Tuberculosis xxx (2014) 1–8

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

REVIEW

The present and future of tuberculosis vaccinations

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ARTICLE INFO

Article history:
Received 18 August 2014
Accepted 13 October 2014

Keywords:
BCG vaccine
Mycobacterium tuberculosis
Prevention
TB
Tuberculosis
Vaccine

SUMMARY

The clinical, social, and economic burden of tuberculosis (TB) remains high worldwide, thereby highlighting the importance of TB prevention. The bacilli Calmette-Guérin (BCG) vaccine that is currently available can protect younger children but is less effective in adults, the major source of TB transmission. In addition, the emergence of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains and the high prevalence of HIV infection have significantly complicated TB prognosis and treatment. Together, these data highlight the need for new and more effective vaccines. Recently, several vaccines containing multiple antigens, including some of those specific for dormant *Mtb* strains, have been developed. These vaccines appear to be the best approach for satisfactory *Mtb* prevention. However, until a new vaccine is proven more effective and safe than BCG, BCG should remain part of the immunization schedules for neonates and children at risk for TB as a fundamental prophylactic measure.

1. Introduction

The global clinical, social, and economic burden of tuberculosis (TB) remains high despite the recent World Health Organization (WHO) TB report indicating that major progress has been made towards the global reduction of the TB burden [1]. There were an estimated 8.6 million new TB cases in 2012 with 1.3 million TB deaths. Most new TB cases and deaths occur among men; however, an estimated 2.9 million cases and 410,000 deaths occurred among women and 530,000 cases and 74,000 deaths among children. More than 80% of the world's TB cases occurs in Southeast Asia, the west Pacific, and Africa [1]. Nonetheless, Europe and the Americas had 79,279 and 63,629 new TB cases, respectively, despite having a generally higher income and more advanced health systems [1]. In addition, the emergence of drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains and the high prevalence of HIV infection have significantly complicated TB prognosis and treatment [2,3]. Together, these data highlight the importance of effective TB prevention and the limits of the available bacilli Calmette-Guérin (BCG) vaccine. This paper summarizes the current status of TB vaccine prevention discussing the attempts to develop new preparations more safe and effective than the traditional BCG vaccine.

PubMed was used to search for all of the studies published over the last 15 years using the key words: “bacillus Calmette Guerin” or “tuberculosis vaccine” or “tuberculosis prevention” or “tuberculosis vaccine therapy” and “children” or “adolescent”. More than 150 articles were found, but only those published in English were included in the evaluation.

2. The BCG vaccine

The BCG vaccine is the only licensed TB vaccine. It is available as an intradermal vaccine that utilizes live attenuated *Mycobacterium bovis* bacillus and was developed without any immunological insight almost a century ago. Today, the BCG vaccine is part of immunization schedules in many countries. Since 1974, the BCG vaccine has been administered to neonates in high-risk TB populations as part of the WHO Expanded Program on Immunization [4]. However, a number of epidemiological studies and controlled clinical trials have shown that the BCG vaccine has several limitations [5]. The BCG vaccine induces an immune response that can prevent miliary and meningeal TB but cannot prevent or eliminate TB infection. The vaccine's efficacy depends on several factors such as: patient age, TB localization, the geographic area in which the vaccine is administered, previous sensitization to mycobacteria, and the patient's immune status. The protection conferred by the BCG vaccine is significantly greater when the vaccine is administered to neonates or school children [6,7] and for miliary or meningeal TB [5]. Protection against pulmonary TB, which accounts for the majority of TB mortality and morbidity worldwide, is

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http://dx.doi.org/10.1016/j.tube.2014.10.004
1472-9792/

significantly lower and clearly age dependent [6,7]. In children, protection against pulmonary TB can reach up to 80% [5]; however, only 50% of adults are protected, and some studies have reported no real preventive effects [8,9]. In addition, previous exposure to environmental mycobacteria appears to be an important limiting factor for the BCG vaccine [8,9]. Subjects with latent TB infection are less protected and the vaccine' efficacy has been shown to be significantly lower in subjects living in countries near the equator where exposure to environmental mycobacteria is more common. Moreover, BCG vaccination can lead to adverse events that, even if local and mild in most of the cases, can be sometimes severe. Incidence of fatal complications, such as osteitis and disseminated BCG infection, usually occur in immunosuppressed subjects [10], and ranges from 0.01 to 43.4 cases per million vaccine's recipients [11]. Finally, an important limit of the BCG vaccine is its inability to induce long-term protection, even when booster doses are administered. Subjects who receive the vaccine soon after birth are not protected during adolescence and adulthood [12]. Thus, the BCG vaccine can protect against TB in younger children but not adults, the major source of TB transmission. Consequently, the performance of the BCG vaccine is far from satisfactory, and this explains why BCG vaccination has been discontinued or has been limited to children at risk in a growing number of countries with low to intermediate TB prevalence. It has been calculated that in settings with prevalence levels below 5 sputum smear positive per 100,000, universal BCG vaccination might lead to an excess of adverse events per case prevented [11]. Consequently, the development of new vaccines more effective and safe than the BCG vaccine is considered a priority.

3. Host immunity against TB and new vaccine development

For many years, cell-mediated immunity, particularly interferon γ (IFNγ) production by $CD4^+$ T cells, has been thought to be the most important factor in host immunity against TB [13–15]. In contrast, the role of antibody-mediated immunity in host defense against TB has been considered marginal since no correlations between antibody titer against mycobacterial antigens during TB and protection or disease severity have been observed [16]. IFNγ has been shown to stimulate macrophage, which kill intracellular *Mt* by activating downstream antimicrobial effector pathways [16]. Both experimental and clinical data support this central role for IFNγ in host defense against TB. Mice that lack IFNγ are extremely susceptible to TB infection [17], and patients who have genetic mutations for the interleukin-12-IFNγ-signal transducer and activator of transcription 1 have an increased risk for disseminated infections due to BCG or other non-tuberculosis mycobacteria [18]. In addition, patients with impaired T-cell responses, such as those who are HIV infected [19] or receiving tumor necrosis factor blockers [20], frequently suffer from severe TB. Thus, attempts to develop new TB vaccines have tried to evoke robust cellular Th1 immune responses with IFNγ secretion [21,22].

Vaccines designers have adopted three different approaches. The first approach replaces the BCG antigen of the BCG vaccine to confer longer protection with increased safety in immunocompromised patients. The second approach prepares vaccines capable of boosting the immune response in subjects who have previously receiving the BCG vaccine. The third approach studies novel therapeutic TB vaccines, i.e. preparations that could increase the effect of pharmacotherapy for active TB. Several strategies for inducing immune responses using several vaccine preparations have been examined. Vaccine preparations for clinical trials have used inactivated whole cell or whole cell extracts, modified mycobacteria, viral vectored *Mtb* genes, fusion protein subunits with Th1-inducing adjuvants, and live recombinant BCG or attenuated *Mtb*. The most important data regarding these vaccines are reviewed in the following sections.

4. New vaccines for tuberculosis

4.1. BCG replacements

4.1.1. Recombinant BCG strains

The first recombinant BCG vaccine is rBCG30; rBCG30 was constructed by inserting the plasmid pMBT30 into BCG, resulting in the overexpression of the 30-kDA protein α antigen or antigen 85b (Ag85b) [23]. This protein is a mycolyl transferase and is the most abundant protein secreted by *Mtb* into broth culture and released into the *Mtb* phagosome in infected human macrophages [24–26]. The immunogenicity and efficacy of rBCG30 have been evaluated in experimental studies. The immune response and protection induced by rBCG30 has been shown to be significantly greater in animals that have received this vaccine compared to those that received the BCG vaccine [26]. Moreover, rBCG30 has been found to be equally safe and well tolerated as the BCG vaccine and also has similar antibiotic susceptibility [27].

In a phase 1 clinical trial of tuberculin-negative adults, rBCG30 was shown to be safe and immunogenic [28]. The vaccine was able to induce increased Ag85b-specific T cell lymphoproliferation, IFNγ secretion, IFNγ enzyme-linked immunospot responses, and direct *ex vivo* intracellular IFNγ responses [28]. Moreover, it significantly enhanced the population of Ag85b-specific $CD4^+$ and $CD8^+$ T cells that were capable of concurrent expansion and effector function. Importantly, rBCG30 significantly increased the number of Ag85b-specific T cells that were capable of inhibiting intracellular mycobacteria. However, despite these promising results, rBCG30 was not pursued further.

The second recombinant BCG vaccine is VPM1002 [29], which is based on live *Mtb* with two modifications. The gene encoding for listeriolysin (Hly) of *Listeria monocytogenes* was integrated into the BCG genome. Hly is responsible for pore formation in the macrophage phagosome after endocytotic pathogen uptake, which allows the bacterium to move into the cytosol. In contrast to BCG, which is trapped inside the phagosome and consequently enters the MHC II pathway, VPM1002 allows BCG to move into the cytosol, which results in MHC I presentation of its peptides [29]. Because MHC I presentation and subsequent $CD8^+$ T cell induction resembles the natural mechanism of protection against *Mtb*, it was thought that this change could improve the induction of host immunity. The second change in VPM1002 was the inactivation of the BCG gene for urease C (ureC). Urease C catalyzes the hydrolysis of urea, leading to ammonia production and a basic environment. Inactivating urease C was necessary since Hly activity is optimal under acidic conditions. During these modifications, hygromycin resistance was incorporated as a selection marker.

The immunogenicity, safety and tolerability of VPM1002 have primarily been tested in animals, including neonates, with very encouraging results [29]. Compared to the BCG vaccine, VPM1002 evoked superior protection due to its abilities of stimulating both type 1 and type 17 cytokine responses [30] and of generating apoptotic vesicles in infected macrophages, which induce more profound CD4 and CD8 T cell responses [31]. In human trials, VPM1002 has been found to be immunogenic (as measured by IFNγ production), safe, and well tolerated in adults. The vaccine was able to induce multifunctional $CD4^+$ and $CD8^+$ T cell subsets and exhibited a trend of being better than a comparable dosage of BCG [32]. A phase 2 study to evaluate the immunogenicity and safety of VPM1002 compared to the BCG vaccine in newborn infants in South Africa has been conducted, but results have not yet been published.

Please cite this article in press as: Principi N, Esposito S, The present and future of tuberculosis vaccinations, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.10.004

Other authors combined the approaches used for rBCG30 and VPM1002 [22,33]. Unfortunately, this vaccine had several problems. They used a BCG strain that was genetically modified to overexpress three proteins (Ag85A, Ag85B, and Rv3407) and to produce perfringolysin O, an enzyme from *Clostridium perfrigens* that, like Hly, can perforate the cell's encapsulating membrane and allow BCG proteins to enter the cytosol. Despite favorable results in animals [22], the administration of this vaccine to humans has recently been halted due to safety concerns in a phase I clinical trial in the US. Two individuals who received the vaccine developed shingles, which is caused by reactivation of dormant varicella-zoster virus; these shingles cases were ascribed to perfringolysin O activity [33].

4.1.2. Attenuated Mtb strains

MTBVAC01 is the only vaccine based on an attenuated *Mt* strain of human origin in phase 1 clinical trials [33,34]. This vaccine should be both safer and more effective than the BCG vaccine. The *PhoP* and *fadD26* genes of the *Mtb* strain were inactivated to increase safety. This prevents the secretion of ESAT-6, the most important *Mtb* virulence factor [34], and reduces the synthesis of phtiocerol dimycocerosates, a component of cell envelope that protects *Mtb* from host defenses [35]. MTBVAC01 does not produce complex lipids regulated by *PhoP*, which interfere with the host immune response [35]. Furthermore, the antigenic properties of MTBVAC01 are enhanced by silencing Mcr7, an antisense RNA that regulates the secretion of the twin arginine translocation (TAT) protein of *Mtb* [35]. Silencing Mcr7 results in the secretion of TAT substrates, which include the Ag85 family; these substrates are considered the most important *Mtb* antigens, and increasing the exposure of these antigens to the immune system is good for developing the host immune response.

MTBVAC01 can be considered the next iteration of the live attenuated *Mtb* vaccine SO_2, which inserted a kanamycin-resistance cassette into *PhoP* [36]. Ten years of rigorous preclinical testing has generated robust data for the safety and improved immunogenicity and protective efficacy of SO_2 compared to BCG in animals, including those with severe combined immunodeficiency [37–40]. In mice, SO_2 was able to induce more differentiation of antigen-specific $CD4^+$ T cells into central memory T cells, which are correlated with longer vaccine efficacy [41]. Despite these promising results, the Geneva consensus for new live mycobacterial vaccines requires the presence of two stable independent mutations without antibiotic-resistance markers for *Mtb*-based vaccine candidates; thus, SO_2 was considered unsuitable for clinical trials [42]. However, data on MTBVAC01 has shown that it is functionally comparable to SO_2. Thus, data generated in preclinical studies of SO_2 were accepted by the Swiss Regulatory Authority (Swissmedic) as support for entering MTBVAC01 phase 1 clinical trials [42].

4.1.3. Engineered mycobacteria

Mice can become infected by mycobacteria containing the esx-3 region, an evolutionarily conserved set of genes that allow bacterium to evade bacterial killing by the innate immune response. Mice infected with a *Mycobacterium smegmatis* Δesx-3 mutant were able to control and clear the infection via a MyD88-dependent bactericidal immune response. This Δesx-3 mutant was called IKEPLUS [43]. IKEPLUS remained susceptible to the innate immune response, was highly attenuated in mice, and was able to stimulate bactericidal immunity against challenges with virulent *Mtb*. This highly protective bactericidal immunity elicited by IKEPLUS was dependent on $CD4^+$ memory T cells and involved a distinct shift in the pattern of $CD4^+$ cell cytokine response [43]. Thus, the esx-3 locus was shown to promote mycobacterial virulence, and IKEPLUS may be a potentially powerful vector for *Mt* vaccines.

4.1.4. Mycobacterium vaccae

Mycobacterium vaccae (*Mv*) is a saprophytic *Mycobacterium* that contains a vast array of antigenic epitopes common to *Mt* such as the heat shock proteins (Hsp; Hsp71, Hsp65, and lipoarabinomannan [LAM]) and some low molecular weight (<40 kDa) secreted antigens [44]. An inactivated whole cell vaccine of *Mv* was prepared since animals that were previously sensitized by soluble *Mtb* and *Mv* antigens exhibited similar delayed hypersensitivity responses [44]. The *Mv* vaccine was initially used as a therapeutic TB vaccine in combination with drug therapy, but it had debatable results and a risk of adverse events that was not marginal [45]

More recently, the *Mv* vaccine has been used as a prophylactic vaccine for TB [46]. In a phase III clinical trial, the *Mv* vaccine was shown to be immunogenic and to confer protection against HIV-associated TB in adults previously immunized with the BCG vaccine who had CD4 counts ≥200 cells/μL [46]. Furthermore, the vaccine was not found to have any risk of significant adverse events. The immunogenicity of the vaccine was evaluated using an IFNγ enzyme-linked immunosorbent assay, a tritiated thymidine lymphocyte proliferation assay, and antibodies to the TB LAM glycolipid. However, post-immunization immune responses did not predict protection from HIV-associated TB [46].

4.2. Booster vaccines

4.2.1. Viral vectored vaccines

Aeras 402/Crucell Ad35 is a vaccine candidate that consists of a replication-deficient adenovirus (Ad35) vector that contains the *Mt* antigens 85A, 85B, and TB10.4 [47–49]. The Ad35 vector was selected because there are relatively little preexisting neutralizing antibodies against this adenovirus in people living in developing countries [47]; furthermore, adeno-vectored vaccines consistently induce high levels of $CD8^+$ T cell responses [48]. Aeras 402 was shown to significantly protect mice against lung challenges of *Mtb* [49]. Similar results were observed in humans by two studies of BCG-vaccinated adults living in areas with different TB endemicity (South Africa [50] and the US [51]). Both studies showed that Aeras 402 could safely induce multifunctional $CD4^+$ and $CD8^+$ T cell responses specific for Ag85A, Ag85B, and TB10.4; in both cases, boosting was more remarkable for $CD8^+$ T cells. The $CD8^+$ T cell response was fiftyfold higher than that at baseline, which was the highest magnitude response seen for any previous TB vaccine candidate. Clinical trials of Aeras 402 in children remain ongoing.

An aerosolized formulation of Aeras 402 has also been evaluated [52]. Aeras 402 was delivered to the lungs of nonhuman primates, which led to lung modifications consistent with the induction of an immune response. Furthermore, a transient, vaccine-specific immune response was observed in the peripheral blood as well as sustained high-level polyfunctional $CD4^+$ and $CD8^+$ T cell responses in the bronchoalveolar lavage fluid. These data suggest that aerosolized delivery of Ad35-based vaccine can be safe and induce potent lung-specific immunity [52].

MVA85A is a recombinant strain of the modified Vaccinia Ankara virus that expresses the immunodominant *Mtb* protein antigen 85A. MVA85A was developed as a heterologous boost for the BCG vaccine in 2002 [53]. Boosting the BCG vaccine with MVA85A improved BCG-induced protection against mycobacterial challenge in several animal studies [39,54,55]. Currently, MVA85A is the *Mtb* vaccine with the most studies in humans. Several clinical trials have shown that MVA85A is safe and well tolerated. However, data on MVA85A immunogenicity and efficacy are conflicting. The first study of MVA85A in humans found that that MVA85A was highly immunogenic in all vaccinated subjects; however, the $CD4^+$ T cell response was significantly higher in BCG-vaccinated subjects

Please cite this article in press as: Principi N, Esposito S, The present and future of tuberculosis vaccinations, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.10.004

than in BCG-naive subjects [53]. This difference was not observed in further studies in Africa, which suggests that the cumulative mycobacterial immunity in adults living in tropical climates was significantly lower than that in adults living in more temperate climates; this may be due to higher exposure to mycobacteria antigens in these tropical climates [56]. Nonetheless, the vaccine-induced responses were higher than baseline in adults, adolescents, and children, although the vaccine's efficacy in children was disappointing. A double-blind, randomized, placebo-controlled phase 2b trial in South Africa enrolled 2797 healthy infants (aged 4–6 months) without HIV infection who had previously received the BCG vaccine [57]. The children were randomly allocated to receive one intradermal dose of MVA85A or an equal volume of placebo and were then actively followed every 3 months for up to 37 months. In this study, the safety of the vaccine was good although more children who received the vaccine experienced local reactions than those who received the placebo. Overall, 32 (2%) of 1399 MVA85A recipients met the primary efficacy endpoint (TB incidence of 1.15 per 100 person-years [95% confidence interval [CI]: 0.79 to 1.62]; with conversion in 178 [13%] of 1398 infants [95% CI: 11.0 to 14.6]) as did 39 (3%) of 1395 controls (1.39 per 100 person-years [95% CI: 1.00 to 1.91]; with conversion in 171 [12%] of 1394 infants [95% CI: 10.6 to 14.1]). Efficacy against TB was 17.3% (95% CI: −31.9–48.2) and against *Mtb* infection was −3.8% (95% CI: −28.1–15.9).

4.2.2. Protein-adjuvanted vaccines

M72/MTB72F is the most advanced adjuvanted subunit vaccine for TB. M72 contains a fusion protein of the *Mtb* antigens 32A and 39A [58–60]. It has been delivered with AS01 (combination of liposomes, MPL and QS21) and AS02 (oil-in water emulsion) as adjuvants, and results suggest that formulation with AS01 induced the highest vaccine-specific responses [58]. Several phase II studies have shown that M72/AS01 has moderate local reactogenicity without serious adverse events [59,60]. Phase I/IIa trials have also found that M72/AS01 has an acceptable safety profile and stimulates both $CD8^+$ and $CD4^+$ T cell responses. In a phase IIa trial, M72/AS01 triggered a T cell response separate from the typical Th1 and Th17 responses observed in other vaccine candidates in 45 *Mtb*-infected and -uninfected adults in South Africa [59]. These novel T cell populations appeared to include Treg cells, which may mediate the inflammation induced by Th1 and Th17 cytokines. T cell counts after M72/AS01 administration were higher in *Mtb*-infected participants than in *Mtb*-uninfected individuals. These results have justified currently ongoing or recently completed phase IIa studies designed to evaluate the safety and immunogenicity of M72/AS01 in: infants in Gambia; HIV-positive adults in Chennai, India; and adults with TB in Taiwan and Estonia [60]. Moreover, a phase IIb study has been planned to evaluate the vaccine's effectiveness for preventing *Mt* infection; this trial will be the largest adult trial of a novel TB vaccine and aims to enroll 4500 HIV-negative adult volunteers in TB endemic communities in sub-Saharan Africa [60].

HyVac4/AERAS-404 (SSI/SP H4-IC31), Hybrid 1/IC31, Hybrid 1/CAF01, and SSI H56-IC31 are a series of TB vaccines that have been developed using protein subunits of different *Mtb* antigens and fusion molecules [61,62]. These preparations have been paired with several different adjuvants.

Hybrid 1 contains the *Mtb* antigens 85B and ESAT6 and has been studied in combination with either IC31 (a combination of an antibacterial peptide and a Toll-like receptor 9 agonist) or CAF01 (a synthetic glycolipid trehalose-dibehenate that is incorporated into cationic dimethyldioctadecylammonium bromide liposomes) as adjuvants [61,62]. Hybrid 1/IC31 has been shown to induce memory T cell responses that were maintained over 2.5 years of follow-up in BCG-naïve volunteers [61]. Moreover, the vaccine enhanced TB-specific immune responses in a study that enrolled individuals who had received the BCG vaccination or had been previously infected with TB [61]. A phase IIa trial of Hybrid 1/IC31 is currently being planned. However, the inclusion of ESAT6 in the vaccine has the potential to confound the new generation of diagnostic tests for latent TB. In one study, 17% of the subjects who received a high dose of Hybrid 1/IC31 were positive by Quantiferon Gold and remained positive more than 2 years later [62].

To address this problem, ESAT6 has been replaced with the antigen 10.4. One such vaccine is HyVac4/AERAS-404, which is also referred to as SSI/SP H4-IC31; this vaccine uses the H4 antigen (a fusion protein of 85B and 10.4) and has been tested before and after TB exposure in preclinical animal models [63]. HyVac4/AERAS-404 is currently being evaluated for TB prevention in infants and adolescents who have received the BCG vaccine.

ID93/GLA-SE is another protein-adjuvanted vaccine that uses ID93, a fusion protein comprised of four *Mtb* antigens (RV2608, RV3619, RV3620, and RV1813), and GLA-SE, an adjuvant (glucopyrasonyl lipid-stable emulsion). This vaccine has been shown to be safe and immunogenic in both BCG-vaccinated and -unvaccinated animals; in these animals, the vaccine was able to evoke $CD4^+$ T cell production of tumor necrosis factor (TNF)α and interleukin (IL) 2 [64]. ID93/GLA-SE was also able to elicit $CD4^+$ and $CD8^+$ T cell responses in human peripheral mononuclear cells [65].

The Hybrid 56 vaccine uses an antigen contained in dormant *Mtb*, which is the most difficult *Mtb* strain to eradicate. Hybrid 56 has recently entered clinical trials [65,66]. The antigen used in Hybrid 56 is poorly immunogenic alone; however, when administered in conjunction with ESAT6 and Ag85B, it is able to induce protective immune responses in experimental animals. Moreover, in nonhuman primate models, Hybrid 56 has been shown to control TB reactivation after animals received the BCG vaccine [66]. Another multistage booster vaccine that contains antigens expressed by *Mtb* (Rv2608, TV3619, Rv3620, and RV1813) is also currently being studied [65].

4.3. Therapeutic vaccines

Several therapeutic TB vaccines, including *Mv*, have been developed; however, only some have entered clinical trials [45]. The most widely studied therapeutic TB vaccine is the RUTI vaccine. RUTI is comprised of detoxified and liposomal cellular *Mt* fragments, which are cultured under stress conditions (intra-granulomatous conditions) to induce latency antigens that are normally hidden from the immune system [67]. Moreover, RUTI is detoxified to decrease the risk of an exacerbated immune response as well as fragmented to facilitate the processing and presentation of cell wall antigens. RUTI contains very low levels of LAM, an endotoxin-like molecule that has been implicated in intra-granulomatous necrosis. RUTI is delivered via liposomes to warrant the homogeneity of the preparation and likely promotes access to intracellular compartments, resulting in MHC class I presentation to $CD8^+$ T cells. Studies in animals and in humans have reported that RUTI is safe, and only mild to moderate local adverse events have been reported [68]. RUTI has been shown to induce a poly-antigenic response against *Mt* antigens. A phase III trial is planned to analyze the protective effect of one 25 μg RUTI vaccination in HIV-positive subjects after standard latent TB treatment has been administered.

5. Criticisms of candidate TB vaccines

Table 1 summarizes the main characteristics of the TB vaccines under development. This global evaluation of current data on the safety, tolerability, and of TB vaccine candidates indicates that a substitute or booster for the BCG vaccine is not yet available. The

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Table 1
Main characteristics of the TB vaccines under development.

Type of vaccine	Name	Characteristics and composition
Recombinant BCG strains	rBCG30	Overexpression of Ag85b
	VPM1002	Deletion in urease gene and expression of listerysin to promote phagosome lysis
	AERAS422	Expression of perfringolysin and several *Mtb* antigens
Attenuated Mtb	MTBVAC	Inactivation of PhoP and fadD26 genes
Engineered Mycobacteria	IKEPLUS	Mutation in *Mycobacterium smegmatis*
Mycobacterium vaccae	*Mycobacterium vaccae*	A strain containing a vast array of antigenic epitopes common to *Mtb*
Viral vectored vaccines	AERAS-402	Replication-deficient adenovirus expressing Ag85a, Ag85b and TB10.4
	MVA85A	Vaccinia virus Ankara expressing Ag85a
Protein-adjuvanted vaccines	MTB72F	Fusion protein of the *Mtb* antigen 32A and 39A
	Hybrid 1	*Mtb* antigens 85B and ESAT6 combined with IC31 or CAF01
	AERAS-404	Fusion protein of *Mtb* Ag85b and TB10.4 antigens
	ID93	Fusion protein of RV2608, RV3619, RV3620 and RV1813 antigens and GLA-SE
	Hybrid 56	Fusion protein of Ag85b,ESAT6 and Rv2660c
Therapeutic vaccines	RUTI	Liposomes containing detoxified fragmented *Mtb* cells

studies that have been performed to date are limited by a poor understanding of the specific human immune response to *Mtb* infection and the mechanisms that lead to TB; furthermore, there are no validated animal models or immunological correlates of protection that can predict vaccine efficacy in humans with certainty [69]. Moreover, clinical trials to obtain reliable data on vaccine efficacy require thousands of participants in multiple regions with different mycobacteria exposure. Finally, some study results were unexpected and completely unsatisfactory. The new TB vaccines were frequently shown to be highly immunogenic but clinically ineffective. These results were slightly inconsistent with the theory that the T cell response and IFNγ secretion are the primary host defense to *Mt* infection and TB disease. The best example of this was the South African study of MVA85A, which enrolled healthy infants who had received the BCG vaccine [57]. In this study, a positive CD4 response and high IFNγ levels was observed in the vaccinated children but not in children who received the placebo; however, these effects were not accompanied by efficacy against *Mtb* infection or TB. The inefficiency of this promising vaccine was considered an indicator that T cells and IFNγ may not be sufficient for preventing active TB despite being essential parts of the host immune response against *Mtb*. Studies in experimental animals have supported this conclusion. Knockout animals that lack different cytokines, such as TNF, IL1, IL 6, or granulocyte-macrophage colony-stimulating factor (GM-CSF), but still produce adequate IFNγ levels are extremely susceptible to *Mtb* infection; if infected, these animals die as rapidly as IFNγ-deficient animals [70–72]. Furthermore, $CD4^+$ cells have been shown to protect mice against *Mtb* independently of IFNγ, which suggests that T cell functions besides IFNγ production are able to defend against *Mtb*. It has been hypothesized that a balance between IFNγ and other cytokines, including Th2 cytokines, can influence disease outcome [73]. In humans, progression from *Mtb* infection to active disease is directly associated with the number of IFNγ-secreting T cells as well as IFNγ levels [74]. Thus, the immune response elicited by immune-dominant *Mtb* antigens could be beneficial for not only the host but also for direct pathogen-induced lung tissue destruction and cavitation, which facilitates the spread of bacilli and contributes to TB transmission [75].

Since studies have shown that IFNγ and $CD4^+$ cells are not the only factors that host defenses depend on to fight *Mtb*, attempts have been made to identify other immune mechanisms that play a major role in TB defense. Several other factors of cell-mediated immunity have been identified. The loss of TNF, GM-CSF, and IL1 β have all been reported to increase susceptibility to *Mtb* infection and TB disease [76–79]. Moreover, cytotoxic T lymphocytes ($CD8^+$ T cells) have been shown to kill *Mtb* via different molecular pathways such as exocytosing cytotoxic proteins (e.g., perforin) and expressing factors like CD95, which mediates apoptosis and TNFα secretion [80–83]. Finally, anti-inflammatory signals and immune cells besides $CD4^+$ and $CD8^+$ have been indicated in host defense against *Mtb*. $CD4^+CD25^+$ regulatory T cells suppress inflammation and limit the immune response by producing immunosuppressive cytokines, such as IL10 and transforming growth factor [84]. Factors that mediate T cell exhaustion after long-term antigen stimulation, which occurs in TB, have been identified; suppressing these factors has been associated with an increase in the number and function of *Mtb*-specific T cells [85]. Invariant natural killer T cells have also been reported to be significantly lower in infected individuals, and increasing their numbers to normal concentrations has been associated with improved treatment outcome and greater synergy with antibiotics [86.87]. Other cells, such as CD1-restricted T cells, γδ T cells, MAIT cells, and Th17 cells, also clearly influence the immune response against *Mtb*, but their specific roles have not been completely defined [88–91].

Humoral immunity has also attracted new interest. Even if many antibodies elicited by *Mtb* are nonfunctional or enhance the inflammatory response, various studies have suggested that certain antibodies are protective against TB [92]. IgG-bound BCG can increase both anti-*Mt* macrophage activity and *Mtb*-specific cell-mediated immunity. Moreover, it can promote the clearance of cell wall immunomodulatory antigens, such as lipoarabinomannan, and modulate inflammation [93]. IgM can also activate the complement system [94], whereas IgG has both pro- or anti-inflammatory properties depending on the antigen and receptor it engages [95].

6. Future perspectives

In the near future, the results of the current ongoing clinical trials for several TB vaccine candidates will become readily available. Hopefully, one or more of these vaccines will prove to be effective and can either replace the BCG vaccine or be used as booster in individuals who have already received the BCG vaccine. However, even if the results of these studies are unsatisfactory, these studies will provide insight into the development of better candidates. Currently, it seems clear that an approach based on only inducing CD4+ and CD8+ cells has limitations. However, the development of an effective TB vaccine is very complex. An effective vaccine required long, protracted and costly clinical trials to evaluate vaccine efficacy, and no correlate or surrogate of protective immunity is available. Thus, the efficacy of each new TB vaccine addresses new hypotheses rather than simply pursuing an approach that has already failed or presented debatable results. It has recently been suggested that characterized *Mtb* surface constituents, particularly the proteins involved in the early stages of infection, will lead to the development of better vaccines against *Mtb* infection. *Mtb* reportedly enters macrophages via the CR3 receptor, which increases bacterial survival [21]. Identifying bacterial components involved in this receptor-mediated entrance may offer new insights into developing antibody-based strategies. This could be accomplished by determining whether *Mtb* surface components are able to evoke protective antibodies in TB patients who remain uninfected despite prolonged exposure. More effective priming or

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booster vaccines will likely be multicomponent vaccines that include proteins that play a role against both active and dormant *Mtb*; these vaccines would limit both the development of active TB and the damage caused by more dangerous active strains. Multivalent TB vaccines that target the esx family are another interesting possibility; the esx antigens are thought to induce a broad-spectrum immune response. A recent vaccine based on 15 esx antigens that were representative of all esx family members was found to elicit a substantial, broad Ag-specific IFNγ response and multifunctional CD4+ and CD8+ T cell responses [96]. Moreover, new laboratory methods will improve studies of vaccine efficacy *in vitro* and *in vivo*. Currently, *Mtb* is grown in liquid culture, which contains detergent to limit bacterial clamping. Antibody-based vaccines should be assessed using bacteria with a well-preserved surface to better mimic what happens *in vivo*. Finally, experimental animals that are exposed to *Mtb* are currently exposed to a single dose with a relatively high *Mtb* concentration; these experiments should be conducted using multiple, consecutive lower doses, which is typically observed in humans.

7. Conclusions

A better understanding of the immune response to *Mtb* infection in humans indicates that multiple mechanisms are involved in host attempts to eliminate *Mtb* and prevent TB development. This explains why vaccines that evoke only a partial immune response have such low efficacy. Unfortunately, what constitutes protective immunity against *Mt* has yet to be precisely defined and remains the most important limitation for TB vaccine development. In particular, why only a minority of infected individuals develop TB remains unknown. *Mtb* also persists in infected subjects but remains dormant unless immunodeficiency occurs. This seems to indicate that the immune system can control but not eliminate *Mtb* after infection, and in most cases, the latent pathogen is unable to become activated and lead to TB. Identifying the immune mechanisms that act on the different stages of *Mtb* infection appears to be essential to vaccine development. Given the complexity of the immune response induced by *Mtb*, it is reasonable to imagine that vaccines containing antigens that evoke simple immune response will have poor efficacy.

In the past decade, impressive strides have been made in the development of new TB vaccines. Several vaccine candidates are currently being studied. However, TB vaccination still faces significant obstacles. There have been several attempts to develop vaccines that are able to prevent *Mtb* infection, reduce the risk of TB development, and have therapeutic ability; however, no vaccine to date can be considered more safe and effective than the BCG vaccine. A definitive TB vaccine is likely far off despite more refined vaccine attempts in recent years that have been based on a better understanding of the host immune response to *Mtb*. This improved understanding has led to the development of vaccines containing multiple antigens. However, until a new vaccine has been proven to be more effective and safe than the BCG vaccine, the BCG vaccine should be maintained in immunization schedules for neonates and children at risk as a fundamental prophylactic measure.

Funding

This study was supported by a grant from the Italian Ministry of Health (Bando Giovani Ricercatori 2009) GR-2009-1596786.

Competing interests

The authors have no conflict of interest to declare.

Ethical approval

This review was approved by the Ethical Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy and all the papers included in this review have been approved by local Ethical Committees.

References

[1] World Health Organization. Global tuberculosis report. 2013.
[2] Günther G. Multidrug-resistant and extensively drug-resistant tuberculosis: a review of current concepts and future challenges. Clin Med 2014;14:279–85.
[3] Davies PD. The world-wide increase in tuberculosis: how demographic changes, HIV infection and increasing numbers in poverty are increasing tuberculosis. Ann Med 2003;35:235–43.
[4] Brenzel L, Claquin P. Immunization programs and their costs. Soc Sci Med 1994;39:527–36.
[5] Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis 2014;58:470–80.
[6] Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E, et al. The efficacy of bacillus Calmette-Guérin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. Pediatrics 1995;96:29–35.
[7] Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA 1994;271:698–702.
[8] No authors listed. Trial of BCG vaccines in south India for tuberculosis prevention: first report 144. Bull World Health Organ 1979;57:819–27.
[9] Fine PE. BCG vaccination against tuberculosis and leprosy. Br Med Bull 1988;44:691–703.
[10] Hesseling AC, Marais BJ, Gie RP, Schaaf HS, Fine PE, Godfrey-Faussett P, et al. The risk of disseminated Bacille Calmette-Guerin (BCG) disease in HIV-infected children. Vaccine 2007;25:14–8.
[11] Manissero D, Lopalco PL, Levy-Bruhl D, Ciofi Degli Atti ML, Giesecke J. Assessing the impact of different BCG vaccination strategies on severe childhood TB in low-intermediate prevalence settings. Vaccine 2008;26:2253–9.
[12] Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet 2005;366:1290–5.
[13] North RJ. Importance of thymus-derived lymphocytes in cell-mediated immunity to infection. Cell Immunol 1973;7:166–76.
[14] Orme IM, Roberts AD, Griffin JP, Abrams JS. Cytokine secretion by CD4 T lymphocytes acquired in response to Mycobacterium tuberculosis infection. J Immunol 1993;151:518–25.
[15] Shimokata K, Kishimoto H, Takagi E, Tsunekawa H. Determination of the T-cell subset producing gamma-interferon in tuberculous pleural effusion. Microbiol Immunol 1986;30:353–61.
[16] Herbst S, Schaible UE, Schneider BE. Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. PLoS One 2011;6:e19105.
[17] Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme M. Disseminated tuberculosis in Interferon γ gene-disrupted mice. J Exp Med 1983;178:2243–7.
[18] Rosenzweig SD, Holland SM. Defects in the interferon-gamma and interleukin-12 pathways. Immunol Rev 2005;203:38–47.
[19] Pawlowski A, Jansson M, Sköld M, Rottenberg ME, Källenius G. Tuberculosis and HIV co-infection. PLoS Pathog 2012;8:e1002464.
[20] Wallis RS. Infectious complications of tumor necrosis factor blockade. Curr Opin Infect Dis 2009;22:403–9.
[21] Delogu G, Manganelli R, Brennan MJ. Critical research concepts in tuberculosis vaccine development. Clin Microbiol Infect 2014;20(Suppl. 5):59–65.
[22] Kaufmann SHE. Tuberculosis vaccine development: strength lies in tenacity. Trends Immunol 2012;33:373–9.
[23] Horwitz MA, Harth G, Dillon BJ, Malesa-Galic S. Recombinant 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;97:13853–8.
[24] Horwitz MA, Lee BY, Dillon BJ, Harth G. Protective immunity against tuberculosis induced by vaccination with major extracellular proteins of Mycobacterium tuberculosis. Proc Natl Acad Sci USA 1995;92:1530–4.
[25] Lee BY, Horwitz MA. Identification of macrophage and stress induced proteins of Mycobacterium tuberculosis. J Clin Invest 1995;96:245–9.
[26] Harth G, Lee BY, Wang J, Clemens DL, Horwitz MA. Novel insights into the genetics, biochemistry, and immunocytochemistry of the 30-kilodalton major extracellular protein of Mycobacterium tuberculosis. Infect Immun 1996;64:3038–47.
[27] Horwitz MA, Harth G. A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the guinea pig model of pulmonary tuberculosis. Infect Immun 2003;71:1672–9.

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[28] Hoft DF, Blazevic A, Abate G, Hanekom WA, Kaplan G, Soler JH, et al. A new recombinant bacille Calmette-Guérin vaccine safely induces significantly enhanced tuberculosis-specific immunity in human volunteers. J Infect Dis 2008;198:1491–501.
[29] Montagnani C, Chiappini E, Galli L, de Martino M. Vaccine against tuberculosis: what's new? BMC Infect Dis 2014;14(Suppl. 1):S2.
[30] Desel C, Dorhoi A, Bandermann S, Grode L, Eisele B, Kaufmann SH. 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;204:1573–84.
[31] Farinacci M, Weber S, Kaufmann SH. The recombinant tuberculosis vaccine rBCG ΔureC:hly(+) induces apoptotic vesicles for improved priming of CD4(+) and CD8(+) T cells. Vaccine 2012;30:7608–14.
[32] Grode L, Ganoza CA, Brohm C, Weiner 3rd J, Eisele B, Kaufmann SH. Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. Vaccine 2013;31:1340–8.
[33] Kupferschmidt K. Infectious disease. Taking a new shot at a TB vaccine. Science 2011;334:1488–90.
[34] Kaufmann SH, Gengenbacher M. Recombinant live vaccine candidates against tuberculosis. Curr Opin Biotechnol 2012;23:900–7.
[35] Perez E, Samper S, Bordas Y, Guilhot C, Gicquel B, Martin C. An essential role for phoP in Mycobacterium tuberculosis virulence. Mol Microbiol 2011;41:179–87.
[36] Arbues A, Aguilo JI, Gonzalo-Asensio J, Marinova D, Uranga S, Puentes E, et al. Construction, characterization and preclinical evaluation of MTBVAC, the first live-attenuated M. tuberculosis-based vaccine to enter clinical trials. Vaccine 2013;31:4867–73.
[37] Aguilar D, Infante A, Martin C, Gormley E, Gicquel B, Hernandez Pando R. Immunological responses and protective immunity against tuberculosis conferred by vaccination of Balb/C mice with the attenuated Mycobacterium tuberculosis (phoP) SO2 strain. Clin Exp Immunol 2007;147:330–8.
[38] Cardona PJ, Asensio JG, Arbues A, Otal I, Lafoz C, Gil O, et al. Extended safety studies of the attenuated live tuberculosis vaccine SO2 based on phoP mutant. Vaccine 2009;27:2499–505.
[39] Verreck FA, Vervenne RA, Kondova I, van Kralingen KW, Remarque EJ, Braskamp G, et al. MVA.85A boosting of BCG and an attenuated, phoP deficient M. tuberculosis vaccine both show protective efficacy against tuberculosis in rhesus macaques. PLoS One 2009;4:e5264.
[40] Martin C, Williams A, Hernandez-Pando R, Cardona PJ, Gormley E, Bordat Y, et al. 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;24:3408–10.
[41] Nambiar JK, Pinto R, Aguilo JI, Takatsu K, Martin C, Britton WJ, et al. Protective immunity afforded by attenuated, PhoP-deficient *Mycobacterium tuberculosis* is associated with sustained generation of CD4(+) T-cell memory. Eur J Immunol 2012;42:385–92.
[42] Kamath AT, Fruth U, Brennan MJ, Dobbelaer R, Hubrechts P, Ho MM, et al. New live mycobacterial vaccines: the Geneva consensus on essential steps towards clinical development. Vaccine 2005;29:3753–60.
[43] Sweeney KA, Dao DN, Goldberg MF, Hsu T, Venkataswamy MM, Henao-Tamayo M, et al. A recombinant *Mycobacterium smegmatis* induces potent bactericidal immunity against Mycobacterium tuberculosis. Nat Med 2011;17:1261–8.
[44] Collins FM. Kinetics of the delayed-type hypersensitivity response in tuberculous guinea pigs and mice tested with several mycobacterial antigen preparations. Am Rev Respir Dis 1983;127:599–604.
[45] Gröschel MI, Prabowo SA, Cardona PJ, Stanford JL, van der Werf TS. Therapeutic vaccines for tuberculosis–a systematic review. Vaccine 2014;32:3162–8.
[46] Lahey T, Arbeit RD, Bakari M, Horsburgh CR, Matee M, Waddell R, et al. Immunogenicity of a protective whole cell mycobacterial vaccine in HIV-infected adults: a phase III study in Tanzania. Vaccine 2010;28:7652–8.
[47] Capone S, D'Alise AM, Ammendola V, Colloca S, Cortese R, Nicosia A, et al. Development of chimpanzee adenoviruses as vaccine vectors: challenges and successes emerging from clinical trials. Expert Rev Vaccines 2013;12:379–93.
[48] Vogels R, Zuijdgeest D, van RR, Hartkoorn E, Damen I, de Bethune MP, et al. Replication-deficient human adenovirus type 35 vectors for gene transfer and vaccination: efficient human cell infection and bypass of preexisting adenovirus immunity. J Virol 2003;77:8263–71.
[49] Radosevic K, Wieland CW, Rodriguez A, Weverling GJ, Mintardjo R, Gillissen G, et al. 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;75:4105–15.
[50] Abel B, Tameris M, Mansoor N, Gelderbloem S, Hughes J, Abrahams D, et al. The novel tuberculosis vaccine, AERAS-402, induces robust and polyfunctional CD4+ and CD8+ T cells in adults. Am J Respir Crit Care Med 2010;181:1407–17.
[51] Hoft DF, Blazevic A, Stanley J, Landry B, Sizemore D, Kpamegan E, et al. A recombinant adenovirus expressing immunodominant TB antigens can significantly enhance BCG-induced human immunity. Vaccine 2012;30:2098–108.
[52] Hokey DA, Wachholder R, Darrah PA, Bolton DL, Barouch DH, Hill K, et al. A nonhuman primate toxicology and immunogenicity study evaluating aerosol delivery of AERAS-402/Ad35 vaccine: evidence for transient T cell responses in peripheral blood and robust sustained responses in the lungs. Hum Vaccin Immunother 2014 [Epub May 8].
[53] McShane H, Pathan A, Sander C, Keating SM, Gilbert SC, Huygen K, et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat Med 2004;10:1240–4.
[54] Vordermeier H, Villarreal-Ramos B, Cockle P, McAulay M, Rhodes SG, Thacker T, et al. Viral booster vaccines improve *Mycobacterium bovis* BCG-induced protection against bovine tuberculosis. Infect Immun 2009;77:3364–73.
[55] Williams A, Goonetilleke NP, McShane H, Clark SO, Hatch G, Gilbert SC, Hill AV. Boosting with poxviruses enhances *Mycobacterium bovis* BCG efficacy against tuberculosis in guinea pigs. Infect Immun 2005;73:3814–6.
[56] Hawkridge T, Scriba TJ, Gelderbloem S, Smit E, Tameris M, Moyo S, et al. Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in healthy adults in South Africa. J Infect Dis 2008;198:544–52.
[57] Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet 2013;381:1021–8.
[58] Leroux-Roels I, Forgus S, De Boever F, Clement F, Demoitié MA, Mettens P, et al. 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;31:2196–206.
[59] Day C, Tameris M, Mansoor N, et al. 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;188:492–502.
[60] Tait D. Progress of the M72/AS01E tuberculosis vaccine candidate into phase IIb efficacy trial. Presented at: TB Vaccines Third Global Forum; 2013 March 25–27; Cape Town, South Africa.
[61] van Dissel JT, Arend SM, Prins C, Bang P, Tingskov PN, Lingnau K, et al. Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in naive human volunteers. Vaccine 2010;28:3571–81.
[62] van Dissel JT, Soonawala D, Joosten SA, Prins C, Arend SM, Bang P, et al. 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;29:2100–9.
[63] Lee J, Kim J, Lee J, Shin SJ, Shin EC. DNA immunization of *Mycobacterium tuberculosis* resuscitation-promoting factor B elicits polyfunctional CD8(+) T cell responses. Clin Exp Vaccine Res 2014;3:235–43.
[64] Baldwin SL, Ching LK, Pine SO, Moutaftsi M, Lucas E, Vallur A, et al. Protection against tuberculosis with homologous or heterologous protein/vector vaccine approaches is not dependent on CD8+ T cells. J Immunol 2013;191:2514–25.
[65] Bertholet S, Ireton GC, Ordway DJ, Windish HP, Pine SO, Kahn M, et al. A defined tuberculosis vaccine candidate boosts BCG and protects against multidrug-resistant Mycobacterium tuberculosis. Sci Transl Med 2010;2:53–74.
[66] Lin PL, Dietrich J, Tan E, Abalos RM, Burgos J, Bigbee C, et al. 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;122:303–14.
[67] Cardona PJ. RUTI: a new chance to shorten the treatment of latent tuberculosis infection. Tuberculosis 2006;86:273–89.
[68] Guirado E, Gil O, Caceres N, Singh M, Vilaplana C, Cardona PJ. Induction of a specific strong polyantigenic cellular immune response after short-term chemotherapy controls bacillary reactivation in murine and guinea pig experimental models of tuberculosis. Clin Vaccine Immunol 2008;15:1229–37.
[69] Nunes-Alves C, Booty MG, Carpenter SM, Jayaraman P, Rothchild AC, Behar SM. In search of a new paradigm for protective immunity to TB. Nat Rev Microbiol 2014;12:289–99.
[70] Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, et al. Tumor necrosis factor-α is required in the protective immune response against *Mycobacterium tuberculosis* in mice. Immunity 1995;2:561–72.
[71] Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, Cheever A, et al. Caspase-1 independent IL-1β production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling in vivo. J Immunol 2010;184:3326–30.
[72] Gonzalez-Juarrero M, Hattle JM, Izzo A, Junqueira-Kipnis AP, Shim TS, Trapnell BC, et al. Disruption of granulocyte macrophage-colony stimulating factor production in the lungs severely affects the ability of mice to control *Mycobacterium tuberculosis* infection. J Leukoc Biol 2005;77:914–22.
[73] Pitt JM, Stavropoulos E, Redford PS, Beebe AM, Bancroft GJ, Young DB, et al. Blockade of IL-10 signaling during bacillus Calmette-Guerin vaccination enhances and sustains TH1, TH17, and innate lymphoid IFN-γ and IL-17 responses and increases protection to *Mycobacterium tuberculosis* infection. J Immunol 2012;189:4079–87.
[74] Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Nienhaus A. Negative and positive predictive value of a whole-blood interferon-γ release assay for developing active tuberculosis: an update. Am J Respir Crit Care Med 2011;183:88–95.
[75] Gagneux S. Host-pathogen coevolution in human tuberculosis. Philos Trans R Soc Lond B Biol Sci 2012;367:850–9.
[76] Saunders BM, Briscoe H, Britton WJ. T cell-derived tumour necrosis factor is essential, but not sufficient, for protection against *Mycobacterium tuberculosis* infection. Clin Exp Immunol 2004;137:279–87.

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[77] Sada-Ovalle I, Chávez-Galán L, Torre-Bouscoulet L, Nava-Gamiño L, Barrera L, Jayaraman P, et al. The Tim3-galectin 9 pathway induces antibacterial activity in human macrophages infected with Mycobacterium tuberculosis. J Immunol 2012;189:5896–902.
[78] Vilcheze C, Hartman T, Weinrick B, Jacobs Jr WR. *Mycobacterium tuberculosis* is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. Nat Commun 2013;4:1881.
[79] Verway M, Bouttier M, Wang TT, Carrier M, Calderon M, An BS, et al. Vitamin D induces interleukin-1β expression: paracrine macrophage epithelial signaling controls *M. tuberculosis* infection. PLoS Pathog 2013;9:e1003407.
[80] Chen CY, Huang D, Wang RC, Shen L, Zeng G, Yao S, et al. A critical role for CD8 T cells in a nonhuman primate model of tuberculosis. PLoS Pathog 2009;5:e1000392.
[81] Skinner MA, Parlane N, McCarthy A, Buddle BM. Cytotoxic T-cell responses to *Mycobacterium bovis* during experimental infection of cattle with bovine tuberculosis. Immunology 2003;110:234–41.
[82] Woodworth JS, Behar SM. *Mycobacterium tuberculosis*-specific $CD8^+$ T cells and their role in immunity. Crit Rev Immunol 2006;26:317–52.
[83] Woodworth JS, Wu Y, Behar SM. *Mycobacterium tuberculosis*-specific $CD8^+$ T cells require perforin to kill target cells and provide protection *in vivo*. J Immunol 2008;181:8595–603.
[84] Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 2012;30:531–64.
[85] Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682–7.
[86] Im JS, Kang TJ, Lee SB, Kim CH, Lee SH, Venkataswamy MM, et al. Alteration of the relative levels of iNKT cell subsets is associated with chronic mycobacterial infections. Clin Immunol 2008;127:214–24.
[87] Montoya CJ, Cataño JC, Ramirez Z, Rugeles MT, Wilson SB, Landay AL. Invariant NKT cells from HIV-1 or Mycobacterium tuberculosis-infected patients express an activated phenotype. Clin Immunol 2008;127:1–6.
[88] Van Rhijn I, Kasmar A, de Jong A, Gras S, Bhati M, Doorenspleet ME, et al. A conserved human T cell population targets mycobacterial antigens presented by CD1b. Nat Immunol 2013;14:706–13.
[89] Havlir DV, Ellner JJ, Chervenak KA, Boom WH. Selective expansion of human gamma delta T cells by monocytes infected with live Mycobacterium tuberculosis. J Clin Invest 1991;87:729–33.
[90] Le Bourhis L, Martin E, Péguillet I, Guihot A, Froux N, Coré M, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol 2010;11:701–8.
[91] Scriba TJ, Kalsdorf B, Abrahams DA, Isaacs F, Hofmeister J, Black G, et al. Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response. J Immunol 2008;180:1962–70.
[92] Achkar JM, Casadevall A. Antibody-mediated immunity against tuberculosis: implications for vaccine development. Cell Host Microbe 2013;13:250–62.
[93] Glatman-Freedman A, Mednick AJ, Lendvai N, Casadevall A. Clearance and organ distribution of *Mycobacterium tuberculosis* lipoarabinomannan (LAM) in the presence and absence of LAM binding immunoglobulin M. Infect Immun 2000;68:335–41.
[94] Ciurana CL, Zwart B, van Mierlo G, Hack CE. Complement activation by necrotic cells in normal plasma environment compares to that by late apoptotic cells and involves predominantly IgM. Eur J Immunol 2004;34:2609–19.
[95] Ballow M. The IgG molecule as a biological immune response modifier: mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory disorders. J Allergy Clin Immunol 2011;127:315–23.
[96] Villarreal DO, Walters J, Laddy DJ, Yan J, Weiner DB. Multivalent TB vaccines targeting the esx gene family generate potent and broad cell-mediated immune responses superior to BCG. Hum Vaccine Immunother 2014 [Epub Jun 23].

Please cite this article in press as: Principi N, Esposito S, The present and future of tuberculosis vaccinations, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.10.004