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REVIEW ARTICLE

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Zhihao Li[1], Changping Zheng[1], Marco Terreni[3], Lisa Tanzi[3], Matthieu Sollogoub[1] and Yongmin Zhang[1,2,*]

[1]*Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire (UMR 8232), 4 Place Jussieu, 75005 Paris, France;* [2]*Key Laboratory of Tropical Medicinal Resource Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou 571158, China;* [3]*Drug Sciences Department, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy*

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Abstract: Ranking above AIDS, Tuberculosis (TB) is the ninth leading cause of death affecting and killing many individuals every year. Drugs' efficacy is limited by a series of problems such as Multi-Drug Resistance (MDR) and Extensively-Drug Resistance (XDR). Meanwhile, the only licensed vaccine BCG (Bacillus Calmette-Guérin) existing for over 90 years is not effective enough. Consequently, it is essential to develop novel vaccines for TB prevention and immunotherapy. This paper provides an overall review of the TB prevalence, immune system response against TB and recent progress of TB vaccine research and development. Several vaccines in clinical trials are described as well as LAM-based candidates.

Keywords: Tuberculosis, vaccine, Lipoarabinomannan, immunotherapy, response, LAM-based candidates.

1. INTRODUCTION

1.1. TB - A Global Health Problem

Tuberculosis (TB) is the ninth leading cause of death in the world because of late diagnosis, lack of access to treatment and associated infections such as HIV, although great efforts have been made to prevent and treat this disease. In 2016, 10.4 million people were infected with TB and there were an estimated 1.3 million TB deaths, with more than 90% of cases occurring in developing countries [1, 2]. According to WHO, this disease killed 1.6 million people in 2017. In addition, it is reported that nearly 2 billion people have been exposed to the tuberculosis bacillus and are at risk of developing active disease [3]. Those whose immune system are damaged by diseases like AIDS, malnutrition or diabetes and long-term smokers are more likely to get TB. Although traditional antibiotic therapy is overall successful for TB treatment, two existing obstacles impede its development. First, it usually needs 6 months or longer time to take the TB drugs, like isoniazid, rifampicin, pyrazinamide and streptomycin. During the time of therapy, Directly Observed Therapy Short Course (DOTS) strategy should be undertaken [2], which costs amounts of money. Second, drug resistance is a serious problem because 6% of new TB cases and 20% of retreatment cases are MDR TB (Fig. **1**). For example, in 2016, there were 600.000 new cases with resistance to the most effective first-line drug rifampicin and 490.000 individuals had MDR TB [2]. Generally, it needs a combination of three specific drugs to avoid drug resistance [4, 5]. So, instead of using medicine to treat the patients, it is better to prevent it by using vaccines.

1.2. History and Drawbacks of BCG Vaccine

Currently, *Mycobacterium bovis* (*M. bovis*) BCG is the only licensed vaccine against TB, which is received by more than 3 billion people worldwide. It is an attenuated strain of *M. bovis* derived from a virulent strain after more than 13 years of continuous *in vitro* passage in the beginning of 1900s [6]. Now the BCG vaccine maintains its position as the world's most

*Address correspondence to this author at Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire (UMR 8232), 4 Place Jussieu, 75005 Paris, France; Key Laboratory of Tropical Medicinal Resource Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou 571158, China; Tel: +33144276153; Fax: +3314427- 5504; E-mail: yongmin.zhang@upmc.fr

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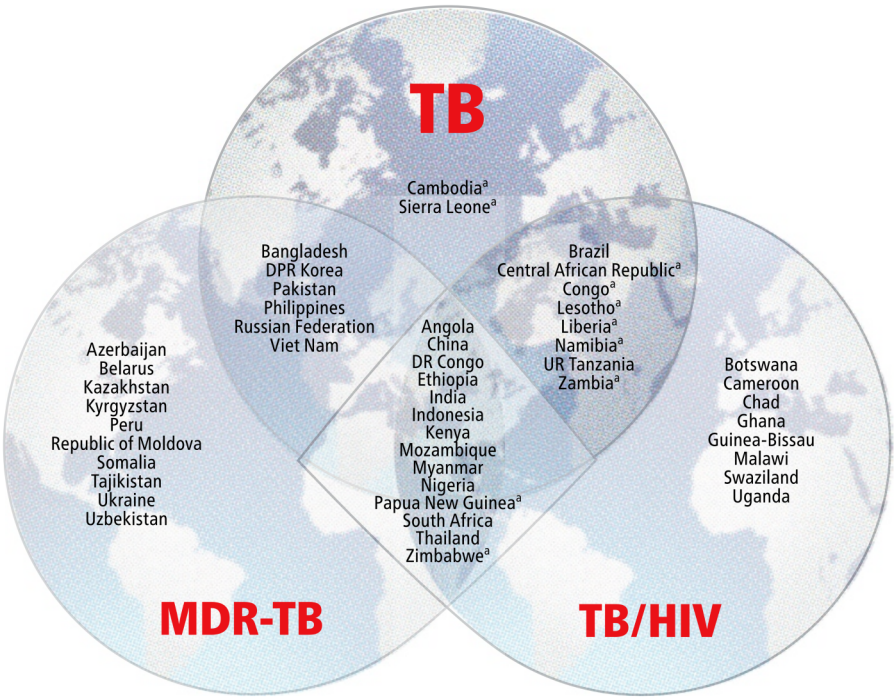

Fig. (1). Main countries suffering from TB; TB/HIV; MDR-TB, and their overlap during the period 2016-2020 [2].
[a]Top 30 countries that suffer from TB according to incidence. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

widely used vaccine, however, it is not effective and safe to satisfy today's needs. Many studies showed that BCG vaccine has a decent effect on children, with a meta-analysis showing that the protective efficacy of BCG on children's tuberculous meningitis and miliary tuberculosis is 73% and 77%, respectively [7]. However, when it comes to teenagers and adults, the protective efficacy is controversial due to the fact that efficacy can vary from 0 to 80% in different countries and areas [8]. Generally, countries in North America can be well protected by BCG, while BCG's efficacy is particularly poor in tropical and subtropical regions [9]. Lalor *et al.* argued that the environmental factors, maturity of the body's immune system, T-cell response, mother-to-child transmission related diseases, and other vaccination effects may lead to the difference [10]. Besides, the effect of BCG vaccine for HIV-infected individuals is problematic. Mansoor N suggested that HIV infection may seriously affect BCG-induced specific cellular responses and increase the risk of BCG-osis in HIV-infected infants. Infants infected with HIV have a risk of disseminated BCG-related diseases after vaccination against BCG, which is hundreds of times more than uninfected HIV infants [11]. Currently, TB is the leading cause of death in HIV-infected people because of the high incidence of dual infections and accompanying inhibition of the immune system by the two diseases. It was reported that co-infection with HIV and *mycobacterium tuberculosis* (*M. tuberculosis*) increases the risk of developing TB 30-fold [12]. Lastly, an adverse reaction is another affair that needs to be well considered. In general, BCG vaccine is relatively safe; the incidence of inflammation is less than 1% and the incidence of life-threatening BCG-related diseases is less than 2 per 1 million. However, with the increase in the diagnosis of immune-deficient diseases recently, the increase in disseminated pulmonary tuberculosis and severe disease-deficient disease, BCG has received much attention in the vaccination of immunodeficient infants [13].

1.3. Immune Response to *M. tuberculosis* Infection

M. tuberculosis is an intracellular parasite, and thus cellular immunity plays a major role in the prevention of infection. When *M. tuberculosis* invades, the host initiates the first line of defense by the natural immune system to fight against the infection. In the system, macrophages, natural killer cells, and neutrophils are important to control TB infection [14-16]. Macrophages can phagocytose and kill the invading *M. tuberculosis* in the body and the phospholipase D released by macrophage can promote phagocytic lysosome maturation and alveolar epithelial type II cells to kill intracellular *M. tuberculosis*. Moreover, it was reported

that antigen carried by exosome from macrophage can protect the mouse from being infected and exo- some can induce antigen-specific CD4 and CD8 T cells to secrete Interferon (IFN)-γ and Interleukin (IL)-2. The killing effect of natural killer cells against *M. tuberculosis* does not depend on the release of IFN-γ and cytotoxic particles, but on cell-cell interact- tions. IL-22 secreted by natural killer cells can effectively enhance phagocytic lysosomal fusion and help cells clear intracellular *M. tuberculosis*. Neutrophils can fight against *M. tuberculosis* in two ways. Firstly, the myeloperoxidase in neutrophil kills *M. tuberculosis* depending on chloride ion and hydrogen peroxide. Neutrophils can also form neutrophil extracellular traps to trap the invading microorganism when it is activated [17]. Not only innate immune cell is essential to fight against *M. tuberculosis*, but T-cell plays an important role. T-cell cytokines including IFN-γ and other T helper 1 (Th1) cytokines can activate antimicrobial activities in macrophages. Then, T cells kill *M. tuberculosis* directly and *Mycobacteria*-reactive T cells can infect macrophages, which seems to be a prerequisite for killing by T cells of microbes residing inside macrophages [12, 18, 19].

It was reported that CD4 T cells are mainly in Th1 type. These cells are judged the most crucial mediators of protection which can produce IFN-γ; Tumor Necrosis Factor α (TNF α); and IL-2 [20-22]. Recent studies showed that CD4 T cell-mediated immune protection against tuberculosis involves controlling the spread of early tuberculosis infection to the lungs, preventing the development of tuberculosis, and assisting the killing of CD8 T cells and natural killer cells against *M. tuberculosis* (two types of antibody-mediated protection mechanism are shown in Figs. (**2**) and (**3**)) [22]. Besides, CD8 T cells are important for protection, because they are an extra producer of cytokines of Th1 type, which can promote the production of opsonization antibodies and block the spread of pathogens from cheese-like necrotic sites to distant tissues. It can also secrete perforin and granulysin to degrade target cells. γδ T cells and CD1-restricted αβ T cells may recognize mycobacterial components and be participants in the protection against tuberculosis, although their precise role is not clear [23].

2. CURRENT PROGRESS OF TB VACCINE

Depending on the time of administration, there are two potential types of vaccines against TB nowadays, those given before and after exposure to the pathogen. Vaccines given before exposure are called pre-exposure or prophylactic vaccines, while those given after exposure are called post-exposure or therapeutic vaccines [24]. Two strategies are commonly considered to develop the pre-exposure vaccine. The first strategy is to design a longer-lasting and more protective vaccine to replace BCG using novel recombinant BCG or attenuated *M. tuberculosis* vaccine. The other strategy is hammering at boosting and prolonging BCG's immunity in individuals who have already been BCG vaccinated [25]. So, using BCG for priming and a subunit vaccine as a booster may provide a direction for pre-exposure of vaccine development. Post-exposure vaccination is targeting to latently infected individuals accounting for over one-third of the world's population. Since tuberculin was once used as a therapeutic vaccine against active disease and caused many deaths from treatment, antigens need to be well considered before using it. However, now it is commonly thought that carefully selected antigens will not cause safety problems as a therapeutic vaccination in latently infected individuals [25, 26].

The reverse vaccinology approach allowed the development of protein-based vaccines and new BCG vaccines over expressing selected antigens [27]. Furthermore, characterization of the epitopes of these proteins inspired the rational design of new vaccine products including chimeric proteins obtained by genetic recombination of different antigens, as well as DNA or RNA based vaccines. The subunit vaccines are commonly composed of one or more antigens selected among the pool of proteins secreted by *M. tuberculosis*. In fact, *M. tuberculosis* secretes more than 30 different proteins [28]. The most abundant proteins-antigen secreted by *M. tuberculosis* is the protein-complex Antigen (Ag) 85, but other predominant antigens are proteins belonging to the ESAT6 family and Mpt64. The Ag85 complex is a 30-32 kDa family of three mycolyl transferases (Ag85 A, B and C) involved in the coupling of mycolic acids with the arabinogalactan in the cell wall [29]. Ag85B is the most powerful *M. tuberculosis* antigen and induces both humoral and cell-mediated immune response. For this reason, it has been considered for the development of most of the new vaccines products under clinical investigation.

Various studies were performed for the characterization of Ag85B epitopes and in some cases, homologs such as Ag85B from *M. Bovis* or *M. Smegnatis* were considered [29-33]. As reported in Table **1**, for T-cell activity, human subjects and different animal models have been investigated, while for B-cell activity, have been indicated as a putative T-cell epitope studies were carried out in the mouse. Different sequences

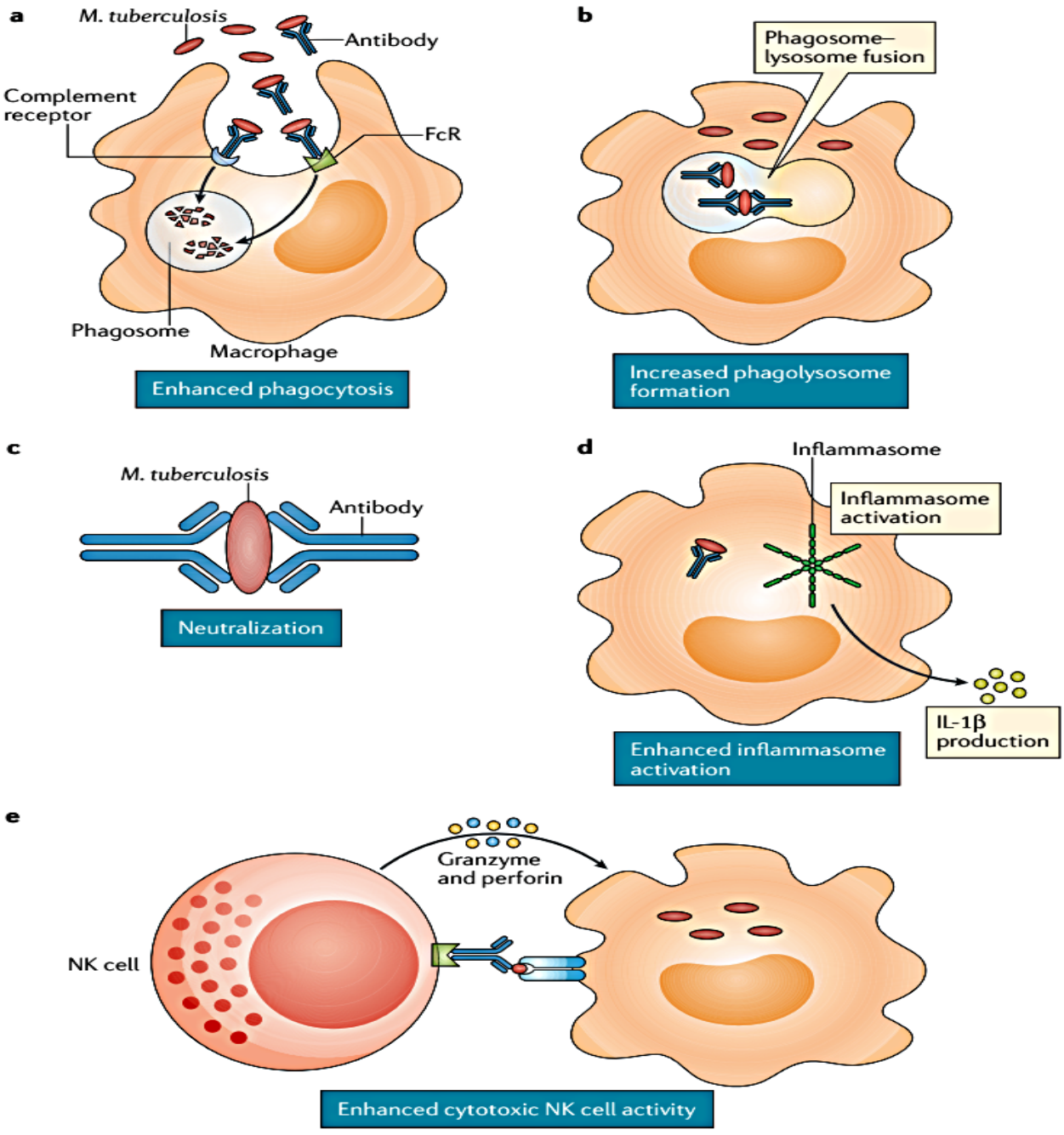

Fig. (2). Potential Mechanisms of Antibody-mediated protection against *M. tuberculosis* [24]. (**a**) The complexes of *M. tuberculosis* and antibody can be phagocytosed by macrophages through Crystallizable Fragment (Fc) Receptors (FcRs) and complement receptors; (**b**) Antibodies against *M. tuberculosis* can increase lysosome-phagosome fusion, in which *M. tuberculosis* usually interferes with and thereby restricts *M. tuberculosis'* growth; (**c**) *M. tuberculosis* antibodies may direct microbicidal or neutralizing activity, or they may prevent the uptake of the bacteria to promote *M. tuberculosis* killing; (**d**) Antibodies against *M. tuberculosis* can promote inflammasome activation in macrophages, which is associated with ASC speck formation and IL-1βsecretion; (**e**) Antibodies against *M. tuberculosis* may stimulate killing of infected cells throug h natural killer (NK) cell mediated antibody dependent cell cytotoxicity [24]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

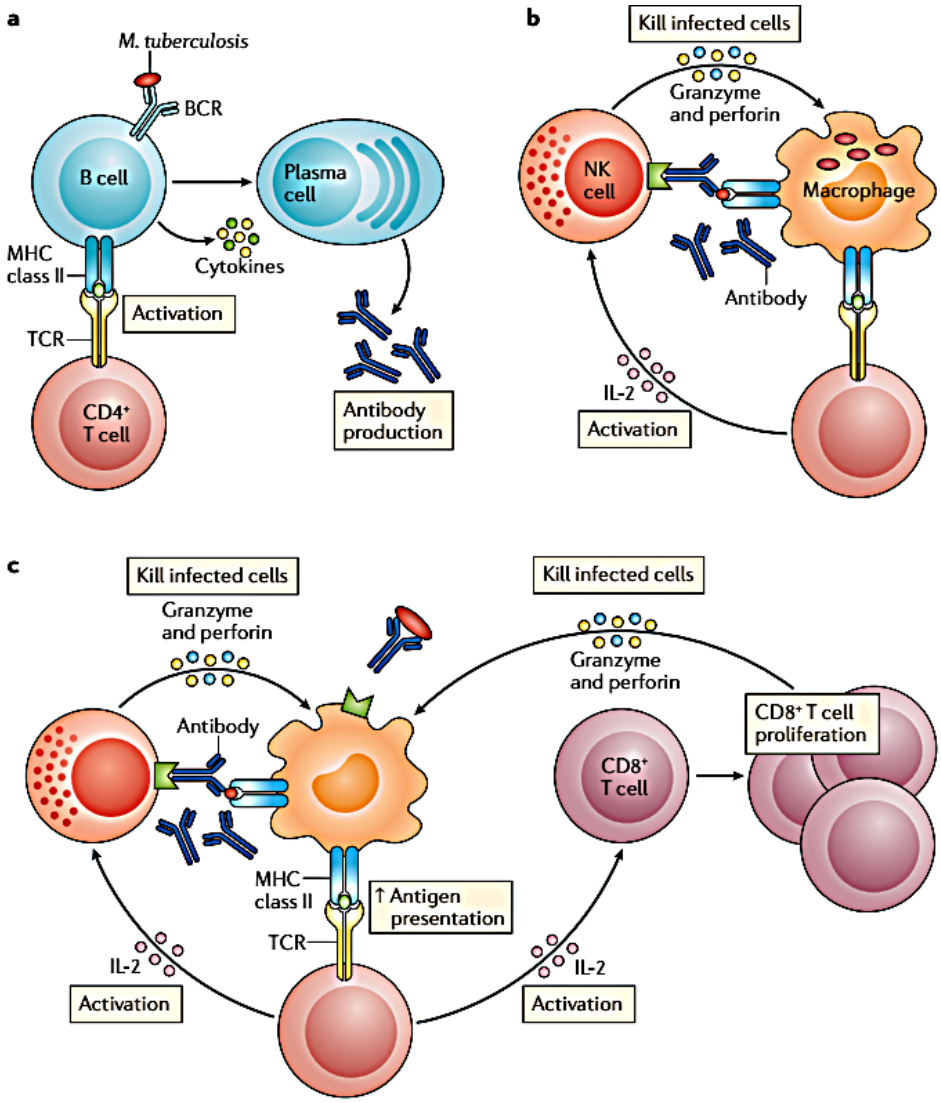

Fig. (3). Potential CD4+ T cell-dependent mechanisms of antibody-mediated protection [24]. (**a**) CD4 T cells may help B cells stimulate the maturation of antibody responses; (**b**) CD4 T cells may stimulate antibody-mediated to produce cytokines which can activate Natural Killer (NK) cells and enhance antibody dependent cell cytotoxicity responses to kill *M. tuberculosis* infected cells; (**c**) Complexes of *M.* tuberculosis and antibody may lead to increased display and presentation of *M. tuberculosis* antigens to CD4 T cells through professional phagocytes, such as macrophages or dendritic cells [24]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

(Table **1**) in the region between positions Leu11-Ala35 and Ala90-Arg113 while two sequences have been indicated to be able to stimulate both the T and B cell antigenic activity against Ag85B (Ser126-Pro140 and Thr261-lys275).

The ESAT6 family includes some low-molecular-mass proteins such as ESAT-6, TB10.4 and CFP10 strongly recognized by T cell [34]. About ESAT-6, nine synthetic 20AA peptides were prepared and tested [35], allowing the identification of two sequences with T-cell activity: ESAT-6_{51-70} (YQGVQQKWDATATE LNNALQN), and ESAT-6_{1-20} (MTEQQWNFAGIEA AASAIQ). It was also discovered that the ESAT-6_{1-20} sequence also included an epitope for B-cells [36].

Recently, in the case of TB10.4, two *in silico* methods indicated the sequences Met2-Met5, His14-Tyr21 and Gly13-Thr24 as putative epitopes in the N-terminal region, Gln37-Gl41 in the central part of this protein and one epitope near the C-terminal region (Val64-Thr88) [37]. Finally, in the ESAT6 family, CFP10 is an important protein because it is not present in BCG and consequently, can be associated with BCG or used as a boost for a subject already vaccinated with BCG. This protein induces both CD4 and CD8 T-cell I mmunity, and Li *et al*. synthesized and tested 26 peptides [38]

Table 1. Epitopes T and B of Ag85B already reported in the literature.

Epitope	Sequence in Ag85B of MTB	*Mycobacterium*	Host
T-Cell (CD4)	LQVPSPSMGRDIKVQFQ (11-27) LQVPSPSMGRDIKVQFQSGG (11-30) GRDIKVQFQSGGNNSPA (19-35) AGCQTYKWETFLTSE (90-104) CQTYKWETFLTSELPQW (92-108)	*M. tuberculosis*	Human
	LTSELPQWLSANR (101-113) SMAGSSAMILAAYHP (126-140) THSWEYWGAQLNAMK (261-275)	*M. tuberculosis*	Mouse
	YYQSGLSIVM (61-70)	*M. bovis*	Cattle
	MPVGGQSSFY (70-79)	*M. bovis*	Human
B-Cell	LTSELPQWLSANR (101-113) SMAGSSAMILAAYHP (126-140) THSWEYWGAQLNAMK (261-275)	*M. smegnatis*	Mouse

allowing the identification of four epitopes: $CFP10_{35–43}$ (TAGSLQGQW), $CFP10_{75-83}$ (NIRQAGV- QY), $CFP10_{3-11}$ (EMKTDAATL) and $CFP10_{13-21}$ (QE-AGNFERI).

There are several studies also for the characterization of T-cell [39] and B-cell epitopes [40] of Mpt64, including in *silico* predictions [41], while the research of other MTB protein-antigens has been reported [42] and is the object of continuous investigation.

Many antigenic proteins have been considered for the development of new vaccine products against MTB. Nowadays, although no vaccine can totally replace BCG's role in vaccination, several vaccine candidates are evaluated in phase I, II or III trials and some of them are likely to be substitutions in the future (Table **2**).

2.1. Prophylactic Vaccines

2.1.1. Live Mycobacterium Vaccines

Live mycobacterium vaccines play important roles in TB vaccines' development. Recombinant BCG vaccines and live attenuated vaccines are the two main types.

2.1.1.1. Recombinant BCG (rBCG) Vaccine

Since BCG has been widely used in clinical practice, it has its own defects, thus the establishment of new vaccines based on BCG is a promising direction. VPM1002 and rBCG30 are two examples of recombinant BCG candidates.

rBCG30

The first recombinant BCG vaccine is rBCG30, which was constructed by recombining the *M. tuberculosis* secretory antigen Ag85B (a 30 kDa enzyme). This protein is involved in outer cell-wall synthesis and it is a key component in several TB vaccines that increase immunity responses [43]. It was reported that rBCG30 vaccine shows better protection efficacy in a highly susceptible guinea pig model than BCG [44]. Although this vaccine has completed phase I trial without serious adverse events and could increase vaccine immunogenicity in healthy adults, it was stopped for further development because it contained an antibiotic resistance marker [45].

VPM1002

Another representative vaccine is VPM1002 ($\Delta ureC Hly^{+} rBCG$), which was developed to enhance major histocompatibility complex class (MHC)-I-related immune responses [46]. It is a rBCG vaccine developed by Max Planck Institute of Germany in which the urease C gene has been replaced by the Listeriolysin O (LLO) encoding gene hly from *Listeria monocytogenes* [47]. Compared with the parental BCG vaccine, it showed better protection efficacy against TB by stimulating CD4 and CD8 T-cell responses as well as type 1 and type 17 cytokines [48-51]. VPM1002

showed excellent result in Trial I in Germany and South Africa in adults and it was reported that VPM1002 vaccine is safe, well-tolerated, and immunogenic vaccine in newborn infants [50]. It has successfully passed two phase I trials and completed the phase IIa randomized clinical trial in healthy infants in South Africa. Now a phase II/III trial for prevention of TB recurrence in adults is undergoing in India [2].

2.1.1.2. Live attenuation of M. tuberculosis

Live attenuated vaccines are derived from *M. tuberculosis*. Its strain is well-tolerated and it is more immunogenic than BCG vaccine [52]. The main advantage of attenuated live *M. tuberculosis* vaccine is that it contains numerous genetic regions encoding important immunodominant *M. tuberculosis* antigens which do not exist in BCG while safety and genetic stability are induced by chromosomal deletions of virulence genes [53, 54].

ΔPhoPΔfad D26(MTBVAC)

MTBVAC is a vaccine, in which the phoP and fadD26 gene of the clinical strains Mt103 is deleted [53]. Mt103 belongs to the modern *M. tuberculosis* Lineage 4, which represents the most geographically widespread lineages of *Mycobacterium tuberculosis* Complex (MTBC) transmitted by the aerosol route among people with Lineage 2 (Beijing strains) [55]. It was reported that MTBVAC vaccine is as safe as BCG in Severe Combined Immune Deficiency (SCID) mice and it shows same or better efficacy in different animal models comparable to BCG [53, 56-58]. It also showed excellent results by evaluating the safety, local tolerance and immunogenicity of three escalating dose of MTBVAC relative to BCG in healthy, BCG-naive, HIV-negative adults in phase I trial. Now MTBVAC is the only live attenuated vaccine that has successfully entered clinical trials as a preventive vaccine in the newborn [54]. Furthermore, it was reported that in clinical evaluation, MTBVAC is the only vaccine which can induce CFP10- and ESAT6-specifc immune responses and these responses are effective in protecting from pulmonary TB, which has obvious impacts on TB transmission [57]. Now, phase IIa trials in neonates adolescents and adults are ongoing this year [2].

ΔlysAΔpanCDΔsecA2

mc26020 (*M. tuberculosis* H37Rv ΔlysAΔpanCD) and mc26030 (*M. tuberculosis* H37Rv ΔRD1ΔpanCD) are two other attenuated live *M. tuberculosis* vaccine candidates. The safety and efficacy of these candidates in mice and guinea pigs were tested, with dose 50- fold higher than the recommended human dose for BCG in non-human primates [52, 59, 60]. Mc26020 was cleared in less than 30 days in C57Bl/6 mice (6 to 8 weeks old) while mc26030 could exist for more than 200 days. Both of the candidates can protect the mice model up to 8 months after infection with virulent *M. tuberculosis*. Different studies also demonstrated that vaccine candidates mc26020 and mc26030 are obviously safer than BCG in immunocompromised mice and it was clearly showed that the two candidates are as safe as BCG in non-human primates. mc26020 vaccine showed similar protective efficacy compared with BCG in immunocompetent and CD4-deficient mice in an *M. tuberculosis* Erdman challenge experiment [61], while mc26030 can prolong the survival of wild-type mice and CD4-deficient mice against an aerosol challenge with virulent *M. tuberculosis*. Although both candidates meet Geneva Consensus requirements, more studies are required to warrant a clinical trial.

2.1.2. Subunit Vaccines

The use of subunit protein vaccine is another approach largely studied for the development of efficient immunization strategies. *M. tuberculosis* culture filtrate contains antigens which can stimulate immune protection and the antigens are essential parts of subunit vaccines. Subunit vaccines can be divided into adjuvant recombinant proteins and viral vector systems [62].

2.1.2.1. Recombinant Subunit Fusion Proteins

Among subunit vaccines, proteins have been mostly investigated and significant progress has been made in their construction and testing. Generally, two or more immunodominant agents are used in fusion proteins of the candidate.

H1/IC31

Over 100 proteins are contained in the *M. tuberculosis* culture filtrates and some of them are apparently immunodominant. Ag85 complex and ESAT-6 are two examples, which exist in all mycobacterial species and only in *M. tuberculosis* complex, respectively. H1 is made up of the two secreted antigens Ag85B and ESAT6 [63]. IC31 that can stimulate robust IFN-γ production by CD4 and Th1 cells in humans and maintain long-lasting memory immune responses has been tested in combination with H1 [64]. In phase I clinical trial, H1/IC31 has been proved to be safe in healthy adults with no adverse events reported and can induce strong and lasting Th1 responses [64-66]. In phase IIa trial, the efficacy and safety of H1/IC31 in HIV-infected

adults were tested showing that this chimeric protein can also induce strong and lasting immunologic responses and it is well tolerated. However, the clinical trial has been terminated for safety issues [67].

H4/IC31

H4/IC31 is another vaccine candidate that is similar to H1/IC31, it contains Ag85B and TB10.4 antigens and the antigens are also combined with IC31. TB10.4 is similar to ESAT6, which has decent immunological properties without avoiding the interference with Interferon-Gamma Release Assays (IGRAS) [68, 69]. H4 given in the adjuvant IC31 or DDA/MPL is well tolerated and protective against pulmonary TB in guinea pigs and mice [68] showing a better protection in a guinea pig model than BCG alone when used in a boost regimen [61]. A phase I study showed that H4/IC31 is safe and can introduce CD4 T cell response in healthy and BCG-vaccinated adults. In phase I randomized, placebo-controlled and double-blind trials have been done to invest the vaccine safety and immunogenicity in healthy BCG-vaccinated individuals [70]. Now the candidate is being tested in a pre-proof-of-concept phase II trial of prevention of infection among IGRA-negative, HIV-negative adolescents and it is also in a phase I/II trial in infants [2].

M72/AS01E

M72 contains Mtb32 (Rv1196) and Mtb39 (Rv0125) antigens, which are strong targets for Th1 cells in Purified Protein Derivative (PPD)-positive individuals. AS01 is an adjuvant system with monophosphoryl lipid A and QS-21 in a liposomal formulation, while AS02 is with the same components in oil-in-water formulation [71]. It was reported that candidate Mtb72F/AS02 revealed good safety and immunogenicity profiles in mice, guinea pigs, rabbits and non-human primate models. It is safe and immunogenic in healthy and BCG vaccinated adults [71-75]. Although some adverse events are reported after M72/AS01's vaccination because of adjuvant AS01E, they are not serious and can be resolved in one week. Moreover, compared with AS02-adjuvanted vaccine, M72/AS01E represents longer-lasting multifunctional CD4 T-cell responses among healthy adults as well as BCG vaccinated or *M. tuberculosis* contacted adults [76, 77]. So, AS01-adjuvanted candidates are more suitable for further study. In a phase IIb trial in HIV-negative adults infected with *M. tuberculosis*, M72/AS01E can reduce the development of active TB disease with 54% efficacy successfully [78].

ID93/GLA-SE

Another recent subunit vaccine is ID93/GLA-SE composed of recombinant fusion protein ID93 and adjuvant GLA-SE from the Infectious Disease Research Institute. ID93 contains 4 *M. tuberculosis* antigens (Rv2608, Rv3619, Rv3620 and Rv1813) and GLA-SE is a TLR4L-containing adjuvant that helps to induce Th1 immune responses [79]. ID93/GLA-SE can induce multifunctional IFN-γ, TNF-α, IL-2 and CD4 T cells in mice and guinea pigs with or without BCG vaccination. The candidate was reported to be safe in both mice and monkeys [80] and it can induce Th1 immune responses inhibiting *M. tuberculosis*-induced lung pathology [81]. Moreover, the safety and immunogenicity of ID93/GLA-SE have been completed in TB infected adults by randomized, double-blind, and placebo-controlled clinical trials. Now a phase IIb trial is ongoing to investigate the prevention of recurrence of disease in South Africa [2].

H56/IC31

Another subunit vaccine is H56/IC31 composed of the fusion protein H56 and adjuvant IC31. H56 contains Ag85B, ESAT6 and Rv2660c, which are antigens essential for the survival of *M. tuberculosis*. The Rv2660c antigen is included as a latent-TB antigen which contributes to efficacy [82]. It was reported that H56/IC31 can release multifunctional CD4 T-cell responses in a mouse model and delay TB disease progression in cynomolgus macaques. It is safe and immunogenic in BCG-vaccinated non-human primate models [83, 84]. H56/IC31 was shown to be safe and well-tolerated, and phase I clinical trial has been completed in HIV-negative patients without serious adverse events reported [85]. Now, a phase Ib trial to evaluate its safety and immunogenicity is in process in adolescents [2].

2.1.2.2. Modified Viral Vector Vaccines

Viral vaccine vectors are like their wild-type parental viruses with highly immunogenic, which are able to elicit both CD4 and CD8 T cell responses [86]. Vaccinia and adenovirus are the most clinically advanced which are immunogenic for boosting BCG responses in clinical trials [62, 87, 88]. Three viral vector candidates are in trials now.

ChAdOx185A - MVA85A

As the first new booster tuberculosis vaccine entering into clinical trials in 2002, MVA85A is a recombinant modified vaccinia virus Ankara expressing the

immunodominant *M. tuberculosis* Ag85A [89]. MVA85A is safe and well tolerated in *M. tuberculosis* and HIV infected patients, adolescents, children and infants with no serious adverse events reported and it can boost BCG-induced immune responses and promote releasing of IFN-γ as well as polyfunctional immune responses [90]. However, it cannot induce significant protection against *M. tuberculosis* in 2797 healthy infants who have been previously vaccinated with BCG in South Africa. Now, ChAdOx185A-MVA85A was used to generate a joint-heterologous prime-boost regimen using both systemic and mucosal routes to improve its efficacy [91]. A phase I trial of ChAdOx185A in BCG-vaccinated individuals with or without a prime-boost strategy by MVA85A has been completed in the UK and two studies of aerosol administration of MVA85A have been completed in individuals with BCG-vaccinated. Now, a further study in people with Latent Tuberculosis Infection (LTBI) is ongoing in phase I trial [2].

Ad5 Ag85A

Ad5 Ag85A is a recombinant 5 non-replicating adenovirus expressing the Ag85A protein. It was reported that Ad5 Ag85A can enhance immune protection in animal models [92, 93]. Its safety and immunogenicity have been done in healthy people with and without BCG-immunized in Canada with results showing that the candidate is safe, well-tolerated and immunogenic [2]. Recent studies reported that Ad5 Ag85A may increase the risk of developing acquired immunodeficiency syndrome against HIV [94]. Now a safety and immunogenicity study of aerosol administration is in progress in phase I trial in BCG-vaccinated healthy individuals [2].

TB/FLU-04L

TB/FLU-04L is a recombinant vaccine developed by Research Institutes from Kazakhstan and Russia. It is a mucosal vectored vaccine containing influenza virus with attenuated replication deficiency and expresses

Table 2. TB vaccine candidates in clinical trials.

Name	Target indication	Composition	Type	Clinical trial status	References
VPM1002	Preventative	rBCG vaccine where urease C gene is replaced by the listeriolysin O	Live *Mycobacterium* vaccines	Phase IIb	[47]
MTBVAC	Preventative	*M. tuberculosis* MT103 strain without the phoP and fadD26 gene	Live *Mycobacterium* vaccines	Phase I	[55]
H4/ IC31	Preventative	Fusion protein Ag85B and TB10.4 in IC31 adjuvant	Subunit vaccines (recombinant fusion protein vaccines)	Phase IIa	[68, 69]
M72/AS01E	Preventative	Fusion protein Mtb32 (Rv1196) and Mtb39(Rv0125) in AS01E adjuvant	Subunit vaccines (recombinant fusion protein vaccines)	Phase IIb	[71]
ID93/GLA-SE	Preventative	Fusion protein Rv2608, Rv3619, Rv3620 and Rv1813 in GLA-SE adjuvant	Subunit vaccines (recombinant fusion protein vaccines)	Phase IIa	[79]
H56/IC31	Preventative	Fusion protein Ag85B, ESAT6 and Rv2660c in IC31 adjuvant	Subunit vaccines (recombinant fusion protein vaccines)	Phase IIa	[82]
ChAdOx185A - MVA85A	Preventative	Mixture of simian adenovirus and pox virus expressing Ag85B	Subunit vaccines (modified viral vector vaccines)	Phase I	[89, 91]
Ad5 Ag85A	Preventative	5 on-replicating adenovirus expressing the Ag85A protein	Subunit vaccines (modified viral vector vaccines)	Phase I	[92, 93]
TB/FLU-04L	Preventative	Influenza virus vector expressing Ag85A and ESAT-6	Subunit vaccines (modified viral vector vaccines)	Phase IIa	[95]
RUTI	Therapeutic	Detoxified and fragmented *M. tuberculosis*	Inactivated TB vaccines	Phase IIa	[98]
Vaccae	Therapeutic	Heat-killed whole *Mycobacterium* vaccae	Inactivated TB vaccines	Phase III	[99, 112]

the Ag85A and ESAT-6 antigens [95]. Protective efficacy of the candidate has been evaluated in mice with results showing that the efficacy induced by BCG was significantly enhanced by a booster immunization with the candidate [96]. The safety and immunogenicity of TB/FLU-04L were explored in healthy adults with BCG vaccination and no serious adverse effects were reported [95, 97]. Now, a phase IIa trial in people with LTBI is being implemented [2].

2.1.3. DNA Vaccines

Due to the disadvantage of some vaccine candidates such as low efficiency and high virulence, researchers pay much attention to DNA vaccines. DNA-based vaccination not only aims at offering cytotoxic T lymphocytes and antibodies, but has engineering of artificial immunogens and co-expression of immunomodulatory proteins [100]. Triccas *et al*. suggested that DNA constructs encoding Ag85 can stimulate substantial humoral and cell-mediated immune responses leading to significant protection against TB [101]. It was reported that injection of naked DNA can stimulate immune responses with high efficiency and long time [102]. Kamath *et al*. studied DNA vaccines efficacy with encoding mycobacterial protein MPT 64, Ag85B and ESAT6 individually and compared their efficacy with the vaccine which contains the same three proteins. They suggested the latter vaccine efficacy is better and multi-subunit DNA vaccination is likely to be a new approach for developing efficient TB vaccines [103]. Accordingly, Yu *et al.* also studied a combined DNA vaccine encoding Ag85B, MPT-64 and MPT-83 and demonstrated that compared to individual antigens, the combined DNA vaccine can stimulate more IFN-γ in mice treated with isoniazid and pyrazinamide [104]. Chauhan *et al.* studied α-crystallin based DNA vaccine (DNAacr) and SodA based DNA vaccine (DNAsod). They suggested that both DNA vaccines increase the production of T_{EM} cells compared with using chemotherapy alone and the overall results showed the DNAacr has a potential role in shortening the duration of TB chemotherapy [105]. Teimourpour *et al.* isolated Mtb32C and heparin-binding haemagglutinin adhesion (HBHA) genes from H37Rv genome and constructed the DNA vaccine encoding these two genes of *M. tuberculosis* [106] allowing efficient expression of Mtb32C-HBHA fusion protein *in vitro*. The immunogenicity of this new DNA vaccine was studied *in-vivo* alone and in combination with BCG and the combination with BCG resulted in better use of DNA vaccine or BCG only, which induced the production of higher amounts of IFN-γ in mice [107]. Ahn *et al.* compared seven well-known TB antigens delivered by DNA vaccine, and studied their immunogenicities and protective efficacies with Flt3-L in pre- and post-exposure mice models, respectively. Among all the antigens, MTb32 is the most effective and Flt3L-Mtb32 DNA vaccine can lead to significant protection in both the spleen and lungs against *M. tuberculosis*, comparable with the protection induced by BCG [108]. Although a host of DNA vaccines can elicit cell-mediated and protective immune responses in clinical trials, until now, no Food and Drug Administration (FDA)-approved DNA vaccine is available for human use because of the low immunogenicity observed in humans [109]. So, more studies need to be done to investigate an effective TB DNA vaccine.

2.2. Therapeutic Vaccines

During the past decades, great efforts have been made to develop therapeutic vaccines. Instead of preventing the disease before infection, the target of therapeutic vaccines is to kill *M. tuberculosis*-infected cells by strengthening individual's immune system. Two vaccines are in clinical trials.

2.2.1. RUTI®

RUTI® is an inactivated TB vaccine constituted by detoxified and fragmented *M. tuberculosis*, which is designed to be used in conjunction with a short intensive antibiotic treatment [98]. Preclinical studies showed that RUTI® is safe and can induce Th1-Th2-Th3 responses in animal models [110, 111]. In a phase I trial, RUTI® was reported to be well tolerated in BCG-naïve healthy adults without serious adverse events occurring [99]. In a phase II clinical trial, people with LTBI have demonstrated that RUTI® has a good safety profile and decent immunogenicity at all studied doses. Currently, a phase IIa study in patients with MDR TB is ongoing [2].

2.2.2. Vaccae

Vaccae is a whole heat-inactivated mycobacterium vaccae evaluated as a therapeutic vaccine [112]. Vaccae was reported to induce strong Th1 immune responses in immunized mice and in phase I and II clinical trials, it is safe and immunogenic in BCG-vaccinated, HIV-infected adults [113, 114]. In a phase III clinical trial, vaccae was shown to be safe, well-tolerated, and protective against TB infection in Tanzania [115]. Now vaccae efficacy and safety in preventing TB disease in people with LTBI are being tested in phase III trial [2].

2.3. Glycoconjugate LAM-based Vaccines

Carbohydrates can be found on the wall of nearly every cell, which is the most complicated and diverse class of biopolymers commonly found in nature [116]. They play important roles in a multitude of biological processes and are essential virulence factors and antigens in most microbial pathogens [117, 118]. Even the simplest monosaccharides can combine in an ocean of ways to form structures more diverse than those formed by naturally occurring amino acids. Besides, carbohydrates can also form antigenic epitopes which can stand high temperatures and other harsh environmental conditions. Thus, polysaccharides have already been successfully considered for the development of efficient vaccines against microbial infections. These products are usually obtained by conjugation of natural or synthetic membrane antigenic sugars with specific immunogenic carrier proteins, required in order to induce a long-term T-cell mediated memory for the antigens [8]. Some of the most successful vaccines based on carbohydrate are pneumococcal vaccines and *H. influenzae* type b [8, 119, 120]. The immunogenic carrier proteins used are Tetanus Toxoid (TT), Diphtheria Toxoid (DT) and a diphtheria toxoid variant protein, Cross-Reactive Material 197 (CRM197) [121-123]. Recently, scientists tried to use glycoconjugates also as vaccine candidates to fight against tuberculosis.

In fact, much progress has been made to study the structure of the mycobacterial cell wall (the structure of *M. tuberculosis* cell wall is given in Fig. (**4**)). *M. tuberculosis* possesses a unique and complex cell wall containing polysaccharides, proteins and lipids [124, 125]. In the wall, the most abundant constituents are mycolic acids playing important roles in innate and adaptive immunity [126]. It is a kind of long-chain fatty acid containing 60-90 carbons, which is up to 60% of the weight of the cell wall. Lipoarabinomannan (LAM), Lipomannan (LM) and Phosphatidyl-Myo-Inositol Mannoside (PIM) are three major lipoglycans in the mycobacterial cell wall. They are attached to the cell plasma membrane and extend to cell wall's exterior [126, 127]. LAM and LM are based on the PIM; they are two carbohydrates with strong antigenicity [128, 129], but LAM is the major lipopolysaccharide component of the outer cell wall of all mycobacterial species. It is the main carbohydrate antigen and it accounts for over 15% of the bacterial weight [130]. The structure of LAM has been well-established (one kind LAM strain is shown in Fig. (**5**)). It has a mannan core with 6- and 2,6-linked mannopyranoses containing multiple branched, arabinofura- nosyl side chains [4, 131]. There are two major chemical forms of LAM: those isolated from *M. tuberculosis* or *M. bovis* BCG are called Mannose capped LAM (ManLAM) and those isolated from rapidly growing avirulent strains of mycobacteria are called AraLAM [127, 128, 132]. As an important immuno-modulating compound, LAM plays important roles in mycobacterial infections. It has been reported that LAM can suppress T-cell proliferation, inhibit IFNγ-mediated activation of macrophages and enhance the production of TNFα by mononuclear cells [133-136]. Nowadays, scientists argued that detection of LAM antigens may be used as a way to diagnose tuberculosis with high accuracy [137].

LAM has been investigated for the development of carbohydrate-based tuberculosis vaccines. Hamasur *et al.* isolated Arabinomannan (AM) from LAM of *M. tuberculosis* H37Rv in order to avoid the immunosuppressive effects of the intact liposaccharide molecule. The AM has been then conjugated to TT, CRM197 and DT. Both types of conjugates induced T helper cell-dependent IgG response showing high immunogenicity [138]. They also conjugated AM with Ag85B or a 75 kDa antigenic protein from *M. tuberculosis.* Both AM oligosaccharide (AMOs)-protein conjugates induced significant IgG response in rabbits and guinea-pigs showing a highly immunogenic property. C57BL/6 mice with respective subcutaneous immunization of AMOs-Ag85B and AMOs-TT got significant protection, being higher compared to that obtained without protein-conjugation, against intravenous challenge with 10^5 H37Rv, which is comparable to BCG vaccine. Mice which received immunization of AMOs-Ag85B in Eurocine™ L3 adjuvant showed better survival rate with the infection. In vaccinated guinea-pigs' lungs and spleens, the numbers of viable *M. tuberculosis* are smaller compared with those without immunization against *M. tuberculosis* H37Rv [139]. Haile *et al.* studied the ability of two new TB vaccine candidates, heat-killed BCG (H-kBCG) and AM-TT. Compared to the non-boosted BCG vaccinated mice, a significant reduction of Colony-Forming Unit (CFU) counts was seen in mice boosted with both of the candidates. Although neither of the candidates can induce a significant reduction in bacterial loads in the lungs, granulomatous inflammation was significantly reduced in lungs of mice receiving both of AM-TT and conventional BCG suggesting that AM-TT can improve primary BCG-induced protection [140]. Kallert *et al.* prepared liposomes containing phosphatidylcholine, cholesterol, stearylated octaarginine and LAM via thin layer hydration method (LIPLAM) which can be

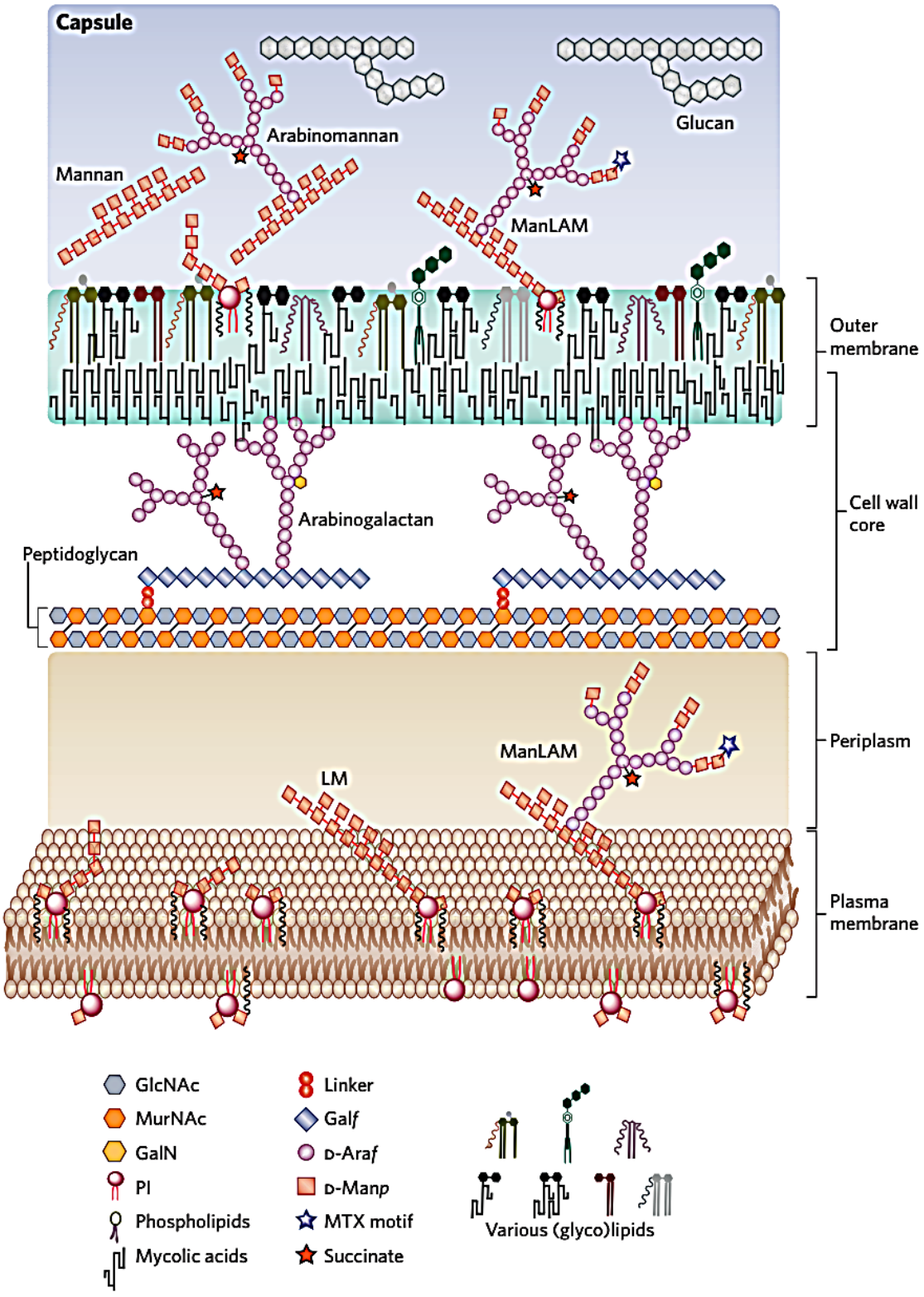

Fig. (4). Structure of Mycobacterial cell wall. The cell wall of *M. tuberculosis* is made up of three parts: plasma membrane, cell wall core and outer membrane. Plasma membrane contains mycobacterium-specific (lipo)proteins, (glyco)lipids and lipoglycans. The cell wall core consists of peptidoglycan covalently attached with arabinogalactan. The outer membrane is made up of an outer membrane consisting of an inner leaflet made of AG-bound mycolic acids, an outer leaflet containing non-covalently bound (glyco)lipids, (lipo)proteins, LM and LAM and an attached capsular-like structure made of proteins, lipids and polysaccharides [124]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

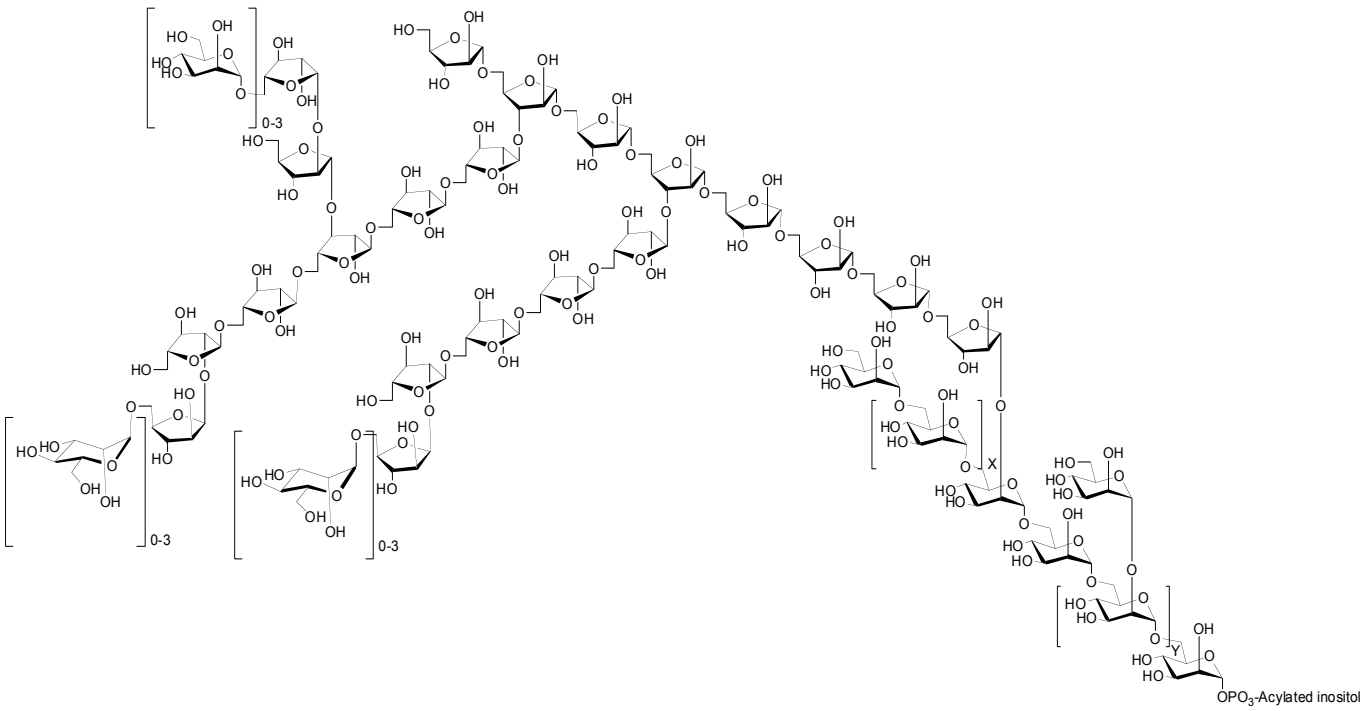

Fig. (5). Structure of LAM. In LAM, phosphatidylinositol moiety is linked to a α-1,6- and α-1,2-linked mannan backbone, which is an arabinan domain containing an α-1,5-linked D-arabinofuranosyl chain. At the end of the arabinose, two types of oligosaccharides are linked to its position 3 and 5. One type is α-1,5 and β-1,2-linked tetraarabinofuranosides, and the other type is α-1,5, α-1,3, and β-1,2-linked hexaarabinofuranosides. The arabinan domains end with different mannopyranoses at the arabinose 5-O-position.

uptaken by antigen presenting cells efficiently. It was reported that higher IFN-γ production from primary human T-lymphocytes was induced by LIPLAM compared with purified LAM or empty liposomes [141]. Glatman-Freedman *et al.* vaccinated AM-*Pseudomonas aeru- ginosa* Exoprotein A (rEPA) conjugated to mice with an increased antibody response occurring and the number of CFU in their organs reducing in 7 days after infection with *M. tuberculosis* [142]. Prados-Rosales *et al.* developed AM-based conjugates by using AM combined with Ag85B or Protective Antigen (PA) from *Bacillus anthracis*. Both conjugates elicited an AM-specific antibody response in mice stimulating transcriptional changes in *M. tuberculosis*. Lower bacterial numbers were seen in the lungs and spleen of immunized mice with Ag85B-AM conjugate and compared to the control mice they lived longer [143]. The conjugation of AM with recombinant *M. tuberculosis* antigenic proteins could be an innovative strategy for the development of highly efficient vaccines, by combining the activity of two different antigens (sugar and protein). However, this approach could be affected by the glycosylation of the epitopes that may reduce the antigenic activity of the protein [144]. It has been demonstrated that the immunogenic activity of Ag85B decreased after glycosylation with mono and disaccharides activated with the iminomethoxyethyl (IME) thioglycoside reactive linker (that selectively reacts with amino groups of the protein). In fact, a strong reduction of the T-cell activity was observed (Fig. **6**) because the two major glycosylation sites, corresponding to K_{23} and K_{275}, are included in two important T-cell epitopes [145]. In order to avoid this problem, Ag85B variants, obtained by site directed mutagenesis replacing the lysine with arginine in position 23 and/or 275, have been proposed as antigenic carriers for the development of efficient AM-AG85B conjugate vaccines. In fact, arginine is not reactive in the glycosylation reaction with the IME-linker and, consequently, the glycosylation of the epitopes in the mutant proteins is avoided. Therefore, these proteins maintained its antigenic activity after glycosylation (Fig. **6**) [146].

Although natural polysaccharides can be used to develop vaccines linked with immunogenic or antigenic carrier proteins, their immunodominant features may be damaged during chemical conjugation. Besides, natural polysaccharides may contain toxic components

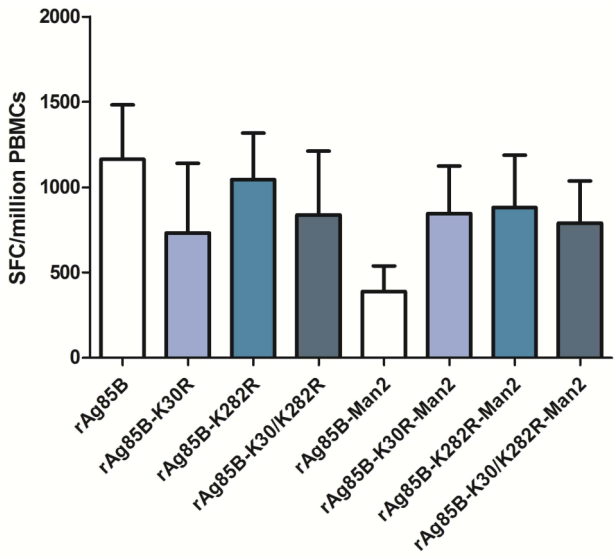

Fig. (6). T-cell responses to rAg85B antigen, its mutants and glycoconjugates. The data are presented as Mean values ± Standard Error Of The Mean (SEM) of the Spot-Forming Cells (SFC) per million of Peripheral Blood Mononuclear Cells (PBMCs) obtained by elaboration of the ELISPOT data in BCG-vaccinated subjects previously published by Bavaro *et al*. in 2017 [145] and Rinaldi *et al.* in 2018 [146]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

that are difficult to remove [147, 148] and the structure of natural antigens such as LAM is too complex to think of the development of a synthetic product. Consequently, much work was reported in the fields of synthetic LAM analogs, but only a few of them evaluated their antigenic activity against tuberculosis [131, 149-152]. Bundle *et al.* synthesized two kinds of β-mannan trisaccharides and an arabinanhexasaccharide, and conjugated them to the polymers by squarate or click chemistry. In ELISA, it was shown that non-protein-based antigens are provided by all the three compounds displaying great characteristics for assay of antibody [153]. Gao *et al.* prepared the analog of mycobacterium tuberculosis which contains the characteristic structures of all three major components (a mannosylated phosphatidylinositol moiety, an oligomannan, and an oligoarabinan). That applied a method to prepare other LAM analogs which can be used as carbohydrate antigen for LAM based vaccine [149]. They synthesized three LAM mimetic compounds **1-3** (Fig. **7**) with decent yields and conjugated them effectively with keyhole limpet hemocyanin (KLH) *via* a bifunctional linker. Immunological studies proved that all three compounds are immunogenic upon conjugation with KLH and the structure of compound plays an role on the immunological property [5]. They also synthesized monophosphoryl lipid A (MPLA) derivative **4** (Fig. **8**) having the 6-OH group substituted with an NH_2 compound **1** and coupled with compound **1** *via* an amide bond. Compound **1** was also coupled with MPLA at the 1-O-position to get **5** (Fig. **8**). Immunological activities of the two synthetic conjugates were evaluated in mice showing that although both conjugates induced IgG antibody responses, the 6′-N-conjugate afforded higher quantity of antibody than the 1-O-conjugate [154, 155]. Wattanasiri *et al.* synthesized LM backbone polysaccharides using rapid synthetic approach and evaluated

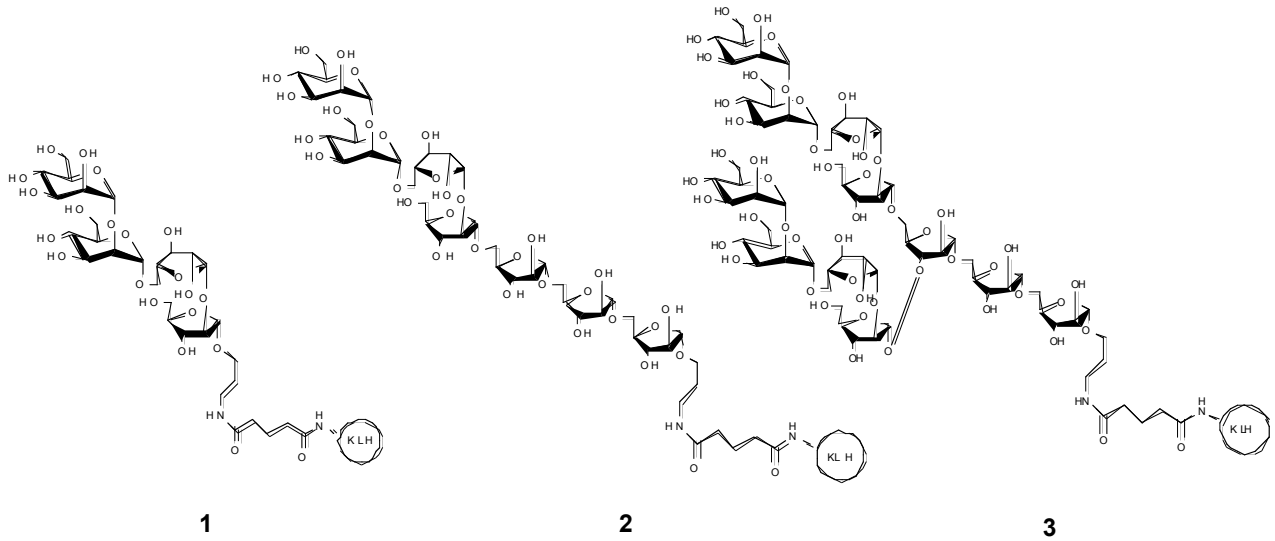

Fig. (7). Structure of LAM mimetic compounds 1-3 [5]. Compounds **1-3** were designed, synthesized and published by Wang *et al*.

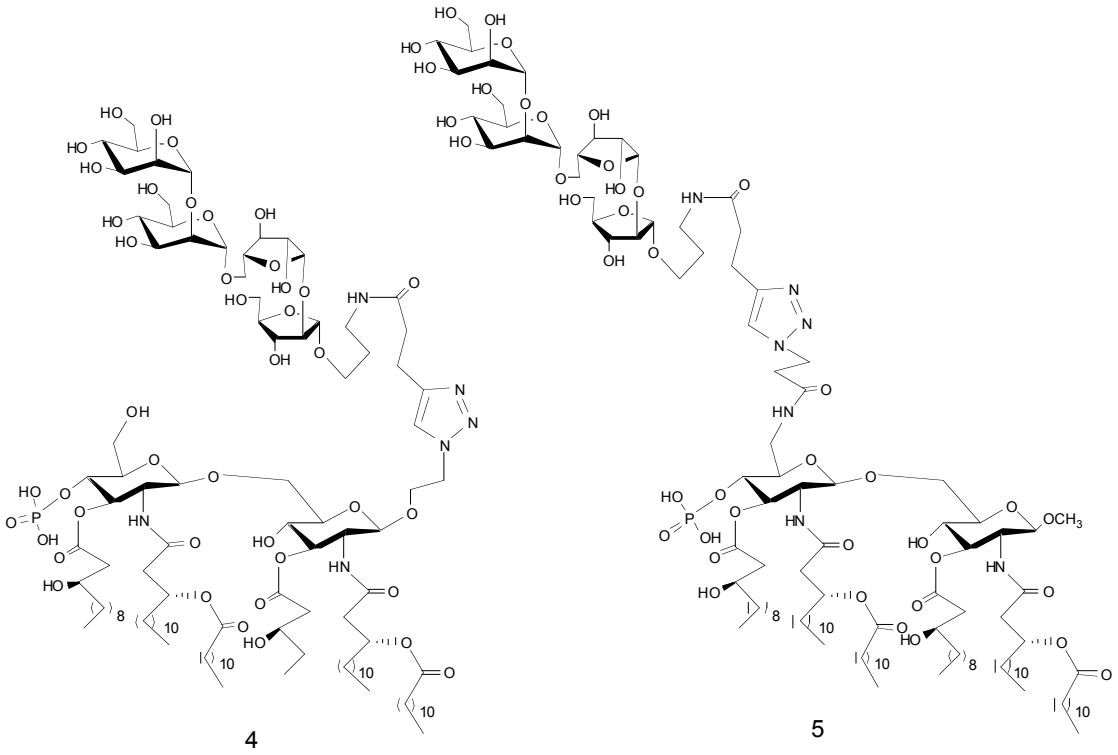

Fig. (8). Structure of LAM mimetic compounds 4, 5 [154]. Compounds **4** and **5** were designed, synthesized and published by Wang *et al.*

its immunological properties showing that the compound can enhance the secretion of TNF-α, IL-12, IL-6, and IL-1β. They suggested that the adjuvanticity mechanism of the compound involves the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and inflammasome pathways [156]. So, AM-based conjugates should be regarded as important forthcoming vaccine candidates in the future.

CONCLUSION

In order to fight against the global TB prevalence and replace the only licensed vaccine BCG, several vaccine candidates have been developed and evaluated in different stages, including more than ten candidates being tested in preclinical and clinical trials, either as priming, booster and therapeutic vaccines. Based on the current research, glycoconjugates may play a key role in the development of novel TB vaccine, especially the LAM-based candidates including glyco- conjugate products designed using *M. tuberculosis* antigenic proteins carriers. To meet the requirement of WHO's goal of eradicating TB by 2030, introduction of novel diagnostics, drugs, and vaccines will provide the only way. However, in order to achieve the goal, further investment needs to be done on the pathogenesis of *M. tuberculosis* and its interaction with host defense system. Also clinical testing and mass vaccination campaigns are essential for disease eradi-cation.

LIST OF ABBREVIATIONS

Ag	=	Antigen
AIDS	=	Acquired Immune Deficiency Syndrome
AM	=	Arabinomannan
B-cell	=	Bursa-derived Lymphocyte
BCG	=	Bacillus Calmette-Guérin
CD	=	Cluster Designation Antigen
CFU	=	Colony-Forming Unit
CRM 197	=	Cross-Reactive Material 197
DT	=	Diphtheria Toxoid
Fc	=	Crystallizable Fragment
FDA	=	Food And Drug Administration
HIV	=	Human Immunodeficiency Virus Infection
IFN	=	Interferon
IGRAS	=	Interferon-Gamma Release Assays

IME	=	Iminomethoxyethyl
IL	=	Interleukin
KLH	=	Keyhole Limpet Hemocyanin
LAM	=	Lipoarabinomannan
LM	=	Lipomannan
LTBI	=	Latent Tuberculosis Infection
M. bovis	=	*Mycobacterium bovi*
MDR	=	Multidrug Resistance
MHC	=	Major Histocompatibility Complex
MPLA	=	Monophosphoryl Lipid A
MTB	=	Mycobacterium Tuberculosis
MTBC	=	Mycobacterium Tuberculosis Complex
NK	=	Natural Killer
PIM	=	Phosphatidyl-Myo-Inositol Mannoside
SCID	=	Severe Combined Immune Deficiency
TB	=	Tuberculosis
T-cell	=	Thymus-derived Lymphocyte
Th 1	=	T Helper 1
TNFα	=	Tumor Necrosis Factor A
TT	=	Tetanus Toxoid
WHO	=	World Health Organization
XDR	=	Extensively-Drug Resistance

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Radosevic, K.; Wieland, C.W.; Rodriguez, A.; Weverling, G.J.; Mintardjo, R.; Gillissen, G.; Vogels, R.; Skeiky, Y.A.; Hone, D.M.; Sadoff, J.C.; van der Poll, T.; Havenga, M.; Goudsmit, J. Protective immune responses to a recombinant adenovirus type 35 tuberculosis vaccine in two mouse strains: CD4 and CD8 T-cell epitope mapping and role of gamma interferon. *Infect. Immun.,* **2007**, *75*(8), 4105-4115. http://dx.doi.org/10.1128/IAI.00004-07 PMID: 17526747

[2] World Health Organization. *Global Tuberculosis Report,* **2019**. *Available at:* http://www.who.int/tb/publications/global_report/ en/

[3] Connell, D.W.; Berry, M.; Cooke, G.; Kon, O.M. Update on tuberculosis: TB in the early 21st century. *Eur. Respir. Rev.,* **2011**, *20*(120), 71-84. http://dx.doi.org/10.1183/09059180.00000511 PMID: 21632795

[4] Joe, M.; Sun, D.; Taha, H.; Completo, G.C.; Croudace, J.E.; Lammas, D.A.; Besra, G.S.; Lowary, T.L. The 5-deoxy-5-methylthio-xylofuranose residue in mycobacterial lipoarabinomannan. Absolute stereochemistry, linkage position, conformation and immunomodulatory activity. *J. Am. Chem. Soc.,* **2006**, *128*(15), 5059-5072. http://dx.doi.org/10.1021/ja057373q PMID: 16608340

[5] Wang, L.; Feng, S.; An, L.; Gu, G.; Guo, Z. Synthetic and immunological studies of mycobacterial lipoarabinomannan oligosaccharides and their protein conjugates. *J. Org. Chem.,* **2015**, *80*(20), 10060-10075. http://dx.doi.org/10.1021/acs.joc.5b01686 PMID: 26375482

[6] Andersen, P.; Doherty, T.M. The success and failure of BCG - implications for a novel tuberculosis vaccine. *Nat. Rev. Microbiol.,* **2005**, *3*(8), 656-662. http://dx.doi.org/10.1038/nrmicro1211 PMID: 16012514

[7] Trunz, B.B.; Fine, P.; Dye, C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet,* **2006**, *367*(9517), 1173-1180. http://dx.doi.org/10.1016/S0140-6736(06)68507-3 PMID: 16616560

[8] Källenius, G.; Pawlowski, A.; Hamasur, B.; Svenson, S.B. Mycobacterial glycoconjugates as vaccine candidates against tuberculosis. *Trends Microbiol.,* **2008**, *16*(10), 456-462. http://dx.doi.org/10.1016/j.tim.2008.07.007 PMID: 18774297

[9] Black, G.F.; Weir, R.E.; Floyd, S.; Bliss, L.; Warndorff, D.K.; Crampin, A.C.; Ngwira, B.; Sichali, L.; Nazareth, B.; Blackwell, J.M.; Branson, K.; Chaguluka, S.D.; Donovan, L.; Jarman, E.; King, E.; Fine, P.E.; Dockrell, H.M. BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies. *Lancet,* **2002**, *359*(9315), 1393-1401. http://dx.doi.org/10.1016/S0140-6736(02)08353-8 PMID: 11978337

[10] Lalor, M.K.; Ben-Smith, A.; Gorak-Stolinska, P.; Weir, R.E.; Floyd, S.; Blitz, R.; Mvula, H.; Newport, M.J.; Branson, K.; McGrath, N.; Crampin, A.C.; Fine, P.E.; Dockrell, H.M. Population differences in immune responses to bacille calmette-guérin vaccination in infancy. *J. Infect. Dis.,* **2009**, *199*(6), 795-800. http://dx.doi.org/10.1086/597069 PMID: 19434928

[11] Mansoor, N.; Scriba, T.J.; de Kock, M.; Tameris, M.; Abel, B.; Keyser, A.; Little, F.; Soares, A.; Gelderbloem, S.; Mlenjeni, S.; Denation, L.; Hawkridge, A.; Boom, W.H.; Kaplan, G.; Hussey, G.D.; Hanekom, W.A. HIV-1 infection in infants severely impairs the immune response in-

duced by bacille calmette-guérin vaccine. *J. Infect. Dis.,* **2009**, *199*(7), 982-990. http://dx.doi.org/10.1086/597304 PMID: 19236280

[12] Kaufmann, S.H. Is the development of a new tuberculosis vaccine possible? *Nat. Med.,* **2000**, *6*(9), 955-960. http://dx.doi.org/10.1038/79631 PMID: 10973302

[13] Govindarajan, K.K.; Chai, F.Y. BCG adenitis-need for increased awareness. *Malays. J. Med. Sci.,* **2011**, *18*(2), 66-69. PMID: 22135589

[14] Abebe, F.; Bjune, G. The protective role of antibody responses during *Mycobacterium tuberculosis* infection. *Clin. Exp. Immunol.,* **2009**, *157*(2), 235-243. http://dx.doi.org/10.1111/j.1365-2249.2009.03967.x PMID: 19604263

[15] Lerner, T.R.; Borel, S.; Gutierrez, M.G. The innate immune response in human tuberculosis. *Cell. Microbiol.,* **2015**, *17*(9), 1277-1285. http://dx.doi.org/10.1111/cmi.12480 PMID: 26135005

[16] Allen, M.; Bailey, C.; Cahatol, I.; Dodge, L.; Yim, J.; Kassissa, C.; Luong, J.; Kasko, S.; Pandya, S.; Venketaraman, V. Mechanisms of control of *Mycobacterium tuberculosis* by NK cells: role of glutathione. *Front. Immunol.,* **2015**, *6*, 508. http://dx.doi.org/10.3389/fimmu.2015.00508 PMID: 26500648

[17] Cheng, Y.; Schorey, J.S. Exosomes carrying mycobacterial antigens can protect mice against *Mycobacterium tuberculosis* infection. *Eur. J. Immunol.,* **2013**, *43*(12), 3279-3290. http://dx.doi.org/10.1002/eji.201343727 PMID: 23943377

[18] Kaufmann, S.H. Fact and fiction in tuberculosis vaccine research: 10 years later. *Lancet Infect. Dis.,* **2011**, *11*(8), 633-640. http://dx.doi.org/10.1016/S1473-3099(11)70146-3 PMID: 21798463

[19] Silva, C.L.; Bonato, V.L.; Lima, K.M.; Coelho-Castelo, A.A.; Faccioli, L.H.; Sartori, A.; De Souza, A.O.; Leão, S.C. Cytotoxic T cells and mycobacteria. *FEMS Microbiol. Lett.,* **2001**, *197*(1), 11-18. http://dx.doi.org/10.1111/j.1574-6968.2001.tb10575.x PMID: 11287139

[20] Kaufmann, S.H.; Parida, S.K. Changing funding patterns in tuberculosis. *Nat. Med.,* **2007**, *13*(3), 299-303. http://dx.doi.org/10.1038/nm0307-299 PMID: 17342144

[21] Kaufmann, S.H.; Hussey, G.; Lambert, P.H. New vaccines for tuberculosis. *Lancet,* **2010**, *375*(9731), 2110-2119. http://dx.doi.org/10.1016/S0140-6736(10)60393-5 PMID: 20488515

[22] Kaufmann, S.H. Future vaccination strategies against tuberculosis: thinking outside the box. *Immunity,* **2010**, *33*(4), 567-577. http://dx.doi.org/10.1016/j.immuni.2010.09.015 PMID: 21029966

[23] van Crevel, R.; Ottenhoff, T.H.; van der Meer, J.W. Innate immunity to *Mycobacterium tuberculosis*. *Clin. Microbiol. Rev.,* **2002**, *15*(2), 294-309. http://dx.doi.org/10.1128/CMR.15.2.294-309.2002 PMID: 11932234

[24] Li, H.; Javid, B. Antibodies and tuberculosis: finally coming of age? *Nat. Rev. Immunol.,* **2018**, *18*(9), 591-596. http://dx.doi.org/10.1038/s41577-018-0028-0 PMID: 29872140

[25] Aagaard, C.; Dietrich, J.; Doherty, M.; Andersen, P. TB vaccines: current status and future perspectives. *Immunol. Cell Biol.,* **2009**, *87*(4), 279-286. http://dx.doi.org/10.1038/icb.2009.14 PMID: 19350048

[26] Doherty, T.M.; Andersen, P. Vaccines for tuberculosis: novel concepts and recent progress. *Clin. Microbiol. Rev.,* **2005**, *18*(4), 687-702. http://dx.doi.org/10.1128/CMR.18.4.687-702.2005 PMID: 16223953

[27] Bavaro, T.; Piubelli, L.; Amicosante, M.; Terreni, M. From new diagnostic targets to recombinant proteins and semi-synthetic protein-based vaccines. *Curr. Org. Synth.,* **2016**, *20*(11), 1150-1168. https://dx.doi.org/10.2174/1385272819666150810204736

[28] Bekmurzayeva, A.; Sypabekova, M.; Kanayeva, D. Tuberculosis diagnosis using immunodominant, secreted antigens of *Mycobacterium tuberculosis*. *Tuberculosis (Edinb.),* **2013**, *93*(4), 381-388. http://dx.doi.org/10.1016/j.tube.2013.03.003 PMID: 23602700

[29] Huygen, K. The immunodominant T-cell epitopes of the mycolyl-transferases of the antigen 85 complex of *M. tuberculosis*. *Front. Immunol.,* **2014**, *5*, 321. http://dx.doi.org/10.3389/fimmu.2014.00321 PMID: 25071781

[30] Lightbody, K.A.; Girvin, R.M.; Pollock, D.A.; Mackie, D.P.; Neill, S.D.; Pollock, J.M. Recognition of a common mycobacterial T-cell epitope in MPB59 of *Mycobacterium bovis*. *Immunology,* **1998**, *93*(3), 314-322. http://dx.doi.org/10.1046/j.1365-2567.1998.00449.x PMID: 9640240

[31] Mustafa, A.S.; Shaban, F.A.; Abal, A.T.; Al-Attiyah, R.; Wiker, H.G.; Lundin, K.E.; Oftung, F.; Huygen, K. Identification and HLA restriction of naturally derived Th1-cell epitopes from the secreted *Mycobacterium tuberculosis* antigen 85B recognized by antigen-specific human CD4(+) T-cell lines. *Infect. Immun.,* **2000**, *68*(7), 3933-3940. http://dx.doi.org/10.1128/IAI.68.7.3933-3940.2000 PMID: 10858206

[32] Valle, M.T.; Megiovanni, A.M.; Merlo, A.; Li Pira, G.; Bottone, L.; Angelini, G.; Bracci, L.; Lozzi, L.; Huygen, K.; Manca, F. Epitope focus, clonal composition and Th1 phenotype of the human CD4 response to the secretory mycobacterial antigen Ag85. *Clin. Exp. Immunol.,* **2001**, *123*(2), 226-232. http://dx.doi.org/10.1046/j.1365-2249.2001.01450.x PMID: 11207652

[33] Kadir, N.A.; Sarmiento, M.E.; Acosta, A.; Norazmi, M-N. Cellular and humoral immunogenicity of recombinant *Mycobacterium smegmatis* expressing Ag85B epitopes in mice. *Int. J. Mycobacteriol.,* **2016**, *5*(1), 7-13. http://dx.doi.org/10.1016/j.ijmyco.2015.09.006 PMID: 26927984

[34] Skjøt, R.L.V.; Oettinger, T.; Rosenkrands, I.; Ravn, P.; Brock, I.; Jacobsen, S.; Andersen, P. Comparative evaluation of low-molecular-mass proteins from *Mycobacterium tuberculosis* identifies members of the ESAT-6 family as immunodominant T-cell antigens. *Infect. Immun.,* **2000**, *68*(1), 214-220. http://dx.doi.org/10.1128/IAI.68.1.214-220.2000 PMID: 10603390

[35] Brandt, L.; Oettinger, T.; Holm, A.; Andersen, A.B.; Andersen, P. Key epitopes on the ESAT-6 antigen recognized in mice during the recall of protective immunity to *Mycobacterium tuberculosis*. *J. Immunol.,* **1996**, *157*(8), 3527-3533. PMID: 8871652

[36] Harboe, M.; Malin, A.S.; Dockrell, H.S.; Wiker, H.G.; Ulvund, G.; Holm, A.; Jørgensen, M.C.; Andersen, P. B-cell epitopes and quantification of the ESAT-6 protein of

Mycobacterium tuberculosis. *Infect. Immun.,* **1998**, *66*(2), 717-723.
http://dx.doi.org/10.1128/IAI.66.2.717-723.1998 PMID: 9453632

[37] Temporini, C.; Bavaro, T.; Tengattini, S.; Serra, I.; Marrubini, G.; Calleri, E.; Fasanella, F.; Piubelli, L.; Marinelli, F.; Pollegioni, L.; Speranza, G.; Massolini, G.; Terreni, M. Liquid chromatography-mass spectrometry structural characterization of neo glycoproteins aiding the rational design and synthesis of a novel glycovaccine for protection against tuberculosis. *J. Chromatogr. A,* **2014**, *1367*, 57-67.
http://dx.doi.org/10.1016/j.chroma.2014.09.041 PMID: 25282312

[38] Li, L.; Yang, B.; Yu, S.; Zhang, X.; Lao, S.; Wu, C. Human CD8+ T cells from TB pleurisy respond to four immunodominant epitopes in Mtb CFP10 restricted by HLA-B alleles. *PLoS One,* **2013**, *8*(12), e82196.
http://dx.doi.org/10.1371/journal.pone.0082196 PMID: 24349220

[39] Roche, P.W.; Feng, C.G.; Britton, W.J. Human T-cell epitopes on the *Mycobacterium tuberculosis* secreted protein MPT64. *Scand. J. Immunol.,* **1996**, *43*(6), 662-670.
http://dx.doi.org/10.1046/j.1365-3083.1996.d01-260.x PMID: 8658056

[40] Oettinger, T.; Andersen, A.B. Cloning and B-cell-epitope mapping of MPT64 from *Mycobacterium tuberculosis* H37Rv. *Infect. Immun.,* **1994**, *62*(5), 2058-2064.
http://dx.doi.org/10.1128/IAI.62.5.2058-2064.1994 PMID: 7513311

[41] Mustafa, A.S. *In silico* binding predictions for identification of HLA-DR-promiscuous regions and epitopes of *Mycobacterium tuberculosis* protein MPT64 (Rv1980c) and their recognition by human Th1 cells. *Med. Princ. Pract.,* **2010**, *19*(5), 367-372.
http://dx.doi.org/10.1159/000316375 PMID: 20639660

[42] Bertholet, S.; Ireton, G.C.; Kahn, M.; Guderian, J.; Mohamath, R.; Stride, N.; Laughlin, E.M.; Baldwin, S.L.; Vedvick, T.S.; Coler, R.N.; Reed, S.G. Identification of human T cell antigens for the development of vaccines against *Mycobacterium tuberculosis*. *J. Immunol.,* **2008**, *181*(11), 7948-7957.
http://dx.doi.org/10.4049/jimmunol.181.11.7948 PMID: 19017986

[43] Zhou, T.; Xu, L.; Dey, B.; Hessell, A.J.; Van Ryk, D.; Xiang, S-H.; Yang, X.; Zhang, M-Y.; Zwick, M.B.; Arthos, J.; Burton, D.R.; Dimitrov, D.S.; Sodroski, J.; Wyatt, R.; Nabel, G.J.; Kwong, P.D. Structural definition of a conserved neutralization epitope on HIV-1 gp120. *Nature,* **2007**, *445*(7129), 732-737.
http://dx.doi.org/10.1038/nature05580 PMID: 17301785

[44] Horwitz, M.A.; Harth, G.; Dillon, B.J.; Masleša-Galić', S. Recombinant bacillus calmette-guerin (BCG) vaccines expressing the *Mycobacterium tuberculosis* 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*(25), 13853-13858.
http://dx.doi.org/10.1073/pnas.250480397 PMID: 11095745

[45] Orme, I.M. Tuberculosis vaccine types and timings. *Clin. Vaccine Immunol.,* **2015**, *22*(3), 249-257.
http://dx.doi.org/10.1128/CVI.00718-14 PMID: 25540272

[46] Grode, L.; Seiler, P.; Baumann, S.; Hess, J.; Brinkmann, V.; Nasser Eddine, A.; Mann, P.; Goosmann, C.; Bandermann, S.; Smith, D.; Bancroft, G.J.; Reyrat, J.M.; van Soolingen, D.; Raupach, B.; Kaufmann, S.H. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille calmette-guérin mutants that secrete listeriolysin. *J. Clin. Invest.,* **2005**, *115*(9), 2472-2479.
http://dx.doi.org/10.1172/JCI24617 PMID: 16110326

[47] Grode, L.; Ganoza, C.A.; Brohm, C.; Weiner, J., III; Eisele, B.; Kaufmann, S.H. Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. *Vaccine,* **2013**, *31*(9), 1340-1348.
http://dx.doi.org/10.1016/j.vaccine.2012.12.053 PMID: 23290835

[48] Saiga, H.; Nieuwenhuizen, N.; Gengenbacher, M.; Koehler, A.B.; Schuerer, S.; Moura-Alves, P.; Wagner, I.; Mollenkopf, H.J.; Dorhoi, A.; Kaufmann, S.H. The Recombinant BCG Δ ureC: HLY vaccine targets the AIM2 inflammasome to induce autophagy and inflammation. *J. Infect. Dis.,* **2015**, *211*(11), 1831-1841.
http://dx.doi.org/10.1093/infdis/jiu675 PMID: 25505299

[49] Kaufmann, S.H.; Cotton, M.F.; Eisele, B.; Gengenbacher, M.; Grode, L.; Hesseling, A.C.; Walzl, G. The BCG replacement vaccine VPM1002: from drawing board to clinical trial. *Expert Rev. Vaccines,* **2014**, *13*(5), 619-630.
http://dx.doi.org/10.1586/14760584.2014.905746 PMID: 24702486

[50] Loxton, A.G.; Knaul, J.K.; Grode, L.; Gutschmidt, A.; Meller, C.; Eisele, B.; Johnstone, H.; van der Spuy, G.; Maertzdorf, J.; Kaufmann, S.H.E.; Hesseling, A.C.; Walzl, G.; Cotton, M.F. Safety and immunogenicity of the recombinant *Mycobacterium bovis* BCG vaccine VPM1002 in HIV-unexposed newborn infants in South Africa. *Clin. Vaccine Immunol.,* **2017**, *24*(2), e00439-e00416.
http://dx.doi.org/10.1128/CVI.00439-16 PMID: 27974398

[51] Desel, C.; Dorhoi, A.; Bandermann, S.; Grode, L.; Eisele, B.; Kaufmann, S.H.; Recombinant, B.C.G. Recombinant BCG ΔureC HLY+ induces superior protection over parental BCG by stimulating a balanced combination of type 1 and type 17 cytokine responses. *J. Infect. Dis.,* **2011**, *204*(10), 1573-1584.
http://dx.doi.org/10.1093/infdis/jir592 PMID: 21933877

[52] Larsen, M.H.; Biermann, K.; Chen, B.; Hsu, T.; Sambandamurthy, V.K.; Lackner, A.A.; Aye, P.P.; Didier, P.; Huang, D.; Shao, L.; Wei, H.; Letvin, N.L.; Frothingham, R.; Haynes, B.F.; Chen, Z.W.; Jacobs, W.R., Jr. Efficacy and safety of live attenuated persistent and rapidly cleared *Mycobacterium tuberculosis* vaccine candidates in non-human primates. *Vaccine,* **2009**, *27*(34), 4709-4717.
http://dx.doi.org/10.1016/j.vaccine.2009.05.050 PMID: 19500524

[53] Arbues, A.; Aguilo, J.I.; Gonzalo-Asensio, J.; Marinova, D.; Uranga, S.; Puentes, E.; Fernandez, C.; Parra, A.; Cardona, P.J.; Vilaplana, C.; Ausina, V.; Williams, A.; Clark, S.; Malaga, W.; Guilhot, C.; Gicquel, B.; Martin, C. Construction, characterization and preclinical evaluation of MTBVAC, the first live-attenuated *M. tuberculosis*-based vaccine to enter clinical trials. *Vaccine,* **2013**, *31*(42), 4867-4873.
http://dx.doi.org/10.1016/j.vaccine.2013.07.051 PMID: 23965219

[54] Gonzalo-Asensio, J.; Marinova, D.; Martin, C.; Aguilo, N. MTBVAC: Attenuating the human pathogen of Tuberculosis (TB) toward a promising vaccine against the TB epidemic. *Front. Immunol.,* **2017**, *8*, 1803.
http://dx.doi.org/10.3389/fimmu.2017.01803 PMID: 29326700

[55] Stucki, D.; Brites, D.; Jeljeli, L.; Coscolla, M.; Liu, Q.; Trauner, A.; Fenner, L.; Rutaihwa, L.; Borrell, S.; Luo, T.; Gao, Q.; Kato-Maeda, M.; Ballif, M.; Egger, M.; Macedo,

R.; Mardassi, H.; Moreno, M.; Tudo Vilanova, G.; Fyfe, J.; Globan, M.; Thomas, J.; Jamieson, F.; Guthrie, J.L.; Asante-Poku, A.; Yeboah-Manu, D.; Wampande, E.; Ssengooba, W.; Joloba, M.; Henry Boom, W.; Basu, I.; Bower, J.; Saraiva, M.; Vaconcellos, S.E.G.; Suffys, P.; Koch, A.; Wilkinson, R.; Gail-Bekker, L.; Malla, B.; Ley, S.D.; Beck, H.P.; de Jong, B.C.; Toit, K.; Sanchez-Padilla, E.; Bonnet, M.; Gil-Brusola, A.; Frank, M.; Penlap Beng, V.N.; Eisenach, K.; Alani, I.; Wangui Ndung'u, P.; Revathi, G.; Gehre, F.; Akter, S.; Ntoumi, F.; Stewart-Isherwood, L.; Ntinginya, N.E.; Rachow, A.; Hoelscher, M.; Cirillo, D.M.; Skenders, G.; Hoffner, S.; Bakonyte, D.; Stakenas, P.; Diel, R.; Crudu, V.; Moldovan, O.; Al-Hajoj, S.; Otero, L.; Barletta, F.; Jane Carter, E.; Diero, L.; Supply, P.; Comas, I.; Niemann, S.; Gagneux, S. Mycobacterium tuberculosis lineage 4 comprises globally distributed and geographically restricted sublineages. *Nat. Genet.,* **2016**, *48*(12), 1535-1543.
http://dx.doi.org/10.1038/ng.3704 PMID: 27798628

[56] Martin, C.; Williams, A.; Hernandez-Pando, R.; Cardona, P.J.; Gormley, E.; Bordat, Y.; Soto, C.Y.; Clark, S.O.; Hatch, G.J.; Aguilar, D.; Ausina, V.; Gicquel, B. The live *Mycobacterium tuberculosis* phoP mutant strain is more attenuated than BCG and confers protective immunity against tuberculosis in mice and guinea pigs. *Vaccine,* **2006**, *24*(17), 3408-3419.
http://dx.doi.org/10.1016/j.vaccine.2006.03.017 PMID: 16564606

[57] Aguilo, N.; Gonzalo-Asensio, J.; Alvarez-Arguedas, S.; Marinova, D.; Gomez, A.B.; Uranga, S.; Spallek, R.; Singh, M.; Audran, R.; Spertini, F.; Martin, C. Reactogenicity to major tuberculosis antigens absent in BCG is linked to improved protection against *Mycobacterium tuberculosis*. *Nat. Commun.,* **2017**, *8*, 16085.
http://dx.doi.org/10.1038/ncomms16085 PMID: 28706226

[58] Clark, S.; Lanni, F.; Marinova, D.; Rayner, E.; Martin, C.; Williams, A. Revaccination of guinea pigs with the live attenuated *Mycobacterium tuberculosis* vaccine MTBVAC improves BCG's protection against tuberculosis. *J. Infect. Dis.,* **2017**, *216*(5), 525-533.
http://dx.doi.org/10.1093/infdis/jix030 PMID: 28329234

[59] Sambandamurthy, V.K.; Derrick, S.C.; Jalapathy, K.V.; Chen, B.; Russell, R.G.; Morris, S.L.; Jacobs, W.R., Jr. Long-term protection against tuberculosis following vaccination with a severely attenuated double lysine and pantothenate auxotroph of *Mycobacterium tuberculosis*. *Infect. Immun.,* **2005**, *73*(2), 1196-1203.
http://dx.doi.org/10.1128/IAI.73.2.1196-1203.2005 PMID: 15664964

[60] Sambandamurthy, V.K.; Derrick, S.C.; Hsu, T.; Chen, B.; Larsen, M.H.; Jalapathy, K.V.; Chen, M.; Kim, J.; Porcelli, S.A.; Chan, J.; Morris, S.L.; Jacobs, W.R., Jr. *Mycobacterium tuberculosis* DeltaRD1 DeltapanCD: a safe and limited replicating mutant strain that protects immunocompetent and immunocompromised mice against experimental tuberculosis. *Vaccine,* **2006**, *24*(37-39), 6309-6320.
http://dx.doi.org/10.1016/j.vaccine.2006.05.097 PMID: 16860907

[61] Tang, J.; Yam, W.C.; Chen, Z. *Mycobacterium tuberculosis* infection and vaccine development. *Tuberculosis (Edinb.),* **2016**, *98*, 30-41.
http://dx.doi.org/10.1016/j.tube.2016.02.005 PMID: 27156616

[62] Andersen, P.; Kaufmann, S.H. Novel vaccination strategies against tuberculosis. *Cold Spring Harb. Perspect. Med.,* **2014**, *4*(6), a018523.
http://dx.doi.org/10.1101/cshperspect.a018523 PMID: 24890836

[63] Andersen, P.; Andersen, A.B.; Sørensen, A.L.; Nagai, S. Recall of long-lived immunity to *Mycobacterium tuberculosis* infection in mice. *J. Immunol.,* **1995**, *154*(7), 3359-3372.
PMID: 7897219

[64] van Dissel, J.T.; Arend, S.M.; Prins, C.; Bang, P.; Tingskov, P.N.; Lingnau, K.; Nouta, J.; Klein, M.R.; Rosenkrands, I.; Ottenhoff, T.H.; Kromann, I.; Doherty, T.M.; Andersen, P. Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in naïve human volunteers. *Vaccine,* **2010**, *28*(20), 3571-3581.
http://dx.doi.org/10.1016/j.vaccine.2010.02.094 PMID: 20226890

[65] van Dissel, J.T.; Soonawala, D.; Joosten, S.A.; Prins, C.; Arend, S.M.; Bang, P.; Tingskov, P.N.; Lingnau, K.; Nouta, J.; Hoff, S.T.; Rosenkrands, I.; Kromann, I.; Ottenhoff, T.H.; Doherty, T.M.; Andersen, P. Ag85B-ESAT-6 adjuvanted with IC31® promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in volunteers with previous BCG vaccination or tuberculosis infection. *Vaccine,* **2011**, *29*(11), 2100-2109.
http://dx.doi.org/10.1016/j.vaccine.2010.12.135 PMID: 21256189

[66] Ottenhoff, T.H.; Doherty, T.M.; van Dissel, J.T.; Bang, P.; Lingnau, K.; Kromann, I.; Andersen, P. First in humans: a new molecularly defined vaccine shows excellent safety and strong induction of long-lived *Mycobacterium tuberculosis*-specific Th1-cell like responses. *Hum. Vaccin.,* **2010**, *6*(12), 1007-1015.
http://dx.doi.org/10.4161/hv.6.12.13143 PMID: 21178394

[67] Gong, W.; Liang, Y.; Wu, X. The current status, challenges, and future developments of new tuberculosis vaccines. *Hum. Vaccin. Immunother.,* **2018**, *14*(7), 1697-1716.
http://dx.doi.org/10.1080/21645515.2018.1458806 PMID: 29601253

[68] Skeiky, Y.A.; Dietrich, J.; Lasco, T.M.; Stagliano, K.; Dheenadhayalan, V.; Goetz, M.A.; Cantarero, L.; Basaraba, R.J.; Bang, P.; Kromann, I.; McMclain, J.B.; Sadoff, J.C.; Andersen, P. Non-clinical efficacy and safety of HyVac4:IC31 vaccine administered in a BCG prime-boost regimen. *Vaccine,* **2010**, *28*(4), 1084-1093.
http://dx.doi.org/10.1016/j.vaccine.2009.10.114 PMID: 19896449

[69] Andersen, P.; Doherty, T.M.; Pai, M.; Weldingh, K. The prognosis of latent tuberculosis: can disease be predicted? *Trends Mol. Med*., **2007**, *13*(5), 175-182. https://dx.doi.org/10.1016/j.molmed.2007.03.004 PMID; 17418641

[70] Norrby, M.; Vesikari, T.; Lindqvist, L.; Maeurer, M.; Ahmed, R.; Mahdavifar, S.; Bennett, S.; McClain, J.B.; Shepherd, B.M.; Li, D.; Hokey, D.A.; Kromann, I.; Hoff, S.T.; Andersen, P.; de Visser, A.W.; Joosten, S.A.; Ottenhoff, T.H.M.; Andersson, J.; Brighenti, S. Safety and immunogenicity of the novel H4:IC31 tuberculosis vaccine candidate in BCG-vaccinated adults: two phase I dose escalation trials. *Vaccine,* **2017**, *35*(12), 1652-1661.
http://dx.doi.org/10.1016/j.vaccine.2017.01.055 PMID: 28216183

[71] Von Eschen, K.; Morrison, R.; Braun, M.; Ofori-Anyinam, O.; De Kock, E.; Pavithran, P.; Koutsoukos, M.; Moris, P.; Cain, D.; Dubois, M-C.; Cohen, J.; Ballou, W.R. The candidate tuberculosis vaccine Mtb72F/AS02a: tolerability and

immunogenicity in humans. *Hum. Vaccin.,* **2009**, *5*(7), 475-482.
http://dx.doi.org/10.4161/hv.8570 PMID: 19587528

[72] Reed, S.G.; Coler, R.N.; Dalemans, W.; Tan, E.V.; DeLa Cruz, E.C.; Basaraba, R.J.; Orme, I.M.; Skeiky, Y.A.; Alderson, M.R.; Cowgill, K.D.; Prieels, J.P.; Abalos, R.M.; Dubois, M.C.; Cohen, J.; Mettens, P.; Lobet, Y. Defined tuberculosis vaccine, Mtb72F/AS02A, evidence of protection in cynomolgus monkeys. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(7), 2301-2306.
http://dx.doi.org/10.1073/pnas.0712077106 PMID: 19188599

[73] Tsenova, L.; Harbacheuski, R.; Moreira, A.L.; Ellison, E.; Dalemans, W.; Alderson, M.R.; Mathema, B.; Reed, S.G.; Skeiky, Y.A.; Kaplan, G. Evaluation of the Mtb72F polyprotein vaccine in a rabbit model of tuberculous meningitis. *Infect. Immun.,* **2006**, *74*(4), 2392-2401.
http://dx.doi.org/10.1128/IAI.74.4.2392-2401.2006 PMID: 16552069

[74] Brandt, L.; Skeiky, Y.A.; Alderson, M.R.; Lobet, Y.; Dalemans, W.; Turner, O.C.; Basaraba, R.J.; Izzo, A.A.; Lasco, T.M.; Chapman, P.L.; Reed, S.G.; Orme, I.M. The protective effect of the *Mycobacterium bovis* BCG vaccine is increased by coadministration with the *Mycobacterium tuberculosis* 72-kilodalton fusion polyprotein Mtb72F in *M. tuberculosis*-infected guinea pigs. *Infect. Immun.,* **2004**, *72*(11), 6622-6632.
http://dx.doi.org/10.1128/IAI.72.11.6622-6632.2004 PMID: 15501795

[75] Spertini, F.; Audran, R.; Lurati, F.; Ofori-Anyinam, O.; Zysset, F.; Vandepapelière, P.; Moris, P.; Demoitié, M.A.; Mettens, P.; Vinals, C.; Vastiau, I.; Jongert, E.; Cohen, J.; Ballou, W.R. The candidate tuberculosis vaccine Mtb72F/AS02 in PPD positive adults: a randomized controlled phase I/II study. *Tuberculosis (Edinb.),* **2013**, *93*(2), 179-188.
http://dx.doi.org/10.1016/j.tube.2012.10.011 PMID: 23219236

[76] Leroux-Roels, I.; Forgus, S.; De Boever, F.; Clement, F.; Demoitié, M.A.; Mettens, P.; Moris, P.; Ledent, E.; Leroux-Roels, G.; Ofori-Anyinam, O. M72 Study Group. Improved CD4$^+$ T cell responses to *Mycobacterium tuberculosis* in PPD-negative adults by M72/AS01 as compared to the M72/AS02 and Mtb72F/AS02 tuberculosis candidate vaccine formulations: a randomized trial. *Vaccine,* **2013**, *31*(17), 2196-2206.
http://dx.doi.org/10.1016/j.vaccine.2012.05.035 PMID: 22643213

[77] Cohen, J.; Hughes, E.; Day, C.; de Kock, M.; Geldenhuys, H.; Gelderbloem, S.; Hawkridge, A.; Hussey, G.; Mahomed, H.; Makhethe, L. Induction and regulation of T cell immunity by the novel TB vaccine M72/AS01 in South African adults. *Am. J Respir. Crit. Care Med.,* **2013**, *188*(4), 492-502. https://dx.doi.org/10.1164/rccm.201208-1385oc PMID: 23306546

[78] Van Der Meeren, O.; Hatherill, M.; Nduba, V.; Wilkinson, R.J.; Muyoyeta, M.; Van Brakel, E.; Ayles, H.M.; Henostroza, G.; Thienemann, F.; Scriba, T.J.; Diacon, A.; Blatner, G.L.; Demoitié, M.A.; Tameris, M.; Malahleha, M.; Innes, J.C.; Hellström, E.; Martinson, N.; Singh, T.; Akite, E.J.; Khatoon Azam, A.; Bollaerts, A.; Ginsberg, A.M.; Evans, T.G.; Gillard, P.; Tait, D.R. Phase 2b controlled trial of M72/AS01E vaccine to prevent tuberculosis. *N. Engl. J. Med.,* **2018**, *379*(17), 1621-1634.
http://dx.doi.org/10.1056/NEJMoa1803484 PMID: 30280651

[79] Kwon, B.E.; Ahn, J.H.; Min, S.; Kim, H.; Seo, J.; Yeo, S.G.; Ko, H.J. Development of new preventive and therapeutic vaccines for tuberculosis. *Immune Netw.,* **2018**, *18*(2), e17.
http://dx.doi.org/10.4110/in.2018.18.e17 PMID: 29732235

[80] Dye, C. Doomsday postponed? Preventing and reversing epidemics of drug-resistant tuberculosis. *Nat. Rev. Microbiol.,* **2009**, *7*(1), 81-87.
http://dx.doi.org/10.1038/nrmicro2048 PMID: 19079354

[81] Coler, R.N.; Bertholet, S.; Pine, S.O.; Orr, M.T.; Reese, V.; Windish, H.P.; Davis, C.; Kahn, M.; Baldwin, S.L.; Reed, S.G. Therapeutic immunization against *Mycobacterium tuberculosis* is an effective adjunct to antibiotic treatment. *J. Infect. Dis.,* **2013**, *207*(8), 1242-1252.
http://dx.doi.org/10.1093/infdis/jis425 PMID: 22891286

[82] Orme, I.M. Vaccine development for tuberculosis: current progress. *Drugs,* **2013**, *73*(10), 1015-1024.
http://dx.doi.org/10.1007/s40265-013-0081-8 PMID: 23794129

[83] Lin, P.L.; Dietrich, J.; Tan, E.; Abalos, R.M.; Burgos, J.; Bigbee, C.; Bigbee, M.; Milk, L.; Gideon, H.P.; Rodgers, M.; Cochran, C.; Guinn, K.M.; Sherman, D.R.; Klein, E.; Janssen, C.; Flynn, J.L.; Andersen, P. The multistage vaccine H56 boosts the effects of BCG to protect *Cynomolgus macaques* against active tuberculosis and reactivation of latent *Mycobacterium tuberculosis* infection. *J. Clin. Invest.,* **2012**, *122*(1), 303-314.
http://dx.doi.org/10.1172/JCI46252 PMID: 22133873

[84] Aagaard, C.; Hoang, T.; Dietrich, J.; Cardona, P.J.; Izzo, A.; Dolganov, G.; Schoolnik, G.K.; Cassidy, J.P.; Billeskov, R.; Andersen, P. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. *Nat. Med.,* **2011**, *17*(2), 189-194.
http://dx.doi.org/10.1038/nm.2285 PMID: 21258338

[85] Luabeya, A.K.K.; Kagina, B.M.; Tameris, M.D.; Geldenhuys, H.; Hoff, S.T.; Shi, Z.; Kromann, I.; Hatherill, M.; Mahomed, H.; Hanekom, W.A.; Andersen, P.; Scriba, T.J.; Schoeman, E.; Krohn, C.; Day, C.L.; Africa, H.; Makhethe, L.; Smit, E.; Brown, Y.; Suliman, S.; Hughes, E.J.; Bang, P.; Snowden, M.A.; McClain, B.; Hussey, G.D. H56-032 Trial Study Group. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in *Mycobacterium tuberculosis* infected and non-infected healthy adults. *Vaccine,* **2015**, *33*(33), 4130-4140.
http://dx.doi.org/10.1016/j.vaccine.2015.06.051 PMID: 26095509

[86] Draper, S.J.; Heeney, J.L. Viruses as vaccine vectors for infectious diseases and cancer. *Nat. Rev. Microbiol.,* **2010**, *8*(1), 62-73.
http://dx.doi.org/10.1038/nrmicro2240 PMID: 19966816

[87] Abel, B.; Tameris, M.; Mansoor, N.; Gelderbloem, S.; Hughes, J.; Abrahams, D.; Makhethe, L.; Erasmus, M.; de Kock, M.; van der Merwe, L.; Hawkridge, A.; Veldsman, A.; Hatherill, M.; Schirru, G.; Pau, M.G.; Hendriks, J.; Weverling, G.J.; Goudsmit, J.; Sizemore, D.; McClain, J.B.; Goetz, M.; Gearhart, J.; Mahomed, H.; Hussey, G.D.; Sadoff, J.C.; Hanekom, W.A. The novel tuberculosis vaccine, AERAS-402, induces robust and polyfunctional CD4+ and CD8+ T cells in adults. *Am. J. Respir. Crit. Care Med.,* **2010**, *181*(12), 1407-1417.
http://dx.doi.org/10.1164/rccm.200910-1484OC PMID: 20167847

[88] McShane, H.; Pathan, A.A.; Sander, C.R.; Keating, S.M.; Gilbert, S.C.; Huygen, K.; Fletcher, H.A.; Hill, A.V. Re-

combinant modified vaccinia virus ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nat. Med.,* **2004**, *10*(11), 1240-1244. http://dx.doi.org/10.1038/nm1128 PMID: 15502839

[89] Goonetilleke, N.P.; McShane, H.; Hannan, C.M.; Anderson, R.J.; Brookes, R.H.; Hill, A.V. Enhanced immunogenicity and protective efficacy against *Mycobacterium tuberculosis* of bacille calmette-guérin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus ankara. *J. Immunol.,* **2003**, *171*(3), 1602-1609. http://dx.doi.org/10.4049/jimmunol.171.3.1602 PMID: 12874255

[90] Beveridge, N.E.; Price, D.A.; Casazza, J.P.; Pathan, A.A.; Sander, C.R.; Asher, T.E.; Ambrozak, D.R.; Precopio, M.L.; Scheinberg, P.; Alder, N.C.; Roederer, M.; Koup, R.A.; Douek, D.C.; Hill, A.V.; McShane, H. Immunisation with BCG and recombinant MVA85A induces long-lasting, polyfunctional *Mycobacterium tuberculosis*-specific CD4+ memory T lymphocyte populations. *Eur. J. Immunol.,* **2007**, *37*(11), 3089-3100. http://dx.doi.org/10.1002/eji.200737504 PMID: 17948267

[91] Chondro, F. New tuberculosis vaccine to support tuberculosis elimination. *Universa Medicina,* **2018**, *37*(2), 85-87. http://dx.doi.org/10.18051/UnivMed.2018.v37.85-87

[92] Santosuosso, M.; McCormick, S.; Zhang, X.; Zganiacz, A.; Xing, Z. Intranasal boosting with an adenovirus-vectored vaccine markedly enhances protection by parenteral *Mycobacterium bovis* BCG immunization against pulmonary tuberculosis. *Infect. Immun.,* **2006**, *74*(8), 4634-4643. http://dx.doi.org/10.1128/IAI.00517-06 PMID: 16861651

[93] Smaill, F.; Jeyanathan, M.; Smieja, M.; Medina, M.F.; Thanthrige-Don, N.; Zganiacz, A.; Yin, C.; Heriazon, A.; Damjanovic, D.; Puri, L.; Hamid, J.; Xie, F.; Foley, R.; Bramson, J.; Gauldie, J.; Xing, Z. A human type 5 adenovirus-based tuberculosis vaccine induces robust T cell responses in humans despite preexisting anti-adenovirus immunity. *Sci. Transl. Med.,* **2013**, *5*(205), 205ra134. http://dx.doi.org/10.1126/scitranslmed.3006843 PMID: 24089406

[94] Méndez-Samperio, P. Global efforts in the development of vaccines for tuberculosis: requirements for improved vaccines against *Mycobacterium tuberculosis*. *Scand. J. Immunol.,* **2016**, *84*(4), 204-210. http://dx.doi.org/10.1111/sji.12465 PMID: 27454335

[95] Evans, T.G.; Schrager, L.; Thole, J. Status of vaccine research and development of vaccines for tuberculosis. *Vaccine,* **2016**, *34*(26), 2911-2914. http://dx.doi.org/10.1016/j.vaccine.2016.02.079 PMID: 26973073

[96] Dockrell, H.M. Towards new TB vaccines: what are the challenges? *Pathog. Dis.,* **2016**, *74*(4), ftw016. http://dx.doi.org/10.1093/femspd/ftw016 PMID: 26960944

[97] Khoshnood, S.; Heidary, M.; Haeili, M.; Drancourt, M.; Darban-Sarokhalil, D.; Nasiri, M.J.; Lohrasbi, V. Novel vaccine candidates against *Mycobacterium tuberculosis*. *Int. J. Biol. Macromol.,* **2018**, *120*(Pt A), 180-188. http://dx.doi.org/10.1016/j.ijbiomac.2018.08.037 PMID: 30098365

[98] Hawkridge, T.; Mahomed, H. Prospects for a new, safer and more effective TB vaccine. *Paediatr. Respir. Rev.,* **2011**, *12*(1), 46-51. http://dx.doi.org/10.1016/j.prrv.2010.09.013 PMID: 21172675

[99] Vilaplana, C.; Montané, E.; Pinto, S.; Barriocanal, A.M.; Domenech, G.; Torres, F.; Cardona, P.J.; Costa, J. Double-blind, randomized, placebo-controlled phase 1 clinical trial of the therapeutical antituberculous vaccine RUTI. *Vaccine,* **2010**, *28*(4), 1106-1116. http://dx.doi.org/10.1016/j.vaccine.2009.09.134 PMID: 19853680

[100] Sharma, A.K.; Khuller, G.K. DNA vaccines: future strategies and relevance to intracellular pathogens. *Immunol. Cell Biol.,* **2001**, *79*(6), 537-546. http://dx.doi.org/10.1046/j.1440-1711.2001.01044.x PMID: 11903613

[101] Triccas, J.A.; Sun, L.; Palendira, U.; Britton, W.J. Comparative affects of plasmid-encoded interleukin 12 and interleukin 18 on the protective efficacy of DNA vaccination against *Mycobacterium tuberculosis*. *Immunol. Cell Biol.,* **2002**, *80*(4), 346-350. http://dx.doi.org/10.1046/j.1440-1711.2002.01087.x PMID: 12121223

[102] Montgomery, D.L.; Huygen, K.; Yawman, A.M.; Deck, R.R.; Dewitt, C.M.; Content, J.; Liu, M.A.; Ulmer, J.B. Induction of humoral and cellular immune responses by vaccination with M. tuberculosis antigen 85 DNA. *Cell. Mol. Biol.,* **1997**, *43*(3), 285-292. PMID: 9193782

[103] Kamath, A.T.; Hanke, T.; Briscoe, H.; Britton, W.J. Co-immunization with DNA vaccines expressing granulocyte-macrophage colony-stimulating factor and mycobacterial secreted proteins enhances T-cell immunity, but not protective efficacy against *Mycobacterium tuberculosis*. *Immunology,* **1999**, *96*(4), 511-516. http://dx.doi.org/10.1046/j.1365-2567.1999.00703.x PMID: 10233735

[104] Yu, D.H.; Hu, X.D.; Cai, H. Efficient tuberculosis treatment in mice using chemotherapy and immunotherapy with the combined DNA vaccine encoding Ag85B, MPT-64 and MPT-83. *Gene Ther.,* **2008**, *15*(9), 652-659. http://dx.doi.org/10.1038/gt.2008.13 PMID: 18288210

[105] Chauhan, P.; Jain, R.; Dey, B.; Tyagi, A.K. Adjunctive immunotherapy with α-crystallin based DNA vaccination reduces tuberculosis chemotherapy period in chronically infected mice. *Sci. Rep.,* **2013**, *3*, 1821. http://dx.doi.org/10.1038/srep01821 PMID: 23660989

[106] Teimourpour, R.; Sadeghian, A.; Meshkat, Z.; Esmaelizad, M.; Sankian, M.; Jabbari, A-R. Construction of a DNA vaccine encoding Mtb32C and HBHA genes of *Mycobacterium tuberculosis*. *Jundishapur J. Microbiol.,* **2015**, *8*(8), e21556. http://dx.doi.org/10.5812/jjm.21556 PMID: 26464766

[107] Teimourpour, R.; Peeridogaheh, H.; Teimourpour, A.; Arzanlou, M.; Meshkat, Z. A study on the immune response induced by a DNA vaccine encoding Mtb32C-HBHA antigen of *Mycobacterium tuberculosis*. *Iran. J. Basic Med. Sci.,* **2017**, *20*(10), 1119-1124. PMID: 29147487

[108] Ahn, S.S.; Jeon, B.Y.; Kim, K.S.; Kwack, J.Y.; Lee, E.G.; Park, K.S.; Sung, Y.C.; Cho, S.N. Mtb32 is a promising tuberculosis antigen for DNA vaccination in pre- and post-exposure mouse models. *Gene Ther.,* **2012**, *19*(5), 570-575. http://dx.doi.org/10.1038/gt.2011.140 PMID: 21956689

[109] Liu, M.A.; Ulmer, J.B. Human clinical trials of plasmid DNA vaccines. *Adv. Genet.,* **2005**, *55*, 25-40. http://dx.doi.org/10.1016/S0065-2660(05)55002-8 PMID: 16291211

[110] Cardona, P-J. RUTI: a new chance to shorten the treatment of latent tuberculosis infection. *Tuberculosis (Edinb.),* **2006**, *86*(3-4), 273-289. http://dx.doi.org/10.1016/j.tube.2006.01.024 PMID: 16545981

[111] Nell, A.S.; D'lom, E.; Bouic, P.; Sabaté, M.; Bosser, R.; Picas, J.; Amat, M.; Churchyard, G.; Cardona, P.J. Safety, tolerability, and immunogenicity of the novel antituberculous vaccine RUTI: randomized, placebo-controlled phase II clinical trial in patients with latent tuberculosis infection. *PLoS One,* **2014**, *9*(2), e89612. http://dx.doi.org/10.1371/journal.pone.0089612 PMID: 24586912

[112] Skinner, M.A.; Prestidge, R.; Yuan, S.; Strabala, T.J.; Tan, P.L. The ability of heat-killed *Mycobacterium vaccae* to stimulate a cytotoxic T-cell response to an unrelated protein is associated with a 65 kilodalton heat-shock protein. *Immunology,* **2001**, *102*(2), 225-233. http://dx.doi.org/10.1046/j.1365-2567.2001.01174.x PMID: 11260328

[113] Hernandez-Pando, R.; Pavön, L.; Arriaga, K.; Orozco, H.; Madrid-Marina, V.; Rook, G. Pathogenesis of tuberculosis in mice exposed to low and high doses of an environmental mycobacterial saprophyte before infection. *Infect. Immun.,* **1997**, *65*(8), 3317-3327. http://dx.doi.org/10.1128/IAI.65.8.3317-3327.1997 PMID: 9234793

[114] Waddell, R.D.; Chintu, C.; Lein, A.D.; Zumla, A.; Karagas, M.R.; Baboo, K.S.; Habbema, J.D.F.; Tosteson, A.N.; Morin, P.; Tvaroha, S.; Arbeit, R.D.; Mwinga, A.; von Reyn, C.F. Safety and immunogenicity of a five-dose series of inactivated *Mycobacterium vaccae* vaccination for the prevention of HIV-associated tuberculosis. *Clin. Infect. Dis.,* **2000**, *30*(Suppl. 3), S309-S315. http://dx.doi.org/10.1086/313880 PMID: 10875806

[115] von Reyn, C.F.; Mtei, L.; Arbeit, R.D.; Waddell, R.; Cole, B.; Mackenzie, T.; Matee, M.; Bakari, M.; Tvaroha, S.; Adams, L.V.; Horsburgh, C.R.; Pallangyo, K. DarDar Study Group. Prevention of tuberculosis in bacille calmette-guérin-primed, HIV-infected adults boosted with an inactivated whole-cell mycobacterial vaccine. *AIDS,* **2010**, *24*(5), 675-685. http://dx.doi.org/10.1097/QAD.0b013e3283350f1b PMID: 20118767

[116] Dennis, J.W.; Granovsky, M.; Warren, C.E. Glycoprotein glycosylation and cancer progression. *Biochim. Biophys. Acta,* **1999**, *1473*(1), 21-34. http://dx.doi.org/10.1016/S0304-4165(99)00167-1 PMID: 10580127

[117] Kato, K.; Ishiwa, A. The role of carbohydrates in infection strategies of enteric pathogens. *Trop. Med. Health,* **2015**, *43*(1), 41-52. http://dx.doi.org/10.2149/tmh.2014-25 PMID: 25859152

[118] Weintraub, A. Immunology of bacterial polysaccharide antigens. *Carbohydr. Res.,* **2003**, *338*(23), 2539-2547. http://dx.doi.org/10.1016/j.carres.2003.07.008 PMID: 14670715

[119] Lockhart, S.P.; Hackell, J.G.; Fritzell, B. Pneumococcal conjugate vaccines: emerging clinical information and its implications. *Expert Rev. Vaccines,* **2006**, *5*(4), 553-564. http://dx.doi.org/10.1586/14760584.5.4.553 PMID: 16989635

[120] Kelly, D.F.; Moxon, E.R.; Pollard, A.J. *Haemophilus influenzae* type B conjugate vaccines. *Immunology,* **2004**, *113*(2), 163-174. http://dx.doi.org/10.1111/j.1365-2567.2004.01971.x PMID: 15379976

[121] Finn, A. Bacterial polysaccharide-protein conjugate vaccines. *Br. Med. Bull.,* **2004**, *70*(1), 1-14. http://dx.doi.org/10.1093/bmb/ldh021 PMID: 15339854

[122] Dagan, R.; Poolman, J.; Siegrist, C.A. Glycoconjugate vaccines and immune interference: a review. *Vaccine,* **2010**, *28*(34), 5513-5523. http://dx.doi.org/10.1016/j.vaccine.2010.06.026 PMID: 20600514

[123] Malito, E.; Bursulaya, B.; Chen, C.; Lo Surdo, P.; Picchianti, M.; Balducci, E.; Biancucci, M.; Brock, A.; Berti, F.; Bottomley, M.J.; Nissum, M.; Costantino, P.; Rappuoli, R.; Spraggon, G. Structural basis for lack of toxicity of the diphtheria toxin mutant CRM197. *Proc. Natl. Acad. Sci. USA,* **2012**, *109*(14), 5229-5234. http://dx.doi.org/10.1073/pnas.1201964109 PMID: 22431623

[124] Angala, S.K.; Palčeková, Z.; Belardinelli, J.M.; Jackson, M. Covalent modifications of polysaccharides in mycobacteria. *Nat. Chem. Biol.,* **2018**, *14*(3), 193-198. http://dx.doi.org/10.1038/nchembio.2571 PMID: 29443974

[125] Brennan, P.J. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. *Tuberculosis (Edinb.),* **2003**, *83*(1-3), 91-97. http://dx.doi.org/10.1016/S1472-9792(02)00089-6 PMID: 12758196

[126] Karakousis, P.C.; Bishai, W.R.; Dorman, S.E. *Mycobacterium tuberculosis* cell envelope lipids and the host immune response. *Cell. Microbiol.,* **2004**, *6*(2), 105-116. http://dx.doi.org/10.1046/j.1462-5822.2003.00351.x PMID: 14706097

[127] Briken, V.; Porcelli, S.A.; Besra, G.S.; Kremer, L. Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. *Mol. Microbiol.,* **2004**, *53*(2), 391-403. http://dx.doi.org/10.1111/j.1365-2958.2004.04183.x PMID: 15228522

[128] Hunter, S.W.; Gaylord, H.; Brennan, P.J. Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. *J. Biol. Chem.,* **1986**, *261*(26), 12345-12351. PMID: 3091602

[129] Mishra, A.K.; Driessen, N.N.; Appelmelk, B.J.; Besra, G.S. Lipoarabinomannan and related glycoconjugates: structure, biogenesis and role in *Mycobacterium tuberculosis* physiology and host-pathogen interaction. *FEMS Microbiol. Rev.,* **2011**, *35*(6), 1126-1157. http://dx.doi.org/10.1111/j.1574-6976.2011.00276.x PMID: 21521247

[130] Moreno, C.; Taverne, J.; Mehlert, A.; Bate, C.A.; Brealey, R.J.; Meager, A.; Rook, G.A.; Playfair, J.H. Lipoarabinomannan from *Mycobacterium tuberculosis* induces the production of tumour necrosis factor from human and murine macrophages. *Clin. Exp. Immunol.,* **1989**, *76*(2), 240-245. PMID: 2503277

[131] Hölemann, A.; Stocker, B.L.; Seeberger, P.H. Synthesis of a core arabinomannan oligosaccharide of *Mycobacterium tuberculosis*. *J. Org. Chem.,* **2006**, *71*(21), 8071-8088. http://dx.doi.org/10.1021/jo061233x PMID: 17025296

[132] Fietta, A.; Francioli, C.; Gialdroni Grassi, G. Mycobacterial lipoarabinomannan affects human polymorphonuclear and mononuclear phagocyte functions differently. *Haematologica,* **2000**, *85*(1), 11-18. PMID: 10629585

[133] Kaplan, G.; Gandhi, R.R.; Weinstein, D.E.; Levis, W.R.; Patarroyo, M.E.; Brennan, P.J.; Cohn, Z.A. *Mycobacterium leprae* antigen-induced suppression of T cell proliferation *in vitro*. *J. Immunol.,* **1987**, *138*(9), 3028-3034. PMID: 3106496

[134] Moreno, C.; Mehlert, A.; Lamb, J. The inhibitory effects of mycobacterial lipoarabinomannan and polysaccharides upon polyclonal and monoclonal human T cell proliferation. *Clin. Exp. Immunol.,* **1988**, *74*(2), 206-210. PMID: 3147152

[135] Chan, J.; Fan, X.D.; Hunter, S.W.; Brennan, P.J.; Bloom, B.R. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. *Infect. Immun.,* **1991**, *59*(5), 1755-1761. http://dx.doi.org/10.1128/IAI.59.5.1755-1761.1991 PMID: 1850379

[136] Barnes, P.F.; Chatterjee, D.; Brennan, P.J.; Rea, T.H.; Modlin, R.L. Tumor necrosis factor production in patients with leprosy. *Infect. Immun.,* **1992**, *60*(4), 1441-1446. http://dx.doi.org/10.1128/IAI.60.4.1441-1446.1992 PMID: 1548069

[137] Minion, J.; Leung, E.; Talbot, E.; Dheda, K.; Pai, M.; Menzies, D. Diagnosing tuberculosis with urine lipoarabinomannan: systematic review and meta-analysis. *Eur. Respir. J.,* **2011**, *38*(6), 1398-1405. http://dx.doi.org/10.1183/09031936.00025711 PMID: 21700601

[138] Hamasur, B.; Källenius, G.; Svenson, S.B. Synthesis and immunologic characterisation of *Mycobacterium tuberculosis* lipoarabinomannan specific oligosaccharide-protein conjugates. *Vaccine,* **1999**, *17*(22), 2853-2861. http://dx.doi.org/10.1016/S0264-410X(99)00124-3 PMID: 10438056

[139] Hamasur, B.; Haile, M.; Pawlowski, A.; Schröder, U.; Williams, A.; Hatch, G.; Hall, G.; Marsh, P.; Källenius, G.; Svenson, S.B. *Mycobacterium tuberculosis* arabinomannan-protein conjugates protect against tuberculosis. *Vaccine,* **2003**, *21*(25-26), 4081-4093. http://dx.doi.org/10.1016/S0264-410X(03)00274-3 PMID: 12922145

[140] Haile, M.; Hamasur, B.; Jaxmar, T.; Gavier-Widen, D.; Chambers, M.A.; Sanchez, B.; Schröder, U.; Källenius, G.; Svenson, S.B.; Pawlowski, A. Nasal boost with adjuvanted heat-killed BCG or arabinomannan-protein conjugate improves primary BCG-induced protection in C57BL/6 mice. *Tuberculosis (Edinb.),* **2005**, *85*(1-2), 107-114. http://dx.doi.org/10.1016/j.tube.2004.09.013 PMID: 15687034

[141] Kallert, S.; Zenk, S.F.; Walther, P.; Grieshober, M.; Weil, T.; Stenger, S. Liposomal delivery of lipoarabinomannan triggers *Mycobacterium tuberculosis* specific T-cells. *Tuberculosis (Edinb.),* **2015**, *95*(4), 452-462. http://dx.doi.org/10.1016/j.tube.2015.04.001 PMID: 26043674

[142] Glatman-Freedman, A.; Casadevall, A.; Dai, Z.; Jacobs, W.R., Jr; Li, A.; Morris, S.L.; Navoa, J.A.D.; Piperdi, S.; Robbins, J.B.; Schneerson, R.; Schwebach, J.R.; Shapiro, M. Antigenic evidence of prevalence and diversity of *Mycobacterium tuberculosis* arabinomannan. *J. Clin. Microbiol.,* **2004**, *42*(7), 3225-3231. http://dx.doi.org/10.1128/JCM.42.7.3225-3231.2004 PMID: 15243086

[143] Prados-Rosales, R.; Carreño, L.; Cheng, T.; Blanc, C.; Weinrick, B.; Malek, A.; Lowary, T.L.; Baena, A.; Joe, M.; Bai, Y.; Kalscheuer, R.; Batista-Gonzalez, A.; Saavedra, N.A.; Sampedro, L.; Tomás, J.; Anguita, J.; Hung, S.C.; Tripathi, A.; Xu, J.; Glatman-Freedman, A.; Jacobs, W.R., Jr; Chan, J.; Porcelli, S.A.; Achkar, J.M.; Casadevall, A. Enhanced control of *Mycobacterium tuberculosis* extrapulmonary dissemination in mice by an arabinomannan-protein conjugate vaccine. *PLoS Pathog.,* **2017**, *13*(3), e1006250. http://dx.doi.org/10.1371/journal.ppat.1006250 PMID: 28278283

[144] McIntosh, J.D.; Brimble, M.A.; Brooks, A.E.S.; Dunbar, P.R.; Kowalczyk, R.; Tomabechi, Y.; Fairbanks, A.J. Convergent chemo-enzymatic synthesis of mannosylated glycopeptides; targeting of putative vaccine candidates to antigen presenting cells. *Chem. Sci. (Camb.),* **2015**, *6*(8), 4636-4642. http://dx.doi.org/10.1039/C5SC00952A PMID: 28717478

[145] Bavaro, T.; Tengattini, S.; Piubelli, L.; Mangione, F.; Bernardini, R.; Monzillo, V.; Calarota, S.; Marone, P.; Amicosante, M.; Pollegioni, L.; Temporini, C.; Terreni, M. Glycosylation of recombinant antigenic proteins from *Mycobacterium tuberculosis*: *in silico* prediction of protein epitopes and *ex vivo* biological evaluation of new semi-synthetic glycoconjugates. *Molecules,* **2017**, *22*(7), 1081. http://dx.doi.org/10.3390/molecules22071081 PMID: 28661444

[146] Rinaldi, F.; Tengattini, S.; Piubelli, L.; Bernardini, R.; Mangione, F.; Bavaro, T.; Paone, G.; Mattei, M.; Pollegioni, L.; Filice, G. Rational design, preparation and characterization of recombinant Ag85B variants and their glycoconjugates with T-cell antigenic activity against *Mycobacterium tuberculosis*. RSC. *Adv.,* **2018**, *8*(41), 23171-23180. http://dx.doi.org/10.1039/C8RA03535K

[147] Vliegenthart, J.F. Carbohydrate based vaccines. *FEBS Lett.,* **2006**, *580*(12), 2945-2950. http://dx.doi.org/10.1016/j.febslet.2006.03.053 PMID: 16630616

[148] Boltje, T.J.; Buskas, T.; Boons, G.J. Opportunities and challenges in synthetic oligosaccharide and glycoconjugate research. *Nat. Chem.,* **2009**, *1*(8), 611-622. http://dx.doi.org/10.1038/nchem.399 PMID: 20161474

[149] Gao, J.; Liao, G.; Wang, L.; Guo, Z. Synthesis of a miniature lipoarabinomannan. *Org. Lett.,* **2014**, *16*(3), 988-991. http://dx.doi.org/10.1021/ol4036903 PMID: 24444032

[150] Ishiwata, A.; Ito, Y. Synthesis of docosasaccharide arabinan motif of mycobacterial cell wall. *J. Am. Chem. Soc.,* **2011**, *133*(7), 2275-2291. http://dx.doi.org/10.1021/ja109932t PMID: 21287985

[151] Kandasamy, J.; Hurevich, M.; Seeberger, P.H. Automated solid phase synthesis of oligoarabinofuranosides. *Chem. Commun. (Camb.),* **2013**, *49*(40), 4453-4455. http://dx.doi.org/10.1039/c3cc00042g PMID: 23370381

[152] Ishiwata, A.; Akao, H.; Ito, Y. Stereoselective synthesis of a fragment of mycobacterial arabinan. *Org. Lett.,* **2006**, *8*(24), 5525-5528. http://dx.doi.org/10.1021/ol062198j PMID: 17107063

[153] Bundle, D.R.; Tam, P-H.; Tran, H-A.; Paszkiewicz, E.; Cartmell, J.; Sadowska, J.M.; Sarkar, S.; Joe, M.; Kitov, P.I. Oligosaccharides and peptide displayed on an amphiphilic polymer enable solid phase assay of hapten specific antibodies. *Bioconjug. Chem.,* **2014**, *25*(4), 685-697. http://dx.doi.org/10.1021/bc400486w PMID: 24601638

[154] Wang, L.; Feng, S.; Wang, S.; Li, H.; Guo, Z.; Gu, G. Synthesis and immunological comparison of differently linked lipoarabinomannan oligosaccharide-monophosphoryl lipid

A conjugates as antituberculosis vaccines. *J. Org. Chem.,* **2017**, *82*(23), 12085-12096. http://dx.doi.org/10.1021/acs.joc.7b01817 PMID: 29112822

[155] Zhou, Z.; Mondal, M.; Liao, G.; Guo, Z. Synthesis and evaluation of monophosphoryl lipid A derivatives as fully synthetic self-adjuvanting glycoconjugate cancer vaccine carriers. *Org. Biomol. Chem.,* **2014**, *12*(20), 3238-3245. http://dx.doi.org/10.1039/C4OB00390J PMID: 24728423

[156] Wattanasiri, C.; Paha, J.; Ponpuak, M.; Ruchirawat, S.; Boonyarattanakalin, S. Synthesis of synthetic mannan backbone polysaccharides found on the surface of *Mycobacterium tuberculosis* as a vaccine adjuvant and their immunological properties. *Carbohydr. Polym.,* **2017**, *175*, 746-755. http://dx.doi.org/10.1016/j.carbpol.2017.07.045 PMID: 28917925