Inhaled Vaccines for the Prevention of Tuberculosis

Garcia Contreras L1, Awashthi S1, Hanif SNM1 and Hickey AJ2,3*
1Department of Pharmaceutical Sciences, The University of Oklahoma Health Science Center, Oklahoma City, OK 73126-0901, USA
2Division of Molecular Pharmaceutics, University of North Carolina at Chapel Hill, NC 27599-7571, USA
3RTI International, 3040 Cornwallis Road, P.O. Box 12194, Research Triangle Park, NC 27709-2194, USA

Abstract

Tuberculosis a major challenge to global health exacerbated by emerging multi drug resistant (MDR) and extensively drug resistant (X-DR) strains of M. tuberculosis and co-infection with HIV. BCG, the only approved vaccine, has variable protection ranging between 0-80%. Compared to the large number of new vaccine candidates a modest effort has been expended to investigate other routes of administration, such as pulmonary and intranasal. Vaccination by these routes is relevant since TB infection is mainly acquired by inhalation of a few aerosol droplets containing as little as 3-5 viable bacilli. The lungs have many attractive immunological features including bronchoalveolar lymphoid tissue (BALT) and local antigen presenting cells (APCs) sampling airborne pathogens. Aerosol vaccination is a noninvasive method of antigen delivery that may facilitate mass vaccination campaigns. Administration by non-medical personnel and the ability to eliminate transmission of blood borne diseases arising from poor practice in injected procedures in remote areas is beneficial. The current dogma does not include mucosal immunity for protection against TB but its contribution may not be ruled out. The selection of the appropriate antigen, aerosol formulation and inhaler will determine the success of this approach. Dry powder formulations of antigen/adjuent combinations have efficiency of delivery, stability and sterility advantages over liquid formulations. Dry powder vaccines can be manufactured as micro particles and nanoparticles prepared with different materials including polymers, sugars and amino acids. Examples of these novel vaccine formulations and their evaluation in animal models are discussed in the present review. The proposed pharmaceutical and clinical advantages of inhaled dry powder vaccines justify further evaluation.

Keywords: Dry Powder; Formulation; Inhaled Vaccines; Tuberculosis

Introduction

Mycobacterium tuberculosis (MTB) is one of the most prominent human pathogens infecting one-third of the world's population [1] and mutates into multidrug-resistant (MDR) and extensively-drug resistant (XDR) strains. Infection with and mutation of MTB, the co-infection with the human immunodeficiency virus (HIV), the limitation in current therapies and the lack of patient compliance for tuberculosis (TB) treatment have all contributed to the significant threat to the global control of TB. Effective vaccination strategies to prevent the disease would be the best intervention for the global control of TB. The current vaccine against TB, bacilli Calmette-Guérin (BCG) was developed by the French scientists Calmette and Guérin after Mycobacterium bovis was isolated from a cow with tuberculous mastitis in 1908 [2]. Small children in many countries worldwide have been routinely vaccinated with BCG for over a century. BCG is considered safe, has minor side effects and it is inexpensive to produce large quantities. Unfortunately, protection conferred by BCG during childhood wanes after 10 or 15 years and has variable degree of protection to pulmonary tuberculosis in adults [3]. Large scale randomized control trials and case-control studies demonstrated a 0% protective efficacy in Chingleput, India and 80% protection in Haiti and Canada [4,5]. In addition to different BCG strains used for these trials (Montreal, Danish, Phillips, Tice, Glazo, Madras and Birkhaug) [5], the efficacy of BCG appeared to be dependent upon temperature and location of the geographical area in which the vaccine was evaluated. Good protection appeared to occur mainly in temperate regions, whereas tropical regions of the globe appeared to show poor protection. Several factors have been implicated in the variability of BCG protection, including nutritional differences in the population, the use of different BCG strains, and the variability in the exposure of different populations to environmental mycobacteria and parasites [4,6].

A major research effort has been expended to develop new TB vaccines that provide the same effective protection despite of the population or the region of the world. Hundreds of TB vaccine candidates have been reported after the publication of the MTB genome in 1998 [7]. These include recombinant BCG or other vectors expressing mycobacterial antigens, attenuated or recombinant strains of mycobacteria, DNA vaccines, protein or peptide vaccines in adjuvants and non-peptide vaccines [8]. Compared to the number of new vaccine candidates produced, little research has gone into investigating other routes of administration, such as the pulmonary, intranasal and oral routes. This is surprising since TB infection is mainly acquired by inhalation of a few aerosol droplets containing as little as 3-5 viable bacilli. Inhaled vaccines are potentially powerful tools to provide immunity since the pulmonary route of immunization follows the natural route of infection, thus closely mimicking the induction of immunity in the respiratory tract by inhaled pathogens. From the perspective of vaccine development, the lungs have many attractive immunological features including organized lymphoid follicles, known as the bronchoalveolar lymphoid tissue (BALT) and local antigen presenting cells (APCs) located ideally to sample antigens entering the airways [9]. In addition, the pulmonary epithelium has a crucial role in host defense against inhaled pathogens as it possesses...
natural defense mechanisms including the mucociliary escalator and secretion of antimicrobial agents, chemokines and cytokines into the mucous layer covering the airway epithelium to prevent colonization of microorganisms [10,11]. Aerosol vaccination is a noninvasive method of antigen delivery that may facilitate mass vaccination campaigns, the administration by non-medical personnel and the ability to eliminate transmission of blood borne diseases arising from poor practice in injected procedures in remote areas [12]. Finally, this route of immunization has the potential to be successful in children, for whom the persistence of maternal antibodies does not appear to interfere with mucosal immunization but does interfere with subcutaneous immunization [13].

The first recorded use of a mass aerosol vaccination can be traced to 1951 when Johnson and Gross immunized a flock of chicken against Newcastle disease by atomization of the B1 strain of the virus [14]. The success of this approach led to the use of aerosol vaccination to immunize other small farm animals against common diseases [15,16]. In 1968, pioneering studies by Rosenthal et al. compared the immunization with aerosolized BCG in guinea pigs and in a few human subjects [17]. However, perhaps the most successful example of a human mass aerosol vaccination has been the immunization of pre-school and school aged children against measles by the inhaled aerosol method undertaken in Mexico between 1988 and 1990 [18]. Fewer side-effects and a larger percentage of seroconversion (52-64%) were observed after aerosol immunization than after injection vaccine (4-23%) [19]. The World Health Organization (WHO) has recognized the potential of immunization using alternative routes of vaccine delivery, such as small-particle aerosol and large-droplet intranasal administration [20] and has considered the use of different devices to administer these vaccines [13]. The pulmonary route of immunization employed to deliver small-particle aerosol is currently preferred, as it has been shown to induce strong and long-lived systemic and mucosal immune responses [21-23].

This review presents the factors that can influence the successful delivery and effectiveness of an inhaled vaccine for tuberculosis including the type of vaccine or antigen used, the relationship between the route of immunization and the nature of the immune response elicited, the formulation and the device employed to deliver the vaccine and the animal model in which the vaccine is tested. Examples of inhaled vaccines for TB will be also discussed.

The Immune Response

Success of a vaccine depends on an efficient presentation of vaccine-antigens by antigen-presenting cells followed by generation of antigen-specific effect or immunity and persistence of immunological memory. As long as the memory response persists, a vaccinated individual can clear the infectious organisms upon contact through a recall immune response. This immunity has to be initiated quickly before the infectious organisms evade the immune mechanisms and get an opportunity to establish pathology. Consequently, delivery of the vaccine-antigens to the lungs should be more effective for prevention of respiratory infectious diseases, such as TB and other microbial infections that are initiated at the pulmonary mucosa [24,25].

The cellular and biochemical milieu in the lung, including surfactant, mucus, proteins, peptides, glycoproteins, glycolipids, will naturally affect the antigen-uptake and presentation by antigen-presenting cells (APCs) [26]. Among different APCs, dendritic cells (DCs) are the most effective because of their flexible morphology, variant phenotypes and unique ability to physically move within the tissue [27,28]. Upon taking up the antigens, the DCs are activated and develop unique features. These activated DCs extend many long dendrites which can cover a considerable zone of cell-cell contacts at targeted site by crossing these natural barriers [29,30]. Therefore, a small number of DCs are required to activate antigen-specific adaptive immune response. Other typical APCs, epithelial cells and macrophages, are also critical even if they are not as flexible and robust in mounting immune response, cross-talk between macrophages and DCs through cross-antigen-presentation, secretion of cytokines, chemokines and trans-signaling can significantly affect this process. The mechanism of phagocytosis and antigen-processing of TB antigens by DCs is temporally different from that in macrophages. DCs first take up the TB antigens directly or by cross-priming. Then, vesicles carrying TB antigens are released from infected macrophages that are taken up by the bystander non-infected APCs, including DCs (Figure 1) [31-33]. Phagocytosis of TB antigens results into maturation and migration of DCs through activation of cytokines, chemokines, release of reactive oxygen intermediates, and increased expression of antigen presentation (MHC class II, CD1) and T cell co-stimulatory molecules (CD40, CD80, CD86) [32].

Migration of mature DCs is mediated by upregulation of receptors such as CCR7. The processed antigen, expressed on the cell-surface MHC molecules as antigenic peptides, is presented to the naïve T cells, NK cells or other immune cells. Dendritic cells show versatility in expressing the antigens on MHC class I or MHC class II molecules, depending on the nature of the epitope, thereby inducing CD8 cytotoxic T cell or CD4 helper T cell response, respectively (Figure 2). The CD4+ T cells have shown to mount protective immune response against TB, while contribution of CD8+ T cells is not confirmed [34].

Depending on the density of the TB antigens presented, types of co-stimulatory molecules, and cytokines secreted by the DCs, naïve T cells differentiate into either Th1 or Th2 cells and B cells produce antibody. A relatively new paradigm suggests that even non-lymphoid organs, such as lung, also have lymphoid structures in their stroma that are efficient in inducing local immune responses [35,36]. This set up will make it more convenient for the local DCs to produce effector immunity and memory in lung against TB antigens. Experimental evidence suggests that pulmonary vaccination is capable of inducing systemic immunity [37,38].

Considering the utility of the pulmonary delivery, a sophisticated and efficient vehicle is needed to carry the vaccine antigen. Viral and some of the non-viral vectors (for example, lipid-based vectors) may

Figure 1: Cross priming mechanism for stimulation of major histocompatibility complex (MHC) I and CD1 restricted T cells. (DC = dendritic cell; ER = endoplasmic reticulum; TAP = transporter associated with antigen presentation) (Adapted from [60]).
cause severe toxic responses including inflammation and mortality as reported in few lung disease-specific clinical trials [39,40]. Thus, unique non-viral vectors are required that can carry the vaccine antigens and target the lungs. For the development of an efficient immune response, stable but not necessarily persistent expression of vaccine antigens is desired [41]. In the lungs, DCs are usually present in a quiescent phase and in a very low number; therefore, adjuvants can be very important in vaccine formulations as a stimulus to the immune cells.

New Vaccine Candidates

Live bacterial vaccines are thought to induce the greatest protection because they carry multiple antigens and they could live in tissues for sufficient time to ensure efficient immunological memory is induced and maintained [42]. However, these assumptions may not apply to all mycobacterial vaccines. Since the earliest use of BCG, there has been debate about the benefits or disadvantages of using this vaccine. BCG may be capable of switching on multiple immune mechanisms in response to compounds in the bacterial cell wall, secreted proteins and naturally occurring antigens (innate, CD4/CD8, and gamma cell responses, respectively). In addition, it has a long track record of safety, protects repeatedly and uniformly against severe childhood forms of TB and leprosy and it is very inexpensive [8]. However after the failure of BCG to protect large populations particularly in the developing world, hundreds of vaccine candidates have been developed to address one or more limitations of BCG. Over the last decades, several types of vaccines have been evaluated in animal models for protection against TB infection including, recombinant BCG, BCG over-expressing native proteins, attenuated MTB, subunit and DNA vaccines. Except for attenuated MTB, none of these vaccines can provide the same or better level of protection as the universal BCG in animal models [43].

In order to develop improved vaccines against TB, BCG has been employed as homologous vector to over express mycobacterial antigens. BCG can supply a number of relevant mycobacterial antigens, recombinant antigens and it also had the capability to express MTB proteins in native form and release them in a manner that makes them being processed similarly to MTB proteins (Table 1). The work of Tullius et al. [44] is noteworthy because recombinant BCG strains were developed specifically to vaccinate immunocompromised persons, such as HIV patients. These vaccines can replicate in vitro, in media containing specific supplements and in cultured macrophages but their replication in animal models is highly restricted; thus avoiding serious disseminated disease in these patients. Other forms of BCG have been developed to express immunomodulatory cytokines (Table 1) or genetically modified to enhance antigen presentation [43]. Two approaches have been used to improve antigen presentation either by enhancing MHC I or MHC II antigen presentation. Grode et al. modified BCG to secrete listeriolysine to promote perforation of the phagosomal membrane and with deleted urease gene to reduce the pH in the phagosome to be optimum for the action of listeriolysine [45].

Another strategy, employing whole bacterium, has been to attenuate MTB by deleting one or more virulence factor genes [46]. In theory, the use of modified MTB is better than BCG because MTB contains over 120 specific genes that are not found in BCG, thus being likely more effective for immune activation. Some of these vaccine candidates are shown in table 1. In addition to public perception and acceptance of these vaccine candidates, the main concerns are the danger of reversion from attenuated to virulent state and the potential release of antibiotic gene markers [47].

Non-viable subunit vaccines have been considered valuable for safety reasons mainly with respect to immunocompromised patients or those who have been previously vaccinated with BCG. Subunit vaccines include one or more immunodominant MTB antigens and can be administered with an adjuvant or a viral vector to increase its potency [46]. Examples of these vaccine candidates are given in table 2.

Potential vaccines for TB have been developed from antigens such as Ag85 and ESAT-6 and their fusion proteins, which have been shown to confer some degree of protection in animal models [48]. More frequently, subunit vaccines have been employed in prime-boost vaccine strategies in animals that are primed with BCG, named heterologous prime-boost immunization strategies. These vaccine strategies can evoke powerful T cell immune responses and may be useful to develop an improved vaccine regimen [1].

The use of viral vectors with MTB antigens, either as prime or boost, also shows great potential to produce a new vaccine. One of the most promising candidates in this category of vaccines is the recombinant strain of the modified vaccinia Ankara virus expressing Ag85A from MTB (MVA85A). Initial clinical studies with MVA85A performed in 2002 were designed to demonstrate its safety in BCG-naive, tuberculin test negative adults [6]. From 2002 to 2008, 258 subjects were vaccinated without signs of adverse events, but in a recent study antigen-specific IL-17A-producing T cells were detected in the systemic circulation of healthy individuals after vaccination with MVA85A [49]. A newer strategy employing MVA expressing IL-15 plus the ESAT-6/Ag85 fusion protein has improved this approach in the mouse model [50].

It is encouraging that several of the vaccine candidates shown in tables 1 and 2 are actually in different stages of clinical trials, making more tangible the promise of a new vaccine against TB.

Animal Models for Vaccine Testing

The use of an animal model relevant to human disease is fundamental to new vaccine development. Overall, there is no ideal animal model of TB, but each of the available models can represent certain aspects of the human disease. However, when analyzing the outcome of a study, it is important to consider the similarities and differences of the animal model selected with respect to those of humans, and their susceptibility to infection with a determined bacterial strain. It is also important to understand that complete protection imparted by a vaccine in terms of complete sterilization of the lungs of immunized animals or reduction in bacterial burden cannot be obtained because the animal model may have a limited “window of protection” [51]. For example, in the mouse this “window of protection” is quite small, with only 1.0-1.2 log protection usually achievable. In the guinea pig, in which the control inoculum grows to higher values because of the inherent susceptibility of this animal, a larger range of 2.0-3.0 logs can be attained [8,52]. Thus, it is of paramount importance to know the advantages and disadvantages of each animal model for vaccine testing.

Among TB animal models, the most frequently used model is the inbred mouse [53] because of the detailed knowledge of its immune system and the large variety of reagents available [51]. In addition, the mouse genome has been completely sequenced and the genetic manipulation of this species is highly advanced so that various strains of knockout mice are accessible [51]. However, the most attractive advantages of the mouse are its cost (for animal purchasing, husbandry and housing) and smaller space requirements. Thus, large numbers of vaccine candidates can be tested in this model, and the most promising candidates can be tested in other more relevant species at a later time. Similarities between the basic immune response of mice and men
include toll-like receptors, NK and NKT cells, and production of antimicrobial molecules such as nitric oxide. However, there are several differences in the response to TB infection and the progression of the disease between mice and man. Besides size, anatomy, vasculature and lymphatic system, the most important differences are in the pathology of the granuloma and the cell mediate immunity. Mice lack CD1b and CD1c cells, there are no visible lesions in the mouse lungs after 10 days of infection, and after 20 days only perivascular cuffing of mononuclear cells with central aggregation of lymphocytes is visible with no evident necrosis [53]. This may be one of the main factors contributing to the concern that vaccines being effective in the mouse may not be effective in humans.

The guinea pig is the smallest animal model that most closely resembles TB infection in humans. Guinea pigs are highly susceptible to TB infection with only a few droplets containing 3-5 bacilli and the progression of the disease is similar to that in humans [52]. Guinea pigs are outbred and in that respect their population is reflective of human populations. Studies in this species have been instrumental to understand the relationship between mycobacteria and their mammalian hosts as well as key elements of their immune response. Discrete lesions can be observed in the guinea pig lungs after 10 days of infection with extra-pulmonary dissemination to the spleen and other organs occurring 10-14 days after infection humans [52]. The “classic” granuloma can be observed 21 days after infection with mineralization of the central core occurring during the chronic phase of the disease.

### Table 1: Summary of new vaccine candidates against TB by modification of the whole bacterium (modified from [43,46]).

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Description</th>
<th>Results (compared to BCG)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant BCG overexpressing native MTB proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rBCG30</td>
<td>BCG over expressing the 30kDa protein (Ag85B)</td>
<td>The first vaccine demonstrating more potency than BCG against TB</td>
<td>[114]</td>
</tr>
<tr>
<td>rBCG/Ag85A</td>
<td>BCG over expressing Ag85B</td>
<td>Fewer CFU in lung and spleen in vaccinated guinea pigs; fewer CFU in lung but not in spleen in cynomolgous monkeys</td>
<td>[115,116]</td>
</tr>
<tr>
<td>rBCG/Ag85C</td>
<td>BCG over expressing Ag85C</td>
<td>In guinea pigs, reduced CFU in lung and spleen; reduced pathology in lung, spleen and liver; reduced pulmonary fibrosis</td>
<td>[117]</td>
</tr>
<tr>
<td>rBCG/ESAT-6 (sCFP10)</td>
<td>BCG secretes both ESAT-6 and CFP10</td>
<td>Tested in mice and guinea pigs; same protection in lung but better in spleen; not good in immunocompromised mice</td>
<td>[118]</td>
</tr>
<tr>
<td>rBCG/38 kDa protein</td>
<td>BCG over expressing 38kDa glycoprotein</td>
<td>Immunized mice survived longer</td>
<td>[119]</td>
</tr>
<tr>
<td>Recombinant BCG overexpressing MTB fusion proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rBCG/72f</td>
<td>BCG secreting a hybrid of proteins Mtb39 +Mtb32 (named 72f)</td>
<td>In cynomolgous monkey induced immune and protective responses but not different than BCG controls</td>
<td>[120]</td>
</tr>
<tr>
<td>rBCG/Ag85B-ESAT-6</td>
<td>BCG secreting fusion proteins of Ag85B and ESAT-6</td>
<td>Protective responses similar to BCG controls in mice</td>
<td>[121]</td>
</tr>
<tr>
<td>rBCG/Ag85B-Mpt64&lt;sub&gt;30-114&lt;/sub&gt;</td>
<td>BCG expressing a fusion protein of Ag85B with immunodominant peptide Mpt64 and Mpt8.4</td>
<td>Comparable or slightly better efficacy than BCG in mice</td>
<td>[61]</td>
</tr>
<tr>
<td>Recombinant BCG overexpressing native proteins and additionally attenuated for safety in HIV-positive persons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rBCG(mtbB)30</td>
<td>Rendered sideropore dependent by deletion of the gene mbtB</td>
<td>Safer than BCG in SCID mice and more potent than BCG in the guinea pig model of TB</td>
<td>[44]</td>
</tr>
<tr>
<td>rBCG(panCD)30</td>
<td>Rendered pantotenate dependent by deletion of the panCD genes</td>
<td>Can multiply in vitro, but multiplication is limited in vivo; safer than BCG in SCID mice in protection comparable to BCG in the guinea pig model of TB</td>
<td>[44]</td>
</tr>
<tr>
<td>Recombinant BCG expressing immunomodulatory cytokines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rBCG/GM-CSF</td>
<td>BCG secreting murine granulocyte macrophage colony stimulating factor</td>
<td>Immunized mice had higher numbers of antigen presenting cells; IFN-gamma secreting cells, and ~1 log fewer CFU in spleen after virulent challenge</td>
<td>[122]</td>
</tr>
<tr>
<td>rBCG/IL-2</td>
<td>BCG secreting murine IL-2 to counter a Type 2 immune response</td>
<td>Immunized mice exhibited greater splenocyte proliferation and IFN-gamma production in response to PPD, comparable protection to BCG</td>
<td>[123]</td>
</tr>
<tr>
<td>rBCG/IL-15</td>
<td>BCG secreting a fusion protein of Ag85B and murine IL-15</td>
<td>Immunized mice had greater absolute numbers of CD4+ and CD8+ T cells in lung and spleen; greater numbers of IFN-gamma secreting CD4+ cells and lower CFU in the lung but not in the spleen</td>
<td>[124]</td>
</tr>
<tr>
<td>Recombinant BCG overexpressing native proteins and escaping the phagosome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rBCG-Aeras403</td>
<td>BCG over expressing Ag85B and TB10.4 with endosome escape</td>
<td>Safer than BCG in SCID mice; induced stronger responses in mice and guinea pigs compared to BCG; mice survived longer than with BCG</td>
<td>[125]</td>
</tr>
<tr>
<td>rBCG ΔureC hly+</td>
<td>BCG that expresses membrane perforating listeriolyisin and is devoid of urease</td>
<td>Induced superior protection in mice than BCG. Proven to be safe and immunogenic in phase 1 clinical trial</td>
<td>[126]</td>
</tr>
<tr>
<td>Modified or attenuated MTB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTB phoP mutant SO2</td>
<td>SO2 strain engineered by a disruption in the phoP gene of MTB</td>
<td>Impaired multiplication in vitro in mouse macrophages and in vivo in infected mice; Enhanced ability to bind human macrophages</td>
<td>[127]</td>
</tr>
<tr>
<td>MTB ΔRD1 ΔpanCD</td>
<td>MTB3H73Rv with deletion of the primary attenuating mutation of BCG (DeltaRD1) and two genes for the synthesis of pantothenate (DeltaPanCD).</td>
<td>Long-lived protective immune responses and longer survival in wild type mice, and CD4-deficient mice against an aerosol challenge with virulent MTB. Safe in guinea pigs and SCID mice</td>
<td>[128]</td>
</tr>
<tr>
<td>MTB ΔleuD Δpan</td>
<td>MTB with two independent attenuating auxotrophic mutations in leucine in vivo</td>
<td>Long-term protection and survival in guinea pigs against challenge with virulent MTB similar to BCG. No vaccine-associated adverse effects (clinical, hematological and bacteriological) in SIV-positive or SIV-negative Rhesus macaques</td>
<td>[129]</td>
</tr>
</tbody>
</table>
The pathology and immune response to TB infection in non-human primates is also very similar to humans, which is a big advantage of this model because most human reagents cross-react with non-human primates [63]. The confirmation of infection and progression of the disease can be tracked by a variety of serological methods, cytokine levels, chest X-rays and weight loss. This model is also unique in that TB co-infection with the human immunodeficiency virus (HIV) can be studied in this species, since they are susceptible to the simian immunodeficiency virus [64]. The disease progression in non-human primates can be classified in three categories [65]: 1) rapid infection/rapid progression, where advanced disease can be observed by 3 months post-infection; 2) active/chronic infection, where the signs of disease can be observed but the progression is slow; 3) latent infection, where no clinical signs of the disease can be observed. Based on this classification, Flynn et al. were the first researchers to develop a tractable model of latent tuberculosis infection [66,67]. In addition, this model has been successfully employed to evaluate long-term protection of experimental vaccines [68]. It is considered that studies in this model should be restricted to final experiments prior to clinical validation.

Table 2: Summary of new subunit vaccine candidates against TB (modified from [49,130]).

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Description</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag85B-TB10.4/C31</td>
<td>Ag85B-TB10.4 delivered in C31 adjuvant</td>
<td>Vaccination resulted in high numbers of polyfunctional CD4+ T cells co-expressing IL-2, IFN-gamma and TNF-alpha. This correlated with protection against virulent challenge in the TB mouse model</td>
<td>[131]</td>
</tr>
<tr>
<td>Mtbb72F</td>
<td>a 72-kDa polypeptide genetically linked in tandem in the linear order Mtb32(C)-Mtbb39-Mtbb32(N)</td>
<td>Immunization of guinea pigs resulted in more than 1 year survival after virulent challenge when compared to BCG.</td>
<td>[132]</td>
</tr>
<tr>
<td>Mtbb72F + AS02</td>
<td>Mtbb72F in the GSK proprietary adjuvant system AS02</td>
<td>Vaccine was immunogenic and caused no adverse reactions in cynomolgus monkeys. Superior protection afforded with BCG prime-Mtbb72F in AS02A boost, compared to that with BCG alone. Monkeys also survived longer and showed reversal of disease progression</td>
<td>[133]</td>
</tr>
<tr>
<td>ID83</td>
<td>fusion protein (ID83), which contains the three Mtb proteins Rv1813, Rv3620 and Rv2608</td>
<td>ID83 + synthetic TLR4 or TLR9 agonists generated a T helper-1 immune response and protected mice against Mtbb7 challenge with regardless of route.</td>
<td>[134]</td>
</tr>
<tr>
<td>MVA85A/Aeras-485</td>
<td>Attenuated vaccinia Ankara virus expressing Ag85A</td>
<td>Induced high levels of antigen-specific IFN-gamma-secreting T cells when used alone in BCG-naïve healthy volunteers. In BCG vaccinated volunteers, substantially higher levels of antigen-specific IFN-gamma-secreting T cells were induced, and at 24 weeks after vaccination these levels were 5-30 times greater than in those receiving a single BCG dose.</td>
<td>[135]</td>
</tr>
<tr>
<td>Crucell Ad35/ Aeras 402</td>
<td>Adenovirus 35 expressing 3 antigens</td>
<td>Confers protection against MTB after intranasal or IM immunization in the mouse model. Histological evaluation revealed differences in immune response between two mouse strains that were elucidated by epitope mapping analysis. A dominant CD8+ T-cell response was detected in BALB/c (H-2(d)) mice whereas a more balanced CD4+CD8+ T-cell responses were observed in C57BL/6 (H-2(b)) mice.</td>
<td>[136]</td>
</tr>
<tr>
<td>AdAg85A</td>
<td>Adenovirus 5 expressing only Ag85A</td>
<td>A single intranasal but not IM immunization was able to induce potent protection from MTB challenge in a mouse model. Also used to boost mice primed with SC BCG, showing enhanced protection that was correlated with the numbers of IFN-gamma CD4+ and CD8+ in airway lumen.</td>
<td>[137]</td>
</tr>
</tbody>
</table>
trials with drugs and vaccines in humans, not only due to the high cost of each animal and its housing but also for ethical reasons.

Lastly, the bovine model of tuberculosis is infrequently employed for the evaluation of experimental vaccines for humans. This model is more commonly used to develop diagnostic reagents to differentiate between vaccinated and infected individuals [69-71]. An advantage of this model is that large volumes of blood can be collected, thus providing the opportunity to measure a large number of immunological parameters [72]. This can be explored to identify immune correlates of protection for TB that are still not defined [73]. Notably, this model has been employed to explore one of the causes attributed to the low efficacy of the current BCG vaccine: exposure to environmental bacteria. In this study [74], a group of calves were vaccinated within 8 h of birth to mimic vaccination policies in African countries. A second group was vaccinated at 6 weeks of age, and a third group was primed at 8 h after birth and boosted at 6 weeks. Non-vaccinated calves were employed as controls. All animals were challenged at 4 months with Mycobacterium bovis and sacrificed 4 months after challenge. The study revealed that calves became responsive to environmental bacteria at 6 weeks and that the responsiveness was lost at 15 weeks of age, which did not appear to hamper the protection provided by BCG. Surprisingly, animals in the prime-boost regimen had significantly less protection than those only vaccinated at birth. The results of this study indicate that the bovine model of tuberculosis may be of use to study novel TB vaccines.

Selection of the animal model employed to evaluate vaccines delivered to the lungs depends upon the characteristic of the aerosol being assessed which includes: efficiency of aerosol delivery; immunogenicity; protection or; toxicity [75]. The anatomy and physiology of species differ significantly and in general the ease of delivery of aerosols increases as the size of the animal increases. Since the method of delivery of the particles to the lungs dictates the efficiency and reproducibility this must be considered in parallel to selection of the animal model [75].

**Pharmaceutical Forms and Delivery Devices**

Successful stimulation of the common mucosal immune system in the airways is a challenge that inhaled vaccines may face at the present stage of development. This is also needed to make the effectiveness of the inhaled vaccines against respiratory pathogens superior to injected conventional formulations. The selection of the appropriate aerosol formulation and the right inhaler device for delivery in concert with the appropriate antigen may address this challenge.

Several methods have been employed to vaccinate different animal models, individual patients or populations. For animal models, these range from direct administration methods such as intra-tracheal instillation, spray instillation and insufflation, to passive inhalation methods such as exposure chambers (whole body or nose only) [75]. For humans, three different inhaler devices can be employed, depending on the vaccine formulation: pressurized metered dose inhalers (pMDIs), nebulizers and dry powder inhalers (DPIs) [76].

Even though most inhaled drugs are delivered by pMDIs, only a few vaccines have been delivered by pMDIs. The main reason is that the formulation ingredients in pMDIs are not compatible with most vaccines, whole attenuated organisms or subunit vaccines. The propellants used to aerosolize pMDI contents as well as the surfactants or co-solvents needed to solubilize the active ingredients are detrimental for most antigens and adjuvants. Brown et al. determined that Streptococcus suis bacteria lost 50-70% of its antigenicity after being delivered with a propellant- driven device similar to a pMDI to the respiratory tract of a swine [77].

Most studies with inhaled vaccines have employed nebulizers and liquid vaccine formulations. However, delivery of therapeutic compounds with typical air-jet nebulizers can be cumbersome, inefficient and expensive. They require the use of compressed air, large volumes of the vaccine solution, long times to inhale the aerosol and it is estimated that approximately 10% of the dose placed in the device will be deposited in the periphery of the lung [78]. More effective nebulizers have been developed in the last few years, including the Pari eFlow, the ActivAero AKITA and APIXNEB system co-developed by Pari and ActivAero appears to deliver more than 80% of the drug loaded into the system [79]. One issue that is not resolved even with these newer technologies is that the sheer force generated by the nebulizer may decrease vaccine potency [80]. This was experienced by Coates et al. when they observed a 71% decrease in the potency of a measles vaccine after 20 minutes of continuous operation of the nebulizer [81]. They also determined that one-third of the vaccine potency was lost for each cycle of 30 seconds on/10 seconds off.

Liquid vaccine aerosols are frequently unstable on storage or during delivery. Manufacture of liquid vaccine formulations requires very stringent controls and the use of refrigeration throughout the process, the shipping and storage. Liquid vaccine formulations are also more prone to contamination when compared to dry formulations. Therefore vaccines such as BCG are freeze dried (lyophilized) to preserve their potency and sterility. Interestingly, the methods used to produce the injectable BCG vaccine used in the present day are quite similar as those described by Calmette many years ago. The few modifications that have been made in different laboratories are largely based on individual laboratory observations, personal preference, and convenience [82]. However, in the case of measles vaccine, the reconstituted form of the lyophilize lost potency rapidly when exposed to temperatures of 25 and 37°C.

Perhaps the best example of inhaled vaccines for the prevention of TB is the pioneer work by Rosenthal et al. in 1968 [83]. Their work was based on the assumption that the sensitization to tuberculin, though not synonymous with immunity, was indicative of the immune state of an animal model. In this study, guinea pigs, children and medical students were exposed to BCG aerosols generated by nebulization of aqueous suspensions of cells. The indirect measure for immunization in this study was the rate and size of the tuberculin reaction following exposure to BCG aerosols. Thus, the results of these early studies were inconclusive due to the measure of effectiveness used and the variability in the nebulizer output [83]. Similar studies conducted by Lagranderie et al. [84] reported the effects of bacterial strain and dose in guinea pigs receiving intradermal or aerosol immunizations with BCG. In comparison with the intradermal route of vaccination, aerogenic vaccination with 10^5 CFU induced higher local cellular immune responses and substantially improved protective effect. As in a study conducted by the authors and presented in the last section of this review, the aerosol vaccination did not result in pathological alterations to the lung tissue at the doses studied [84].

Delivery of vaccines in a dry powder form by means of dry powder inhalers (DPIs) has several advantages over pressurized metered dose inhalers and nebulizers. DPIs are portable, inexpensive, and give a better control on the dose delivered. There are single dose devices (dose in a capsule or single reservoir) and multi-dose devices (doses in blister packs). The dose deposited in the lung among common devices ranges from 12-40% for passive DPIs, but is higher for active (battery driven)
DPIs [85]. Dry powder formulations of drugs or vaccines have greater stability than liquid formulations and do not require reconstitution or refrigeration to preserve their potency. They are usually packed in a manner that protects the powder from light and humidity, thus enhancing their stability. However, one of the most desirable properties of dry powder vaccines for inhalation is that APCs in the lung (macrophages and DCs) tend to uptake dry particles. This event elicits an additional immune response and can make particles act as adjuvants, depending on their composition. In general, microparticulate systems are known to enhance the immune response greatly in comparison to soluble antigens because they act as adjuvants and stimulate the immune response to help Th1 induction [86]. Antigens alone or with other adjuvants have been associated or encapsulated in micro- or nanoparticle made of lipids, polymers, sugars, aminoacids or viral vectors. In addition to providing adjuvancy, the encapsulation of antigens into particles has other advantages for pulmonary delivery such as protecting the integrity of antigens, improving the aerosol dispersion of the particles and lung deposition, and in cases even control the time and amount of antigen released from these particles.

A group in Switzerland, led by Bachman developed novel virus-like particles (VLPs) to induce potent B cell responses in the absence of adjuvants [87]. The authors attributed this property to the highly repetitive and organized array of epitopes on viral surfaces efficiently cross-links B cell receptors constituting a strong activation signal that may even overcome B cell tolerance. A novel vaccine for tuberculosis was prepared from Influenza A VLPs were produced displaying a 20 amino acid sequence from MTB ESAT-6 [88]. Immunization of mice with this vaccine resulted in high serum antibody titers against ESAT-6, indicating that VLPs can be an efficient platform for epitope presentation.

Several experimental vaccines against TB have been formulated in liposomes and showed enhanced protection compared to the vaccine alone. Dimethyl diocatadecyl ammonium bromide (DDA) is a cationic, micelle-forming surfactant that has been used as adjuvant in few experimental TB vaccine, live or subunit, because of its Th1-stimulating micelle-forming surfactant that has been used as adjuvant in few liposomes and showed enhanced protection compared to the vaccine alone. Dimethyl diocatadecyl ammonium bromide (DDA) is a cationic, micelle-forming surfactant that has been used as adjuvant in few experimental TB vaccine, live or subunit, because of its Th1-stimulating effect and elicited protective immunity in mice [89-91]. Higher levels of protection have been obtained in this animal model when immunomodulators have been tested in combination with DDA. Some of these include trehalose 6,6 dibenenate (TDB), monophosphoryl lipid A (MPL), muramyl dipeptide (MDP), saponin, calcitrol, betagalcan and n-hexadecane. The highest advantage was obtained after vaccination of C57BL/6 mice with the DDA-MPL and the DDA-TDB combinations when the early secretory antigenic target 6 protein (ESAT-6) was used as the antigen [90]. All these experimental liposomal vaccines were administered by parenteral route. To the best of our knowledge, no liposomal vaccine has been administered by inhalation yet. However, it is possible to generate aerosols of liposomal vaccines from liquid or dry-powder forms. The latter is deemed to be more stable since the shear produced during nebulization can compromise the integrity of liposomes [92] and the antigen as described before. Dry powder liposomal vaccines can be prepared by spray drying the liquid liposomal formulation [93], or by freeze drying it followed by milling the resulting cake to obtain particles in the respirable size [94].

Polymeric micro- or nano- particles are the most used vehicles to encapsulate antigens for tuberculosis vaccines. Bivas-Benita et al. [95] prepared a chitosan-DNA nanoparticle vaccine encoding eight known HLA-A*0201-restricted T-cell epitopes derived from M. tuberculosis antigens: 19 KD, Ag85B (2 epitopes), Ag85A, PstA1, ThyA & RpoB and ESAT-6. The levels of IFN-gamma after spray instillation of a suspension of these nanoparticles were much higher in a transgenic mouse model compared to those after spray instillation of the nanoparticles alone of the plasmid by the intramuscular route. In another study, Zhu et al. produced chitosan microspheres containing an Ag85-MPT64-Mhb8.4 fusion protein made from MTB genes. Similarly, to the Bivas-Benita study, higher IFN-gamma levels were obtained after subcutaneous administration of the chitosan microspheres containing the plasmid, compared to administration of the plasmid in solution. These two studies suggest that chitosan particles can be effective carriers for potential tuberculosis vaccines. However, this should be confirmed by studies challenging immunized animals with viral strains of MTB. Polyethyleneimine (PEI) has been used in the same way as chitosan to produce nanoparticle subunit vaccines [96,97]. However, studies by Yu et al. showed a modest efficacy with Fe(3)O(4)-Glue-PEI in the MTB-infected mouse model [97].

Perhaps the polymers that have been most frequently used to encapsulate vaccines to be delivered by different routes are the derivatives of the poly-lactic and poly-glycolic acids and their co-polymers. They are popular because they are biodegradable, biocompatible and their toxicity, if any, is minimal [9]. The poly-lactic-co-glycolic acid copolymer is probably the most used from this family of polymers. Micro- or nano particles can be produced by different techniques using these polymers and their efficiency to encapsulate compounds depends on the molecular weight of the compound, its physicochemical properties and the intended size of the particle. Some of the vaccine candidates against TB that have been formulated into PLGA particles include: MTB 38KDa protein [98], MTB 71KDa protein [99], DNA encoding mycobacterial hsp65 protein +TDM [100], Ag85 DNA [101], DNA encoding Ag85B+MPT-64+MPT83+DAA [102], Ag85+TDB [103], antigen TB10.4-Ag85B fusion protein [104], and RHP65 protein+ artificial antimicrobial peptide KLK [105]. Invariable, the protection conferred by the vaccines encapsulated into particles is superior to that of the vaccine in its liquid form. As for other formulations, only one of these studies has delivered the particles in dry form by the pulmonary route [103].

As previously described (section 3), despite the many efforts to develop an effective vaccine, BCG still performs well, apparently better than most new TB vaccines, including some recombinant forms of BCG, modified forms of MTB or subunit vaccines. Several of these whole cell vaccines have shown to have advantages over subunit vaccines. The preparation of these whole cell vaccines into dry powders for pulmonary delivery requires a totally different formulation approach from the ones described for subunit vaccines. To our knowledge, only one group has prepared dry powders for inhalation containing a whole cell vaccine. Wong et al. [106] reported an alternative method to prepare BCG with leucin, by spray drying to provide a dry, flowable powder that is more viable and more stable over time at room temperature conditions than conventionally lyophilized BCG. It was demonstrated that by limiting osmotic stress on bacterial membranes during drying, i.e., by removing extracellular salts and cryoprotectants from suspensions of bacteria, bacterial viability on drying can be significantly improved relative to standard spray drying (with salts and cryoprotectants) and relative to lyophilization. BCG dried by this method also exhibited improved room-temperature shelf-life stability than with the lyophilized formulation. The size and form of the resulting particles (a mix of rods and small spheres) was thought to have a role not only in aerosol deposition but in extent of adjuvant effect achieved: the rod –shaped coated bacterium is associated with leucine spheres. The size of these particles (MMAD=2.1-3.7 μm) makes them ideal candidates for phagocytosis by APCs.
More recently the same approach was employed to manufacture a powder vaccine intended for aerosol delivery by spray drying the Ad5-vectored tuberculosis (TB) AERAS-402 vaccine with mannitol-based stabilizers. Physicochemical properties of the powder vaccine were determinate as well as the integrity of the virus during spray drying and storage [107]. The resulting powder vaccine formulated with a combination of mannitol-cyclodextrin-trehalose-dextran was deemed appropriate for pulmonary vaccination of humans and stable when stored at different temperatures.

**Evaluation of Inhaled Vaccines against Tuberculosis**

Despite the success shown with the inhaled measles vaccine in human population, immunization by the pulmonary route in the prophylaxis and therapy of infectious diseases is still in its early stages [12]. Besides the influenza vaccine, a few other studies have achieved successful immunization by the nasal route, including papilloma virus and cytomegalovirus [108]. Given the large number of new vaccine candidates against tuberculosis and the variety of novel formulations and aerosol delivery systems, there are only a few studies formulating these new vaccine candidates for inhalation, and fewer evaluating their efficacy in animal models.

Our laboratory evaluated the BCG powder vaccine, manufactured by Wong et al. [106], administered by the pulmonary route to prevent tuberculosis in the guinea pig model [109]. Animals were divided in different groups immunized by different routes (pulmonary, intradermal and subcutaneous routes) with commercial BCG solution, BCG powders in suspension BCG dry powders. Ten weeks after immunization and 4 weeks after aerosol infectious challenge with MTB H37Rv, the bacterial burden in the lungs of all immunized animals was, as expected, significantly lower than that of unimmunized controls, regardless of the BCG dose or the route of immunization (Figure 3). Most notably, the bacterial burden of lungs of animals immunized by the pulmonary route with BCG particles was significantly lower than that of animals immunized by the parenteral route (with either solution or particles). Histopathological analysis mirrored the bacteriology results, showing less lung tissue damage (in the form of granulomas) in animals vaccinated by parenteral routes whereas in animals immunized by the pulmonary route, lung tissue appeared almost normal (Figure 4). Studies that may give an insight into the way that BCG replicates and disseminates when delivered by the parenteral or pulmonary routes and possible differences in the nature of the immune response have been completed by our group and the data is being analyzed. At this point we postulate that delivering BCG in dry particle form rather than in aqueous bacterial suspension may have increased the residence time in the lungs, thus increasing the possibility of uptake by APCs. Lagranderie et al. have shown that activation of alveolar macrophages occurs more readily after inhalation than after intradermal vaccination [84]. Dannenberg suggested that at the local site, products of bacilli and those from sensitized lymphocytes are at higher concentration than occur systemically, and suggested that macrophages in the local lesion achieve much higher antimicrobial resistance that that observed for systemic macrophages [62]. It is possible that delivery of the vaccine to the lungs would favorably influence the mucosal response in the lungs and improve the local immunity. A decade ago, Kauffmann suggested that a vaccine that could generate neutralizing antibodies in the respiratory tract, such that it will kill rather than just phagocitize MTB before macrophage uptake would solve the problems of the present vaccines [51,108]. For years the dogma has been that the cellular
immune response is the only defense against MTB, but the importance of the mucosal immune response has not been established. However, it is interesting to question how the absence of a mucosal response can be postulated when mucosal routes have not been used for immunization.

Microspheres delivered as aerosols to the lungs in the size range 1-5 mm can reside at the site of deposition for extended periods of time prior to uptake by APCs [26,58]. Microspheres are mainly taken up by macrophages, which are mobile and number almost a billion in the periphery of the lungs [110]. Given the efficient targeting of microspheres to APCs, the particles have the ability to elicit strong immune responses even with small amounts of antigen [86]. Therefore, role of alveolar macrophages as initial host cells for MTB was employed by our group in a second vaccine strategy with a subunit antigen. Poly (lactide-co-glycolide) (PLGA) microspheres were manufactured in respirable sizes as carriers for recombinant Ag85B to be delivered by pulmonary route as a novel vaccine against TB [103]. Continuous release of antigens from microspheres has been shown to provide a prolonged immunological response in animals and avoids the need for multiple boosting [111,112]. Recombinant Ag85B was expressed from two Escherichia coli strains and encapsulated it by spray-drying in PLGA microspheres (rAg85-PLGA) with/without adjuvants. The ability of these rAg85-PLGA microspheres to deliver antigen to macrophages for subsequent processing and presentation to the specific CD4 was assessed in the T-hybridoma cells DB-1. These cells recognize the Ag85B97-112 epitope presented in the context of MHC class II and secrete IL-2 as the cytokine marker. The rAg85-PLGA microspheres (3.4 - 4.3 µm) were suitable for aerosol delivery to the lungs and targeting alveolar macrophages. THP-1 macrophage-like cells exposed with PLGA rAg85B microspheres induced the DB-1 cells to produce IL-2 at a level that was two orders of magnitude larger than the response elicited by soluble rAg85B. These results demonstrated that rAg85-PLGA microspheres in respirable sizes were effective in delivering rAg85B in an immunologically relevant manner to macrophages [103]. They also demonstrated that dry powder delivery of rAg85B using a microsphere formulation has potential as a vaccine strategy for preventing TB or to be employed as a promising boosting vaccine.

It is important to recognize that adult populations in many developing countries have been immunized previously with BCG or exposed to MTB, which constitutes a priming exposure, and a boost approach is more appropriate for these populations. Therefore, there is a need to develop an effective boosting immunization that could enhance and prolong the protective immunity based on BCG, or prior MTB, initiated immunity.

Based on the results obtained with rAg85B-PLGA, our group designed and performed studies to evaluate the protection afforded in the guinea pig model against virulent MTB challenge by combinations of prime-boost strategies with BCG delivered by the pulmonary and intradermal routes [113]. PLGA microspheres containing rAg85B in sizes suitable for delivery by inhalation were prepared as described above and delivered as dry powders to the lungs of guinea pigs in single or multiple doses of homologous and heterologous immunization strategies. BCG was delivered subcutaneously as the positive control and as part of heterologous immunization strategies. Immunized animals were challenged with a low-dose aerosol of MTB H37Rv to assess the extent of protection measured as reduction in bacterial burden (CFU) in the lungs and spleens of guinea pigs. Histopathological examination and morphometric analysis of those tissues was also performed. The heterologous strategy of BCG prime-P-rAg85B aerosol boosts appeared to enhance protection from bacterial infection, as indicated by a reduction in CFU in both the lungs and spleens compared with untreated controls (Figure 5). Although the CFU data were not statistically different from the BCG and BCG–BCG groups, the histopathological and morphometric analyses indicated the positive effect of BCG-P-rAg85B in terms of differences in area of tissue affected and number and size of granulomas observed in tissues. Therefore, it was suggested that direct pulmonary immunization using these particles enhanced the protection afforded by primary immunization with BCG against infection in guinea pigs. Since a large proportion of the population of the world has been immunized with BCG as infants, an aerosol boost would be a promising strategy to enhance and prolong the protective immunity based on BCG-initiated immunity [113].

An alternative prime-boost strategy was proposed by Bivas-Benita et al. [96]. They evaluated the immunogenicity of a DNA vaccine encoding the MTB latency antigen Rv1733c and to evaluate the effect of pulmonary delivery and co-formulation with PLGA–polyethyleneimine (PEI) nanoparticles on host immunity in mice. Characterization of the PLGA–PEI nanoparticles indicated they were positively charged and that their nanometer size was preserved after concentration. The nanoparticles were able to mature human dendritic cells and stimulated them to secrete IL-12 and TNF-α a comparable to levels observed after lipopolysaccharide (LPS) stimulation. MTB latency antigen Rv1733c DNA prime combined with Rv1733c protein boost enhanced T cell proliferation and IFN-γ secretion in mice in response to Rv1733c and MTB hypoxic lysate. Rv1733c DNA adsorbed to PLGA–PEI nanoparticles and applied to the lungs increased T cell proliferation and IFN-γ production more potently compared to the same vaccinations given intramuscularly. The strongest immunogenicity was obtained by pulmonary priming with nanoparticles-adsorbed Rv1733c DNA followed by boosting with Rv1733c protein. Their results confirmed that PLGA–PEI nanoparticles are an efficient DNA vaccine delivery system to enhance T cell responses through pulmonary delivery in a DNA prime/protein boost vaccine regimen and the immunogenicity of DNA vaccines can be strongly enhanced in case of pulmonary delivery by formulating the DNA with PLGA–PEI nanoparticles, followed by protein boosting.

In summary, the lungs could be a suitable route of administration for novel dry powder vaccines that may offer not only clinical advantages but also the pharmaceutical advantages of absence of needles in a mass immunization program and absence of a cold chain for delivery globally and also will address the issue of the need to increase efficacy that confers better local mucosal as well as systemic immunity; the need
to demonstrate safety during administration by reducing the risk of contamination by sharps and needles; the need to eliminate powder reconstitution, thus increasing stability during transport, storage, administration; and the need to improve cost effectiveness.

References


Inhaled Vaccines for the Prevention of Tuberculosis

- Rv1733c associated to PLGA-PEI nanoparticles enhances T cell responses in a Pulmonary delivery of DNA encoding Mycobacterium tuberculosis
- Combined DNA vaccine encapsulated in microparticles enhances protective efficacy against Mycobacterium tuberculosis infection of mice. Vaccine 23: 4167-4174.
- A subunit vaccine based on biodegradable microcarriers carrying rHsp65 protein and KLK protects BALB/c mice against tuberculosis infection. Hum Vaccin 6: 1047-1053.
- Aerosol vaccines for tuberculosis: a fine line between protection and pathology. Tuberculosis (Edinb) 91: 82-85.


