

Education and information can promote understanding, respect, tolerance, and non-discrimination in relation to people with TB, and the day will arrive in which these values will be considered a basic human right in any part of the World



Untitled – Alfredo Sosa Bravo
Mixed/X-ray film

'During the incoming moon
you will lie to deliver
and your womb will irradiate
the clarity over me.'

To the Incoming Moon (Poem) – Miguel Hernández

Alfredo Sosa Bravo
Sagua la Grande, Cuba

Alfredo Sosa Bravo is a painter, designer, engraver, and ceramist. Although he studied at the Academy of Fine Arts in San Alejandro, Cuba, he was mostly self taught. He started exhibiting his works in 1958.

Since 1960, he has received a number of awards in Cuba and abroad, among them the Gold Medal at the XXXIV International Competition of Contemporary Ceramic Art, Faenza, Italy, 1976.

He has also been accorded the highest cultural awards in Cuba like the Orden Félix Varela and the National Prize of Fine Arts; as well as being conferred with an honorary doctorate in Art.

CHAPTER 16

Respiratory Mucosal Immunization against Pulmonary Tuberculosis

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Introduction

Tuberculosis 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 8 million of these go on to develop the disease and 1.5–2.0 million succumb to TB. 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. One of the major problems associated with BCG immunization is that the protective immunity by BCG can only last for 10–15 years (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 vaccines for 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 Immunity

The lungs are in constant contact with the exterior world. The surface of the airway contains many substances that can have antimycobactericidal properties including lysozyme and defensins (5–7). The epithelium provides a physical barrier and can produce a variety of pro-inflammatory cytokine 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 it can facilitate phagocytosis by human alveolar macrophages (9–11). Alveolar macrophages are also the important first line of host defense in the lungs and are the first target cells that MTB infects. Evidence suggests that the macrophage surface CD14 molecules do not play a role in uptake of MTB by human alveolar macrophages whereas TLR can play a role in phagocytosis as well as direct killing (12). TLR activation leads to direct killing by 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 macrophages are able to produce 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 aid in 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 the immune response (15). B cell deficient mice (μ MT) have been shown to be more susceptible to primary TB infection by increased numbers of viable bacteria found in lungs, spleens, and livers compared to littermate controls. The authors also showed that T cells from B cell deficient mice could not react against as many TB antigenic peptides as compared to littermate controls, suggesting that B cells play a role in activating T cells of certain specificity by acting as APC (15). A more recent study has shown that MTB infected B cell deficient mice had heightened immunopathology and IL-10 responses while they demonstrated similar IFN- γ and TNF- α responses (16). While neutrophils are not considered to be potent professional APCs, there is evidence to suggest their antigen presentation capability (17). Recently, it has been shown that neutrophils could shuttle live mycobacterial bacilli from the site of infection to the draining lymph nodes in vivo (18). Whether these infected neutrophils could directly present mycobacterial antigens to T cells in the lymph nodes still remains to be elucidated.

Alveolar macrophage is believed to be the first cells to be infected by MTB. The MTB bacillus has the ability to prevent phago-lysosomal fusion and take up residence within the alveolar macrophage. We have shown that alveolar macrophage can be stimulated by mycobacteria to produce IL-12, TNF- α , and IFN- γ , which can then lead to activation and production of reactive nitrogen metabolites such as nitric oxide (13, 14). Alveolar macrophage is also a critical cellular component of granuloma (19). Alveolar macrophages can thus play an important immune regulatory role in type 1 T cell immunity and we have

found that one of such regulatory mechanisms is mediated through the biologic activities of alveolar macrophage production of TNF- α (20). However, it is believed that alveolar macrophage is a weak APC (21) and this may suggest its small direct role in activating MTB specific naïve T cells. Mycobacterial species including MTB have also evolved ways to interfere with MHC expression and antigen presentation (22–24). Nevertheless, our recent data have suggested that while indeed alveolar macrophage is ineffective in activating naïve CD4 and CD8 T cells compared to lung interstitial and splenic CD11c+ APCs, it could potentially activate mycobacterial antigen-primed T cells (25).

Dendritic cells are the most potent APCs and can express high levels of MHC class I and II as well as co-stimulatory molecules (26). Dendritic cells are widely distributed throughout the airway epithelium and in uninfected lungs 500–1,000 dendritic cells per mm² can be found (27). Dendritic cells can also be found at the alveolar surface and the lung parenchyma (28). 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 (29, 30). We have found that lung mucosal dendritic cells are potent APCs able to activate not only the antigen-experienced but also the naïve T cells (25). 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 activation. 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 (31). This observation supports the findings from other studies (32, 33) 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.

T Cell Trafficking and Tissue Distribution upon Activation

Some of the T cells activated in lymphoid organs recirculate preferentially back to the 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). In a separate study, it was found that after respiratory mucosal or parenteral immunization, CTL responses could be found systemically and mucosally. However, as time

progressed the mucosal CTL waned after parenteral immunization while it is sustained after mucosal immunization (36). 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 quite 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 $\alpha 4\beta 7$ integrin-bearing T cells preferentially homed to the intestinal lymph nodes (37), pulmonary T cells do not express $\alpha 4\beta 7$ integrin (38) nor does the pulmonary vasculature express its ligand, MAdCAM-1 (39). A second integrin, $\alpha E\beta 7$ 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 $\alpha 4$ integrin and vascular cell adhesion molecule-1 (VCAM-1) interaction was blocked by using a monoclonal antibody (41).

Tuberculosis 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 forms of TB. However, BCG-triggered protection lasts only for 10–15 years and unfortunately repeated BCG immunization does not enhance/prolong (boost) protective immunity by BCG prime immunization (42–44). 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 have been fairly effective experimentally. Cytokines have been co-administered with BCG vaccination, including IL-12, IFN- γ , IL-2, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (45–51).

Enhanced protection when compared to unmodified BCG has been observed with both IL-12 and GM-CSF (4, 45). 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 bacterial DNA, known as CpG, or recombinant IL-12 protein (45). 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 the 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 prime immunization.

Antigen-Based Vaccines

Modern recombinant DNA technology and the known sequences of the MTB genome have allowed for a variety of antigen-based vaccines to be developed and evaluated experimentally. Bacterial DNA plasmids, adjuvanted proteins, and viral vectors have all been utilized 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 many 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 level of protective efficacy against TB challenge that is lower than that conferred by BCG immunization. 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, 61). Our studies comparing mucosal and 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, 62). Indeed, very recently 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 (63).

Protein-based subunit TB vaccines are also safe and, when formulated with adequate immune adjuvants, 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 (64) or CTA1–DD–ISCOMs (65), Ag85B–TB10.4 in the adjuvant DDA–MPL (66) or MTB72F in the adjuvant ASO2A (67). 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 are promising to be used as boosters for BCG prime immunization and three formulations have entered early clinical trials (68).

Recombinant virus-vectored TB vaccines hold great promise for boosting protective immunity after BCG prime immunization (42). 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) (69–75). While when used alone, MVA is weakly immunogenic (75), it seems very immunogenic when used as a booster. MVA_{Ag85A} has been successfully evaluated in Phase I clinical trials (76, 77) and is now entering Phase II trials in South Africa. 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, 44, 70). A Phase I clinical trial has recently been launched to evaluate Ad5Ag85A in healthy volunteers in Canada. Human type 35 adenovirus-based TB vaccine expressing a fusion protein of Ag85A, Ag85B, and TB10.4 is being evaluated in two Phase I clinical trials (68, 78).

Regimen and Route of Immunization

Prime-Boost Immunization

Boosting immunity by giving multiple doses has been widely implemented in human immunization programmes. 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 potentially boost immunity triggered by BCG priming (44, 79–82). MVA_{Ag85A} was able to significantly boost T cell responses in BCG-vaccinated humans (76, 77). Ag85B–ESAT-6 fusion protein vaccine in IC31 adjuvant is currently being evaluated for its boosting effects in BCG-vaccinated humans (83).

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 (84). 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 CD8 T cells (42, 84). Continuing efforts to compare parenteral with intranasal immunization remains important. Contrary to the potency by its intranasal delivery, genetic parenteral vaccination (priming or boosting) has proven ineffective in rodent models (35, 59, 62, 63). 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 (85). 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. Recently a Phase I trial has begun to evaluate the safety and immunogenicity of intranasally delivered Ag85B–ESAT-6 fusion protein vaccine in LTK63 adjuvant (83) and undoubtedly this trial will provide important information to guide future respiratory mucosal TB immunization trials.

Respiratory Mucosal TB Vaccination

BCG

The current BCG or an improved BCG vaccine will unlikely be used intranasally (i.n.) in humans due to its potent proinflammatory nature. In particular, direct delivery of BCG organisms to the respiratory tract will inevitably elicit granulomatous tissue 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 was shown to provide better protection than subcutaneous immunization and improved protection was associated with quicker T cell responses in the airway following secondary mycobacterial challenge (85). Similar observations were also made in other studies using not only murine but guinea pig and monkey models (86).

Plasmid DNA Vaccines

It has been a challenge to intranasal mucosal immunization with plasmid DNA vaccines due to low transfection efficiency. Up to date, there are very few successful examples. A DNA vaccine was administered via the intranasal route in a cationic lipid adjuvant (87) 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 (87).

Given the 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 (88). Furthermore, intranasally delivered recombinant *E. coli* expressing MTB FbpA or HtpX induced antigen specific T cell responses and enhanced protection (89).

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). 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, recently 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 (82).

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 (65). 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 (90). 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 (91). 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 (92).

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. 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. 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 (93). 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 (69, 70). 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, or still under development, include fowlpox (81) and influenza viruses (73). In addition to recombinant Ad-vectored TB vaccines, we have also developed and evaluated recombinant VSV-vectored TB vaccines (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 (93). In addition, because of its inability to replicate in mammalian cells, recombinant Vaccinia virus is safe to use in immunocompromised individuals (94). As a result, 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 (95). Intranasal delivery of MVA_{Ag85A} boosted T cell responses and protection by intranasal BCG prime immunization in a murine model (86). The intranasal boosting effects of MVA_{Ag85A} in other animal models still remain to be established. MVA_{Ag85A} has been successfully evaluated for intramuscular immunization in Phase I clinical trials (76, 77) and is entering Phase II clinical trials in South Africa.

Ad5-Vectored Vaccines

Genetic manipulations of this virus have been widely applied for the purpose of gene transfer (96, 97). 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 (93). Ad was later used as a gene transfer vector for *in vivo* functional studies of specific proteins, such as cytokines (98). More recently, the potential use of Ad as a vaccine vector has been widely explored. 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 (99) and professional APCs. 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 (98, 100). However, it remains to be understood whether small amounts of MTB antigens in Ad5 vector-infected lungs may be available within the lung mucosal tissue for a much prolonged period of time. Indeed, competent antigen presentation was detectable for at least 40 days within the draining lymph nodes following intramuscular administration of Ad5 vectors even though gene expression could be detected only up to day 14 (101).

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 CTLs within the respiratory tract (34). These airway lumenally induced T cells were clearly immunoprotective as they, upon adoptively transferred to the lungs of SCID mice, protect these mice from pulmonary MTB challenge. This important knowledge has recently been applied to restore potent protection in the lungs of intramuscularly immunized mice with AdAg85A (102) or a plasmid DNA vaccine expressing Ag85A (63).

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 as well as parenteral BCG immunization (35). This is most likely due to its expression of only one MTB antigen. Bivalent or multivalent Ad-based TB vaccines are expected to rectify such weakness but these vaccines will still be unsuitable for primary immunization. Thus, such antigen-based recombinant viral TB vaccines will mostly be suitable to boost immunization. Based on this consideration, it was shown that intranasal AdAg85A delivery markedly boosted protection by BCG priming in a murine model (44).

Furthermore, it was also shown to be effective in boosting protective T cells in BCG-primed calves (79). Similarly, intranasal boosting with AdAg85A markedly enhanced the survival of BCG-primed guinea pigs following low dose MTB aerosol challenge (manuscript submitted). 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 an important limitation (103–106). 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 (107). One way to ameliorate this concern is to use rare human Ad serotypes such as Ad35 and Ad11 as vectors in place of Ad5 (108). However, some of these Ad vectors of rare serotypes may suffer the drawback of low transduction and immunogenicity (109).

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 and enhance immune protection following intramuscular AdAg85A priming (74).

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.

Conclusion

The failure of BCG vaccine to control the global TB epidemic 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 programmes. And such boost vaccines will most likely take on the form of protein or recombinant virus-vectored vaccines. While it is possible that boosting vaccine will still be carried out preferably via the skin or muscle in humans, it remains to be determined whether the intranasal route will gather sufficient justification for boosting immunization. The safety of heterologous boost immunization in humans also remains to be fully established in BCG-primed humans with latent MTB infection. Furthermore, little has been done about identifying safe and effective vaccines to replace replicable BCG vaccine for neonatal/childhood prime immunization of HIV+ children as BCG has proven unsafe to such children.

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