CHAPTER 6.2

RESPIRATORY MUCOSAL IMMUNIZATION AGAINST PULMONARY TB

Zhou Xing, Fiona Smaill, Carly Horvath, Christopher R. Shaler, Xuerong Chen, Jingyu Mu, Mangalakumari Jeyanathan

The entry of MTB is via mucosal route, therefore a reinforcement of the mucosal immune response could be an advantage for the prophylaxis of the infection.

‘I only pray to God that the pain never be indifferent to me.’

_I Only Pray to God_  
Leon Gieco

Untitled  
Ivo Saglietti  
Photography
INTRODUCTION

TB remains one of the leading causes of death worldwide, secondary only to AIDS (1). One-third of the world’s population is latently infected by MTB and every year approximately 9 million of these go on to develop the disease and 2 million succumb to it. These staggering numbers suggest that there is a lack of protective efficacy provided by BCG which has been administered to humans for more than 85 years. BCG immunization is reasonably effective in protecting from disseminated forms of childhood TB but is less effective in protecting from pulmonary TB which is a major global disease burden in adults (2). Furthermore, it has been recently found that BCG is unsafe when given to HIV + children (3). These facts suggest the need not only for developing effective boost vaccination strategies for parenteral BCG prime immunization but for identifying safe and effective vaccines to replace the current replication-efficient BCG vaccine for immunizing HIV + children (4). This chapter will focus on the prospect of respiratory mucosal immunization strategies.

Respiratory mucosal anti-mycobacterial immunity: innate immune mechanisms in the lung

The lungs are in constant contact with the exterior environment. The surface of the airway contains many substances that can have anticyclobacterial properties including lysozyme and defensins (5–7). The epithelium provides a physical barrier and can produce a variety of pro-inflammatory cytokines including TNF-α (8). Both immunoglobulins and complement have been shown to play a role in innate defence against TB. For example, the complement component C3 has been shown to be produced by bronchiole epithelial cells and that facilitate MTB phagocytosis by human alveolar macrophages (9–11). Alveolar macrophages are the important first line of host defence in the lungs and are the first target cells of inhaled MTB. Evidence suggests that the macrophage surface CD14 molecules do not play a role in uptake of MTB by human alveolar macrophages whereas toll-like receptors (TLR) can play a role in phagocytosis as well as direct killing (12).
TLR activation leads to direct mycobacterial killing in murine and human alveolar macrophages by nitric oxide-dependent and nitric oxide-independent mechanisms, respectively (12). The macrophage mannose receptor can also facilitate phagocytosis of MTB. Furthermore, the alveolar macrophage produces significant quantities of type 1 cytokines including IL-12, IFN-γ, and TNF-α (13). Cytokines produced by alveolar macrophages such as IL-12 and IFN-γ can also mediate the production of reactive metabolites such as nitric oxide that have mycobactericidal properties (14).

B cells do not seem to play a role in the memory immune response against TB. However, these cells may play a role in priming T cells as antigen presenting cells (15) whereas another study suggests that B cells are not required for T cell priming but rather they are involved in the regulation of immunopathology (16). While neutrophils are not considered to be potent professional APCs, there is evidence to suggest their role in facilitating antigen presentation by dendritic cells (17). It has been shown that neutrophils could shuttle live mycobacterial bacilli from the site of infection to the draining lymph nodes in vivo (18). The role of alveolar macrophages in mycobacterial antigen presentation and T cell priming still remains to be elucidated. However, we have recently shown that compared to lung dendritic cells, alveolar macrophages are incapable of T cell priming contrasting to their strong capability to activate the mycobacterial antigen-primed or experienced T cells (19). It is also possible that infected alveolar macrophages may acquire the property of dendritic cells and go on to migrate the local lymphoid tissues whereby to prime T cells (20). Alveolar macrophage is also a critical cellular component of granuloma (21) and a major source of TNF-α. We have found that TNF-α is a critical type 1 immune regulatory cytokine which operates not only to induce nitric oxide production but also to negatively control the level of potentially immunopathogenic CD4 T cells during pulmonary mycobacterial infection (22). Nonetheless, the mechanisms by which TNF-α controls the level of anti-mycobacterial type 1 CD4 T cells remain to be elucidated. Traditionally, mycobacterial granuloma has been believed to be an active immune microenvironment solely serving the benefit of the host but recent emerging evidence suggests it to be beneficial to both the mycobacterium and the host (23). Indeed, we have recently found that compared to airway luminal alveolar macrophages and dendritic cells, granuloma counterparts display a suppressed type 1 immune phenotype due to heightened production of immune suppressive cytokine IL-10 (24).
Dendritic cells are the most potent APCs and can express high levels of MHC class I and II as well as co-stimulatory molecules (25). Dendritic cells are widely distributed throughout the airway epithelium and approximately 500–1,000 dendritic cells per mm² can be found in uninfected lungs (26). Dendritic cells can also be found at the alveolar surface and the lung parenchyma (27). Unfortunately the respiratory dendritic cells have been poorly studied in the context of TB and a few in vitro studies used only bone marrow-derived dendritic cells and macrophages for comparison upon infection with mycobacteria (28, 29). Traditionally, it is believed that upon capturing antigens or pathogens from lung sites of exposure, dendritic cells migrate to the local draining lymph nodes for naïve T cell priming. However, we have recently found, by using IL-12p40-deficient dendritic cells, that dendritic cells transferred to the respiratory tract of mice are incapable of migrating to the local draining lymph nodes but remain fully capable of naïve CD8 and CD4 T cell activation (30). This observation supports the findings from other studies (31, 32) and suggests that antigen presentation to naïve T cells and subsequent T cell priming may also occur in the local lung lymphoid tissues in addition to the draining lymph nodes. Continuing dissection of the role of dendritic cells in T cell priming will be critical to much needed understanding of the reason behind delayed T cell priming and lung recruitment following primary mycobacterial infection in the lung (20, 33).

**T cell trafficking and lung tissue homing**

Some of the T cells activated in lymphoid organs re-circulate preferentially back to the lung mucosa. We have observed that, following intramuscular genetic immunization, antigen specific T cells repopulate not only the spleen but also the lung interstitium although only a few of such T cells repopulate the airway luminal space (34). On the contrary, intranasal mucosal genetic immunization activated T cells preferentially repopulate the airway luminal space while some of them get distributed in the lung interstitium and spleen (35). Following a single intranasal vaccination, the airway luminal MTB antigen-specific T cells, particularly CD8 T cells, may persist for at least six months (34). Together these results suggest that respiratory mucosal immunization-activated T cells could survive and maintain their function outside of the lymphoid tissues for prolonged periods of time. While some information has been generated about the cell surface molecules involved in T cell mucosal homing, less is known about the mechanisms of pulmonary mucosal homing. It was found that while the α4β7 integrin-bearing T cells preferentially homed to the intestinal lymph nodes (36), pulmonary T cells do not express α4β7
integrin (37) nor does the pulmonary vasculature express its ligand, MAdCAM-1 (38). A second integrin, αEβ7 whose ligand is E-cadherin, was found to be expressed on T cells in the gut as well as the lungs and reproductive tract, which may represent the molecule shared by the common mucosal immune system (40). The migration of memory T cells to the bronchus-associated lymphoid tissue (BALT) was inhibited when the α4 integrin and vascular cell adhesion molecule-1 (VCAM-1) interaction was blocked by using a monoclonal antibody (40,41). Recently, α4β1 expressed on T cells has been implicated in the airway luminal recruitment of pheripherally located mycobacterium-specific type 1 CD4 T cells in humans (42).

**TB VACCINE PLATFORMS**

**Mycobacterial organism-based vaccines**

Although BCG is ineffective in preventing adolescent and adult pulmonary TB, there is no doubt that it is an effective vaccine to protect children against severe disseminated forms of TB. Unfortunately repeated BCG immunization does not enhance/prolong (boost) protective immunity by BCG prime immunization (43-45). Three main approaches have been attempted to improve the immunogenicity or protective efficacy of BCG: BCG administered with immune adjuvant, auxotrophic MTB and BCG, and use of naturally attenuated mycobacteria. Co-administration of immune adjuvant or use of recombinant BCG strains has been fairly effective experimentally. Cytokines have also been used as an immune adjuvant and co-administered with BCG vaccination. These include IL-12, IFN-γ, IL-2, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF) (45–52). Enhanced protection when compared to unmodified BCG has been observed with both IL-12 and GM-CSF (4, 46). These cytokine adjuvants have been delivered by using viral vectors, plasmid DNA vectors, or even cytokine-expressing recombinant BCG. Immunity induced by BCG can also be enhanced by co-administering synthetic unmethylated oligodeoxynucleotides, known as CpG, or recombinant IL-12 protein (46). Another study showed that recombinant BCG engineered to secrete more Ag85B enhanced protection against TB challenge in a guinea pig model, which is likely due to the enhancement of T cell responses against a dominant T cell antigen (52). These data suggest that immune adjuvant may play a role in modulating the immune response to BCG. Auxotrophic BCG or MTB vaccine may be able to replace replicating BCG vaccine to immunize HIV-positive infants due to its enhanced safety associated with auxotrophic vaccines since the current BCG vaccine proves...
unsafe to HIV-infected infants (3). In any event, a heterologous boost vaccine will be needed to prolong protective immunity triggered by mycobacterial organism-based prime immunization.

**Antigen-based vaccines**

Modern recombinant DNA technology and the known sequences of the MTB genome have allowed the development and experimental evaluation for a variety of antigen-based vaccines. Bacterial plasmid DNA, adjuvanted proteins and viral vectors have all been evaluated experimentally. A wide range of antigens have been used or expressed and these include Ag85A, Ag85B, ESAT-6, TB10.4, MTB39, MTB32, and MTB 8.4 (53–59). DNA vaccines have several advantages including ease of production, low cost, and the ability to activate a broad range of CD4 and CD8 T cells (53, 60). Unfortunately, the majority of studies have shown a modest level of protective efficacy against TB challenge that is much lower than that conferred by BCG immunization. This is the reason why there has not been any plasmid DNA vaccine entering clinical trials (61). While this lack of potency was previously attributed to the lack of efficient uptake of DNA plasmids by cells and low levels of MTB antigen production, recent findings from us and others suggest that enhancement of transfection by electroporation and/or of immunogenicity by GM-CSF co-expression could only moderately improve the protective efficacy of intramuscular DNA immunization (59, 62). Our studies comparing mucosal with intramuscular adenovirus TB immunization have provided a plausible explanation for the ineffectiveness of parenteral genetic TB immunization in eliciting airway luminal T cell activity (34, 35, 63). Indeed, we have found that simple intranasal mucosal delivery of soluble MTB antigens to intramuscular DNA-immunized animals represents a powerful solution to enhancing immune protection from pulmonary MTB challenge via elicitation of airway luminal T cells (64). Protein-based subunit TB vaccines are also found to be safe and, when formulated with adequate immune adjuvants (65), are able to provide potent immune protection following parenteral or intranasal delivery. The most encouraging results were obtained from the studies involving the use of the fusion protein Ag85B–ESAT-6 in the adjuvant DDA-MPL (66) or CTA1-DD–ISCOMs (67), Ag85B–TB10.4 in the adjuvant DDA–MPL (68) or MTB72F in the adjuvant AS02A (69). A drawback with protein vaccines is that they have to be administered more than once and they primarily activate CD4 T cells and are poor CD8 T cell stimulators. Nonetheless, these vaccines have appeared to be promising as boosters for BCG prime immunization and three formulations have entered clinical trials (61).
Recombinant virus-vectored TB vaccines hold great promise for boosting protective immunity after BCG prime immunization (43). Several viral vectors have been utilized for vaccination against TB and these include MVA, human type 5 and 35 adenoviruses, influenza A, and vesicular stomatitis virus (VSV) (70–75). Though MVA is weakly immunogenic when used alone (75), it seems very immunogenic when used as a booster. MVAAg85A has been successfully evaluated in several Phase I clinical trials (76, 77) and has recently completed a Phase IIb trial (78). Human type 5 adenovirus-based TB vaccine expressing Ag85A (Ad5Ag85A) has been evaluated in murine, guinea pig, and bovine models, demonstrating great potential to be both a primary and boost vaccine (35, 45, 79, 80), and has completed a phase I clinical trial in Canada (61). Human type 35 adenovirus-based TB vaccine expressing a fusion protein of Ag85A, Ag85B, and TB10.4 is being evaluated in Phase I/II clinical trials (61).

REGIMENS AND ROUTES OF IMMUNIZATION

Prime-boost immunization

Boosting immunity by giving multiple doses has been widely implemented in human immunization program. As aforementioned, the current BCG or an improved BCG vaccine will likely continue to be used as a priming vaccine for infants and BCG is ineffective for boosting purposes. Therefore, an effective heterologous prime-boost vaccination strategy is urgently needed involving BCG priming and boosting with a heterologous non-mycobacterial vaccine such as recombinant MVA- or adenovirus-based or adjuvanted protein-based TB vaccines. Experimental studies have provided good evidence that such genetic or subunit vaccines could potently boost immunity triggered by BCG priming (45, 79,80-83). MVAAg85A was able to significantly boost T cell responses in BCG-vaccinated humans (76, 77, 84). Ag85B–ESAT-6 fusion protein vaccine in IC31 adjuvant is currently being evaluated for its boosting effects in BCG-vaccinated humans after it was shown to be safe and immunogenic in naïve human volunteers (85). Human type 5 adenovirus-based TB vaccine (AdAg85A) was evaluated in healthy naïve or BCG-vaccinated human volunteers and the results indicate that it is safe and immunogenic (86).
Consideration of routes of immunization

Most of the human vaccines are given parenterally via the skin or muscle and there is only one viral vaccine (cold-adapted live flu virus) that has been given intranasally to humans. While there is no doubt that respiratory mucosal route of immunization would provide better protection from pulmonary TB, to date the majority of TB vaccines have been evaluated parenterally via the skin or muscle (87). Furthermore, the intranasal route of immunization has the advantage of being needle/pain-free. Mounting evidence suggests that the mechanism underlying improved protection by respiratory mucosal immunization is via eliciting and sustaining airway luminal MTB antigen-specific T cells (43,87-89). Contrary to the potency by its intranasal delivery, genetic parenteral vaccination (priming or boosting) has proven ineffective in rodent models (35, 59, 63, 64). This clearly contrasts the established efficacy by parenteral route of immunization with BCG or protein-based vaccines. However, intranasal BCG immunization provided better protection than subcutaneous BCG administration (90). Whether the intranasal route of immunization with protein-based vaccine is better than parenteral immunization remains unclear as there is a lack of side-by-side comparison studies and this is in part due to a different adjuvant formulation requirement depending on the route of administration (65). A Phase I trial initially set out to evaluate the safety and immunogenicity of intranasally deliverable Ag85B–ESAT-6 fusion protein vaccine in LTK63 adjuvant was unfortunately terminated due to safety concerns (91).

Respiratory mucosal TB vaccination

BCG

The current BCG or an improved BCG vaccine will unlikely be used intranasally in humans due to its potent proinflammatory nature. In particular, direct delivery of BCG organisms to the respiratory tract will inevitably elicit granulomatous inflammatory responses which may cause undesired respiratory symptoms. However, experimental evaluation of intranasal BCG immunization helps establish the proof of principle and appraise the advantages and disadvantages of intranasal versus parenteral TB immunization. Intranasal immunization with BCG in a murine model has been shown to provide better protection than subcutaneous immunization and improved protection was observed following secondary mycobacterial challenge, which is associated with quicker T cell responses in the airway (90). Similar observations were also made in other studies using not only murine but guinea pig and monkey models (92). The improved protection by mucosal BCG immunization
may be linked to enhanced dendritic cell activation and preferential accumulation of activated T cells in lung mucosa (93). However, in a badger model, subcutaneous and respiratory mucosal BCG immunization was found to induce a comparable level of protection (94).

**Plasmid DNA-vectored vaccines**

It has been a challenge to intranasal mucosal immunization with plasmid DNA vaccines due to low transfection efficiency. Up to date, there have been a few successful examples. A DNA vaccine was administered via the intranasal route in a cationic lipid adjuvant (95) and it was not as effective as those delivered via the intramuscular route. Unfortunately, in this study protection against TB challenge was only described for intramuscular immunization (95). On the other hand, a recent study has compared intramuscular and intranasal routes of immunization with a DNA-hsp65 vaccine complexed with cationic liposomes and finds only the intranasal route to be effective (96).

Given the general low efficiency of DNA vaccine mucosal delivery, attenuated or naturally invasive bacteria have been explored to shuttle DNA TB vaccines to the respiratory mucosa for the purpose of immunization. Intranasally delivered recombinant *Salmonella typhimurium* expressing Ag85A provided a level of protection similar to BCG (97). Furthermore, intranasally delivered recombinant *E. coli* expressing MTB FbpA or HtpX induced antigen specific T cell responses and enhanced protection (98).

**Protein vaccines**

Purified or recombinant MTB proteins on their own are mostly poorly immunogenic due to their short lives and lack of built-in immunoadjuvanticity, only able to activate very limited T cell clones even with repeated deliveries (58, 99). As a result, immune adjuvant formulations must be used for protein-based TB vaccines and repeated administrations are required. Furthermore, protein vaccines primarily target the MHC class II pathway and hence predominantly activate CD4 T cells.

Most protein-based TB vaccines have been given parenterally in experimental models. However, intranasal administration of a fusion protein vaccine of Ag85B–ESAT-6 in LTK63 adjuvant by itself induced potent T cell activation and protection which was comparable to that by parenteral BCG and, when used as an intranasal booster, it also enhanced protection by BCG prime immunization (83). A fusion protein vaccine of Ag85B–ESAT-6 in CTA1-DD–ISCOMs adjuvant was also used
intranasally to boost BCG-primed mice with much enhanced protection (67). On the other hand, intranasal delivery of an arabinomannan–tetanus toxoid conjugate (AM–TT) vaccine in an Eurocine adjuvant to BCG primed mice only enhanced protection in the spleen but not in the lungs (100). In a separate study, ESAT-6 encapsulated in polylactide (PLA) microspheres was delivered intranasally to BALB/c mice and was found to lead to T cell activation (101). Ag85A protein coupled with the outer membrane lipoprotein I (OprI) of *Pseudomonas aeruginosa* was delivered intranasally to boost T cell responses elicited by intranasal BCG prime immunization but it failed to enhance protection (102). Mycobacterial HBHA protein co-delivered intranasally with cholera toxin induced both antigen-specific humoral and cellular responses and reduced systemic dissemination following pulmonary BCG challenge (103).

**Virus-vectored vaccines**

In general, viral vectors possess natural tropism to the respiratory mucosal epithelium and thus can induce high levels of transgene product in a wide range of cell types, an advantage over plasmid DNA vectors or protein-based vaccines (87,104). Therefore, virus vectors have the flexibility to be used either parenterally or mucosally. Furthermore, viral-vectored TB vaccines are able to activate both CD4 and CD8 T cells, a clear advantage over protein-based vaccines and the activation of protective CD8 T cells without the CD4 T cell help (105). The safety of virus-vectored vaccines is enhanced by genetically rendering them replication-defective without affecting their infectivity. These features make virus-vectored TB vaccines attractive candidate boost vaccines for enhancing BCG prime immunization (104). However, compared to protein- or plasmid DNA-based vaccines, the major limitation to virus-vectored vaccines is that a strong antibody response is engendered against the virus which may hinder repeated administrations of the same virus vector to the same host. This shortcoming may be circumvented by using different viral platforms or different serotypes of the same virus to express the same MTB antigen. To date two virus vectors, MVA and replication-deficient human type 5 adenovirus (Ad5), have been extensively explored for their applications in TB vaccine development (70,87,104). Both vectors have an excellent safety record as well as demonstrated to boost CD4 and CD8 T cells in humans. The other viral TB vaccines that have been developed include fowlpox virus (82), influenza virus (73), human type 35 adenovirus (Ad35) (72) and vesicular stomatitis virus (74).
**MVA-vectored vaccines**

MVA is a genetically modified vaccinia virus strain which is highly attenuated. The virus has a very narrow restricted host range and as such it cannot replicate in normal mammalian cells although it can replicate well in some avian cell lines. MVA has an excellent safety record as it has been used in the final stages of the smallpox eradication campaign (104). In addition, because of its inability to replicate in mammalian cells, recombinant vaccinia virus is safe to use in immunocompromised individuals. MVA can accommodate more than 25kb of foreign genetic material without consequence, which makes it a very attractive vector for gene delivery. A number of MTB antigens have been inserted into MVA, including Ag85A, Ag85B, ESAT-6, and MPT63 (56, 75). As a stand-alone vaccine, MVA was not able to induce a very robust immune response in mice, demonstrating its low potency (75). Nevertheless, when used in combination with BCG or DNA plasmid vaccines in heterologous prime-boost regimens, MVA-vectored TB vaccines conferred significantly enhanced immune activation and protection against MTB challenge in a variety of animal models (106). Intranasal delivery of MVAAg85A boosted T cell responses and protection by intranasal BCG prime immunization in a murine model (107). MVAAg85A has been successfully evaluated for intramuscular immunization in Phase I clinical trials (76, 77) and is entering Phase II clinical trials in South Africa (61,78). Whether this vaccine will also be evaluated via intranasal route in clinical trials remain to be seen.

**Ad5-vectored vaccines**

Genetic manipulations of this virus have been widely applied for the purpose of gene transfer (108). Ad has an excellent safety record for human use since a live wild-type Ad has been given to about 10 million army recruits in North America (104). Ad was later used as a gene transfer vector for *in vivo* functional studies of specific proteins, such as cytokines (109). More recently, the potential use of Ad as a vaccine vector has been widely explored (108). Its advantages in this capacity are numerous: Ad has limited pathogenic virulence; it is able to induce transient but high level transgene expression; and its cellular receptor, the Coxsackie-adenovirus receptor, is expressed on a wide variety of cells allowing the virus to gain entry to the liver, kidney, muscle, bronchial epithelial cells (108) and professional antigen presenting cells. Furthermore, its strong immunogenicity serves as an adjuvant and enhances the innate and adaptive immune responses towards the foreign antigen being expressed. Another attractive characteristic of Ad for use as a vaccine vector against TB is its natural tropism for the respiratory epithelium enabling mucosal immunization.
Following intranasal inoculation of Ad5 vector, raised transgene protein levels could be readily detected for 10–18 days (109,110). However, we have recently found that MTB antigens in intranasal Ad5 vector-infected lungs may be still available to maintaining antigen-specific memory T cells within the lung mucosal tissue several months after the initial intranasal delivery of Ad5 TB vaccine (111).

The first replication-defective Ad5-vectored TB vaccine was developed to express Ag85A (AdAg85A) (35). Contrary to its lack of protection by intramuscular immunization in murine models, intranasal AdAg85A inoculation provided a robust and durable level of protection against pulmonary MTB challenge and systemic dissemination, which was superior to subcutaneous BCG vaccination (35). Potent immune protection by intranasal immunization was associated with a marked increase and long-term retention of Ag-specific IFN-γ secreting and CTL within the respiratory tract (34).

These airway luminally induced T cells were clearly immunoprotective as they, upon adoptively transferred to the lungs of SCID mice, protected these mice from pulmonary MTB challenge. While a single intranasal AdAg85A immunization provides an impressive level of protection in mouse models, it is unlikely that such a vaccine will be a suitable candidate to replace BCG as a primary vaccine for human application. Indeed, we found that AdAg85A-mediated protection was unable to hold MTB infection in check over time as well as parenteral BCG immunization did (35). This is most likely due to its expression of only one MTB antigen. In this regard, we have recently developed a robust bivalent Ad-based TB vaccine (Ad5Ag85A-TB10.4) (112) and is expected to confer not only enhanced but also prolonged protection. Such antigen-based recombinant viral TB vaccines will be suitable to boost immunization following parenteral BCG priming. Indeed, it was shown that intranasal, but not intramuscular, delivery of AdAg85A, markedly boosted protection by BCG priming in a murine model (44, 113,114). Furthermore, it was also shown to be effective in boosting protective T cells in BCG-primed calves (80). Similarly, intranasal boosting with AdAg85A markedly enhanced the survival of BCG-primed guinea pigs following low dose MTB aerosol challenge (79). It is noteworthy that the astonishing lack of boosting effects on protection by intramuscular AdAg85A immunization observed in murine models contrasts the markedly boosted protection by parenteral AdAg85A immunization in BCG-primed bovine and guinea pig models. These observations keep the hope high that parenteral viral-mediated boosting strategies may still be applicable to humans. Although Ad5 is a promising vector for TB vaccine development, the high prevalence of neutralizing antibodies towards this serotype within the human population is a potential limitation (104, 105). While anti-Ad5 antibodies significantly dampened
Ad5-based vaccine modalities, as indicated by ~threefold lower T cell response in subjects with pre-existing Ad5 immunity than those of Ad5-seronegative subjects recent information suggests that this represents a much smaller concern than previously thought (61). In any case, an alternative approach is to use rare human Ad serotypes such as Ad35 and Ad11 as vectors (72). However, some of these Ad vectors of rare serotypes may suffer the drawback of low transduction and immunogenicity (115). A further approach is to use non-human Ad vectors such as chimpanzee Ad-based vectors that share the similar immunologic properties with human Ad5 but humans do not carry the antibodies against it (108).

**VSV-vectored vaccines**

A recombinant VSV-based TB vaccine (VSVAg85A) was recently developed and tested in a murine model for intranasal mucosal immunization (74). This VSV vector carries a mutation in the M gene, leading to attenuation in its ability to evade host interferon responses while it remains replication-competent. Compared to AdAg85A, VSVAg85A represents a much less potent vaccine when used alone intranasally as a priming vaccine. However, it is able to potently boost airway luminal T cell responses following intramuscular AdAg85A priming (74). Whether such VSVAg85A-boosted T cell responses can translate into improved and lasting immune protection is currently under investigation.

Such heterologous viral TB vaccine may be potentially used as a second boost vaccine following the initial Ad-based boost immunization. It remains to be understood whether a replication-defective VSV vector will be advantageous in its immunogenicity over its replication-competent counterpart.

**AIRWAY LUMINAL T CELLS AND THE STRATEGIES TO ENHANCE THE EFFICACY OF PARENTERAL IMMUNIZATION BY ALTERING T CELL DISTRIBUTION**

Over the last several years, basic vaccine immunology research has provided a wealth of new knowledge to help us understand the fundamental differences between parenteral and respiratory mucosal TB immunization (88,89,116). The findings from these studies have rekindled the realization of the importance of T cell location and indicated that having antigen-specific T cells residing at the airway luminal site before or at the time of MTB entry, provides the best
protection. The lack of such airway luminal T cells has been linked to the lack of MTB protection following parenteral plasmid DNA- or recombinant viral-based immunization (34,63). Furthermore, the lack of airway luminal T cells is also associated with delayed immune protection following parenteral BCG immunization (unpublished data). The presence of vaccine-derived MTB antigens within the airway mucosal tissue was found to be critical to maintaining airway luminal antigen-specific T cells via stimulation of continual T cell proliferation (111). On one hand, such important new knowledge shall continue to fuel the concept and investigation of respiratory mucosal immunization strategies. On the other hand, it helps develop the strategies to enhance the protective efficacy of parenteral TB vaccination (89). Hence, any strategies designed to recruit and maintain the peripherally activated T cells within the airway lumen, are expected to enhance respiratory mucosal protection in parenterally immunized hosts. In this regard, in addition to traditional intranasal boosting vaccination strategies, intranasal delivery of “vaccineless” non-adjuvant soluble MTB proteins was found to be particularly effective in the hosts intramuscularly immunized with AdAg85A (63) or DNAAg85A (64). Furthermore, intranasal delivery of such MTB proteins was also found to recruit the peripheral T cells into the airway lumen whereby to restore immune protection in the lung that is otherwise missing within the first two weeks of time in parenteral BCG-immunized animals (117). Whether such “vaccineless”-based mucosal boosting strategies will be effective to BCG-immunized humans remain to be investigated.

CONCLUSIONS

The failure of BCG vaccine to effective control pulmonary TB in adult humans is now widely accepted. The current conviction is that the current or an improved BCG will continue to be a childhood priming vaccine and 1–2 heterologous boost vaccines need to be identified and ultimately introduced into human immunization program. And such boost vaccines will most likely take on the form of protein or recombinant virus vectored vaccines. While it is very likely that a boosting vaccine will still be given via the skin or muscle in humans, its limitation in positioning the peripheral immune protective T cells to the airway lumen and lung interstitium should be noted. Furthermore, mere assessment of circulating antigen-specific T cells in the current TB vaccine clinical trials may be insufficient to correlating with protective potency of a given vaccine in question. It also remains to be seen whether the intranasal route will gather sufficient justification for boosting immunization in humans.
ACKNOWLEDGEMENTS

Our own work cited in this chapter is supported in part by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR) and WHO Initiative for Vaccine Research (IVR) and the Canadian Institutes of Health Research (CIHR).

REFERENCES


70. McShane H, Pathan AA, Sander CR, Goonetilleke NP, Fletcher HA, and Hill AV. 
Boosting BCG with MVA85A: the first candidate subunit vaccine for tuberculosis 

71. Santosuosso M, McCormick S, and Xing Z. Adenoviral vectors for mucosal 

G, et al. Protective immune responses to a recombinant adenovirus type 35 
tuberculosis vaccine in two mouse strains: CD4 and CD8 T-cell epitope mapping 

Vaccine potential of influenza vectors expressing *Mycobacterium tuberculosis* 

74. Roediger EK, Kugathasan K, Zhang X, Lichty BD, and Xing Z. Heterologous 
boosting of recombinant adenoviral prime immunization with a novel vesicular 

75. McShane H, Brookes R, Gilbert SC, and Hill AV. Enhanced immunogenicity of 
CD4(+) T-cell responses and protective efficacy of a DNA-modified vaccinia 
virus Ankara prime boost vaccination regimen for murine tuberculosis. *Infect 

Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts 
BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nat 

Boosting BCG with recombinant modified vaccinia ankara expressing antigen 
85A: different boosting intervals and implications for efficacy trials. *PLoS ONE*, 

78. Tameris MD, Hathenil M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, Shea 
JE, McClain JB, Hussey GD, Hanekom WA, Mahomed H, McShane H. Softy and 
efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated 
with BCG: a randomised, placebo-controlled phase 2b trial. MVA85A O2O Trial 
Study Team.

mucosal boosting with an adenovirus-vectored vaccine markedly enhances the


91. “A Phase I Trial of a LTK63 Adjuvanted Tuberculosis Nasal Subunit Vaccine (Ag85B-ESAT6) (TMUVA-01). This study has been terminated. (Safety Issues)” http://clinicaltrials.gov/ct2/show/results/NCT00440544


