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Review article

Tuberculosis vaccine: A journey from BCG to present

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ABSTRACT

Tuberculosis (TB) is the leading cause of death worldwide due to an infectious disease, causing around 1.6 million deaths each year. This situation has become more complicated by the emergence of drug-resistant *Mycobacterium tuberculosis* (*M.tb*) and HIV-TB co-infection, which has significantly worsened TB prognosis and treatment. Despite years of intensive research, Bacille Calmette-Guerin (BCG) remains the only licensed vaccine and has variable efficacy. It provides protection against childhood TB but is not effective in adult pulmonary TB. As a result of intense research in understanding TB vaccinology, there are many new vaccine candidates in clinical development and many more in pre-clinical trials which aim either to replace or boost BCG vaccine. This review discusses the history of BCG vaccine development and summarizes limitations of the current vaccine strategy and recent advances in improving BCG immunization along with other new vaccines in clinical trials which are promising candidates for the future tuberculosis vaccinology program.

1. Introduction

Tuberculosis is a major health concern globally that claims almost 2 million lives annually with the developing countries bearing the major brunt of the disease. In March 1993, the World Health Organization (WHO), declared TB, a “global public health emergency” [1]. The situation has deteriorated further due to the emergence of multidrug-resistant TB and HIV epidemic. One-fourth of the global population gets latently infected with tuberculosis, and 5–10% of this latently infected group develops active disease during their lifetime [2]. At present, TB immunology and vaccinology together is the most evolving field in medical research. After its advent in 1921, BCG remains the most widely used vaccine until today despite its limitations and highly variable efficacy. BCG is an attenuated vaccine derived from *M. bovis* after 13 years of continuous in-vitro passage. It provides successful protection against childhood pulmonary TB but fails in protection against adult pulmonary TB. The protection offered by BCG wanes off with time (approximately 10–20 years post-immunization), the time when the person is at the maximum risk of exposure to the disease [3]. The last decade has witnessed remarkable success in the field of TB vaccine research, but the search for a better vaccine candidate still remains a dream owing to the lack in knowledge of the role of the protective immunity against *Mycobacterium tuberculosis*. The only available tuberculosis therapy is Directly Observed Treatment Short-course (DOTS), which too is very lengthy and possesses various side effects including the dampening of immune responses resulting in the vulnerability to TB reactivation and reinfection [4]. The countries in which there is maximum TB onset, people have limited access to TB treatment and suffer from the drug-resistant form of TB due to non-compliance with the treatment procedure or complete withdrawal from it. Therefore, owing to the limited protection offered by BCG and safety concerns involved due to BCG being as an attenuated vaccine, with a high risk of becoming virulent, we are in immediate need of a vaccine which may able to prevent both childhood and adult TB, has to be better than BCG, both in safety and efficacy. There are many vaccine candidates, which are in clinical trials which are aimed at improving the BCG vaccine by manipulating the host immune responses. The sequencing of *M.tb* genome has further led to a rapid increase in the identification of novel antigens which have the scope of being used as a vaccine candidate. Vaccine development strategy mainly groups the TB vaccines based on their route of administration and nature into prime-boost vaccines, subunit vaccines, DNA vaccines, and attenuated vaccines. Therefore, continuous research, employing different novel strategies, is going throughout the world to eliminate the TB by 2050 as part of the Stop TB partnership of WHO [5].

2. History of TB and onset of BCG vaccine

MTB has been postulated to have originated > 150 million years ago and an early progenitor of *M.tb* might have infected early hominids

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in East Africa approximately 3 million years ago. But the common ancestor of modern strains of *M.tb* might have appeared around 20,000–15,000 BCE [6]. Tuberculosis is a deadly infectious disease that has been victimizing the human race since ages. Its deadly effects reached widespread magnitudes in North America and Europe during the 18th and 19th centuries, which led to the condition being addressed as "Captain Among these Men of Death" [7]. The work of The'ophile Laennec around the beginning of the 19th century led to the initial understanding of the pathogenesis of tuberculosis which took further progress by the explanation of the contagiousness and spread of the infection by Jean-Antoine Villemin in 1865. In 1882, Robert Koch identified the tubercle bacillus, as the causative agent of tuberculosis. Soon after, public health measures to combat the spread of tuberculosis emerged. After its discovery in the 1920s, BCG vaccination was widely employed following TB endemic after World War I.

The following flow-chart depicts the chronological history of *M.tb*, from its hypothesized origin to the extraordinary milestone of isolation of tubercle bacillus by a German microbiologist, Robert Koch (Table 1) [8].

3. The BCG vaccine

The BCG vaccine was discovered by Calmette and Guerin at the Institut Pasteur, France in 1921, albeit without any knowledge of its immunological insight [9]. It was developed from a live-attenuated strain of *Mycobacterium bovis*, a related subspecies of *M. tuberculosis* (> 90% homology). Calmette and Guerin discovered that adding beef bile to reduce the clumping in *M. bovis* decreases the virulence of the strain. After 231 serial passages between 1908 and 1924, it offered some protection in animal models [10,11]. Genomic studies have elucidated that deletion of RD1 region, is crucial in the loss of virulence in BCG. The Region of Deletion (RD) is a 10.7 kb fragment that contains 9 Open Reading Frames (ORFs) and is found in virulent strains of *M. tuberculosis* and *M. bovis*. RD1 encodes for a secretion system known as ESX-1 which codes for two major immunogenic proteins of *M.tb*, ESAT-6 and CPF-10. It has been studied that lack of RD1 region is the major reason for the lack of virulence. Restoration of the RD1 region does not completely return the virulence phenotype the presence of other genetic factors which may also play a role [12]. Clinical immunization with BCG started as early as in 1921 and its use was supported by early trials conducted by Heimbeck among nursing students in Norway, where students vaccinated with BCG had lower rates of active TB as compared to unvaccinated students [13]. Additional support came from the first formal trials organized by the North American Indians during the 1930s [14–16]. Clinical trials that followed in Belgium and France led to the implementation of BCG vaccination throughout Europe. By 1940s several studies and clinical trials established the protective role of BCG vaccine in tuberculosis, among children. As the tuberculosis rates increased after World War II, several international health organizations recommended BCG vaccination among the population. In the 1960s, WHO developed strategies for routine BCG vaccinations in the population. In 1974, BCG was also included in the Expanded Program on Immunization (EPI) infant vaccination schedule [17].

Today, BCG immunization is one of the widest implemented vaccination strategies in the world. The success of BCG is mainly due to its effectiveness against TB meningitis in children, its cost-effectiveness for being used in poor countries of the world as well as safety of use among humans. Currently, BCG vaccines are being produced worldwide by > 40 manufacturers [18].

4. BCG failure and the need for the development of new vaccine candidates

Several hypotheses have emerged to explain the failure of BCG vaccination. These include climatic conditions, geographic latitude, the genetic background of the host and the strain of BCG used [19–21]. Pre-exposure to environmental mycobacteria, which are quite common in tropical regions leads to compromise in the protection offered by BCG vaccination [19]. The following two hypotheses have been proposed to explain the adverse effects of prior sensitization to environmental mycobacteria before BCG immunization.

4.1. The masking hypothesis

The masking hypothesis proposes that exposure of the host to environmental mycobacteria provides some level of protective immunity against tuberculosis and subsequent immunization of the pre-exposed host with BCG does not improve the level of protection any further. So, BCG, which is administered to neonates immediately after birth, has a protective contribution since no prior sensitization of the infants occurs to mask the effect of the vaccine. On the contrary, due to prior sensitization with environmental mycobacteria in adults, BCG vaccination is not effective. Therefore, a novel vaccine is needed which has to be remarkably superior to BCG in terms of efficacy to have a measurable effect in adults [21].

4.2. The blocking hypothesis

The blocking hypothesis suggests that the replication of BCG is inhibited in the presence of prior sensitization with the environmental mycobacteria, due to the presence of a pre-existing immune response to the antigens common to the mycobacteria. The replication of BCG is a prerequisite to eliciting an immune memory response since it is a live vaccine. In such a scenario, the new vaccine needs to be as good as BCG when it comes to efficacy, until it is not blocked by pre-exposure. A vaccine based on non-mycobacterial vectors such as virus, recombinant protein or naked DNA is required to diminish the effect of pre-sensitization and provide effective protection to the host [22].

Moreover, different BCG strains have different efficacies because, in the 1920s, the original strain at Institute Pasteur was distributed to different regions of the world. Lack of properly established culture protocols at that time led to BCG strains which have different antigenic and immunologic effectiveness which may affect their efficacy worldwide.

These limitations associated with the BCG vaccine call for urgent development of novel and improved vaccine candidates that are effective, safe as well as not effected by pre-sensitization with the environmental mycobacteria.

5. The immune response against *M.tb* and approach to vaccine development

M.tb is transmitted through aerosol droplets and infects the lungs of an individual. Once the bacilli reach the alveoli, they are ingested by alveolar macrophages. Alveolar macrophages are competent enough to kill the bacteria by recruiting an immune response against the pathogen. It has been estimated that in 20–50% of humans exposed to *M.tb*, the bacilli resists the immune response mediated by alveolar macrophages and multiply within them, infecting nearby cells and activating the immune response [23,24]. Cell-mediated immune response is mounted by the host leading to cellular infiltration at the site of primary infection which leads to the formation of granuloma. Formation of granuloma in the lung with the infected macrophages in the centre and lymphocytes, stem cells and epithelial cells in the marginal zone is the hallmark of cellular immunity against tuberculosis. This immune response is capable of reducing bacterial multiplication and leading to a clinically silent, asymptomatic form of infection, known as latent tuberculosis. Latent TB infected individuals have a 5–10% chance of developing active TB during their lifetime [25].

Studies of TB infection in the murine model have demonstrated that immune response against TB is characterized by control of bacterial multiplication, the formation of granulomas and recruitment of

Table 1
Flow-chart depicting the chronological history of *M.tb*, from its origin to the isolation of tubercle bacillus by Robert.

	150 Million Yrs Ago	
The genus Mycobacterium originated		
	3 Million Yrs Ago	An early progenitor of Mycobacterium tuberculosis might have infected early hominids in East Africa
The common ancestor of modern strains of MT might have appeared	**20000 BCE**	
	2400 BCE	Egyptian mummies reveal skeletal deformities typical of tuberculosis
Peruvian mummies depict archaeological evidence of early TB	**600 BCE**	
	400 BCE	Ancient Greeks address TB as Phtisis. Hippocrates described its symptoms
Personal physician of the Roman Emperor Marcus Aurelius, the Greek Clarissimus Galen detailed symptoms of TB including fever, sweating, coughing and blood-stained sputum	**174 CE**	
	~ 600 CE	Byzantine doctors Aetius, Alexander and Paul described the pulmonary and glandular forms of TB, while in Arabic, Avicenna supposed the contagious nature of TB
Guy de Chauliac proposed the removal of scrofulous gland as a cure	**1363 CE**	
	1679 CE	Francis Sylvius gave the exact pathological and anatomical description of TB describing tubercles, their progression to abscesses, cavities and empyema in the lungs and in other sites of consumptive patients
Sir Percivall Pott, a British surgeon, recognized Extra-pulmonary phthisic tubercles naming TB as Pott's disease, linking vertebral collapse and spinal cord paralysis with TB	**1779 CE**	
	1793 CE	Scottish pathologist Matthew Baille named the caseous necrosis, "cheese-like", phthisic abscesses as "Tubercles"
French physician Gaspard-Laurent Bayle described the disseminated "miliary" TB only as a disease affecting the lung, but a generalized one, clinically defined by coughing, difficulty in breathing, fever and purulent expectoration	**1810 CE**	
	1819 CE	French Theophile Laennac identified the presence of consolidation, pleurisy and pulmonary cavitation as pathognomonic signs of pulmonary or extra-pulmonary TB
German physician Philipp Friedrich Hermann Klencke succeeded in the experimental reproduction of human and bovine forms of TB, causing generalized TB in rabbits, through a successful inoculation of material from a miliary tubercle into their liver and lungs	**1843 CE**	
	1850 CE	Johann Lukas Schönlein coined the term "**Tuberculosis**"
The first successful remedy against TB was introduced as the sanatorium cure by Hermann Brehmer, a botany student suffering himself from TB, in the doctoral dissertation "Tuberculosis is a curable disease"	**1854 CE**	
	1865 CE	Jean-Antoine Villemin, a French military surgeon, demonstrated the infectious nature of TB
Theodor Albrecht Edwin Klebs became one of the early scientists to isolate the TB bacillus, sowing tuberculous material on egg white, stored in	**1867 CE**	
	1882 CE	Robert Koch isolated the tubercle bacillus. Using methylene blue staining, he identified, isolated and cultivated the bacillus in animal serum reproducing the disease by inoculating the bacillus into laboratory animals and presented this extraordinary result to the Society of Physiology in Berlin
The Pirquet and Mantoux tuberculin skin tests, Albert Calmette and Camille Guérin (BCG) vaccine, Selman Waksman streptomycin and other anti-tuberculous drugs were developed	**A few decades later**	
	Present day	

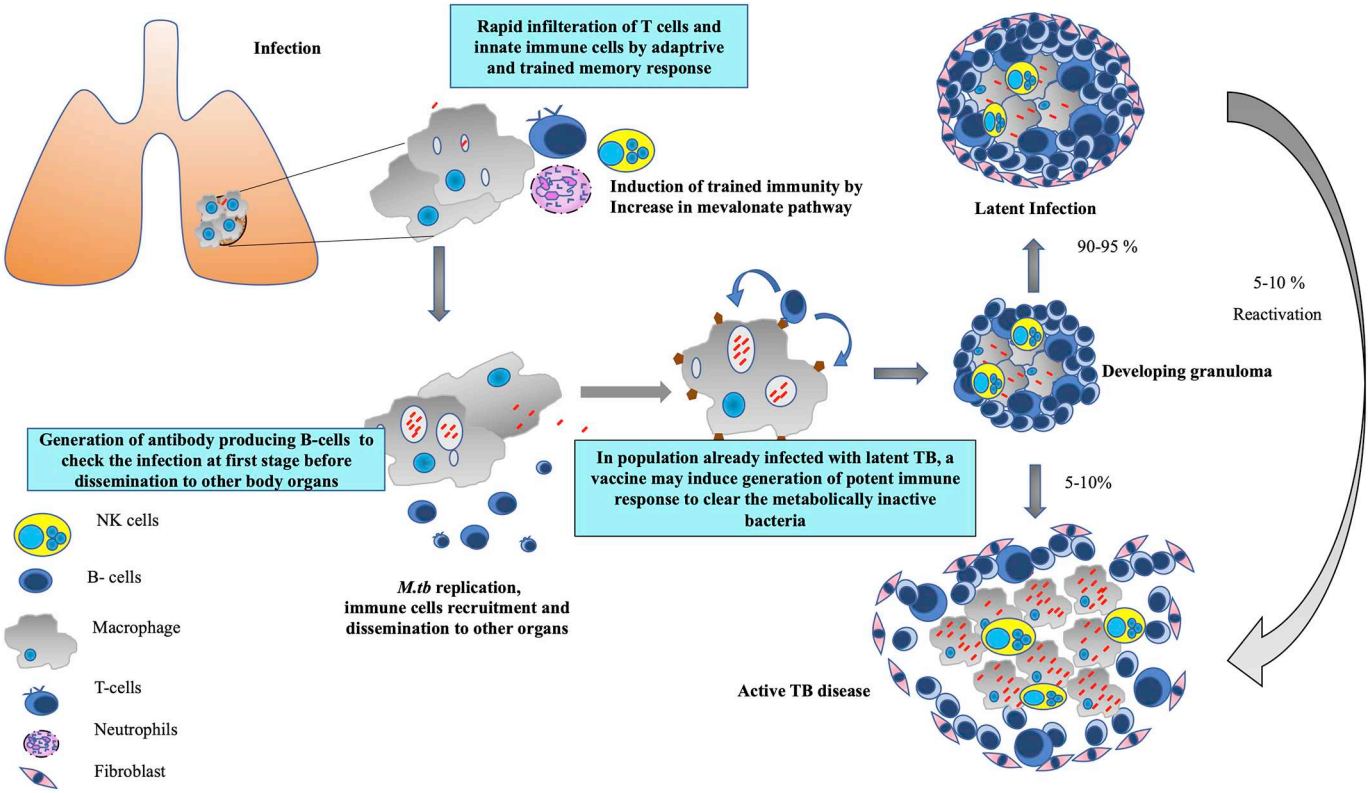

Fig. 1. *M.tb* infection cycle and stages of the infectious process that may serve as potential targets of the vaccine-induced immune response (highlighted in light blue boxes). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

memory cells at the site of infection (Fig. 1).

Studies in both humans and mice have demonstrated that T-helper cells (Th1) basically $CD4^+$, $CD8^+$ T-cells as well as Th17 cells provide protection against TB. IFN-γ and TNF-α produced by Th1 cells act on macrophages infected with *M.tb* and help in the killing of the bacteria. IL-12p70 is required for controlling *M.tb* replication by inducing IFN-γ response. Both IL-23 and IL-17 play a role in vaccine-induced protection but do not have a role in the primary protection in the lungs [26]. IL-17, a pro-inflammatory cytokine which induces recruitment of immune cells to the site of infection by cytokine release is modulated by IL-23. Hence, both Th1 and Th17 are important for protection against *M.tb*. In vaccinated animals loss of Th17 response leads to the absence of protective memory Th1 response. Therefore, Th1 and Th17 response both cross-regulate each other for providing effective immunity against TB [27]. Apoptotic vesicles engulfed by dendritic cells secrete TGF-β, IL-6 and Il-23 leading to Th17 differentiation [28]. Apoptotic vesicles derived from macrophages infected with TB bacilli induce improved $CD4^+$ and $CD8^+$ T-cell stimulation by cross-priming [29]. IL-18 also plays a protective role against TB. $CD8^+$ T-cells mediated killing is through secretion of cytokine and synthesis of perforin and granzyme-granulysin pathway [30]. Humoral immunity against M.tb has also received some attention due to the presence of notable differences in the plasma levels of natural mycobacteria-specific IgG or IgA and their glycosylation profiles and Fc functions when comparing individuals with LTBI and patients with active TB [31,32]. IL-27 is required for long term survival by the bacteria limiting protective immunity in the lungs [33].

The primary goal of any vaccination strategy is to augment an efficient and long-lived immune memory response with the objective of minimizing the time duration between infection and the inception of an adaptive immune response at the site of infection, so as to control the infection at the earliest and avoid its spreading to other sites. Although most research put impetus to the role of Th1 cells and its secreted cytokine IFN-γ for the development of a successful vaccine, recent findings hint at the presence of other immune cells which may help in the identification of new biomarkers as a target for vaccine research.

6. Importance of diversity in $CD4^+$ T cell response in vaccine development

CD4+ T-cell subsets are the major target of TB vaccine development strategies owing to their central role in the generation of a long-lasting memory response. CD4+ T-cells differentiate into T central memory (T_{CM}) cells that reside in the secondary lymphoid organs and bronchus-associated lymphoid tissue (BALT) in the lungs. Upon re-exposure, to antigens, T_{CM} differentiate to T effector memory (T_{EM}) and T effector (T_{EFF}) cells of Th1 and Th17 lineage which migrate to the infection sites. A fraction of these cells reside in the lungs as T tissue-resident memory (T_{RM}) cells. Equilibrium between T_{RM} and T_{EFF} cells decide the efficacy of the host immune response. Long-time exposure of T-cells with the same antigen leads to exhaustion of T_{EM} and T_{EFF} characterized by the expression of inhibitory receptors, TIM3 and increase in the expression of exhaustion markers such as killer-like lectin receptor G1 (KLRG1). A major goal of an efficient vaccine is to maintain enough pool of T_{EM} subset so as to avoid T-cell exhaustion. T stem cell memory (T_{SCM}) cells and T_{CM} cells play a vital role because of their proliferative potential in maintaining the supply of tissue-resident T cells (Fig. 2).

M.tb infection leads to the generation of a diverse pool of $CD4^+$ T-cells at different stages of differentiation, from less differentiated T_{SCM} and T_{CM} secreting IL-2 to more differentiated T_{EFF} secreting IFN-γ. The antigen, stage of disease progression and the cytokine milieu where the particular T-cell subset is localized, maintains this range of T-cell differentiation. It has also been reported that a less differentiated memory T cell subset, expressing the checkpoint molecule PD1 and the chemokine receptor CXCR3 is more capable of entering the *M.tb*-infected lung parenchyma compared to the more differentiated T_{EFF} cell subsets, characterized by the expression of KLRG1 and the fractalkine receptor CX3CR1 [34]. Recently discovered adjuvant vaccines channelize T-cell

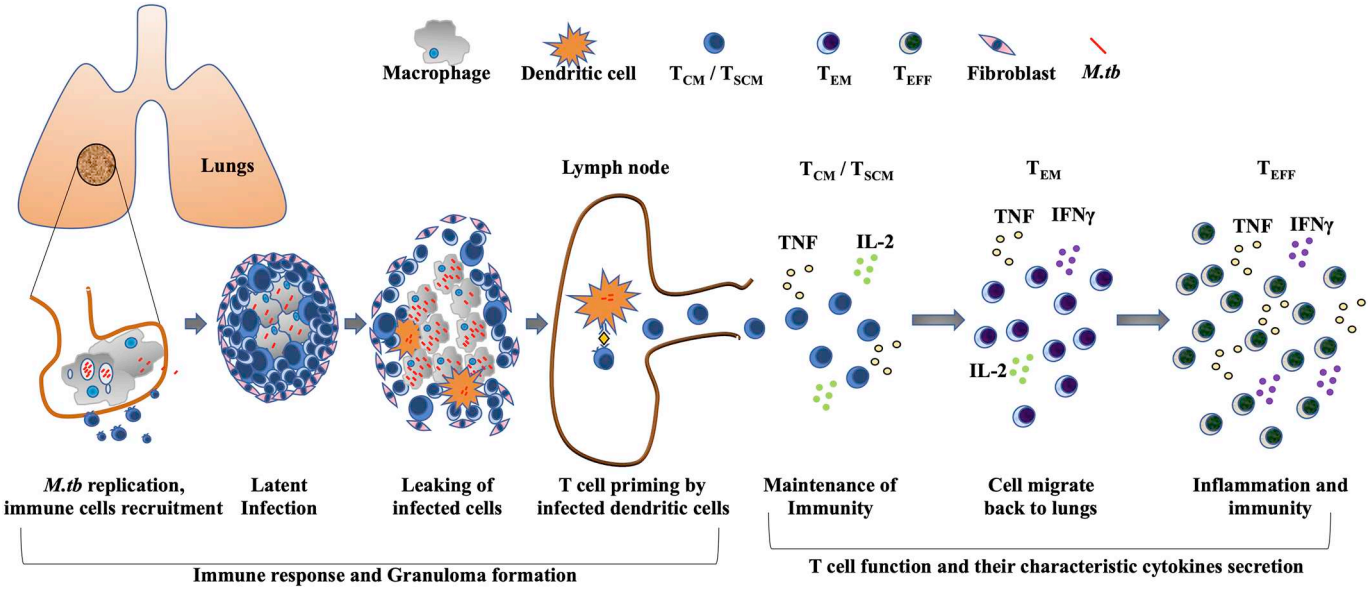

Fig. 2. The diverse pool of *M.tb* specific T-cells generated upon antigen exposure and their effector cytokines-target of vaccine designing strategies.

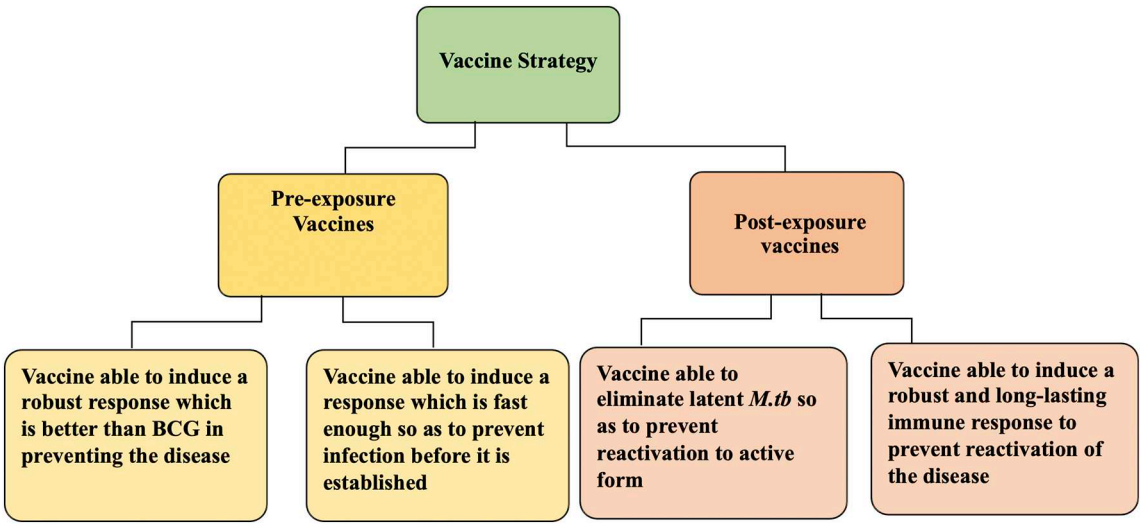

Fig. 3. Schematic diagram showing vaccine development strategies for the development of a potential vaccine candidate against neonatal and adult TB.

responses towards T_{SCM} and T_{EM} unlike *M.tb* and BCG, which direct it towards exhausting T_{EM} and T_{EFF}. Therefore, these new vaccines avoid T-cell exhaustion and provide long-lasting memory response against TB. TB vaccine development strategies, therefore, need to give careful consideration to maintaining the balance of T-cell subset diversity, which the vaccine is generating. Vaccine development approach against *M.tb* falls into four major categories (Fig. 3).

7. Role of innate immunity in TB vaccine development

Vaccine development mainly depends on the induction of adaptive immune response which elicits long term memory against *M.tb* antigen. However, recently it has been shown that innate immunity also plays an important role mainly, myeloid and NK cells undergo functional adaptation via metabolic rewiring and epigenetic reprogramming, which constitutes trained immunity, which elicits memory upon infection. Recent studies have provided enough evidence to prove that innate immune system, particularly, adjuvant activity and induction of trained immunity contribute in the protection-efficacy of any vaccine and therefore a vaccine that targets both trained immunity (innate immunity) as well as adaptive immunity would be more effective against *M.tb* [35].

8. Challenges in the development of new vaccine candidates

There has been no major development in the Tuberculosis Vaccine development over the past few decades due to severe under-funding. Only 25% of the projected TB vaccine research investments have been met since 2011–2015. Even today, after 98 years of its development in 1921, the BCG vaccine remains the only WHO-approved vaccine against TB. Several factors have contributed towards the stunted progress in the search for a better TB vaccine candidate. Which are:

1. The lack of incentives to invest in a disease that majorly affects people in low-income developing countries.
2. The absence of reliable biomarkers that could be used as prospective signatures of tuberculosis risk or as correlates of protection
3. A lack of mechanisms to reduce the inherent uncertainties associated with the process of vaccine development—it is unclear whether animal models predict protection in humans. Also, large

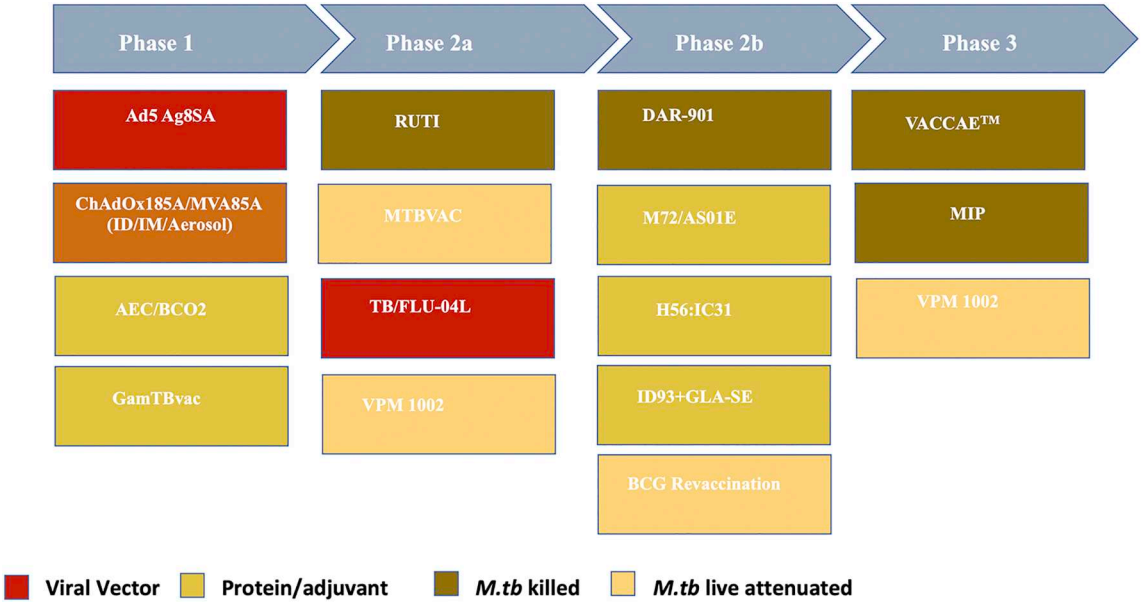

Fig. 4. TB vaccine candidates at different phases in clinical trials all over the globe.

sample sizes are needed to demonstrate vaccine efficacy during the later stages of tuberculosis vaccine development.

4. Any new TB vaccine should be appropriate for use in individuals pre-exposure to prevent the onset of infection, and also post-exposure, to avoid the development of the disease and provide therapeutic interventions for clearance of the bacteria through immunomodulators and action of antibiotics in combination.
5. As most of the population is vaccinated with BCG after birth, the effect of which wanes off as the person reaches in adulthood. The new generation vaccine should be such that is able to provide protection efficiently in the population vaccinated with BCG.

The combination of these factors acts as a major discouragement for sponsors of new TB vaccines candidates' research and development.

9. New TB vaccine candidates in trials

TB vaccine development approaches must strike an appropriate equilibrium between the self-renewing and undifferentiated T-stem cell memory (T_{SCM}) and T-central memory (T_{CM}) cell subsets and fully differentiated T-effector memory (T_{EM}) cells and T-effector (T_{EFF}) cell subsets. This has to be ensured to avoid immunopathology. In accordance with the above considerations, novel TB vaccine candidates have been developed. These TB vaccine candidates can be characterized into the following groups

1. *Preventive pre-exposure vaccines or prophylactic vaccines*
   These vaccines are administered prior to the first encounter with *M. tuberculosis* bacteria, typically to infants immediately after birth. They can be either priming vaccines such as *Mycobacterium bovis* (BCG) or booster vaccines for future administration.
2. *Preventive post-exposure vaccines*
   These vaccines are directed at protecting adolescents and adults with latent TB infection (LTBI) and who are BCG immunized. They can boost up the naturally occurring infection-promoted reactions.
3. *Therapeutic vaccines*
   These vaccines are administered to a population already infected with *M.tb*, in addition to established anti-TB drugs, particularly to prevent recurrence of the disease after successful treatment.

Preventive vaccines are classified into three broad categories: viable whole-cell vaccines, inactivated whole-cell vaccines and subunit vaccines.

9.1. Viable whole-cell vaccines

Live attenuated whole-cell vaccines which were originally developed as prophylactic pre-exposure vaccines (priming vaccines) for 'replacing' BCG vaccination in neonates, are now also being assessed as post-exposure vaccines in adults. Two examples of these vaccines are, VPM1002, a recombinant BCG (rBCG) vaccine and MTBVAC, live, attenuated *M.tb* vaccine. In VPM1002, the addition of a listeriolysin gene is accompanied by the deletion of a urease gene, allowing the rBCG to escape the macrophage lysosome, similar to *M.tb* infection [35]. For developing MTBVAC, two stable deletions particularly in *phoP* gene, required for the transcription of key *M.tb* virulence genes; and *fadD*26 gene, important for the synthesis of cell surface lipids which control *M.tb* pathogenicity were made in the genome of *M.tb* strain [36]. Both are currently in clinical trials. They mount a complex and diverse immune response to a wide range of antigens, which gives them an edge over the subunit vaccines which have a restricted response to limited antigens. The limitations of such live vaccines, however, are that they are prone to inhibitions caused due to pre-sensitization by environmental mycobacteria as stated for BCG.

9.2. Inactivated whole-cell vaccines

Inactivated and viable live attenuated vaccines constitute whole-cell vaccines. These vaccine candidates utilize killed whole Mycobacteria or mycobacterial cell wall extracts to mount an immune response against multiple *M.tb* antigens. These include *Mycobacterium obuense*-based DAR-901 vaccines, RUTI, and *Mycobacterium vaccae*-based vaccines. RUTI is synthesized from the cell wall of *M.tb* formulated in a liposome suspension [37]. The Vaccae™ vaccine is derived from heat-killed *Mycobacterium vaccae*, a non-tuberculosis mycobacteria (NTM) closely related to *M. obuense*. RUTI and *M. vaccae* based vaccines are primarily being used as therapeutic vaccines for reducing the duration of drug treatment in active TB patients, while DAR-901 is being developed as both a prophylactic and a therapeutic vaccine. Since whole-cell

vaccines are polyantigenic, they are more capable of including the crucial epitopes than subunit vaccines. Thus, they may provide better protective efficacy.

9.3. Adjuvanted protein subunit vaccine

Subunit vaccines are based on combining one or more protein antigens. To increase the vaccine efficacy, antigens are either administered along with an adjuvant or expressed by a recombinant viral vector. These are primarily developed as prophylactic or post-exposure vaccines that boost immune responses, initially primed by BCG or *M.tb* infection. Subunit vaccines presently in clinical trials include H56: IC3, H4: IC3, ID93 + M72/AS01E and GLA-SE [38]. These vaccines are also being evaluated for their therapeutic value for prevention against recurrence of the disease in individuals who have successfully completed the DOTS treatment. In general, these subunit vaccines are given as boosters after a BCG prime, for improving BCG-mediated protection or increasing the duration of the protection offered by BCG prime vaccine.

M72/AS01E vaccine composed of a recombinant fusion protein derived from two *M.tb* antigens (*M.tb32A* and *M.tb39A*) in combination with the AS01 adjuvant system has proved to be a very promising candidate against pulmonary TB in adults. This vaccine has shown to provide 54.0% protection against active pulmonary disease without any safety issues involved. Studies have shown that vaccination with M72/AS01E elicits both antibody and T cell-mediated immune response and has shown to be efficacious for up to 3 years [39].

9.4. Viral-vectored vaccines

Live vector vaccines based on recombinant viral vectors are among the most immunogenic vaccines for induction of effector T cells independent of the antigen, inducing strong $CD8^+$ and $CD4^+$ Th1-type immune response [39]. They can be used as a booster vaccine for producing augmented immune responses to the primed T-cell responses against encoded antigens and epitopes. Based on such principle, live, non-replicating, attenuated viruses can be genetically modified to deliver genes encoding the antigens of importance into the host cells. Viral-based vaccines lead to the intracellular production of the antigen activating the innate immune system. They do not require adjuvants to mount a high immune response. Viral-vectored vaccines are being developed both as prophylactic vaccines and post-exposure vaccines. Limitation of viral vector-based vaccines is the generation of immunity against the vector that may limit later booster vaccinations. Example of two viral vectored TB vaccines are MVA85A and Ad5Ag85A (Rv3804) [40].

The list of all new TB vaccine candidates at different phases in clinical trials is depicted in Fig. 4.

10. BCG revaccination strategy - evaluation of booster dose efficiency

Two large-scale cluster-randomized controlled trials were conducted in Brazil and Malawi to access BCG revaccination potential against TB disease but none proved efficacious leading to the World Health Organization (WHO) recommending against this strategy [41]. These and subsequent studies indicated that prior sensitization with environmental mycobacteria is a major factor in preventing BCG revaccination efficacy [42]. Recently, clinical trial with the H4:IC31 subunit vaccine or BCG revaccination conducted in South Africa, BCG revaccination provided 45% efficacy against persistent *M.tb* infection. BCG revaccination resulted in a surprisingly high efficacy in providing protection against TB. A reasonable justification for this observation was that Cape Town has low NTM exposure levels [43]. The H4:IC31–BCG revaccination study suggested that in absence of prior exposure to environmental mycobacteria (NTM) or LTBI, the immunity conferred from child BCG vaccination does not block or mask the efficacy of BCG revaccination in adults. This success of BCG revaccination opens up doors for the consideration of BCG revaccination in certain settings as part of an overall improved TB vaccination strategy.

11. The revival of BCG by changing the route of immunization

BCG vaccine shows variable efficacy against pulmonary TB when given intradermal [44]. This is a major drawback of BCG. The earliest report that suggested that BCG delivered intravenously (IV) gave superior protection than when it was delivered by other routes in rhesus macaques in 1972 [45]. Recent studies using BCG in the macaque model have shown tremendous potential against TB, when given intravenously (IV) [46]. IV BCG vaccination led to huge infiltration of T cells into the lungs compared to intradermal and aerosol vaccination route. Upon exposure of the animals to *M.tb* six months after vaccination, the presence of long-surviving T cells was still observed which could be rapidly activated upon infection, producing many 'effector' T cells. The explanation for this rapid influx and expansion of T cells could be that IV vaccination leads to the delivery of a high dose of BCG to the lung [47]. Nine out of ten macaques that received IV BCG vaccination were highly protected, with six macaques showing no detectable levels of infection [46]. Therefore this study has clearly brought BCG back in the limelight, stating that the route of BCG injection markedly affects the immunity that it confers, and the IV route provides the most powerful protection against TB (Table 2).

12. Conclusions

After almost a century after the development of BCG vaccine to combat the deadly TB disease we are approaching towards a large number of potential vaccine candidates which are in advanced stages of clinical trials. These vaccine candidates along with a large number of vaccine candidates in pre-clinical trials present the most exciting era in TB vaccine development. The recent successful clinical trials are a milestone in the efforts to develop a novel efficacious TB vaccine with protective response against both neonatal and adult TB. With the success of various subunit vaccine and BCG revaccination strategies, there is hope for the development of vaccine providing protection to adults and having long-lasting memory response. Studies involving including ours, a combination of BCG and subunit vaccine may provide a new vaccine strategy that has such high efficacy which may trigger clinical implementation internationally, which is a goal worth achieving. An effective vaccine that prevents pulmonary TB in adults could contribute significantly to the aim of reducing TB morbidity and mortality by 90% and 95%, respectively, by the year 2035.

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Table 2
TB vaccines in clinical trials.

Candidate/mode of immunization	Efficacy	T cell response	Antibody response	Antigens, vector or formulation	Clinical trial status	References
Whole cell vaccines — inactivated						
RUTI/therapeutic	NA	IFNγ-expressing $CD4^+$ T cells directed to different purified mycobacterial antigens	No changes observed in IgG responses to 16 kDa or 38 kDa antigens	Detoxified, fragmented *M.tb* cells delivered in liposomes	Phase II completed	[37]
DAR-901/prophylactic, post exposure and therapeutic	39% (95% CI 4–61%) against TB in HIV-positive Patients with $CD4^+$ count > 200 cells/μl and BCG scar	Elevated IFN-γ levels and lymphoproliferative responses to stimulation with sonicated *M. vaccae*	IgG responses to lipoarabinomannan	Whole cell, heat-inactivated *Mycobacterium obuense*, a nontuberculous mycobacterium closely related to *M. vaccae*	Phase II; phase IIb trial of DAR-901 for prevention of infection ongoing (ClinicalTrials.gov NC T02712424)	[50]
MIP/therapeutic	Not known; no efficacy against pericardial TB	Not known	Not known	Whole cell, heat-inactivated *Mycobacterium indicus pranii*	Phase III	[51]
M. vaccae-based vaccines/ therapeutic	Not known	Not reported	Not reported	Whole cell, heat-killed *M. vaccae*	Phase III results expected in 2019 (ClinicalTrials.gov NC T01979900)	[52]
Whole cell vaccines — live						
MTBVAC/prophylactic and postexposure	NA	$CD4^+$ and $CD8^+$ T cells that express IFN-γ, TNF and IL-2	Not tested	Live, attenuated *M.tb* vaccine with two independent and stable deletions in genes encoding the virulence factors phoP and fadD26	Phase II completed; phase III trial in newborn babies to commence soon	[53]
VPM1002/ prophylactic, postexposure and therapeutic	NA	$CD4^+$ and $CD8^+$ T cells expressing different combinations of IFN-γ, TNF or IL-2; unusual subset of IL-17-expressing $CD8^+$ T cells	Not reported	Recombinant BCG (BCG ΔureC::hly: expresses the listeriolysin gene to promote lysosome escape, while the urease C-encoding gene ureC, which reduces acidification of the phagosomal compartment, has been deleted)	Phase II completed; phase III trial in newborn babies to commence soon	[35]
Viral-vectored vaccines						
Ad5Ag85A/ prophylactic and postexposure	NA	$CD4^+$ and $CD8^+$ T cells expressing TH1-type cytokines	Not reported	Ag85A (Rv3804c; mycolyl transferase) and recombinant adenovirus serotype 5	Phase I/II trials ongoing (ClinicalTrials.gov NC T02337270)	[54]
MVA85A/prophylactic, prophylactic and postexposure	17.3% (95% CI – 31.9% to 48.2%) against TB disease; −3.8% (95% CI – 28.1% to 15.9%) against IGRA conversion	$CD4^+$ T cells co-expressing IFN-γ, TNF and IL-2; absent or very low $CD8^+$ T cell responses	Not reported	Ag85A (Rv3804c; mycolyl transferase) and recombinant vaccinia virus	Phase II aerosol administration trials ongoing (ClinicalTrials.gov NC T02532036)	[41]
Adjuvant protein subunit vaccine						
H4: IC31/prophylactic and postexposure	30.5% (95% CI – 15.8% to 58.3%) against sustained	$CD4^+$ T cells co-expressing TNF and IL-2 or IFN-γ, TNF and IL-2; absent or very	Not reported	Ag85B (Rv1886c; mycolyl transferase) and TB10.4 (Rv0288; ESAT family protein) and IC31 adjuvant, consisting of	Phase IIb trials completed	[55]

(*continued on next page*)

Table 2 (*continued*)

Candidate/mode of immunization	Efficacy	T cell response	Antibody response	Antigens, vector or formulation	Clinical trial status	References
	IGRA conversion	low $CD8^+$ T cell responses		positively charged peptide-based particles and the non-CpG immunostimulatory oligonucleotide ODN1a		
ID93 + GL ASE/ prophylactic, postexposure and therapeutic	NA	$CD4^+$ T cells expressing IFN-γ, TNF and IL-2; absent or very low $CD8^+$ T cell responses	High levels of IgG1 and IgG3 responses to Rv1813 (most immunogenic) as well as the other three antigens	Rv1813 (hypothesized to be a secreted protein), Rv2608 (belongs to the PE and/or PPE family of proteins), Rv3619 and Rv3620 (ESAT6 family members) and GL ASE (TLR4 agonist) in a squalene-water emulsion	Phase II trial in adults with cured TB disease completed (ClinicalTrials.gov NC T02465216)	[56]
H56:IC31/prophylactic, postexposure and therapeutic	NA	$CD4^+$ T cells co-expressing TNF and IL-2 or IFN-γ, TNF and IL-2. In *M.tb*-infected individuals, IFNγ, TNF and IL-2 co-expressing $CD4^+$ T cells; absent or very low $CD8^+$ T cell responses	Not reported	Ag85B (Rv1886c; mycolyl transferase), ESAT6 (Rv3875; ESAT family protein) and Rv2660c (hypothesized to be stress-related protein and IC31 adjuvant)	Phase II prevention of TB recurrence trial ongoing (ClinicalTrials.gov NC T03512249)	[57]
M72/AS01E/booster, prophylactic and postexposure	54.0% (95% CI 13.9–75.4%) against pulmonary TB in IGRA positive adults	Efficient induction of $CD4^+$ T cells co-expressing IFNγ, TNF and IL-2; detectable $CD8^+$ T cell responses	High-level antigen-specific IgG	*M.tb*39A (serine protease), *M.tb*32A adjuvant consisting of liposomes, monophosphoryl lipid A and Quillaja saponaria fraction	Phase IIb ongoing (ClinicalTrials.gov NC T01755598)	[58]

Declaration of competing interest

The authors declare no competing financial interests.

Author contribution

SF collected the literature. SF, AK, GD and VPD hypothesized and wrote the manuscript.

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