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REVIEW

Aerosol vaccines for tuberculosis: A fine line between protection and pathology

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SUMMARY

Pulmonary delivery of vaccines against airborne infection is being investigated worldwide, but there is limited effort directed at developing inhaled vaccines for tuberculosis (TB). This review addresses some of the challenges confronting vaccine development for TB and attempts to link these challenges to the promises of mucosal immunity offered by pulmonary delivery. There are several approaches working toward this goal including subunit vaccines, recombinant strains, a novel vaccine strain *Mycobacterium w*, and DNA vaccine approaches. While it is clear that lung-resident adaptive immunity is an attainable goal, vaccine platforms must ensure that damage to the lung is limited during both vaccination and when memory cells respond to pathogenic infection.

1. Introduction

Bacilli Calmette Guérin (BCG), the only licensed vaccine against tuberculosis (TB), has been shown to be effective in preventing TB meningitis and miliary TB in children. However, the efficacy of this vaccine in preventing adult pulmonary TB is questionable.[1,2] Despite widespread vaccination with BCG, nearly 2 million people die each year from TB.[2] Furthermore, the World Health Organization no longer recommends BCG vaccination of children with HIV or HIV$^+$ mothers due to safety concerns, leaving many infants without any protection against this disease.[3] While drug therapies exist to combat TB infection, the availability of these drugs is limited in the countries hardest hit by the disease and have limited effectiveness at treating drug resistant strains of TB. The best hope for the control or elimination of TB is a safer and more effective vaccine.

There are several opportunities for the improvement of the current vaccine for TB. Modern vaccination applications, such as the use of recombinant bacteria or viruses, utilization of subunit vaccines, or specific targeting of the innate immune system for enhancement of adaptive immunity, can greatly enhance the immunogenicity of vaccines and potentially reduce safety concerns compared to BCG immunization.[2–4] Another interesting avenue under exploration is to alter the route of vaccination.

Vaccine delivery by inhalation has been suggested for eliciting mucosal and systemic immunity to airborne viruses including measles,[5–7] influenza,[8] papilloma virus,[9] and cytomegalovirus.[10] Prophylaxis of airborne bacterial infections leading to pneumonia,[11] against potential biological weapons,[12–14] and even of secondary infection in chronic occlusive pulmonary disease[15] is also under investigation.

TB shares several characteristics with other pulmonary infections, but is also characterized by a set of distinguishing features. Thus, whereas most viral lung infections target lung parenchymal and epithelial cells, pathogenic TB bacilli (and CDC Category A agents such as the etiological agents of pneumonic plague[16] or tularemia[17]) establish infection in professional antigen-presenting cells (APCs) of the lung: alveolar macrophages (AMφ) and, to a lesser extent, dendritic cells (DCs).[18,19] Establishment of *Mycobacterium tuberculosis* infection leads to 'alternative activation' of infected AMφ[20] and suppression of DC function,[21] subverting innate bactericidal, immunological and signaling mechanisms.

About 10 years back, Kaufmann questioned whether a new vaccine for TB was even possible.[22] He did suggest, however, that a vaccine that could generate neutralizing antibodies in the respiratory tract that would kill rather than just opsonize TB bacilli before their uptake by AMφ would "*solve all problems. Unfortunately, this scenario remains a dream…* A more likely prospect is vaccine-induced immunity that attacks the pathogen after it has established itself inside macrophages—a task that is the exclusive realm of T lymphocytes." Recent preclinical work on the AERAS-402 adenovirally vectored inhalable vaccine[23] promises adaptive immune responses in the respiratory tract, suggesting that the 'dream' is attainable. Further, an appreciation of the coordinated involvement of not only B-cell immunity, but also of innate immunity, classical, CD1-restricted[24,25] and γδ T cells,[26] CTL, and NK

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cells can now be brought into the context of the response of the respiratory tract and lung tissue to inhaled material.

2. Development of acquired immunity to inhaled antigens

The immune system is composed of two primary compartments: the systemic immune system and the mucosal immune system. Studies have demonstrated that vaccination of mucosal tissue, such as through the intranasal route, leads to mucosal immunity—immune responses localized to mucosal surfaces that are not observed in the systemic immune system.[27,28] Similarly, systemic immunizations, such as through intramuscular injections, are optimal for the induction of systemic immunity and suboptimal for induction of immunity in mucosal tissues. These differences in localization of the immune responses are largely attributed to tissue resident DCs.[28–31]

DCs are professional APCs that sample antigens from surrounding tissues.[32,33] During steady–state interactions, DCs in an immature/semi-mature state will migrate to draining lymph nodes and present antigens to naïve T cells and help to maintain tolerance.[34,35] However, when DCs are stimulated, such as through ligation of pattern recognition receptors, they become activated and undergo maturation, resulting in the up regulation of antigen processing and presentation machinery and stimulatory capacity. Mature DCs are potent stimulators of adaptive immunity with the capacity not only to activate both $CD4^+$ and $CD8^+$ T cells, but also to program T cells to respond with specific patterns of cytokines depending on the environmental stimuli the DCs received.[36–41] Studies have also revealed that DCs from different tissues will imprint specific homing characteristics in antigen-specific T cells such that they home back to the tissue where the antigen was encountered.[28–31] While antigen-specific T cells will migrate to areas of inflammation regardless of the imprinting acquired during priming, having resident memory T cells at the site of potential infection may increase the speed at which the immune response is initiated following infection. Infection with TB occurs when aerosolized droplets containing the bacteria are coughed into the air by an infected individual and then inhaled by another individual. Therefore, vaccines that target the lungs and generate lung-resident immunity may be particularly advantageous for preventing or controlling TB infection.

Bhaskar et al. have been investigating aerosol immunization with *Mycobacterium w* (M.w.) as a potential vaccine for TB.[42] M.w. shares cross-reactive antigens with TB and has been used in humans as an immunomodulatory agent for treatment of leprosy. M.w. has been evaluated using subcutaneous and as well as aerosol delivery using either live or heat-killed bacteria in protection studies in mice. Bhaskar's studies revealed that mice immunized by aerosol immunization using M.w. and subsequently challenged with TB had far fewer CFU in the lungs compared to mice that received subcutaneous immunizations with M.w. (live or heat-killed) or BCG. Aerosol M.w.-immunized mice also had greater levels of IgA antibodies, antigen-specific cytotoxic T lymphocytes, and the Th1 promoting cytokines IL-12 and IFN-γ in BAL compared to subcutaneous vaccination with either M.w. or BCG.

Studies on inhaled vaccines for other diseases fail to provide a clear path for aerosol vaccinations. For instance, a 23-valent pneumococcal vaccine was demonstrated to be no more efficient at eliciting a mucosal response when inhaled, as compared to delivery using the intramuscular route.[11] Further, instilling a vaccine in the nostrils was observed to provide equivalent efficacy as compared to deep lung delivery in the case of a viral vaccine.[8] However, intranasal delivery and deep lung delivery both target the mucosal immune system.

3. Inhalation delivery options

Unlike drug delivery in TB (reviewed elsewhere in this issue), vaccines need not be administered repeatedly in large doses over long periods. Thus, vaccines may be administered through either nasal inhalation or oral inhalation, or both (Figure 1).

Nasally administered vaccines measles vaccines have been extensively tested in human infants and children, with encouraging results. Orally inhaled vaccines are still in developmental stages, but offer potential advantages over nasal vaccines in terms of safety.[43] For experiments in animals, however, one of the most preferred approaches is intra-tracheal delivery using a variety of devices supplied by PennCentury.[44]

Vaccines and vaccine candidates for TB range from whole bacilli[42] to DNA sequences.[44] Most candidates require some degree of compounding or formulation so that they can be administered to the airways. General principles of formulation of therapeutic agents, reviewed by Misra et al. (this issue) are very similar in the case of vaccines, except for the need to pay due attention to the sensitivity of the immunizing antigen (Ag) to denaturation by high and low temperature, exposure to solvents and on account of turbulence stress[45] during liquid aerosolization. Thus, preparation of microparticles using multiple emulsion methods for example, requires the use of innocuous protectants such as albumin to stabilize Ag.[46] Considerations of allergenicity and adjuvanticity must go hand-in-hand when designing and testing formulations for pulmonary delivery of vaccines.

For instance, material that has potential to ligate Toll-like receptors (TLRs), such as alginate,[47] chitosan,[48] or repetitive nucleic acid sequences such as CpG[49] can induce a pro-inflammatory innate response and enhance vaccine efficacy.[50] Heuking has been investigating the use of a TLR2 agonist to enhance DNA vaccines for TB.[51,52] For this approach a chitosan polymer, which is mucoadhesive, is tagged with the TLR2 agonist and then complexed with plasmid DNA expressing TB antigens. The chitosan polymer serves to both protect the DNA from degradative enzymes and to promote

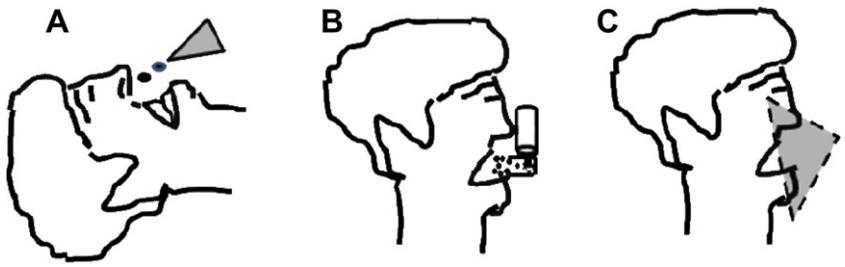

Figure 1. (A) Nasally inhaled vaccines may be sprayed or instilled into the nares, for access to the respiratory tract through the nasopharynx. (B) Orally inhaled vaccines require to pass through the oropharynx. (C) Nebulized vaccines may be administered using a face mask or cone that covers both nose and mouth, and could thus be administered to infants who are obligate nose-breathers.