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REVIEW

TB vaccine development and the End TB Strategy: importance and current status

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TB is now the leading, global cause of death due to a single infectious microbe. To achieve the End TB vision of reducing TB by 90% by 2035 we will need new interventions. The objectives of this manuscript are to summarize the status of the clinical TB vaccine pipeline; to assess the challenges facing the TB development field; and to discuss some of the key strategies being embraced by the field to overcome these challenges. Currently, 8 of the 13 vaccines in clinical development are subunit vaccines; 6 of these contain or express either Ag85A or Ag85B proteins. A major challenge to TB vaccine development is the lack of diversity in both the antigens included in TB vaccines, and the immune responses elicited by TB vaccine candidates. Both will need to be expanded to maximise the potential for developing a successful candidate by 2025. Current research efforts are focused on broadening both antigen selection and the range of vaccine-mediated immune responses. Previous and ongoing TB vaccine efficacy trials have built capacity, generated high quality data on TB incidence and prevalence, and provided insight into immune correlates of risk of TB disease. These gains will enable the design of better TB vaccines and, importantly, move these vaccines into efficacy trials more rapidly and at a lower cost than was possible for previous TB vaccine candidates.

Keywords: BCG, Biomarker, T cell, Tuberculosis, Vaccine

Introduction

In 2014, TB became the leading, global cause of death due to a single infectious disease.[1] Globally there were 9.6 million new cases and 1.5 million deaths in 2014. The greatest incidence of TB disease is in sub-Saharan Africa, largely driven by the HIV epidemic. To achieve the End TB vision of a 90% reduction in TB incidence by 2035, we will need new interventions including shorter, less toxic drug treatments, improved diagnostics and more effective vaccines.[2,3] It is estimated that with current biomedical interventions and improved social protection if fully implemented, including global universal health coverage, we can halve the current rate of TB cases from 100 to 50 per 100 000 per year by 2025. To reach the End TB vision of less than 10 per 100 000 by 2035, however, we will need new interventions (Figure 1).

Vaccination is the most effective intervention for the control of infectious disease. The eradication of smallpox and rinderpest and the near eradication of polio have only been possible due to the availability of highly effective vaccines. While the BCG vaccine, first introduced in 1921, has been used worldwide to prevent life-threatening TB disease in infants and children, it has demonstrated limited and variable effectiveness in preventing pulmonary TB and the transmission of *Mycobacterium tuberculosis* (Mtb), the causal agent of TB, in adolescents and adults. The possibility of reaching the End TB vision of less than 10 cases per 100 000 per year in 2035 would be greatly enhanced if a more effective vaccine for protection against the development of TB disease in adolescents and adults, the prime sources of TB transmission, were available by the year 2025.

An additional imperative to developing TB vaccines is the emerging threat of drug-resistant Mtb strains; including multidrug-resistant TB (MDR-TB), Mtb strains resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second-line drug. Although the global incidence of MDR-TB appears to be holding steady at approximately 3% of newly diagnosed TB cases, the devastating, debilitating, toxicity associated with treating MDR-TB and extensively drug resistant TB (XDR-TB), drug resistance of these types carry with them extraordinary economic costs. The direct cost of treating MDR-TB and XDR-TB has been found to be 8-fold and 25-fold higher, respectively, than treating drug-sensitive TB.[5] When considering the indirect cost

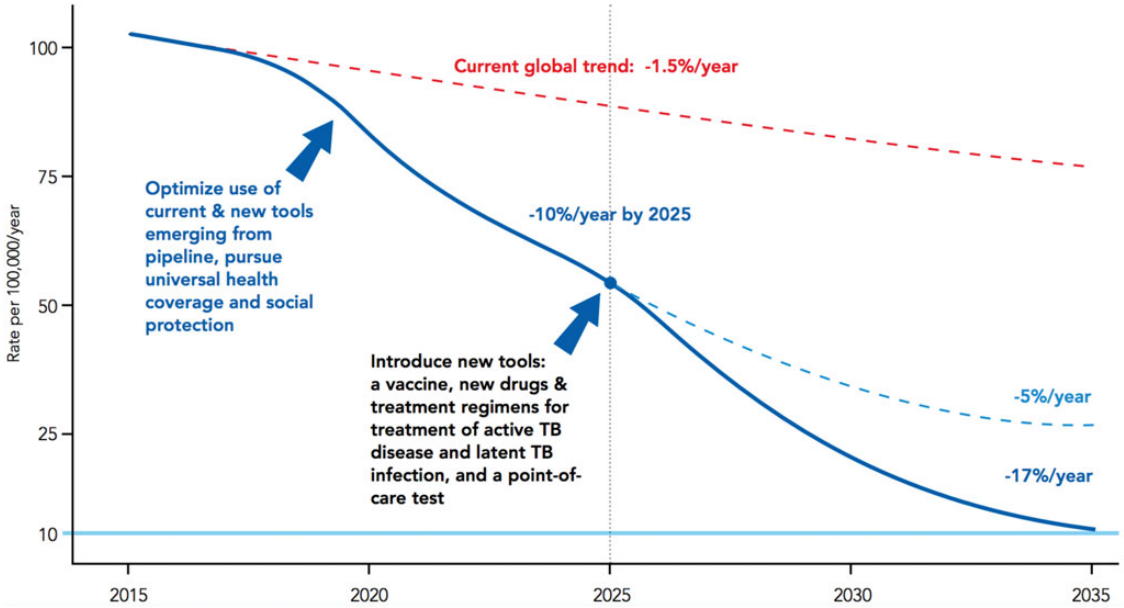

Figure 1. The importance of new tools in achieving the End TB Strategy. The projected acceleration of TB incidence decline needed to reach the target of the End TB Strategy.

of these diseases, including loss of productivity during convalescence, these costs rise to 15-fold and 35-fold the cost of drug-sensitive TB, respectively. TB vaccines demonstrated to be effective against drug-sensitive strains should also be effective against drug-resistant strains, as the nature of the drug-resistant mutations would not be expected to change the immunological profile of the organism. As there are no animal challenge models involving drug-resistant Mtb strains, given the danger of their use in laboratory settings, the ultimate proof of this postulate will need to be derived from clinical trials of vaccine with demonstrated efficacy against drug-sensitive strains in populations with an elevated incidence of Mtb drug-resistance.

The risk of failure of new TB vaccines in development is perceived to be high due to both the complexity of the biology and previous failure of a TB vaccine candidate in clinical testing.[6,7] Due to the risk of failure and low commercial value, TB vaccine development is unattractive to industry.[6] The public health benefits of an improved TB vaccine, however, would be high, and when considered in a cost-benefit analysis investment in TB vaccine development represents a cost-effective public health intervention even if efficacy is only partial.[8–10] Funders have responded with renewed investment in TB vaccine development, resulting in the 2014 launch of two major TB vaccine consortiums funded through the Horizon 2020 EU Framework Programme for Research and Innovation. In 2015 the Bill and Melinda Gates Foundation and The European & Developing Countries Clinical Trials Partnership (EDCTP) also committed funding for TB vaccine development. The next few years should see a reinvigoration of the TB vaccine development pipeline.

Current vaccines in clinical development

There are 13 vaccines currently in clinical development (self-reported by vaccine sponsors). These can be divided into whole cell-derived vaccines, viral vectored subunit vaccines and adjuvanted protein subunit vaccines (see Supplementary file).

Whole cell-derived vaccines

The strategy of utilizing whole cell vaccines for TB has gained increased interest due to the ongoing difficulties in identifying individual antigens critical to generating protective immune responses to Mtb. Additionally, as whole organisms, these vaccines induce a more diversified immune response than do subunit-based vaccines, including both humoral and cellular immune responses to a range of protein, lipid and antigens.

The candidate currently at the most advanced stage of clinical trials is *Mycobacterium vaccae* (Vaccae™, AnHui Longcom Biologic Pharmacy Co., Ltd [Longcom], Beijing, China), a non-TB mycobacteria (NTM) currently being assessed in a phase 3 trial for safety and efficacy in preventing TB disease in purified protein derivative (PPD) skin test positive adults in China.[11] Vaccae™ already has been licensed in China as an adjunct to therapy in persons with TB disease.[12] The phase 3 trial, in which the vaccine is administered six times, has enrolled 10 000 subjects and is due for completion in mid-2016.

VPM1002 is the only recombinant BCG vaccine candidate currently in clinical trials. VPM1002 is designed to provide enhanced immunogenicity as compared to BCG due to the insertion in the BCG DNA of a gene for listeriolysin and the deletion of a urease gene. This candidate, being developed by Vakzine Projekt Management of Germany and the Serum Institute of India, is being assessed as both a possible BCG replacement in infants and as a vaccine to prevent recurrent TB disease in adults following successful completion of treatment for active pulmonary TB. It is also being developed as a replacement for BCG in the treatment of bladder cancer.

MTBVAC represents the only whole cell candidate currently in clinical trials that is derived from Mtb. Stable deletions of genes

coding for two different Mtb virulence factors, phoP and fadD26, have been made to ensure the safety of the vaccine. This vaccine, being developed by investigators at the University of Zaragoza, Spain, and BioFabri, primarily is being developed as a BCG replacement vaccine in infants and is in phase 2 trials.

Like *M. vaccae*, DAR-901 is derived from a heat-inactivated whole cell, NTM. When a heat-inactivated, agar-cultured preparation of this NTM was administered in multiple doses during a phase 3 trial for prevention of disseminated TB disease in HIV-infected participants (the DAR-DAR trial), significant protection against definite TB disease was observed (p=0.03, 95% CI 0.39–0.96).[13] Protection against disseminated disease was not demonstrated, although the number of cases overall was small. DAR-901, a broth-cultured preparation of this NTM, is being developed by investigators at Dartmouth University, USA, to prevent TB disease in adolescents and adults. A phase 1 safety and immunogenicity trial recently was completed and a phase 2 trial in Tanzania is being planned. The DAR-DAR trial, which included HIV-infected participants, illustrates the importance of developing vaccines both safe and effective for use in individuals at high-risk for Mtb infection and TB disease.

RUTI® (Archivel Farma, Barcelona, Spain), developed by Dr. Pere-Joan Cardona, Barcelona, Spain, consists of detoxified, fragmented Mtb contained in liposomes and comprises a range of protein, lipid and glycolipid antigens. It is being developed as an immunotherapeutic agent for use in persons with active TB to reduce the extent and duration of drug treatment.

Although not currently being assessed clinically, a killed preparation of *Mycobacterium indicus pranii* (Mip), a vaccine originally used as a vaccine against leprosy, was demonstrated to have provided some protection against Mtb in a retrospective analysis of a phase 3 leprosy trial.[14] Plans for the further development of Mip, however, are unclear.

Viral vectored vaccines

Ad5Ag85A, ChAdOx185A, MVA85A and TB/FLU-04L are all viral vectors expressing antigen 85A (Ag85A) from Mtb. Ag85A is a mycolyl transferase enzyme important for cell wall synthesis. Ag85A is also involved in lipid accumulation and storage, potentially important in Mtb dormancy.[15] The Ag85A protein is conserved across mycobacterial species including Mtb, BCG and environmental mycobacteria and induces a strong Th1 type cellular immune response. Viral vectors can induce high levels of Ag85A antigen specific CD4+ and CD8+ T-cells in those with pre-exisiting immunity to Ag85A primed by BCG vaccination or exposure to mycobacteria in the environment. TB/FLU-04L additionally contains ESAT-6, an immunodominant antigen secreted by Mtb but not BCG.

Adjuvanted protein subunit vaccines

M72 + AS01E, H4 + IC31, H1 + IC31, H56 + IC31 and ID93 + GLA-SE are adjuvanted protein subunit vaccines. These vaccines are being developed as boosts to BCG to prevent de novo infection with Mtb and/or reactivation in those already infected.

The Glaxo Smith Kline (GSK) product M72, a fusion protein containing the Mtb genes MTB 32A and MTB 39A, in combination with the GSK proprietary adjuvant AS01E, currently is the adjuvanted protein subunit vaccine in the most advanced level of clinical testing. An ongoing, phase 2b study of M72 + AS01E, cosponsored by GSK and Aeras, is designed to assess the efficacy of this candidate to prevent the development of active TB disease among persons already infected with Mtb. Enrolement is complete and a total of 3573 adults have been enrolled in this study from South Africa, Kenya and Zambia. Results from this study are expected in 2018.

The H4 + IC31 adjuvanted fusion protein vaccine candidate, containing the Mtb antigens 85B and TB10.4, originally created by SSI (Denmark) but now being developed by Sanofi Pasteur in collaboration with Aeras, and administered in combination with the Valneva IC31 adjuvant, currently is in a novel, phase 2 study designed to assess the ability of this vaccine to prevent the establishment of Mtb infection in a cohort of South African adolescents at high-risk of Mtb acquisition. The study, cosponsored by Aeras, is fully enrolled; results are expected in 2017. Another IC31-adjuvanted SSI vaccine candidate, H56, containing the Mtb antigens 85B, ESAT6 and Rv2660c, is being assessed for safety and immunogenicity in a phase 2 trial in South African adults.

ID93 + GLA-SE, developed by the Infectious Disease Research Institute (IDRI), Seattle, USA, is composed of four Mtb antigens, RV2608, Rv3619, Rv3620 and Rv1813, in combination with IDRI's proprietary adjuvant, GLA-SE. A phase 2 trial of the safety and immunogenicity of the vaccine is ongoing.

Exploring new ideas in TB vaccine design

Broadening antigen selection

There is a dominance of vaccines that express contain Ag85 proteins with six of the eight subunit vaccines containing an Ag85 protein (Ag85A or Ag85B). There is limited data from clinical efficacy trials to determine whether a focus on this antigen or any other individual antigen is an effective approach or not. However, Mtb is a complex with a large number of protein, lipid and glycolipid antigens that are known to be immunogenic in humans. Induction of immunity across a broad range of molecules is an alternative strategy to the highly focused subunit vaccine approach. As previously noted, the whole cell mycobacterial vaccines DAR-901, Vaccae™, VPM1002 and MTBVAC contain a broader range of immunogenic molecules, as compared to viral vectored and protein and adjuvant vaccines, and have the potential advantage of inducing a broader immune response. Increasing the breadth of an immune response, however, frequently has a cost of lowering the magnitude of the immune response to any one individual antigen. Accordingly, strategies that increase the breadth of immune response while maximizing immune strength may be required.

Sequencing has shown that T-cell epitopes in almost all antigens across different strains of Mtb are hyperconserved; across 1226 sequenced T-cell epitopes variation was only found in seven genes.[16,17] Hyperconservation indicates that the majority of epitopes in Mtb are not under direct T-cell immune pressure. This finding suggests that inclusion of one of the rare, variable genes in a vaccine candidate may help to boost immunity to one of the few antigens naturally exposed and pressured by a T-cell immune response.

An alternative antigen selection strategy is to choose a conserved antigen but redirect the immune response towards an epitope within that antigen, which is either not usually recognized or only weakly recognized during natural immunity. Such epitopes are referred to as subdominant. The ability to induce immunity and protection against challenge with Mtb subdominant epitopes

has been demonstrated in mouse models.[18–20] Translating this approach to humans will require the identification and characterization of subdominant epitopes, a challenging task given the extent of human leucocyte diversity as compared to epitope identification in less diverse, clonal mouse strains.

Broadening immune responses

We do not have a validated immune correlate of protection from TB disease to aid the development of TB vaccine candidates. However, the importance of Th1 type cellular immunity has been demonstrated through human genetic studies and murine Mtb challenge experiments;[21–27] therefore, vaccines that focus on the induction of a CD4+ and CD8+ Th1 type immune response dominate the current TB vaccine pipeline. Although we have neither proved nor disproved the concept of Th1 type vaccine induced immunity for protection against TB disease in clinical trials, it is important to support the development of vaccines that enhance further aspects of the immune response if we are to increase the chances of obtaining a successful TB vaccine by 2025.

Humoral immunity

It is increasingly recognized that B cells and antibodies can modulate the immune response to intracellular bacteria including Mtb.[28] Mechanisms of antibody-mediated protection are likely to be both classical, including opsonization and complement activation, and non-classical, including modulation of immunity through Fc receptor (FcR) engagement. FcRs can either inhibit or activate immune responses, and inhibitory FcRs, such as FcϒR ϒ-chain, have been shown to limit immune pathology in active TB disease.[29] BCG can induce antibody, although only at modest levels.[30–33] When pre-coated with sera from BCG vaccinated people, however, the uptake of BCG by phagocytic cells is increased, indicating that although modest in titre, BCG-induced antibodies can have anti-mycobacterial effects.[34] Designing vaccines to boost antibody responses primed by the BCG vaccine is one strategy open to TB vaccine developers, although care must be taken to consider the pattern of FcR engagement of the boosted antibodies. In addition, antibody avidity should be considered, given the finding that, among patients with active TB, antibody was present but avidity was lower than that of uninfected controls.[35] It will also be important to consider the impact of pre-existing antibody responses induced by environmental mycobacteria. A study of non-BCG vaccinated UK school children found high titres of mycobacterial specific IgG antibodies at birth and in older children.[36] Others have also found significant antibody responses cross-reactive with NTM in healthy UK populations.[37] The impact of pre-existing responses on the immunogenicity and efficacy of an antibody inducing vaccine should be assessed during clinical development.

Non-classical T-cells

In recent years there have been new discoveries and greater understanding of T-cells that do not express the classical T-cell receptor, which recognises complexes of peptide antigen, in association with MHC molecules. Unconventional T-cells include CD1, MR1, HLA-E and $\gamma\delta$ T-cells.[38] These cells bear receptors able to bind non-protein antigens and are present in large numbers in the circulation and tissue, such as mucosa, making them capable of reacting rapidly to infection. Innate lymphoid cells (ILCs) do not bear any antigen receptor but react rapidly to inflammation through cytokine receptors and secrete large quantities of cytokine, which can shape the development of an adaptive immune response.[39] Natural killer (NK) cells can be considered a Type 1 ILC as they do not have an antigen receptor yet are quickly recruited and secrete large quantities of IFN-γ and cytotoxic molecules following infection or vaccination.[39,40]

The subunit vaccines in the current TB vaccine pipeline are designed to enhance classical T-cell responses against peptides presented in association with MHC class I and II, respectively. Innate lymphoid cells and unconventional T-cells such as NK cells and $\gamma\delta$ T-cells, however, are induced by both BCG and viral vector vaccines in humans.[41–45] Current research is focused on optimal antigen selection for enhancement of an unconventional T-cell response relevant for long-term, vaccine-induced protection against TB disease.

Vaccine design and the End TB strategy

There is a need for greater diversity in the antigens targeted and immune responses elicited by TB vaccine candidates if we are to maximize our chances of obtaining a successful candidate by 2025. In recognition of this, there has been renewed investment in TB vaccine development by funders, such as the EU Horizon 2020 Programme and the Bill and Melinda Gates Foundation. These funders have specifically encouraged the development of TB vaccine candidates that would broaden the current vaccine portfolio to include mucosal immunity inducing, antibody inducing, and non-conventional T-cell inducing vaccines.[46]

TB vaccine clinical trials and the End TB Strategy

There are ongoing Vaccae™, VPM1002, M72 + AS01E and H4 + IC31 TB vaccine efficacy trials. Even if these vaccines do not demonstrate sufficient efficacy to merit further development, conducting these and other later stage clinical trials will hasten the development of a TB vaccine that ultimately will prove to be successful. Direct results of conducting later stage clinical trials include building capacity; generating high quality data on TB incidence and prevalence; gathering data on TB vaccine safety; gaining insight into immune correlates of risk of TB disease. Also, if conducted in parallel with properly designed NHP or other animal model studies, will help determine if the animal model is predictive. Even vaccines that showed very limited efficacy have potential to be used to identify correlates of protection.

These gains will enable us to design better TB vaccines and, importantly, to move these vaccines into advanced efficacy trials more rapidly and at lower cost than was possible for previous TB vaccine candidates.

Impact of host-immunity on vaccine response

Factors specific to the host can impact on the response to a vaccine and may interfere with vaccine efficacy. Coinfection with pathogens is one factor known to modulate the immune response.[47] TB vaccine trial participants are therefore screened for symptomatic evidence of active infection and specifically

screened for infection with HIV and hepatitis B virus (HBV). For some infections, such as cytomegalovirus (CMV) and influenza, however, the immune response may be altered for substantial periods of time after symptoms are no longer apparent and could impact on vaccine immunogenicity and efficacy in clinical trials.[48–50] Gene signatures associated with a Type 1 interferon immune response are increased in those at risk of progressing to TB disease (Zak, Penn-Nicholson, Scriba et al; Forthcoming) and increased frequencies of monocytes relative to lymphocytes have been associated with risk of progression to TB disease.[51–53] How these and other factors could impact TB vaccine efficacy have not been determined but care should be taken to collect data on cofactors during clinical efficacy trials.

Vaccines and social interventions: working together to End TB

There is no doubt that TB transmission and progression are driven by social factors, including crowded, poorly ventilated housing, transportation and classrooms in areas where TB is otherwise endemic, and exogenous situations that increase a person's susceptibility to Mtb infection and TB disease, such as poor nutrition. Addressing the socioeconomic determinants of TB disease are necessary components in any comprehensive strategy designed to contain the global TB epidemic.[54] Unfortunately, the case for social interventions is sometimes presented as an alternative to investment in TB vaccine development. It is critical that these complementary strategies not be viewed from the perspective of a 'zero-sum game': furtherance of both approaches will be imperative for a strategy to end the global TB epidemic to succeed. As noted in the Stop TB Strategy, TB vaccine research and development efforts are vastly underfunded, with an annual funding shortfall of approximately US$250 million.[55] Clearly, further depleting TB vaccine R&D funding, even in support of otherwise well-intentioned TB control initiatives, would be short-sighted and would have a devastating effect on the TB vaccine development effort.

Conclusions

Developing new vaccines capable of preventing active TB disease and thereby decreasing Mtb transmission represents a critical strategy in the overall efforts to end the global TB epidemic. Although the current TB vaccine clinical pipeline contains a degree of diversity in terms of vaccine delivery platforms, ranging from whole mycobacterial cell vaccines to adjuvanted proteins and vectored vaccines, these candidates generate CD4+ and, to a lesser extent, CD8+ T-cell responses. Clearly, there is a need to develop greater immunologic diversity in the current TB vaccine pipeline. In recognition of this, funders have invested in TB vaccine development with a particular focus on efforts to induce unconventional T-cell responses (e.g. CD1, MR1, HLA-E and $\gamma\delta$ T-cells) and antibody responses to broaden vaccine immunogenicity. By conducting later stage clinical trials, which include preliminary assessments of efficacy, we have built research capacity, generated high quality data on TB incidence and prevalence, and gained insight into immune correlates of risk of TB disease. These gains will enable the design of better TB vaccines and the acceleration of the move into efficacy trials more rapidly than was possible for previous TB vaccine candidates. Further investment is needed to enable the conduct of additional, robust trials of TB vaccine safety, immunogenicity and efficacy. Potential cofactors, such as diabetes mellitus and malnutrition, have been identified which could impact TB disease risk and vaccine efficacy. These cofactors should be assessed during efficacy trials of TB vaccines.

Supplementary data

Supplementary data are available at Transactions online (http://trstmh.oxfordjournals.org/).

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