted bacterial proteins, including Fbps, can provide insights into the growth stage of *Mtb*. Currently, only one vaccine, H56:1C31, uses Ag85B as the antigen.

5.3. PPE18 (mtb39a, Rv1196)

PPE18 is a member of the PPE family, characterized by a conserved N-terminal domain that contains a proline-proline-glutamic acid (PPE) motif. These proteins have been proposed to be involved in antigenic variation and disease pathogenesis (Gey van Pittius et al., 2006). Proteomics results identified eight PE and PPE family proteins are *Mtb* culture filtrate proteins (CFP), including PPE18 in low abundance, which suggests its potential secretion (Tucci et al., 2020). However, another investigation found PPE18 in the lysate of *Mtb* but not in the CFP, indicating that it may not be a secreted antigen (Dillon et al., 1999). Additional research indicates that PPE18 is associated with the cell wall of *Mtb* and exposed on the cell surface. It has been suggested that PPE18 can trigger an anti-inflammatory response by interacting with TLR2 in macrophage, resulting in the production of interleukin-10 (IL-10) (Nair et al., 2009; Wolfe et al., 2010). During the innate immune response, the cytokines IL-12 and TNF-α, secreted by macrophages, play crucial roles in promoting the development of Th1 T cells and enhancing cell-mediated immune responses. These immune responses are essential for combating intracellular infections, such as tuberculosis. However, PPE18 has demonstrated the capability to suppress the production of proinflammatory cytokines in macrophages upon LPS stimulation. This is achieved by upregulating the expression and tyrosine phosphorylation of suppressor of cytokine signaling 3 (SOCS3), subsequently inhibiting the nuclear translocation of transcription factors p50, p65, and c-rel (Nair et al., 2011). Furthermore, PPE18 has the ability to hinder MHC class II-mediated antigen presentation by macrophages. This leads to impaired activation of $CD4^+$ T cells and subsequently response of B cells (Dolasia et al., 2021). Collectively, these findings suggest that PPE18 acts as an immune suppressor and plays a critical role in the immune evasion of *Mtb*. The vaccine M72/AS01E selects PPE18 as one antigen.

5.4. PPE42 (Rv2608)

PPE42 is a member of the PPE protein family of *Mtb*. PPE42 serves as a potent B cell antigen, it can induce a robust humoral immune response while eliciting a relatively low level of T cell response (Chakhaiyar et al., 2004). PPE42 is the antigen of ID93+GLA-SE/QTP101.

5.5. pepA (mtb32a, Rv0125)

PepA is a putative serine protease with an unknown function and is believed to be a secreted protein (Mattow et al., 2003). It has the capacity to promote the proliferation of peripheral blood mononuclear cells (PBMC) and induce the secretion of IFN-γ (Skeiky et al., 1999). The high conservation of PepA among different *Mycobacterium* species suggests its potential as a promising vaccine target (McNamara et al., 2010). PepA is fused with PPE18 in M72/AS01E vaccine.

5.6. esxA (esat-6, Rv3875)

EsxA, also referred to as the early secreted antigenic target 6 kDa (ESAT-6). It belongs to the WXG100 family, which consists of 23 proteins that are around 100 amino acids in size. The proteins in this family exhibit a helical structure, and display a distinctive hairpin bend created by the conserved W-X-G motif (Pallen, 2002). This protein is secreted through the ESAT-6 secretion system 1 (ESX-1), which is also called type VII secretion system (T7S) (Bitter et al., 2009; Samten et al., 2009). The genes responsible for encoding ESAT-6 as well as a 10-kDa culture filtrate protein (CFP10), are located in the RD1 region. As mentioned earlier, this region exists in all virulent *Mtb* and *M. bovis* strains but is absent in BCG strains. The reintroduction of ESAT-6 and CPF10 into BCG strains leads to noticeable changes in colonial morphology and an increase in virulence within the host (Majlessi et al., 2005).

ESAT-6 plays a crucial role as a virulence factor in *Mtb*, aiding immune evasion through several mechanisms. First, ESAT-6 hinders the antigen presentation process in host cells. It interacts with the host protein beta-2-microglobulin (β2M) in endoplasmic reticulum, sequestering β2M, and inhibiting the display of MHC-I-β2M complexes on the cell surface, thus downregulating MHC-I-mediated antigen presentation (Sreejit et al., 2014). Secondly, ESAT-6 is associated with decreased innate immune responses to *Mtb*. It inhibits the activation of the transcription factor NF-κB and interferon-regulatory factors (IRFs) by impeding Toll-like receptor (TLR) signaling (Pathak et al., 2007). Additionally, ESAT-6 directly suppresses the production of IFN-γ and the proliferation of human T cells in a p38 MAPK-dependent manner (Peng et al., 2011). Third, ESAT-6 can modulate host cell death. It induces necrosis in neutrophils as well as apoptosis in THP-1 human macrophages (Derrick & Morris, 2007; Francis et al., 2014). ESAT-6 is a potent antigen that triggers T cell immune responses and is recognized by T cells during the early stages of tuberculosis infection (Brodin et al., 2004; Ravn et al., 1999). Moreover, it can stimulate B cells to produce specific antibodies (Mohamud et al., 2013). As a result, ESAT-6 is frequently utilized as a diagnostic tool and included in vaccine formulations. AEC/BC02, H56:1C31, GamTBvac, TB/FLU-01/4L 4 vaccines choose ESAT6 as a common antigen.

5.7. esxB (cfp10, Rv3874)

CFP10 is another WXG100 family protein that can form a tight 1:1 complex with ESAT-6 and secreted by the ESX-1 (Renshaw et al., 2005). Phosphoproteome and secretome data have shown that phosphorylation of CFP10 at T49 by Mtb serine/threonine kinases PknA and PknB promotes the formation of CFP10 homodimers while inhibiting the secretion of ESAT6 (Malakar et al., 2023). The CFP10/ESAT6 complex can interact with cell surface receptors to influence host cell behavior. It has been shown to inhibit or promote TNF-α production in macrophages, thereby modulating macrophage cell death (Guo et al., 2012). Additionally, the complex inhibits the production of reactive oxidative species (ROS) in RAW264.7 cells and downregulates LPS-induced NF-κB activation (Ganguly et al., 2008). Furthermore, CFP-10, rather than ESAT-6, specifically triggers a Ca^{2+} response in human neutrophils, potentially contributing to their recruitment and activation during *Mtb* infection (Welin et al., 2015). Both CFP10 and ESAT-6 are highly immunogenic and associated with virulence in humans. Notably, CFP10 elicits a robust cytotoxic T lymphocytes (CTL) response that is recruited to the lungs, accounting for up to 30% of pulmonary $CD8^+$ T cells in *Mtb*-infected mice (Kamath et al., 2004). These findings hold promise for vaccination strategies aimed at eliciting $CD8^+$ T cell responses. AEC/BC02 and GamTBvac vaccines select EsxB as the antigen.

5.8. Rv1813c

Rv1813c is a secreted protein whose expression is regulated by the two-component signal systems (TCSS) of *Mtb*. The DosR and MprA proteins of TCSS can bind to the upstream promoter of Rv1813c and enhance its transcription in reaction to hypoxia, nitric oxide, and carbon monoxide (Bretl et al., 2012). Rv1813c targets mitochondria to manipulate host metabolism. Upon expression in eukaryotic cells, it is transported to the intermembrane space of mitochondria, thereby boosting ATP production through the enhancement of the oxidative phosphorylation metabolic pathway. This, in turn, influences the metabolic and apoptotic responses in macrophages infected with *Mtb* (Martin et al., 2023). As a conserved latency antigen, Rv1813c represents a promising candidate for vaccine development. The protein is capable of being recognized by T cells and activates the expression of IFN-γ/TNF and TNF/IL-2 cytokines in mice, resulting in a strong $CD4^+$ T cell response. Notably, compared to Rv3619c, Rv3620c, and Rv2608, Rv1813c stimulates the highest number of $CD8^+$ T cells positive for IFN-γ and/or TNF (Fatma et al., 2021). ID93+GLA-SE/QTP101 selects Rv1813c as the antigen.

5.9. esxV (Rv3619c) and esxW (Rv3620c)

Rv3619c and Rv3620c are belong to the WXG100 family and excreted by T7S in *Mtb*. Similar with ESAT-6/CFP10, Rv3619c and Rv3620c interact with each other to form a 1:1 heterodimeric complex (Mahmood et al., 2011). Both Rv3619c and Rv3620c can induce IFN-γ production and T cell response, indicating they are nice vaccine candidates of *Mtb* with multiple T cell immunogenic epitopes (Safar et al., 2022). Both Rv3619c and Rv3620c are the antigens in ID93+GLA-SE/QTP101 vaccine.

5.10. Rv2660c

Rv2660 is a latency-associated protein with unknown functions (Perez-Martinez et al., 2017). Studies have demonstrated that Rv2660 can induce increased production of IFN-γ, higher numbers of viable T cells, and enhanced specific $CD4^+$ T cell proliferation in individuals with LTBI (Govender et al., 2010). H56:1C31 uses Rv2660c as an antigen.

6. Conclusions and perspectives

Tuberculosis is one of the most ancient and deadliest diseases in human history, causing significant health, social, and economic burdens, particularly in low- and middle-income countries. Active pulmonary TB patients can spread tuberculosis bacilli into the air through coughing or sneezing. The primary target of *Mtb* infection is alveolar macrophages. If not cleared promptly, the bacteria replicate within macrophages and then infect other cells, triggering a robust immune response. Neutrophils, lymphocytes, and other immune cells migrate to the site of infection, forming cellular infiltrates that gradually develop into the characteristic granuloma structure, which eventually calcifies (Delogu et al., 2013). Within these granulomas, *Mtb* can remain in a dormant, non-metabolically active state for years, decades, or even a lifetime. Currently, there are over 10 million people worldwide with LTBI, which can progress to highly contagious and potentially fatal active TB at any time.

The treatment of tuberculosis primarily relies on antibiotics. However, the emergence of MDR-TB has rendered two of the most effective first-line drugs, isoniazid and rifampicin, ineffective (Ormerod, 2005). Substitute second-line treatment regimens require a large number of expensive and toxic drugs. Additionally, the management of tuberculosis becomes more challenging in cases of HIV-TB coinfection. The risk of developing active TB is 26–31 times higher in HIV-positive patients than in uninfected individuals (Leite et al., 2023). HIV-TB coinfection necessitates long-term treatment, which is further complicated by complex drug interactions and adverse reactions, posing additional challenges in simultaneously addressing both diseases.

In this context, the development of novel vaccines for the prevention and treatment of tuberculosis is both urgent and necessary. With

advancements in vaccine research technologies, several new vaccine candidates have undergone clinical trials. However, current clinical research indicates that most of these novel vaccines do not demonstrate significant advantages over BCG in inducing immune responses and clearing pathogen infections. Additionally, the efficacy and safety of these vaccines in HIV patients and immunocompromised individuals still need to be evaluated. Clearly, there are notable issues to consider in vaccine design and antigen selection strategies: (1) The interaction between *Mtb* and the host, as well as the mechanisms regulating the immune system, are not yet fully understood. The *Mtb* genome consists of 4.4 Mb and contains approximately 4000 genes, but the functions of most genes remain unclear (Galagan, 2014). Investigating genes related to *Mtb* proliferation, virulence, and dormancy may provide new targets for vaccine design or drug development. (2) Developing both preventive and therapeutic tuberculosis vaccines is equally important. On one hand, we need to develop alternatives to BCG vaccine to achieve more durable and specific protection. On the other hand, there is a need for therapeutic vaccines targeting active and latent TB infections to eliminate pathogens within the body and combat drug-resistant strains. (3) *Mtb* can secrete multiple antigens, functioning as both immunogenic proteins and virulence factors, involved in various physiological activities such as regulating host cell death, suppressing host immune signaling pathways, modulating host cell antigen presentation, and regulating host cell cytokine production. Modifying these antigens to enhance their immunogenicity and reduce their side effects is an issue that needs to be addressed. (4) Current vaccine evaluations mainly focus on helper T cell responses and IFN-γ secretion, with limited research on humoral immunity in combating TB infections. Considering the incorporation of B cell epitopes in vaccine antigen design may lead to a more balanced cellular and humoral immune response, potentially yielding improved efficacy.

With the COVID-19 pandemic, the development of mRNA vaccines seems to have entered its golden age. mRNA vaccines have introduced a new type of vaccine that delivers messenger RNA encoding pathogen antigens into mammalian cells via lipid nanoparticles, triggering strong cellular and humoral immune responses. mRNA vaccines offer flexibility and adaptability, requiring only a change in coding sequence to express different antigens. Multiple mRNA vaccines can share same purification methods and production lines, significantly reducing the development cycle. Furthermore, mRNA vaccines have advantages in the fusion expression of multiple antigens. Developing a multivalent vaccine for TB to enhance its immunogenicity is also a promising direction. If mRNA vaccines can address issues related to production costs, storage, and transportation stability, they may have even greater prospects for application. In conclusion, new research theories and technological advancements have injected fresh vitality into vaccine development, urging us to continue the fight against tuberculosis and further reduce the losses caused by TB in human society.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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