10TH ANNIVERSARY SERIES

Exacting Edward Jenner's revenge: The quest for a new tuberculosis vaccine

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Recent experimental and clinical work has reinvigorated the pursuit of a better tuberculosis vaccine.

The power of vaccines was first realized with the eradication of smallpox. Ironically, Edward Jenner, the inventor of the first smallpox vaccine, lost his wife and son to tuberculosis (TB), a major global infection that continues to thwart efforts to create a highly effective vaccine. In this fourth installment of the 10th-anniversary Focus series, we examine progress in the decade since Bertholet *et al.* reported in *Science Translational Medicine* their strategy to boost immunity in animal models of TB (*1*). Although progress on developing new and more effective TB vaccines has been slow and uncertain, recent advances have generated renewed optimism.

Disease caused by infection with the bacterium *Mycobacterium tuberculosis* remains a substantial global infectious disease problem. This bacterium primarily infects macrophages and other phagocytic cells in the lungs as a result of inhalation of aerosolized infectious microdroplets, and quickly spreads to adjacent lymph nodes and other tissues. Current estimates suggest that at least 1 billion people harbor latent *M. tuberculosis*. *M. tuberculosis* bacilli can persist in tissues as viable organisms without causing apparent disease for the lifetime of the host and can eventually progress or become reactivated in a fraction of individuals to cause active TB and transmission to new hosts. Although a well-developed public health infrastructure combined with effective chemotherapy regimens has effectively controlled and largely eliminated TB from most developed nations, the disease continues to rage unchecked in underdeveloped and resource-poor countries. In these settings, the availability of effective vaccines for prevention of *M. tuberculosis* infection and active disease is widely viewed to be a key strategy for breaking the cycle of transmission and finally controlling the ongoing epidemic (Fig. 1).

An attenuated strain of *Mycobacterium bovis* known as Bacille Calmette-Guérin (BCG) has been available as a TB vaccine since 1921, and it is the only TB vaccine currently licensed for use in humans. Vaccination with BCG is widely used in many areas of the world and is routinely administered to newborns within a few days after birth in most countries with high rates of *M. tuberculosis* infection. Despite rates of newborn vaccination with BCG exceeding 90% in many of the countries most seriously affected by TB, rates of infection have not been consistently declining. This is in line with the generally accepted view that infant BCG vaccination reduces the risk of severe disseminated TB in infants and young children but does not provide consistent or durable protection against pulmonary TB in adolescents and adults. In addition, given that infants and young children rarely transmit TB, the protection afforded them by BCG contributes relatively little to halting the cycle of transmission that perpetuates the global TB epidemic. Over the last several decades, major effort has been directed toward generating new candidate TB vaccines and to improving the potential impact of the existing BCG vaccine. Very recently, these efforts have begun to yield tangible evidence of success.

CAN BCG BE IMPROVED OR SHOULD IT BE REPLACED?

It is surprising that a vaccine developed 100 years ago that shows at most partial efficacy has not been replaced by now with something more effective, particularly given the extraordinary advances achieved in the fields of immunology and vaccinology. Much effort has been expended on reengineering BCG to improve its immunogenicity, and to developing live attenuated vaccine strains of *M. tuberculosis* to replace BCG entirely. Very few, if any, of these vaccine strains have shown more than marginal evidence of improved protective immunity compared to that induced by BCG in standard animal models of *M. tuberculosis* infection (*2*). Relatively few of these new vaccine strains have advanced to clinical trials in humans, and even those candidates have not yet been shown to be clearly better than the standard BCG vaccine.

Because of the standard practice of BCG vaccination in many TB-endemic areas, diagnostic methods have been developed to sensitively and specifically detect *M. tuberculosis* infection even in BCG-vaccinated subjects, for which the standard tuberculin skin test can often yield false-positive results. Most notable has been the increasing use of the whole blood interferon-γ release assay (IGRA), which detects latent or active *M. tuberculosis* infection based on T cell responses to a small number of antigens that are present in all *M. tuberculosis* isolates but absent from BCG (*3*). The IGRA is now well established as a valuable test for diagnosing *M. tuberculosis* infection and for guiding treatment decisions, particularly in cases of suspected latent infection. However, one consequence of the increasing use of this test to guide treatment decisions is that it will become increasingly difficult to test or implement new vaccine strains that generate false-positive results for TB infection. Indeed, in ongoing clinical trials, the live attenuated *M. tuberculosis* vaccine strain MTBVAC (*2*) has been reported to cause IGRA conversions from negative to positive that cannot be distinguished from actual TB infection, confounding decisions on when to apply prophylactic antibiotic treatment.

MOVING FORWARD WITH IMPROVED BCG-BASED STRATEGIES

Given the enormous experience with BCG, the substantial investment into implementing its widespread use, and the validation of diagnostic approaches designed for use in the context of previous BCG vaccination, it is likely that BCG vaccination of newborns will remain as a component of any TB vaccination regimens introduced in the foreseeable future. With these realities in

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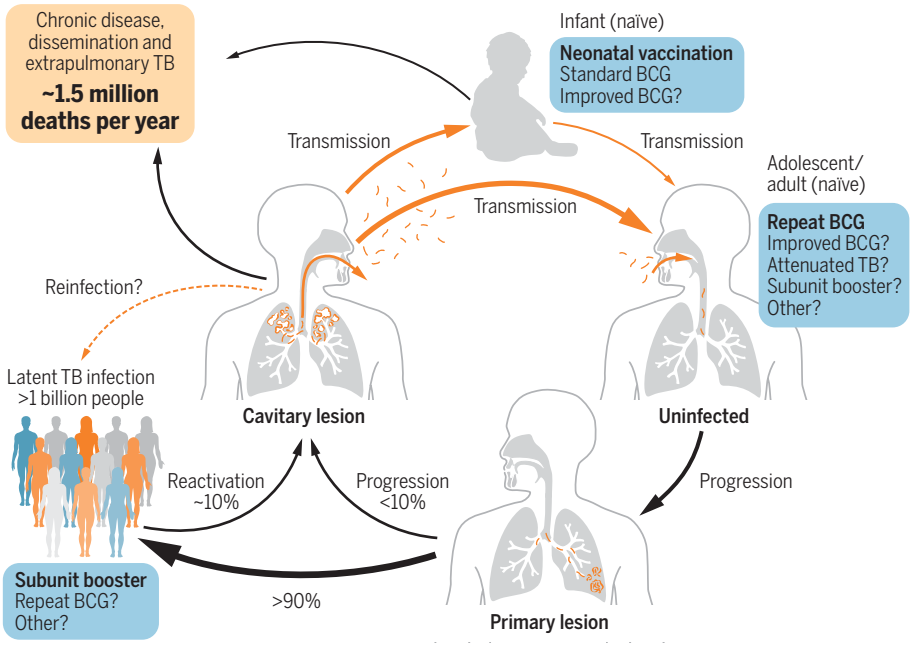

Fig. 1. Breaking the cycle of TB transmission. *M. tuberculosis*, the pathogen causing TB, is transmitted by inhalation of aerosolized microdroplets that are released by the coughing of infected individuals, usually those with cavitary lung lesions that have advanced into the bronchi. Infants and young children exposed to such infectious aerosols are highly susceptible to infection and have a high risk of developing severe, progressive disease with dissemination beyond the lungs to other organs. Cumulative deaths from TB currently total about 1.5 million globally per year. The blue boxes indicate key points in the infectious cycle where vaccination could have the greatest impact. Neonatal BCG vaccination is already well established in most countries with a high prevalence of TB. Improvements to this vaccine will likely involve introduction of modified BCG strains with better safety and immunogenicity profiles. Vaccination of uninfected or latently infected adults or older children may involve a more diverse array of new vaccine candidates such as subunit or live attenuated vaccines. Recent reports suggest benefit from revaccinating adults with BCG, and one trial has shown efficacy of the M72 subunit vaccine in prevention of disease in adults with latent TB infection.

mind, greater attention has turned toward rationally engineering BCG to improve its safety and immunogenicity. Although BCG has generally been accepted as safe, it retains its replicative potential and can lead to disseminated disease in substantially immunodeficient neonates or adults. This risk can be eliminated by genetic manipulation of the parent BCG strain to generate auxotrophs that propagate normally in culture but cannot grow at all in the body of an animal host because of an inability to synthesize required nutrients. Many such auxotrophies have been introduced into both *M. tuberculosis* and BCG (*4*), although their effects on immunogenicity and vaccine efficacy are incompletely studied. It remains unclear whether the marginal advantage in safety with such strongly attenuated BCG strains will provide sufficient justification to advance these into further studies in nonhuman primate models or in humans.

Various strategies to improve the immunogenicity, and hence the vaccine efficacy, of BCG are also being considered. As a mutated version of a pathogenic mycobacterium, BCG retains many immune evasion mechanisms that prevent the development of optimal host immune responses. By disabling some of these mechanisms, it may be possible to create BCG strains that stimulate greater protective immunity. One such modified BCG strain that has progressed into clinical trials is the VPM 1002 strain (*2*). This is a variant of standard BCG with genetic changes that increase phagosomal acidification and release of antigens into the cytosol of infected macrophages and dendritic cells, thus enhancing antigen presentation and T cell responses. Initial phase 2 clinical trials to determine whether VPM 1002 should be adopted as a replacement for standard BCG in vaccination of newborns are currently ongoing.

BOOSTING BCG-INDUCED IMMUNITY

Various approaches to boost preexisting BCG-induced immunity have emerged as perhaps the most promising area for delivering practical solutions in the near term for improving immunity to prevent TB. An important study published in 2018 reexamined the impact of homologous prime-boost regimens for BCG, in which human subjects who had received neonatal BCG priming underwent a second BCG immunization as adolescents to boost the waning protective effects of the initial vaccination (*5*). Evaluation during 2 years of follow-up after the second vaccination in a high transmission setting revealed evidence for differences in the acquisition of latent TB infection. Although initial conversions to IGRA positivity (indicating new TB infections) were similar in BCG-boosted versus placebo-boosted groups, a greater proportion of these reverted to negative in the BCG-boosted group. One interpretation of these results is that reversion of the positive IGRA test indicates an immunologically mediated clearance of recent infection, consistent with benefits from homologous boosting. This study raises many questions about the potential for repeated administration of BCG to reduce rates of actual disease (particularly in light of previous large BCG revaccination trials that failed to show effects) but is likely to encourage further studies to evaluate the impact of BCG revaccination.

A second approach to boosting protective immunity in BCG-vaccinated hosts involves the use of specific protein antigens of *M. tuberculosis*, delivered either with viral vectors or as purified recombinant proteins formulated with adjuvants. This approach provides many options, and the questions of which adjuvants to use and which specific antigens to select from the ~4000 proteins encoded by the *M. tuberculosis* genome are currently major areas of study. Initial efforts to create subunit booster vaccines have focused mainly on the relatively small group of immunodominant secreted protein antigens, such as the members of the Antigen 85 family (e.g., Ag85A and Ag85B) and secreted substrates of type VII secretion systems (e.g., ESAT-6 and CFP-10) (*2*). However, a major phase 2b clinical trial using an attenuated vaccinia virus producing Ag85A to boost immunity in BCG-vaccinated infants revealed no impact on subsequent acquisition of clinical TB infection (*6*). This finding raises serious doubts about using immunodominant secreted antigens to stimulate protective immune responses against *M. tuberculosis*. Indeed, given that strong immune responses are generally seen against these antigens in animals or humans with active TB infection, they may be unlikely to serve as points of vulnerability for *M. tuberculosis*.

A second approach that has developed in parallel over the last decade is that of targeting less immunodominant antigens for the design of BCG-boosting subunit vaccines, often with two or more antigens linked as recombinant fusion proteins. In general, this approach has emphasized protein antigens associated with immune responses in subjects with latent TB who have successfully controlled their infections. One important early demonstration of this approach involved the creation of a single recombinant fusion protein called ID93, composed of four *M. tuberculosis* antigens associated with bacterial virulence or latency (*1*). Studies of ID93 in multiple animal models demonstrated the ability of this antigen combined with a suitable adjuvant to stimulate polyfunctional CD4 T cell responses against *M. tuberculosis*, with effective control of bacterial growth in the lungs of infected mice. Perhaps most striking was the ability of ID93 to strongly enhance control of *M. tuberculosis* infection when administered to previously BCG-vaccinated guinea pigs (*1*). This finding established the concept of using properly selected *M. tuberculosis* protein antigens to boost BCG to increase immune control of infection in a highly susceptible host and represents an important landmark achievement in the TB vaccine field.

The evaluation of ID93 in humans for its ability to increase protective immunity against primary TB or reactivation is currently at an early stage with at least one phase 2 study being planned (*2*), but a major success was recently reported using another recombinant fusion protein antigen. This fusion protein, known as M72, was produced by combining two *M. tuberculosis* antigens: one a secreted protease and the other a putative immune evasion or virulence mediator. M72 has been tested in formulation with the adjuvant $AS01_E$ in a major phase 2b clinical trial for boosting immunity to prevent active TB in adults with latent TB infection documented by a positive IGRA test (*7*). The vast majority of subjects in this study had received infant BCG vaccination, so the protocol could be viewed as one for boosting immunity induced by subclinical TB infection in the context of previous childhood BCG vaccination for prevention of subsequent clinical disease. After 2 years of follow-up, a statistically significant reduction in the proportion of subjects free of TB disease was observed in the M72/$AS01_E$ group compared to the placebo group, with an estimated vaccine efficacy of 54%. Further follow-up on these study populations is planned to determine the durability of the observed protective effect.

FUTURE PERSPECTIVES

The recent progress in preclinical studies relevant to principles for design of TB vaccines will undoubtedly maintain a steady flow of new candidates for testing in the current validation pipeline. Now, with a notable glimmer of positive results on improving vaccination against *M. tuberculosis* from recent clinical trials, there is sure to be renewed interest in accelerating the testing of additional new products and protocols in this space. Although vaccine regimens that are compatible with continued neonatal BCG vaccination will likely be emphasized, we also will see continued experimentation with radically different platforms that could further enhance or ultimately replace BCG. One such approach that has already achieved remarkable early success in nonhuman primate studies is the cytomegalovirus-based polyepitope vaccine, which achieves high numbers of sustained circulating effector T cells through persistent antigen delivery (*8*). Another area that is likely to continue to drive preclinical experimentation is the delivery of BCG or other live vaccines through alternate routes of administration, particularly by inhalation or possibly intravenously, which may lead to greater immunogenicity (*9*). In addition, improvements in tractable animal models such as mice for higher throughput of early-stage vaccine testing (*10*), as well as development of more sophisticated in silico models, should accelerate the design and testing of new TB vaccines. Although we are still at a relatively early phase in the discovery of a vaccine that will truly make a major contribution to eliminating TB as a leading global health problem, the stage may now be set for finally achieving Edward Jenner's long-awaited revenge.

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