VIEWPOINT

Sterilizing immunity: New opportunities for rational TB vaccine design

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Recent studies have revealed situations of high-level naturally acquired and vaccine-induced immunity against *Mycobacterium tuberculosis* in animal models along with examples of significantly protective immunization in humans. These discoveries offer immunologists new opportunities to define effector mechanisms that when triggered by appropriately engineered vaccines could end TB's deadly reign.

The contrast couldn't be more striking. In response to a deadly global pandemic, highly effective vaccines against SARS-Cov-2 were developed in a matter of a few months, while after more than a century of research no vaccines exist for preventing the major forms of tuberculosis (TB), a disease estimated to have killed over a billion people in the last two millennia and over 1.5 million in the last year alone (World Health Organization, 2020). The explanation is not complicated. From prior work on SARS1 and other coronaviruses, COVID-19 vaccinologists had a proven humoral effector function as well as a likely viral target antigen upon which to base their vaccines. In contrast, TB immunologists still have not agreed on the appropriate effector mechanism(s) or antigenic target(s) needed to rationally design a fully protective TB vaccine. BCG vaccination, now celebrating its centennial year, is still administered intradermally to infants in countries with high transmission rates to prevent extrapulmonary TB but has limited efficacy in adults. Indeed, most adults who acquire TB worldwide today were BCG vaccinated as neonates, and even in infants BCG is not universally protective (Mangtani et al., 2014). Nevertheless, while failing to halt the TB pandemic, BCG continues to yield important secrets about the nature of host resistance to mycobacteria.

With the repeated disappointing outcomes of early empirical approaches to TB vaccine development, it became clear that *Mycobacterium tuberculosis* (Mtb) differed from many other pathogens in its interaction with the immune system and that successful immunization was likely to depend on the delineation of that relationship. An important step in this pursuit was the definition in the mid 20th century by Mackaness and colleagues of a new immunological effector mechanism for controlling intracellular pathogens within macrophages, Mtb's major niche, and the role of lymphocyte products in activating them for this nonspecific killing function (Van Epps, 2005). With the definition of both the cytokine activators (IFN-γ, TNF) and their major source (T lymphocytes), a strategy for TB immunization based on this "central dogma" emerged involving the triggering of strong Mtb-specific CD4 Th1 responses, leaving the induction of humoral immunity largely ignored as irrelevant. Although providing some protection in animal models, vaccine constructs that stimulate strong Mtb-specifc Th1 responses have failed to confer significant protective immunity in clinical trials, indicating that the original central dogma is either an incorrect or oversimplified mechanism upon which to base human TB vaccine development (Zeng et al., 2018).

Since many of the prior mechanistic studies and much of the preclinical vaccine testing had been performed in murine Mtb infection experimental models, a knee-jerk response to the failure of central dogma–based vaccines was to declare the mouse (along with other small animal models) irrelevant for studying human immunity. Indeed, murine and human TB have several important dissimilarities. A major consequence was to stimulate further interest in the use of nonhuman primates (NHPs) as experimental models for both mechanistic and vaccine research. As shown in pioneering studies (Flynn et al., 2015), in addition to possessing a more human-like immune system, Mtb-infected NHPs develop granulomas and other pathologies that mimic those seen in TB patients and importantly (and in direct contrast to inbred mice) mirror at the individual level the diversity in disease outcome observed in infected humans.

Somewhat unexpectedly, the new emphasis on NHP-based vaccine research has led to the demonstration of high and sometimes sterilizing levels of protection against TB induced by several immunization protocols. These include: (1) vaccination with BCG administered by the unconventional intravenous route (Darrah et al., 2020) or delivered via bronchoscope directly into the lower lung lobe (Dijkman et al., 2019); (2)

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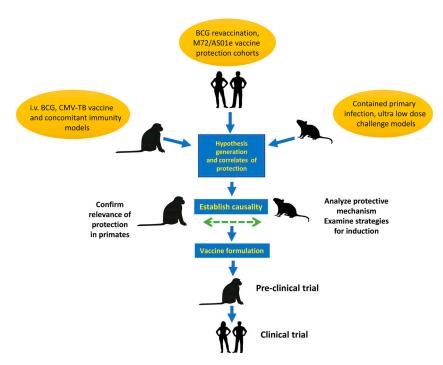Alan Sher: asher@niaid.nih.gov;   Joanne L. Flynn: joanne@pitt.edu.

immunization with CMV expressing Mtb antigens (Hansen et al., 2018); and (3) concomitant Mtb infection (Cadena et al., 2018), thus providing both proof of principle and new hope for protective immunization in humans. This optimism was reinforced by the publication of clinical trials reporting over 49% protection from TB in previously exposed individuals (i.e., those with clinically latent Mtb infection) given an adjuvanted protein subunit vaccine (M72/AS01E; Tait et al., 2019) and signals of enhanced immunity in BCG revaccinated adolescents (Nemes et al., 2018).

The above breakthroughs have reenergized TB immunologists by providing them models of robust immunity to infection/disease in which to identify effector mechanisms of host protection (Scriba et al., 2020). Indeed, many of the previous mechanistic concepts concerning TB immunity derived either from experimental model and clinical studies on the control of existing infection (as opposed to resistance to challenge) or from the analysis of immunization protocols (e.g., intradermal BCG) conferring lower levels of protection, distinctions that may have been important factors underlying their poor translation into effective vaccines. Now highly sophisticated correlates of protection (CoPs) studies can be performed in both vaccinated monkeys and humans displaying solid levels of immunity.

At the same time, the mouse, with the development of newer models, has regained its status as a relevant and now even more powerful experimental platform for studying protective immune mechanisms against Mtb. High levels of concomitant immunity to challenge have now been demonstrated in mice with contained primary infections (Nemeth et al., 2020), and it is now clear that with the appropriate genetic background and challenge dose, mice can develop pulmonary pathology that, if still not identical with, more closely resembles that seen in humans. With the use of powerful new technology for analyzing genetic influences on host resistance (e.g., collaborative cross mice) and its cellular requirements, the mouse can perform its function of "hypothesis generator" in settings more directly linked with the type of high-level protection sought for with human vaccines (Bucsan et al., 2019).

As discussed above, modern TB immunologists now have three hosts (mouse, NHP, and human) for comparative analysis of solid and in some cases sterilizing levels

The tri-host species strategy for generating TB vaccine design from correlates of high-level protection. The text within ovals beside each host refers to current clinical and experimental situations of sterilizing or unusually high levels of vaccine or concomitant immunity that can be mined for correlates shared between two or more species.

of protective immunity. Justification for such a three-pronged approach comes from gene expression profiling studies comparing the response to Mtb in the three species that revealed a surprising level of commonality in the transcriptional signatures induced in response to infection among the three hosts (Ahmed et al., 2020). Together, these findings suggest a strategy in which shared CoPs between humans, NHPs, and mice developing robust levels of immunity as a result of vaccination or ongoing primary infection are used to identify candidate effector functions that can be examined mechanistically and their protective potential validated in mice and then (or simultaneously with) NHPs before clinical trial (see figure). The critical distinctions from prior approaches of this kind are the use of CoPs from highly immune hosts as "hypothesis generators" and the major role of NHP both as a source of CoPs and for validating their relevance to protective immunity in humans.

While the field awaits the completion of ongoing CoP analyses from the promising BCG revaccination and M72/AS-01 clinical trials, considerable progress has been made in the in-depth dissection of immune correlates of resistance and mechanisms of protection in the NHP models. These studies

have employed cutting-edge technologies, including single-cell RNA sequencing (RNAseq), multiparameter flow cytometry, and cytometry by time of flight, systems serology, and serial positron emission tomography–computed tomography imaging. While confirming CD4 T cells as a significant component of immunity, this new work has revealed a striking association of cytotoxic lymphocytes, including innate and adaptive CD8+ cells, with the high level of protection induced by vaccination with CMV-Mtb (Hansen et al., 2018) and i.v. BCG, as well as concomitant immunity. As noted above, BCG administered i.v. triggers a sterilizing immunity to Mtb challenge, and interestingly, a prominent airway CD8 T lymphocyte response was observed in i.v.-vaccinated monkeys but not in animals immunized with BCG by the less-protective intradermal and aerosol routes (Darrah et al., 2020). In related work, single-cell RNAseq has revealed that within individual macaques, granulomas that control infection, in comparison with those that do not, are characterized by the presence of a diverse set of cytotoxic lymphocytes (Gideon, 2021 Preprint). Systems serology has also revealed IgM antibody responses that correlate with sterilizing protection in vaccinated NHPs (Irvine,

2021 *Preprint*), suggesting the possible involvement of humoral immunity. Going forward, depletion of key subsets, such as CD4 or CD8 T cells, innate CD8+ cells, and B cells in the context of infection, concomitant immunity, and vaccination in NHP models should provide critical information about the mechanisms of natural and vaccine-induced control of Mtb infection.

At the same time, studies in mice have associated the high-level immunity to challenge resulting from ongoing contained Mtb infection with both an augmented and accelerated influx of antigen specific T cells into the lung parenchyma and a change in the activation status of alveolar macrophages (Nemeth et al., 2020). The latter observation is an important example of a large body of current work in the field examining the role of innate myeloid and lymphoid cells in host resistance to TB, and indeed it may be that a successful TB vaccine will require the induction of appropriate innate as well as adaptive responses. Together, these animal model studies point to new mechanisms that can be targeted in vaccine development and assist in the choice of adjuvants and routes of administration that will selectively enhance protective immune cell functions.

Will the study of situations of high resistance to TB lead directly to an effective vaccine or simply add additional layers of complexity? Time will tell. In the meantime, the current COVID-19 pandemic has had a severe impact on international TB control efforts as well as clinical trials and drawn TB investigators and their BSL-3 resources away from their own vaccine efforts. Nevertheless, it is hoped that the ongoing massive investment in combating pandemic respiratory disease will spin off new interest, funding, and research infrastructure for tackling the ever-challenging but now more reachable goal of TB vaccine development.

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