EXPERT OPINION

An update on vaccines for tuberculosis – there is more to it than just waning of BCG efficacy with time

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Introduction: Apart from better diagnostics and new anti-microbial drugs, an effective vaccine for tuberculosis is urgently needed to halt this poverty-related disease, afflicting millions of people worldwide.
Areas covered: After a general introduction on the global threat of tuberculosis, the pros and cons of the existing *M. bovis* BCG vaccine are discussed. As the correlates of protection against tuberculosis remain largely unknown, new findings in biomarker research are described. Next, an update on the ongoing Phase I and Phase II clinical trials is given. Finally, some of the most promising novel pre-clinical developments using live attenuated vaccines, sub-unit vaccines, prime-boost strategies, and new vaccination routes are discussed. The field has made considerable progress and 12 vaccine candidates have now actually entered Phase I or Phase IIa and IIb clinical trials.
Expert opinion: It is argued that the variable protection conferred by the existing BCG vaccine against reactivation of latent TB is caused not only by waning of its efficacy with time but also by its weak induction of MHC class I restricted responses. Prime-boost strategies based on the actual BCG vaccine may not be sufficient to overcome this hurdle. The use of plasmid DNA vaccination might offer a solution.

Keywords: clinical trials, cytolytic t cells, dna vaccine, latency, prime-boost protocols, tuberculosis, vaccine

Expert Opin. Biol. Ther. (2012) 12(12):1601-1610

1. Introduction

Tuberculosis, once also called consumption, phthisis, scrofula, Pott's disease, or white plague is a major source of morbidity and mortality that has afflicted mankind since its origin [1]. This contagious disease is caused by infection with bacteria belonging to the *Mycobacterium tuberculosis* complex. To this day, tuberculosis remains a major health problem, which caused disease to 8.8 million people and the death of 1.4 million individuals in 2010 [2]. Infection with *Mycobacterium tuberculosis* is transmitted by individuals suffering from open cavitary pulmonary TB before onset of combination chemotherapy. It is estimated that one untreated patient can infect up to 10–15 individuals/year through close contact. By sneezing or coughing, TB patients can spread *Mtb* bacilli, which can infect individuals in near proximity. Immunocompetent persons inhaling the bacilli will be able to prevent the establishment of an infection in an estimated 70% of cases. The other 30% will become infected and develop acquired immunity to *M. tuberculosis* proteins (resulting in a positive PPD skin test) [3]. Among these infected persons, about 5–10% will develop active TB within 1 or 2 years after infection and the remaining 90–95% will remain asymptomatic but latently infected. These individuals have a

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Article highlights.

- Tuberculosis is a poverty-related disease, afflicting millions of people worldwide.
- The existing Bacille Calmette-Guérin (BCG) vaccine protects against extrapulmonary TB in young children but not against pulmonary TB in adults.
- Twelve new TB vaccine candidates have now progressed from the preclinical to the clinical phase.
- They can be divided in two groups: live, attenuated vaccines, replacing the actual BCG vaccine, and viral or protein sub-unit booster vaccines administered after BCG vaccination.
- Cell mediated immunity is essential in the control of an infection with the intracellular pathogen *M. tuberculosis* infection, but satisfactory correlates of protection are lacking so far.
- The relative importance of $CD4^+$ and $CD8^+$ T cells in protection against TB is unknown, but the latter population is particularly important for protection against reactivation of latent *M. tuberculosis* infection.
- BCG induces only weak $CD8^+$ responses and latency antigen specific T cells; combination of BCG with plasmid DNA vaccines may overcome these two hurdles.

This box summarizes key points contained in the article.

residual population of viable mycobacteria, which are mainly contained in well-organized pulmonary structures called granulomas. Latently infected persons are a reservoir of quiescent TB bacilli, because they are potentially at risk to develop TB at some stage of their life, following reactivation. It is estimated that one-third of the world's population is latently infected with *M. tuberculosis*. Co-morbidity factors resulting in a state of immunodepression increase the risk of active TB. Besides malnutrition and stress, HIV co-infection is probably the most severe factor causing this immunodepression. Thus, in persons infected only with *M. tuberculosis*, annual risk to develop active TB is 2.5% in the first 2 years after infection and 0.1% thereafter [4], whereas this annual risk is 10% for HIV-infected persons [5] and TB is responsible for more than a quarter of deaths in people living with HIV [6].

Bacille Calmette–Guérin (BCG), still the only vaccine available against TB, was developed by attenuation of virulent *M. bovis* through 230 passages in glycerin-bile-potato medium over the course of 13 years, and was first tested in humans in 1921 [7]. At that time, no antibiotics for the treatment of TB were yet available and a newborn child from a household of TB patients had a mortality risk of 25%. Trials with the BCG vaccine performed between 1921 and 1924 showed a decrease to 1% of this mortality risk [8]. Hence, vaccination with BCG was progressively implemented worldwide and currently – in TB endemic countries – BCG is part of the vaccines administered in the context of the Expanded Program on Immunization (EPI). In non-endemic countries, BCG is recommended only to populations at increased risk of exposure to *Mtb*. BCG vaccination protects children against TB meningitis and against disseminated, miliary disease, but has been found to be of variable efficacy against pulmonary TB (ranging from 0 to 80%) in a number of clinical trials [9] [10] [11]. Therefore, although efficient against extrapulmonary forms of childhood TB, BCG has a minor impact on transmission of *Mtb* infection. The ideal TB vaccine would be a pre-exposure vaccine able to elicit sterilizing immunity preferably at the time of initial infection. Nevertheless, given the complex interactions between *Mtb* and its host and our poor understanding of the correlates of protection, current vaccine research rather aims at developing a vaccination protocol impacting the transmission of the infection by inducing levels of immunity able to prevent the development of active TB, to be administered before *Mtb* exposure or to latently infected individuals. It could be argued that a sterilizing vaccine would not even be required to eradicate the disease, provided that reactivation of all cases of latent infection (and subsequent transmission) could be prevented.

2. Correlates of protection/biomarker research

A better understanding of the protective immune response against *M. tuberculosis* is very important in the quest for better TB vaccines, but the actual "correlates of protection" remain unknown so far and will perhaps only be identified from successful vaccine trials. Conversely, unsuccessful vaccine trials may also give valuable information, even if they might negatively impact support for subsequent trials. A major role in protection against this intracellular pathogen is played by the cellular arm of the adaptive immune system [12], particularly by CD4+ Th1 type T-helper cells producing IFN-γ, TNF-α and IL-2. This is underscored by the clinical association between HIV and TB [13], by the genetic susceptibility to TB and opportunistic mycobacterial disease of individuals bearing mutations in the IL-12/IL-23–IFN-γ pathway [14] and by the increased risk to develop reactivation of TB in individuals treated with anti-TNF-α agents used for a range of inflammatory/autoimmune diseases, such as rheumatoid arthritis and Crohn's disease [15]. Besides MHC class II restricted CD4+ T cells, also MHC class I restricted CD8+ T cells play a role in the immune response against *Mtb* through production of cytokines and their lytic activity targeting infected cells. This has been demonstrated by a number of studies in pre-clinical models and with samples isolated from humans [12,16]. The importance of CD8+ T cells in the control of latent TB infection and immune protection against reactivation of TB in humans was convincingly demonstrated by Bruns *et al.*, who have reported that anti-TNF-α immunotherapy with infliximab reduced CD8+ T cell-mediated anti-microbial activity against *Mtb* in humans through the interaction of the antibody with cell surface TNF on the CD8+ T cell and their subsequent complement-mediated lysis [17]. Clearly, CD4+ and CD8+ T cells play an important

role in protective immunity against *Mtb*. However, the picture is far more complex as indicated a.o. by the results obtained by Kagina *et al.* who analyzed a large cohort of BCG-vaccinated South African infants. Indeed –and in contrast to the prevailing dogma– the frequency of multi-functional CD4+ T cells producing IFN-γ, TNF-α, IL-17 and IL-2, measured 10 weeks after BCG vaccination, was not directly associated with protection against active TB in this population [18]. Interestingly, more detailed transcriptional profiling of samples isolated from this cohort of BCG-vaccinated infants has shown that a wide spectrum of immune reactivities were generated and that children both with very low or very high inflammatory responses were at increased risk for developing TB. A better understanding of the immune correlates of protection will possibly be generated by this type of biomarker research [19]. Transcriptional profiling has already enabled identification of TB biosignatures that discriminate latently infected individuals controlling the infection from active TB patients [20] [21], but identification of TB biomarkers/biosignatures goes also beyond. Indeed, also conceivable are similar studies that could identify exposed individuals who will not become infected; infected individuals at risk to develop active TB within 1 or 2 years after infection; those who will be able to control the infection lifelong and finally those who will experience reactivation of a remotely acquired latent infection [19]. Research on biomarkers may also give clues for the development of more rapid diagnostic tests [22]. Biomarkers of sterilizing immunity could possibly be identified by analyzing innate lung immune mechanisms in TB case-contacts recurrently exposed to *Mtb* but who never develop T-cell sensitization to mycobacterial antigens. Although logistically and technically challenging, the latter type of studies could provide an important comprehension of mycobactericidal immunity at the site of infection, that could be applied for the development of better TB vaccines [23].

Twelve TB vaccine candidates are currently evaluated in clinical trials, but so far, none of the preventive vaccine candidates has reached the Phase III level of efficacy testing [24] [25]. Given our poor understanding of what type of immunity a TB vaccine should induce to be protective at least against development of active TB, it is impossible to anticipate if one of these candidates, and which one will potentially impact the TB epidemic. For that purpose, Phase III clinical trials in large cohorts of volunteers in TB-endemic countries are needed and will take several years to complete. As success of these candidates in unpredictable, it is essential to continue fundamental and pre-clinical research to understand better the protective immunity to *Mtb* and to identify novel TB vaccine candidates that might prove more efficient. Currently ongoing clinical trials will be discussed in section 3 as well as challenges faced and ongoing research that might facilitate vaccine efficacy evaluation. Fundamental research and novel pre-clinical TB vaccine candidates will be discussed in section 4.

3. Update on current clinical trials

As already mentioned, several TB vaccine candidates are currently at different stages of clinical trial development and have been listed in the 2011 Tuberculosis Vaccine Pipeline of the STOP-TB partnership. Twelve vaccines have actually progressed from the pre-clinical to the clinical phase (See **Figure 1**). When performing a basic search on "ClinicalTrials.gov" with the terms "Tuberculosis AND vaccine", we found 87 studies, of which the status was *not yet recruiting, recruiting, active not recruiting, unknown, completed, terminated* or *withdrawn*. Among these, 28 are "false-positive" unrelated to TB vaccine development or related to clinical testing of adjuvants that could be exploited for TB vaccines or to clinical evaluation of BCG for the treatment of diseases such as autoimmune diseases. Among the remaining 59, two clinical trials are devoted to the study of the immune response to *Mtb* (respectively ClinicalTrials.gov identifier numbers NCT00340990 and NCT00257907). Being tested in five trials as an adjunct to chemotherapy for latent TB or active TB is the RUTI vaccine (fragmented *Mtb* cells in liposomes – 2 completed trials : NCT00546273 and NCT01136161) and *M. indicus pranii* (whole cell saprophytic non-TB mycobacterium – 2 trials with "*unknown*" status: NCT00265226 and NCT00341328 and one "*recruiting*" trial NCT00810849). *Mycobacterium vaccae* has been tested in a Phase III trial as a vaccine designed to prevent disseminated TB in HIV-infected patients. In addition, seven trials are evaluating different outcomes of BCG administration such as BCG-induced immunity when administered after clearance of LTBI by isoniazid preventive therapy (ClinicalTrials.gov identifier number NCT01119521); effect of vitamin D supplementation on BCG-induced immunity, safety of BCG administration in low-birth-weight infants or in children born from HIV-positive mothers and finally three trials are evaluating different administration routes of BCG such as the oral route (first route used in humans) and a comparison of the intradermal versus the percutaneous route (the latter used in Japan since the end of the 1960's). The remaining 44 trials are related to the clinical evaluation of novel preventive TB vaccine candidates. They can be divided into two groups. The first group consists of candidates that have been conceived for the replacement of BCG by more efficient live (attenuated) vaccines and the second group is the one of the "booster" sub-unit vaccines. Regarding the clinical testing of genetically modified BCG strains that are being tested to replace BCG, the VPM1002 candidate (a recombinant BCG Prague strain expressing listeriolysin and carrying a urease deletion mutation) has been tested in two completed Phase I trials and a Phase II trial to evaluate its safety and immunogenicity in comparison with BCG in newborn infants is "active, not recruiting" (NCT01479972). The VPM1002 BCG strain has been modified to be safer and to mimic better the immune responses induced by natural infection with *Mtb*, especially the induction of CD8+ responses. A Phase I trial with

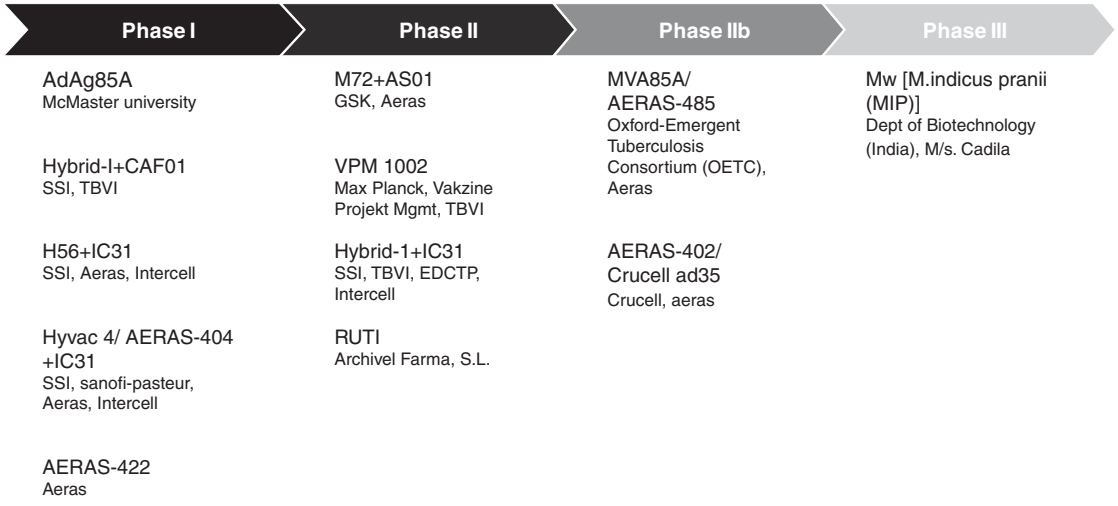

Figure 1. TB Vaccine candidates currently evaluated in clinical trials.
Adapted from http://www.stoptb.org/wg/new_vaccines/documents.asp.

AERAS-422 (NCT01340820) (a recombinant BCG expressing perfringolysin and overexpressing antigens 85A, 85B, and Rv3407) had to be terminated due to side effects (reactivation of shingles) [26]. *M. tuberculosis*-based live attenuated vaccines have also been engineered and tested in pre-clinical models. The most promising of these candidates, a mutant *M. tuberculosis* strain genetically inactivated in the transcriptional regulator *phoP* and *fadD26* [27], is currently produced under good manufacturing practices and is expected to shortly enter a Phase I clinical trial [25].

The second group of novel preventive TB "booster" sub-unit vaccines – comprising either protein–adjuvant combinations or recombinant viral vectors – have been conceived to be administered in a vaccine regimen involving BCG vaccination at birth followed by a boost vaccination. These booster sub-unit vaccines are also tested in individuals latently infected with *Mtb*. Clinical trials are ongoing or have been completed for MVA-Ag85A (Modified Vaccinia Ankara vector expressing *Mtb* antigen 85A - 19 trials listed on clinicaltrials.gov); the GSK-692342 candidate (recombinant M72 protein composed of a fusion of *Mtb* antigens Rv1196 and Rv0125 in adjuvant - 11 trials on clinicaltrials.gov); Hybrid-1 formulated in different adjuvants (recombinant protein composed of *Mtb* antigens 85B and ESAT-6 - 5 trials on clinicaltrials.gov); AERAS-402 (a replication-deficient adenovirus 35 vector expressing *Mtb* antigens 85A, 85B, TB10.4 – 3 trials on clinicaltrials.gov), AdAg85A (a replication-deficient adenovirus 5 vector expressing *Mtb* antigen 85A – 1 trial on clinicaltrials.gov) and finally ID93 (a sub-unit fusion protein composed of 4 *Mtb* antigens Rv1813-Rv2608-Rv3619- Rv3620) formulated in GLA-SE adjuvant, a synthetic TLR-4 agonist, glucopyranosyl lipid adjuvant (GLA) in an o/w emulsion (1 trial on clinicaltrials.gov).

The rationale behind all the booster vaccines is the notion that BCG-induced immunity wanes with time. BCG-induced protective efficacy is thought to last 10–15 years [28], but at least one study has reported on much longer persistence of protection [29]. Indeed, studies by Aronson *et al.* have reported on a protection that persisted for 50–60 years in American Indians and Alaska Natives [30]. It has been proposed by C. Dye that a persistently high level of incidence or infection risk may have contributed to the maintenance of BCG efficacy in this population [31], but this seems to us a "counter-intuitive" argument. On the other hand, it is intriguing to read that in Aronson's study, the 13 lots of BCG vaccine were prepared from live cultures of BCG in a *mobile* laboratory, and the vaccine was used within 3 days of preparation. One could speculate that this "fresh" non- lyophilized BCG vaccine had a better CD8+-inducing potential than the freeze-dried BCG vaccine preparations routinely used in the other trials. Finally, besides waning and weak CD8+ responses, other factors may influence BCG efficacy such as co-infection with helminths, which may dampen the induction of a protective T helper1 response by induction of T helper 2 and regulatory T cells [26], interference of environmental, non-tuberculous mycobacteria NTM [32] and nutritional factors such as iron status and anti-microbial peptide-inducing vitamin D [33].

None of the novel TB vaccine candidates have reached the Phase III level of efficacy testing against development of active TB. As already mentioned in the introduction, given our poor understanding of which immunity a TB vaccine should induce to be protective, it is impossible to anticipate if one of these candidates will potentially impact the TB epidemic. A major limitation in the clinical evaluation of protective efficacy of TB vaccine candidates is linked to the lack of an experimental human challenge model in which to assess

candidate vaccine efficacy in small scale proof-of-concept Phase II challenge studies prior to expensive Phase III clinical studies [34]. Such human experimental infection challenge models exist for other infectious diseases such as malaria, influenza, dengue fever, and typhoid fever. Recently, Minassian *et al.* have reported on the intradermal administration of *Mycobacterium bovis* BCG as a surrogate for *M. tuberculosis* infection, based on the assumption that an effective vaccine against *M. tuberculosis* should also reduce the replication of BCG [35]. This approach will be tested in a currently recruiting Phase I clinical trial (ClinicalTrials.gov identifier: NCT01194180). However, attenuated *M. bovis* BCG is not virulent *Mtb* and an immunocompetent host can clear BCG bacilli effectively. Challenge studies with recombinant *Mtb* strains engineered specifically for this purpose could represent a better alternative, although very challenging to develop [19]. A valid alternative may be the use of *in vitro* models of *Mtb* killing to measure vaccine efficacy. As reported by Kampmann *et al.,* these type of *in vitro* models are perfectly feasible [36]. Indeed, using a whole-blood luminescence assay based on a reporter gene-tagged BCG used to infect human whole-blood cultures derived from volunteers before and at different time points after BCG vaccination, Kampmann *et al.* observed that BCG vaccination reduced growth of reporter BCG and this growth was restricted better by PBMC from BCG-vaccinated than from unvaccinated controls. Similar types of assays could be developed with reporter *Mtb* replacing BCG. In addition, such *in vitro* human models could also further be expanded to an even more complicated system such as the MIMIC (Modular Immune In vitro Construct) system that was developed by VaxDesign [37]. In these types of systems, vaccine efficacy is tested using immune cells isolated from volunteers that are incubated in *in vitro* systems mimicking the infection (such as artificial granulomas).

4. Update on novel pre-clinical TB vaccines

Despite the relatively numerous clinical trials related to the evaluation of novel preventive TB vaccine candidates, it is impossible to predict when and if a new efficient vaccine regimen against TB will result from these studies. It is therefore essential to continue fundamental and pre-clinical research to understand better the protective immunity to *Mtb* and to identify novel TB vaccine candidates or vaccination protocols that might prove more efficient than BCG or the novel candidates currently under clinical evaluation. Several promising pre-clinical candidates are listed with their references in the 2011 Tuberculosis Vaccine Pipeline of the STOP-TB partnership [25]. Concerning live-attenuated vaccines that could possibly be used to replace BCG, we will only focus our discussion on some that are particularly attractive to us. Regarding the sub-unit vaccines, we will discuss possible benefits of co-administration of BCG with sub-unit vaccines (particularly plasmid DNA vaccines). Finally, promising alternative routes of vaccine administration eliciting both systemic and mucosal immune responses that warrant further investigation will be described.

4.1. Live-attenuated TB vaccine candidates

The potential of recombinant live vaccine candidates against TB has been discussed extensively in two recent reviews, [8,26], and we will therefore only elaborate here on some novel approaches that seem particularly attractive to us. First, promising results have been obtained with a recombinant *M. smegmatis* strain called IKEPLUS [38]. Originally, the study aimed to analyze the influence of the ESX-3 secretion system on innate and adaptive immune responses induced by infection. In IKEPLUS, the ESX-3 secretion system of *M. smegmatis* was replaced with the orthologous ESX-3 secretion system from *M. tuberculosis*. An effective protective efficacy was observed in mice administered IKEPLUS intravenously 8 weeks before intravenous challenge with a high dose of virulent *Mtb* H37Rv. When tested in more conventional/physiological conditions, using the sub-cutaneous route for vaccination and the aerosol route for experimental challenge infection, IKEPLUS resulted in a protective efficacy more similar to the one achieved by vaccination with BCG. Collectively, these encouraging data indicate that IKEPLUS has a potential as novel live-attenuated TB vaccine candidate, but clearly, additional research is needed.

Other interesting new avenues in the development of live, attenuated TB vaccines are studies targeting the mycobacterial cell wall, more in particular its mycolic acid composition. *M. tuberculosis* has three classes of mycolic acid, the α-mycolates and two oxygenated forms the methoxy- and ketomycolates respectively. These major lipids of the *M. tuberculosis* cell wall, are modified by cyclopropane rings, methyl branches, and oxygenation through the action of eight mycolic acid methyl transferases (MAMTs), located in four genetic loci. Through characterization of *M. tuberculosis* strains lacking individual MAMTs, mycolic acid modification has been shown to be important for *M. tuberculosis* pathogenesis in mice, in part through effects on the inflammatory activity of Trehalose Dimycolate (cord factor). However, it was unknown whether a cyclopropane-deficient strain of *M. tuberculosis* would be viable, and what the effect of cyclopropane deficiency on virulence would be. These questions were addressed by sequentially deleting all functional MAMTs from the *M. tuberculosis* chromosome and analyzing these strains *in vitro* and *in vivo* [39]. Results showed that *M. tuberculosis* is viable either with or without cyclopropanation and any oxygenated mycolates. These mutants are severely attenuated after aerosol infection of the mouse, but induce strong pro-inflammatory immune responses, making them interesting new vaccine candidates. These observations expanded on a previous report by Dao *et al.* who identified the *mmaA4* gene, which encodes for a methyl transferase required for introducing the distal oxygen-containing modifications of

mycolic acids, as a key locus involved in the repression of IL-12p40. Mutants in which *mmaA4* (*hma*) was inactivated, stimulated macrophages to produce significantly more IL-12p40 and TNF-alpha than wild-type *M. tuberculosis* and were attenuated for virulence [40]. Using different synthetic mycolic acid isomers formulated in liposomes, Vanderbeken *et al.* also found strong variations in pro-inflammatory patterns following intratracheal instillations in mice [41]. Besides their relevance for vaccine development, these findings indicate that enzymes involved in the mycolic acid modification pathway are also attractive targets for anti-mycobacterial drug development [39]. Recently, the therapeutic potential of an inhibitor of the mycolyl transferase Ag85C was reported [42].

Another interesting live attenuated vaccine candidate is a BCG mutant, genetically inactivated in the secreted acid phosphatase SapM, which plays a critical role during phagosome maturation [43]. Effective delivery of Ag to sites of T-cell activation may be a key requirement for optimal T-cell responses to control mycobacterial infection. Recruitment of antigen-loaded dendritic cells to the draining lymph nodes following BCG vaccination is inhibited by the secreted acid phosphatase SapM. Indeed, it was found that a mutant BCG, genetically inactivated in SapM, induced a better protection against TB when tested in long term survival experiments than parental BCG and that this improved efficacy was related to increased recruitment and activation of CD11c(+) MHC-II(int) CD40(int) dendritic cells (DCs) to the draining lymph nodes [43]. Deletion of the *SapM* gene was also reported in a $\triangle$*fbpA* mutant of *M. tuberculosis* H37Rv that lacks expression of Ag85A and that is attenuated in mice but retains its vaccinogenic potential [44]. Compared to the $\triangle$*fbpA* mutant, macrophages infected with this $\triangle$*fbpA*/*SapM* double knock-out strain showed increased antigen presentation capacity as measured in an *in vitro* Ag85B peptide presentation assay. Furthermore, the $\triangle$*fbpA*/*SapM* double knock-out strain elicited a better Th1 response in mice than the single mutants, suggesting indeed that immunogenicity of mycobacterial vaccines can be enhanced by rational deletion of mycobacterial genes that adversely regulate phagosome traffic [45].

4.2. Sub-unit vaccines

A very comprehensive literature review on the pre-clinical studies that are related to the development of a prime/boost vaccine strategy for TB comprising a live-attenuated vaccine (mainly BCG) was recently published by Brennan and colleagues [46]. Hereafter, we would like to focus our discussion on the potential of plasmid DNA vaccines in such vaccine regimen. DNA vaccines are easy to manipulate and produce sub-unit vaccines in which the gene encoding an antigen is inserted into a bacterial plasmid vector. Plasmid is amplified in transformed bacteria and the purified pDNA is administered to an immunocompetent host. Further manipulation of the bacterial plasmid vector can result in vectors encoding more than one antigen, fusion of several antigens or antigens plus other proteins leading to more efficient vaccines. Following DNA vaccination, antigenic material is generated in the myocyte/keratinocyte and within the antigen presenting cells and exogenous and endogenous antigen processing can proceed in much the same way as following infection with intracellular pathogens [47]. By virtue of the strong Th1-biased and particularly the MHC class I restricted immune responses that DNA vaccines can induce, they are very attractive as priming agents in prime/boost regimens for infectious diseases in which this type of immunity correlates with protection. It is well known that BCG vaccination is only a weak inducer of CD8+ T cells as compared to tuberculosis infection and a 200-fold higher dose of BCG is needed to induce CD8+ responses comparable to those induced with *M. tuberculosis* [48]. Immune responses induced by the existing BCG vaccine can be augmented by combinations with plasmid DNA in mice, guinea pigs and cattle either by priming with DNA or by boosting with DNA (reviewed in [46] and [49]). In most of these studies, increased efficacy of BCG has been measured by CFU counting or pathological scoring in infected organs, but effects on long term survival were rarely reported. Performing long term survival studies in BALB/c mice infected intravenously with *M. tuberculosis*, we have shown that priming with DNA prior to BCG, but not boosting BCG with DNA encoding Ag85A, could increase the potency of the BCG vaccine, resulting in seven to nine weeks longer mean survival times [50]. The number of Ag85A specific cytolytic and IFN-γ secreting CD8+ T cells were significantly increased in animals primed by the DNA vaccine as compared to animals that only received the BCG vaccine. Natural Killer cell activity can also be increased by DNA priming before BCG [51]. However, priming with DNA and boosting with BCG is an unrealistic vaccine regimen certainly for developing countries, where neonatal BCG vaccination is part of the expanded program of immunization by WHO. We are therefore currently analyzing the vaccine potential of approaches in which BCG is co-administered with pDNA vaccines encoding *Mtb* antigens. Our first results show that this BCG-DNA combo is capable of increasing mycobacteria-specific MHC class II restricted Th1 responses and more importantly, to induce also strong MHC class I restricted CD8+ responses. This BCG-DNA combo could be used to generate an immune response with a broader antigenic repertoire. More specifically, in contrast to *M. tuberculosis* infection, BCG vaccination induces only very weak responses against so-called latency associated antigens, the expression of which is upregulated in *Mtb* grown in conditions of starvation and dormancy [52]. We have previously shown that vaccination of mice with plasmid DNA encoding latency antigens of the DosR dormancy regulon can induce strong Th1 and CD8+ mediated immune response [53]. Hence, besides inducing stronger CD8+ responses, the BCG-DNA combo may target antigens produced not only by actively replicating *Mtb* but also by

dormant *Mtb* present in lung granulomas of latently infected individuals (N. Bruffaerts, unpublished results).

4.3. Alternative routes of vaccine administration

Numerous studies in animal models indicate that an important aspect of the interaction of *Mtb* with its host is that induction of naïve T cell activation is slow and takes more than a week after lung infection before *Mtb* is carried to the draining lymph nodes. Vaccine-induced immunity needs to be either resident in the lungs or able to be rapidly recruited following infection to be effective in limiting bacterial growth [54]. A possible means to achieve such a vaccines inducing immunity is represented by vaccines inducing immune responses localized to mucosal surfaces. Classically, pre-clinical TB vaccines are evaluated after parenteral administration, thus mainly inducing systemic immunity and sub-optimal mucosal immunity. By modifying the route of vaccination improved protective efficacy could be achieved [55,56]. For example, Z. Xing and his colleagues have shown that airway delivery of soluble mycobacterial antigens restores protective mucosal immunity by single intramuscular plasmid DNA tuberculosis vaccination [57]. Gupta *et al.* have reported that aerosol vaccination with live *M. w* (*M. indicus pranii*, a whole cell saprophytic non-TB mycobacterium clinically tested as an adjunct to TB chemotherapy) results in a better protective efficacy as compared to mice that have been vaccinated sub-cutaneously with *M.w* or BCG [58]. Delivering *M. w* by aerosol, increased the levels of IgA antibodies, of antigen-specific cytotoxic T lymphocytes and of IL-12 and IFN-γ in BAL compartment as compared to levels observed in mice vaccinated sub-cutaneously. Regarding IgA antibodies, it is of interest to note that IgA might play an important function in protection against *Mtb* as indicated by the fact that IgA-deficient mice have an increased susceptibility to intranasal infection with *Mycobacterium bovis* BCG, [59], and potential of human single-chain IgA1 specific for mycobacterial α-crystallin for passive immunotherapy of TB was reported [60]. Another interesting approach leading to both systemic and mucosal vaccine-induced immune responses was described by Tchilian *et al.* [61]. Indeed, in that study mice were immunized *simultaneously* by the parenteral and intranasal routes with BCG or with recombinant mycobacterial antigens formulated in adjuvants and this simultaneous immunization resulted in increased protective efficacy against an intranasal experimental challenge with *Mtb*. This improved protective efficacy was attributed to increased inhibition of the growth of *Mtb* in the early phase of infection in simultaneously immunized animals. The protective immune mechanism of simultaneous parenteral and mucosal administration warrants further investigation, but it is certainly a promising vaccination regimen for testing novel pre-clinical TB vaccines.

5. Expert opinion

Tuberculosis is a poverty-related disease, afflicting millions of people world-wide, particularly in low-income countries. Improving the socio-economic situation of people living in the South, solving the problem of malnutrition and improving housing conditions are key factors that could have a major impact. One must not forget that before the era of antibiotic treatment, incidence of tuberculosis had already dramatically decreased in Europe and the U.S.A (from 250/100.000 in 1880 tot 33/100.000 in 1947) precisely through these improved living conditions and "hygienic" measures [62]. From a medical point of view, development of an effective vaccine is by far the most cost-effective health measure to control and eventually eradicate an infectious disease. It is estimated that one untreated TB patient can infect up to 10–15 individuals/year through close contact. As opposed to the diagnosis-treatment approach of the individual patient, effective prophylaxis can halt the transmission chain at a population level. This has obvious advantages for public health. Moreover, there is no issue of antibiotic resistance (which will develop sooner or later in response to even the most powerful drugs).

Through concerted efforts of governmental and non-governmental bodies (National Institutes of Health, Framework V-VII Programs of the European Union, Bill & Melinda Gates Foundation, Aeras and Tuberculosis Vaccine Initiative TBVI, to name the most important ones), the tuberculosis vaccine field has seen a major breakthrough in the last ten years. Since the report of the first clinical trial using a new generation of TB vaccines by Mc Shane and her colleagues on boosting BCG-induced responses with Modified Vaccinia Ankara encoding the mycolyl-transferase Ag85A in 2004 [63], the field has made a considerable progress and 12 vaccine candidates have now entered Phase I or Phase IIa and IIb clinical trials.

As the correlates of protection against tuberculosis are still not fully understood, outcome of Phase IIb and Phase III trials are eagerly waited for, to confirm or falsify the premises on which these new vaccines are based. The first results of the MVA85A/AERAS-485 Phase IIb trial are expected by the end of 2012. The rationale behind the booster vaccines that are administered to BCG vaccinees is the notion that BCG-induced immunity wanes with time and lasts only 10–15 years [28]. However, at least one study has reported on much longer persistence of protection [30]. Whereas the boosting protocols have a well-reported property to increase MHC class II restricted CD4+ responses, their effect on MHC class I restricted CD8+ T cells (which are poorly induced by the BCG vaccine) is much less pronounced. This latter population is particularly important for the control of latent TB and the prevention of its reactivation. Use of attenuated, live *M. tuberculosis* mutants will likely have a better CD8+ generating potential, although experimental data have not been provided so far. Plasmid DNA vaccines are the most powerful sub-unit vaccines capable of triggering CD8+ responses. In contrast to viral vectors, they do not induce vector immunity and can be used for repeated boosting when

needed. Combination of plasmid DNA encoding early-secreted and particularly latency-associated antigens with the existing BCG vaccine may be a new vaccination approach that could help to overcome both the weak potential of BCG to trigger MHC class I restricted immune responses and broaden its antigenic repertoire.

Declaration of interest

The authors state no conflict of interest to declare. This work was partially funded by grants of European Union (FP7-NewTBVAC, contract no. HEALTHF3-2009-241745) and FWO-Vlaanderen (G.0063-09N).

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