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Tuberculosis vaccines- perspectives from the NIH/NIAID Mycobacteria vaccine testing program

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Abstract

The development of novel vaccine candidates against infections with *Mycobacterium tuberculosis* has highlighted our limited understanding of immune mechanisms required to kill *M. tuberculosis*. The induction of a Th1 immunity is vital, but new studies are required to identify other mechanisms that may be necessary. Novel vaccine formulations that invoke effector cells such as innate lymphoid cells may provide an environment that promote effector mechanisms including T cell and B cell mediated immunity. Identifying pathways associated with killing this highly successful infectious agent has become critical to achieving the goal of reducing the global tuberculosis burden.

Mycobacterium tuberculosis vaccination- the old and the new

Chronic infection due to *Mycobacterium tuberculosis*

Aerosol transmission of *Mycobacterium tuberculosis* infection is initiated through the interaction of the pathogen with antigen presenting cells, most commonly macrophages, that line the alveolar sacs in the lungs. In the majority of cases, healthy individuals limit the infection to the lung and draining lymph nodes. The development of granulomas is a compromise between the pathogen and adaptive immunity and the only long-lasting physical evidence of infection [1]. Infected individuals display immunity, as determined by skin test reaction and/or increased interferon (IFN)- γ production, when stimulated with *M. tuberculosis* antigens. Despite this, sterile immunity is rarely achieved [2]. The chronic nature of this infection is associated with persistence of the pathogen within the granulomatous lesions where it undergoes reduced replication and metabolism [3], and considerable research is devoted to better understanding the life cycle of *M. tuberculosis* in this environment [4]. Vaccine candidates have been developed targeting antigens expressed during the various phases of its life cycle [5]. Given that much of our existing understanding of resistance to natural infection is mainly studied in animal models, developing a vaccine

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against an infection by a well-adapted, persistent pathogen represents a situation with many complications.

Animals used to model *M. tuberculosis* infection and test vaccine candidates have recently been summarized [6]. Studying immunity to infection while also modeling infection outcomes in the same animals has proven difficult and has limited the quality of data that can be obtained before vaccine candidates are selected for advancement. Some animal hosts are good models for granuloma formation and the chronicity of the infection (guinea pig) while others are far more amenable to the tracking and elucidation of immune mechanisms (mouse). Additional variables, like the effect of the microbiota, can affect both bacterial growth and the immune response [7], and how parameters such as this will influence vaccine response and efficacy remain unclear [8, 9]. A number of new tuberculosis vaccine candidates have progressed through various stages of the developmental pipeline and have been summarized elsewhere [10].

Vaccines against tuberculosis – The old

For the majority of public health programs, elimination and eradication are the ultimate goals of vaccination programs [11, 12]. Developing vaccines against *M. tuberculosis* infection is limited by our incomplete understanding of protective immunity in humans. The only available vaccine against tuberculosis, the attenuated *Mycobacterium bovis* strain, bacillus Calmette Guérin (BCG) is essential for many vaccination programs throughout the world [13]. BCG was developed in the 1920's as a vaccine for veterinary tuberculosis, and then used in humans [14]. However, since its inception BCG vaccination has had limited and variable impact on transmissible pulmonary tuberculosis [15]. Studying BCG mediated protection has initially taught us about T cell based mechanisms that were studied extensively in humans and mice [16]. More recently, studies have shed light on the concept of “trained immunity”- a long lasting modification of innate cells which provides better overall protection against infections unrelated to tuberculosis [17, 18]- suggesting that BCG can elicit protection independent of adaptive responses [19, 20]. Mycobacteria such as BCG carry biological response modifiers that interact with multiple pathogen recognition receptors (PRR) [21]. These exert potent pro- and anti-inflammatory effects on the host and probably facilitate the capacity of BCG to induce heterologous protection [22], even against non-tuberculosis challenges as diverse as bladder cancer [23]. That being said, our limited understanding of the mechanisms by which BCG accomplishes these various avenues of protection has hindered vaccine development, and has created the expectation that new vaccines need to be “better than BCG” [24].

Is BCG vaccination dead?

The variability of BCG to protect adult humans from tuberculosis is well documented and there is considerable controversy about whether it will continue to be used to immunize young children once a more effective vaccine is available [10, 13]. Disagreement about its use remains mainly due to the fact that BCG induced immunity is poorly understood. BCG has been shown to be effective as a neonatal vaccine not only against *M. tuberculosis*, but against other endemic infectious agents in many developing countries [25] as it enhances the

activity of co-administered pediatric vaccines [26]. To improve upon its effectiveness, a number of new vaccines use BCG as a backbone, either incorporating genes to over-express an existing or new *M. tuberculosis* antigens [27], or modifying the biology of BCG to alter its life cycle to enhance T cell mediated immunity [28]. These recombinant BCG strains (reviewed by Kaufmann and colleagues in this issue) are developed with the assumption that BCG can mediate protective immunity through induction of adaptive immunity. Assuming continued use of BCG as a pediatric vaccine, new candidates may need to be developed to boost or augment BCG [29].

Boosting BCG? A mix of the old and the new

The variability of BCG as a vaccine to protect against *M. tuberculosis* infection may be offset by the possibility that existing BCG mediated immunity can be boosted [29] to produce potent long-term immunity [30]. Boosting neonatal BCG vaccination with a second BCG immunization later in life has not delivered significant improvements in protection against pulmonary tuberculosis [31], arguing again for the need to better understand the mechanisms by which BCG elicits immunity. Commonly used small animal models have only been able to provide limited information about whether a vaccine can successfully boost BCG immunity due to several factors. Booster vaccines stimulate pre-existing antigen specific immune cells to induce a greater, more rapid response similar to that expected during infection. BCG priming in mice is already able to eliminate approximately 90% of the mycobacterial burden, suggesting it may be difficult to observe boosting, using this host and microbiological endpoint [32, 33]. Boosting BCG with a second vaccine will hopefully result in faster and stronger immune responses than what is seen with BCG alone. The immune cells most commonly analyzed are multi-IFN- γ /TNF- α /IL-2 secreting T cells, although it is not clear which combination of these is required for effective mycobacterial clearance. Studies in both adults [34] infants [35] demonstrated the ability of MVA85A to boost pre-existing BCG immunity [34]. However protective efficacy in infants over BCG alone could not be discerned in the boosted cohort as BCG may have provided better protection than anticipated. Testing diverse scenarios in animal models by boosting at variable times or via alternative routes after BCG priming may be able to identify schedules that increase protective immunity [36–38]. However, given that the dynamics of immune responses to *M. tuberculosis* infection are likely different in small animals than humans, how boosting intervals can be translated from these studies to clinical trials is unclear. While information about the extent and nature of immune responses to a vaccine in animal models can provide guidance about the potential immune response a vaccine generates in humans, clinical trials are eventually required to assess safety and protective immunity of candidate vaccines. Indeed, it could be argued that efficacy animal models may be best used to identify vaccine mechanisms rather than provide data to predict human efficacy.

Vaccines against tuberculosis – The new

A recent review [10] summarized the current vaccine candidate pipeline, and provided a comparison of the number of different formulations tested in pre-clinical studies versus those in clinical trials (Figure 1). Pre-clinical standardized testing of vaccine candidates and formulations in mice and guinea pigs has been conducted in the same laboratory at Colorado

State University under an NIAID/NIH contract since 2004, which complements vaccine testing at other sites around the world. The number of candidates that are tested in animal models but do not advance to more complex studies and human clinical trials is significant, emphasizing the difficulty of vaccine development. Furthermore, development of vaccines that are either able to prevent infection or disease has raised the question whether it will be necessary to develop T cells that recognize infected antigen presenting cells or help in antibody production. It has also been recognized from studies using non-parenteral vaccination routes [39] that lung immunity and the engagement of cells that are not traditionally regarded as important for resolution of infection [36, 40–42] are critical.

Critical factors in developing vaccine elicited protection

The role of non-traditional mechanisms in vaccine mediated immunity

Defining the role of CD4⁺ [43–47] and CD8⁺ T cells [46, 48] continues to be of great importance. However, the study of non-traditional immune cells for the control of *M. tuberculosis* infection may also provide important new insight. Outside the question of “trained immunity”, interest has been directed at understanding the mechanism of innate lymphoid cells (ILC) such as mucosal associated invariant T (MAIT) cells, [37, 42, 49–52] invariant natural killer T (iNKT) cells [49, 53, 54] and NK T cells [55]. Whether these cells perform a direct effector function during infection is unclear, but they may be an early requirement for induction of protective immunity. The integral role of ILC in disease pathogenesis is unclear, and may be important in vaccine-mediated immunity [56–58].

Adjuvants

The number of immunostimulatory agonists targeting PRR in recombinant antigenic peptide vaccines is increasing [59]. Anti-tuberculosis vaccines have used a diverse array of molecular adjuvants directed against multiple Toll-like receptors (TLR) and have elicited a surprisingly diverse set of responses [59–66]. Comparative analysis between novel adjuvants and BCG suggests that stimulation of antigen presenting cells via a single PRR may in some cases be sufficient to induce activated T cells [21]. Whether these cells are sufficient to induce protective immunity is not fully understood, leaving the critical role of a T cell during *M. tuberculosis* infection, cytokine production, cytolytic activity and antibody engagement to be determined. Given the diverse type and functions of dendritic cells (DC) the need to combine an adjuvant with an appropriate route of administration is important in directing subsequent adaptive immunity [47, 67, 68]. For example during mucosal vaccine delivery, defining the role of TLRs in DC activation is important [60], though other pulmonary cells including epithelial cells and macrophages may also be involved and contribute to a network of interactions that lead to productive adaptive immunity [69].

Vaccine induced antibodies as a means of preventing infection

Recent attention has been directed towards vaccine-induced antibodies to prevent infection. How and whether antibodies that are produced during infection may contribute to protective mechanisms remain unclear [70]. However, if produced prior to infection, antibodies may become significant in prevention of infection. Vaccines studied in mice have reported

induction of post-vaccination antibodies, but could not demonstrate contribution to protective immunity [33, 71] since no effect on the mycobacterial burden was seen despite antibody production to vaccine antigens [72]. This is also highlighted by the fact that maternal antibodies do not seem to play a role in protecting new born against infection, although maternal antibodies were shown to inhibit PPD-specific T cell responses in BCG vaccinated infants [73]. However, more recent studies in mice [74] and to a limited extent, humans [75] have indicated roles for antibodies, in particular mucosal IgA [76]. Producing the correct antibody through vaccination may be as important as inducing the correct T cell response and care must be taken to ensure that novel vaccines do not elicit pathology inducing antibody responses.

Post-exposure vaccination

The number of infected individuals worldwide that carry latent *M. tuberculosis* infection indicate the need for a post-exposure vaccine to prevent the development of active tuberculosis. Each person with latent tuberculosis represents a potential future tuberculosis patient if any number of immunosuppressive events [77] take place. Thus including latently infected individuals in a broad vaccination program will have enormous advantages [78]. Identification of latency-associated antigens such as those in the DosR regulon expressed by *M. tuberculosis* during times of stress are being incorporated into vaccines [79]. Developing animal models that create relevant data on the efficacy of post-exposure vaccines is becoming critical [80, 81] and it remains to be seen whether these models are able to generate relevant data that translate to humans. Non-human primates are gaining increasing importance in the study of vaccines, but the fact that only a subset of infected animals develops latent infection limits their use and increases costs of this model [82–84]. Guinea pigs are known to be highly sensitive to infection and reliably develop acute tuberculosis and latency, albeit in a small proportion of infected animals (approximately 1–5%; AAI unpublished).

Conclusions

It is increasingly clear that there are multiple mechanisms that can be utilized to kill *M. tuberculosis* and each may be useful at the various stages of the infection process. Vaccines remain the most critical tool for tuberculosis control since they promise to reduce transmission and a subsequent reduction in the global burden of tuberculosis. As vaccines advance through clinical trials, we have the unique opportunity to compare results from human trials with data obtained in animal models. Programs such as the Advanced Small Animal Models for the Testing of Candidate Therapeutic and Preventative Interventions against Mycobacteria (NIH/NIAID) will undoubtedly provide data supporting this goal.

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- Expanding knowledge of the immune response to *M. tuberculosis* infection is improving the design of vaccine candidates
- The identification of novel pathways that may be involved in killing *M. tuberculosis* is critical for vaccine development
- Understanding the network of interactions between immune cells and signals will provide a significant breakthrough in developing an effective vaccine

Figure 1.

The number of protein, recombinant BCG, attenuated *M. tuberculosis*, DNA/vector and adjuvant formulations submitted for testing by the National Institutes of Health at Colorado State University from 2004 to 2017 in the mouse and guinea pig models of tuberculosis, compared to those currently in clinical trials. The number tested in animal models does not reflect samples tested by individual investigators or at other testing sites.

Figure 2.

Designing a novel vaccine against *M. tuberculosis* infection will require the contribution of a network of cells and signals to promote effective killing mechanisms. Novel vaccine adjuvants stimulate PRR on antigen presenting cells (such as dendritic cells) to augment immunity to antigenic proteins and peptides. The contribution of innate lymphoid cells and non-lymphoid early response cells (e.g. neutrophils), remains to be elucidated. The role of antibody in preventing infection is under extensive investigation, but requires T cell help to ensure the induction of the appropriate antibody.