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Pursuing Edward Jenner's Revenge: The Quest for the New TB Vaccine

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Short summary

The power of vaccines was first realized with the eradication of smallpox. It is ironic that Edward Jenner, the inventor of the first smallpox vaccine, lost his wife and son to tuberculosis, a major global infection that continues to thwart our efforts to create highly effective vaccines. Although progress on developing new and more effective tuberculosis vaccines has been slow and uncertain, recent incremental advances from pre-clinical research and encouraging successes in clinical trials reported in the past year have generated renewed optimism.

Disease caused by infection with *Mycobacterium tuberculosis* (Mtb) remains one of the most significant global infectious disease problems. Current estimates are that at least 1 billion people are infected with and harbor latent Mtb bacilli in their bodies. These organisms can persist in a dormant state in tissues for the lifetime of the host, and reactivate in a low but significant fraction of individuals to cause active tuberculosis (TB) and transmission to new hosts. While a well-developed public health infrastructure combined with effective chemotherapy regimens has effectively controlled and largely eliminated TB from most of the developed nations, the disease continues to rage unchecked in under developed and resource poor countries. In these settings, the availability of effective vaccines for prevention of Mtb infection and active disease is widely viewed to be a key strategy for breaking the cycle of transmission and finally controlling the ongoing pandemic (Figure 1).

An attenuated strain of *Mycobacterium bovis* known as Bacille Calmette-Guerin (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 has achieved wide usage 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 Mtb 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 consistent with the generally accepted belief that infant BCG vaccination reduces the risk of severe disseminated TB in infants and young

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children, but does not generally provide consistent or durable protection against pulmonary TB in adolescents and adults. Over the last several decades, a major research 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 nearly 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. Many attempts and much effort has been expended on re-engineering BCG to improve its immunogenicity, and to developing live attenuated vaccine strains of *Mtb* to replace BCG entirely, but very few if any have shown even marginal evidence of improving on the protective immunity induced by BCG in standard animal models of *Mtb* infection (1). Relatively few of these new vaccine strains have advanced to clinical trials in humans, and those few that have not yet been shown to be clearly better than the standard BCG vaccine.

It is likely to be important for future efforts to replace BCG that, due to the standard and widely accepted practice of BCG vaccination in many TB endemic areas, improvements in diagnostic methods have been developing that sensitively and specifically detect *Mtb* infection even in previously BCG vaccinated subjects. Most notable in this area has been the increasing use of whole blood interferon- γ release assay (IGRA), most recently the QuantiFERON-TB Gold In-Tube test approved by the US FDA in 2007, which detects latent or active *Mtb* infection based on T lymphocyte responses to a small number of antigens that are present in all *Mtb* isolates but absent from BCG (2). The IGRA test is now well established as a valuable test for diagnosing *Mtb* infection and for guiding treatment decisions, particularly in cases of suspected latent disease. One consequence of increasing use of IGRA tests 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, this has already emerged as an issue with one live attenuated *Mtb* vaccine strain, MTBVAC (1), which in ongoing clinical trials has been reported to cause IGRA conversions, leading to serious difficulties in diagnosing true latent TB infections and confounding decisions on when to apply prophylactic antibiotic treatment.

Moving forward with improved BCG-based strategies

Given the enormous background experience with BCG, the substantial investment into implementing its widespread use, and the validation of new diagnostic approaches designed for use in the context of prior BCG vaccination, it is likely that BCG vaccination of newborns will remain as a component of any new TB vaccination regimens introduced in the foreseeable future. With these realities in mind, greater attention has turned toward rationally engineering BCG to improve its safety and immunogenicity. Although BCG has generally been accepted as extremely safe, it retains its replicative potential and can lead to disseminated disease in significantly immunosuppressed neonates or adults. This risk can be completely 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. Many such auxotrophs have been introduced into both Mtb and BCG (3), although the effects on immunogenicity and vaccine efficacy are incompletely studied. At this time, 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 of their immunogenicity 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 mycobacteria (*M. bovis*), BCG retains many immune evasion mechanisms that prevent the development of optimal host responses. It is generally believed that by disabling some of these, it may be possible to create BCG strains that stimulate greater protective immunity. One such modified BCG strain that has progressed into phase II clinical studies is the VPM 1002 strain (1). This is a genetically modified variant of standard BCG that incorporates genetic changes that increase phagosomal acidification and release of antigens into the cytosol of infected cells, thus enhancing antigen presentation and T cell responses. Initial phase II 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 boosting pre-existing BCG induced immunity have emerged in recent years 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 re-examined the impact of homologous prime-boost regimens for BCG, in which human subjects that had received neonatal BCG priming underwent a second BCG immunization as adolescents to boost the waning protective effects of the initial vaccination (4). Evaluation during two years of follow up in a high transmission setting revealed a significant effect of BCG boosting on results of serial IGRA tests that were used to assess acquisition of latent TB infection. Thus, while initial conversions to IGRA positivity (indicating new TB infections) were similar in BCG versus placebo boosted groups, a significantly 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 a significant benefit from homologous boosting. This study raises many questions about the potential for repeated administration of BCG to reduce rates of actual disease, but is likely to encourage further studies to evaluate the impact of BCG revaccination in a variety of settings.

A second approach to boosting protective immunity in BCG vaccinated hosts involves the use of specific protein antigens of Mtb, 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 ~4,000 proteins encoded by the Mtb genome are currently major areas of study. Initial efforts to create subunit boosters 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) (1). However, a major phase IIb 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 (5). This finding raised serious doubts about the use immunodominant secreted antigens to stimulate protective immune responses against Mtb. 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 Mtb.

A second approach that has developed in parallel over the last decade is that of targeting less immunodominant antigens for the design of subunit vaccines for boosting BCG. In general, this approach has emphasized protein antigens associated with immune responses in subjects with latent TB who have successfully controlled their infections. Rather than using single protein antigens for boosting, proponents of this approach have often preferred to link together two or more proteins in recombinant fusion proteins. One important early demonstration of this approach involved the creation of a single recombinant fusion protein called ID93, composed of four Mtb antigens associated with bacterial virulence or latency (6). Studies of ID93 in a variety of animal models demonstrated the ability of this antigen combined with a suitable adjuvant to stimulate polyfunctional CD4 T cell responses against Mtb, with significant control of Mtb growth in the lungs of infected mice. Perhaps most striking was the ability of ID93 to strongly enhance control of Mtb infection when administered to previously BCG vaccinated guinea pigs. This finding strongly established the concept of using properly selected Mtb 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 area.

The evaluation of ID93 in humans for its ability to increase protective immunity against TB is currently at an early phase, but a major success was recently reported using another recombinant fusion protein antigen as a boosting vaccine for BCG in humans. This fusion protein, known as M72, was produced by combining two Mtb antigens, one a secreted protease and the other a putative immune evasion or virulence mediator. This has been tested in formulation with the adjuvant AS01_E in a major phase IIb 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 that received saline injections only (0.3 cases vs. 0.6 cases per 100 person-years for a vaccine efficacy of 54%. $P = 0.04$). Further follow up on these study populations is planned to determine the durability of the observed protective effect of M72/AS01_E boosting of immunity on prevention of clinical TB.

Future perspectives

The recent progress in preclinical studies relevant to new 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 TB from recent clinical trials for the first time in the period of modern scientific vaccinology, there is sure to be renewed interest in accelerating the testing of additional novel products and protocols in this space. While it seems likely that vaccine regimens that are compatible with continued neonatal BCG vaccination will be emphasized, we are likely to see continued experimentation with radically different platforms that could further enhance or ultimately replace BCG. One such new approach that has already achieved some remarkable early success in nonhuman primate studies is the cytomegalovirus based polyepitope vaccine, which achieves high levels of sustained circulating effector T cells through persistent antigen delivery (8). Another area that is likely to continue to drive pre-clinical experimentation is the delivery of BCG or other live vaccines through alternate routes of administration, particularly by aerosol or possibly intravenously, which may be associated with greater immunogenicity (9). In addition, many improvements in tractable animal models such as mice for higher throughput of early stage vaccine testing, as well as development of more sophisticated *in silico* models, should accelerate the design and testing of new TB vaccines (10). Although we seem to be still at a relatively early stage in discovery of a vaccine that will truly make a major contribution to eliminating TB as a leading global health issue, the stage may now be set for finally achieving Edward Jenner's long awaited revenge.

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Figure 1. Breaking the cycle of TB transmission with improved vaccination regimens.

The TB bacillus is transmitted by inhalation of aerosolized microdroplets that are released by coughing of infected individuals, usually those with advanced cavitory lung lesions that erode 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 approximately 1.5 million globally per year. The yellow 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 high prevalence of TB, and improvements at this stage 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. Recent reports suggest benefit from revaccinating adults with BCG, and one trial has shown significant efficacy of the M72 subunit vaccine in prevention of disease in adults with latent TB infection.