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Review

A new TB vaccine: Fact or fiction?

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ABSTRACT

Vaccination has been spectacularly successful in eradicating or controlling some infectious diseases, and is particularly attractive as an approach to tackling other infectious diseases. Although vaccination against tuberculosis has been done for nearly 100 years, it is clearly not that successful since the disease still occurs at epidemic levels in animals and humans in many areas. New approaches to vaccination against TB in humans and animals are currently in the pipeline, but none show either complete protection or sterilization. However, there is evidence to suggest that vaccination may deliver some positive outcomes. Not only should we be investigating new vaccines, but also how vaccines and candidates are used and delivered. There are many reasons to think that this task will not be simple, or perhaps not possible in some cases. We present different aspects of the development of vaccines against TB, outline some complications and suggest some new ways to consider this problem.

Contents

1. Background/introduction

Mycobacteriosis, or tuberculosis (TB) is still common today in both humans and animals. At present, we can treat the disease in humans with antibiotic treatment. This is generally not an option for animals, with the exception of the occasional animal in captivity. For TB, as with other diseases, prevention is better than cure, and thus attempts have been made to produce a vaccine against TB for over 100 years. This paper aims to briefly review the field in terms of human and animal reaction to vaccination against TB and highlights some of the difficulties and progress in the field.

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It is often said that *Mycobacterium bovis* BCG, the current vaccine against tuberculosis, is arguably the most frequently given human vaccine in the world today. It has been in use since the 1920s, despite ongoing argument about its efficacy and complications in its usage [1,2]. Owing to this, some low incidence countries such as the USA, do not routinely vaccinate with BCG [3]. One of the factors in this controversy is that there are now many different derivatives of BCG [4], which may not be comparable in their effect, making any analysis of outcome extremely complex, and inter-trial or meta-analyses impossible owing to lack of power [1,2,5]. Furthermore, BCG can be administered in different ways and at different ages [3], complicating any comparative analysis. Existing meta-analysis of BCG vaccination suggests efficacy ranging from negative to positive effects, or protection preferentially against TB meningitis and military TB, although it has been concluded that overall the risk of TB in humans are reduced by 50% [1,2]. Despite problems of analysis it cannot be claimed that BCG vaccination against TB is highly effective. It is probably safe to say that BCG performs best at limiting disseminated disease and mortality in children [1,6]. It cannot reliably prevent infection or protect against pulmonary TB disease in adults. Thus, BCG as a vaccine is not sterilizing and there is a clear reason for development of new vaccine candidates (likewise, BCG is not a sterilizing vaccine in animals). Any new candidate will have to be demonstrably better than the current live BCG, in terms of efficacy and safety, the latter being a problem with BCG use, particularly in HIV positive neonates [7]. BCG is also a pre-exposure vaccine which does not stop infection, latent TB or reactivation, or guarantee sterilizing immunity and we may perhaps find better solutions using a post-exposure or therapeutic vaccine [8].

Perhaps the most difficult problem with TB or mycobacterial immunity is that the organism hides very successfully inside the macrophage or granuloma. For this reason, it is commonly thought that T-cell immunity is critical and that B-cells are of lesser importance [9–12]. Developing a vaccine to deliver immunity based on this premise is of course complex, unlike the well-known successful humoral response vaccines against viral diseases.

2. Prior or acquired immunity?

Since the overwhelming majority of infected humans never develop symptomatic (TB) disease, one may conclude that they have inherent or innate immunity which can cope with the infection, rendering them resistant. However, it is also clear that at least in some communities, BCG vaccination provides some protection, thus one may reasonably conclude that vaccination can confer some degree of acquired immunity. One may thus postulate that in the absence of immunosuppression, most humans are inherently resistant via innate immunity; some may become resistant with the correct stimulus, but that some may not be able to acquire immunity to TB. In the absence of a sound body of research and data concerning different species of animals, we cannot make statements with the same level of confidence for these species. However, we know that different mouse strains show varying levels of resistance or susceptibility to mycobacterial infection and we also know that many species appear to be quite susceptible since TB is commonly reported in them, e.g. badgers, possums, cattle, buffalo, lions, lechwe, deer [13–16]. On the other hand, TB (or mycobacterial disease) has never or rarely been reported in many other species, such as dogs or horses. In some susceptible species, vaccination studies have been done, with some studies reporting various levels of protection, using BCG or new candidate vaccines [17–26]. However, it is not clear whether some animal strains or species or a proportion of any given species is resistant to TB, or whether a proportion can acquire immunity under the appropriate conditions.

3. Animal models

Various options exist, but the mouse model is still the most frequently used. Mice are relatively cheap and can be housed reasonably easily under containment for pathogen challenge. Many well-characterized strains of mice are available, and there is a variety of reagents and kits available for mouse research. Few if any specific reagents or lines exist for other animal models. However, there are some major differences between mice, other animals and humans. One notable difference is that mice do not form the classic granuloma seen in most other vertebrates with TB. For this and many other reasons, this model may therefore have major deficiencies. Noting this, some researchers have invested in non-human primates as a model system [27–29], but this is an expensive and time consuming route, albeit perhaps self-evidently better than others. Ideally, potential vaccines for a particular species should be tested in either the same animal, or at least a closely related species such as bovids for cattle or buffalo vaccines. Each existing model has its own problems and deficiencies, the details of which are beyond the scope of this paper to discuss in detail. However, a common limitation is that many lab-based models are not exposed to the environmental stimuli experienced by free-living creatures, which include organisms such as other bacteria, fungi and parasites, which alone may render their hosts deficient as models. In addition, most animal models are inbred strains. If we postulate that genetics is a major determinant of resistance or susceptibility [30–33], this alone makes such models tricky to understand, since the strain may be inherently resistant or susceptible. Even if not inbred, certain animal species may be innately resistant, where vaccination is unnecessary, or innately susceptible, being perhaps intractable to vaccination. This variation may of course also be found within an out-bred population, due to genetic variation. Recognizing the limitations in laboratory based animal models and the importance of dealing with research close to the real problem, a number of groups have investigated larger animals such as deer or cattle for trial vaccination. These animals are arguably a good choice, since they are potentially susceptible, are at risk for disease, are economically important and impact on ecosystems. In addition, they present with large genetic and environmental heterogeneity, which better reflects what we may expect in trials under real life conditions.

For this reason, apart from laboratory-based animal models, a number of attempts to vaccinate free-living

larger mammals have been made [17–26,34]. Some success in badgers, possums, deer and cattle has been claimed, but total protection has not been evident. The advantage is that in these cases, a controlled challenge can be given to directly assess protection. However, it can always be argued that the challenge is very unlikely to reflect the real, or field condition and therefore the evaluation outcome of vaccination may not reflect the true efficacy of the vaccine. Currently, TB in animals is either dealt with by a test-and-slaughter policy or, as is generally the case with wildlife in many parts of the globe, left unmanaged and untreated. These two approaches are both unsatisfactory for a variety of reasons which will not be the subject of discussion in this paper. At this time, it would seem that the best theoretical options for control would be test and slaughter, or vaccination. Neither is simple nor clear-cut, but nevertheless they are options to consider. Since *M. bovis* is generally an introduced or "alien invader" pathogen, it may exert serious negative effects on ecosystems, which does require attention. This is particularly important in free-ranging animals, where treatment is not possible, particularly in the case of wildlife.

4. Choice of vaccine candidates

For nearly a century, the vaccine given (BCG) has been a live one. There are good intellectual reasons to consider a live vaccine, not least of which is that we know that the live vaccine BCG has some effect and that it reflects the pathogen in that it is an intracellular organism. Newer live vaccine candidates may come in the form of modified BCG (for example strongly expressing a particular antigen) [11,35–37], or attenuated *Mycobacterium tuberculosis* constructs (deletion mutants) [20,38–40]. In this case, it is thought to be necessary to knock out at least 2 genes to negate any possibility that a pathogenic strain may regain virulence. However, live vaccines may also be the source of adverse effects: in immunocompromised humans for example, a live vaccine can cause disease (BCGosis is seen frequently in HIV positive neonates) [7]. However, the same situation may not be the case in immunocompromised individuals vaccinated with double knock-out live attenuated vaccines. It was shown that such a vaccine candidate elicited good responses in FIV+ and FIV− cats, albeit weaker responses in those that were FIV+, with no adverse events noted. This work suggests that live vaccination may be possible in immunocompromised hosts [41]. Despite this finding there are good reasons to explore non-viable preparations for a vaccine. Much attention has been given to this, ranging from individual antigens [11,12,18,42], DNA or heat killed organisms or crude preparations of mycobacterial extracts [24,43], which may also lead to adverse responses (tried by Robert Koch, where the adverse reaction has become known as the Koch phenomenon). Most work has probably been devoted to protein antigens, perhaps because these can be cloned, expressed and produced relatively easily. However, there is published evidence in animal models to suggest that lipids or carbohydrates also hold potential as candidates [22,42,44]. Finally, a new-generation vaccine that targets host response can be an option. Such vaccines can be immune system stimulants, which directly affect cytokine response for example [5,9,11,12,45], or alternatively, target the phagosome [9,35], or interfere with the host cell cycle. The use of a simple antigen may seem counterintuitive, since it is widely believed that humoral immunity may not be important in mycobacterial infection or disease. However, we do not know this for certain, and immune boosters or vaccination with antigens alone have shown promising effects [17,18,36,42]. Hence the rationale to vaccinate first with a live vaccine and follow with a boost of antigen. Trials currently underway in primates [46] and humans may show whether this approach suggests any advantage.

5. Possible complications?

Perhaps one of the most distressing problems is that the preferred location of pathogenic mycobacteria is intracellular and pathogen survival is achieved by subverting normal macrophage function. It is thus difficult to imagine how we may overpower these hidden organisms. Quite apart from this problem, there are also many other problems to consider. For example, the Koch phenomenon suggests possible complications for a good vaccine against tuberculosis: in endemic areas, many individuals may have been infected and if they are exposed to a strong antigen, the ensuing host response may result in the exacerbation of occult disease leading to severe toxicities [47].

If we use a new live vaccine, what danger will this pose to immunocompromised individuals, such as HIV positive individuals? We already know BCG is problematic in this regard.

Even if we overcome these problems, there is evidence that any protection will wane [48,49], which then begs the question of when to vaccinate for maximum protection [10] and whether a booster will be necessary or effective. All these difficulties are compounded by the lack of comprehensive information on the effect of dosage [23], use of adjuvants and timing of prime or boost vaccination. In addition, it seems that there will be species-specific differences, making extrapolation risky. Finally, it is possible that vaccination may interfere with some diagnostic tests for TB, complicating diagnosis [50]. This is particularly relevant for control of TB in domestic and production animals at present.

6. Where should we target our intervention step?

Generally, vaccination is considered to be a preventative activity. However, one may envisage the possibilities of a pre-exposure vaccine as is currently done, or a post-exposure vaccine, a therapeutic vaccine, or one that will suppress reactivation disease. The use of *Mycobacterium vaccae* as a post-exposure vaccine has been tried, but not been convincingly demonstrated to be of importance for general use [51], although there is some evidence to suggest improved conversion during antibiotic treatment in humans [51], but no efficacy on its own. One might also envisage that if we find that all active cases of TB lack an adequate concentration of a biomolecule, such as a

cytokine for example, then a vaccine that will deliver this could be considered as a therapeutic.

7. Vaccine administration and delivery

We do not yet know how changing given parameters of administration of any given candidate TB vaccine will impact on its efficacy. We are not even certain that current practices with respect to BCG vaccination are optimal. For example, giving BCG by the oral, intra-dermal route or by multi-puncture can vary success rate of vaccination "take" considerably. Although BCG is given as a live vaccine, many bacilli in the vials as packaged are not live at the time of administration. Thus, there is no exact controlled dose of live bacilli and simultaneous administration of dead bacilli. We have little idea of the effect of this uncontrolled mix. Should we continue to give it as we do currently with BCG, i.e. a single administration at birth or later [6,10,52], or do we follow a "prime-boost" using the same vaccine twice [52]? This approach has failed with BCG in humans for those time points tried [52], but does not guarantee that it will fail at all time points. There is some evidence (from a deer model [50]) that a second vaccination of BCG may have strongly positive effects, but that the timing is important.

One of the strategies being tried at present is a prime-boost design (see Fig. 1). Given the strong possibility that, at least for humans, there will be enormous resistance or reluctance to abandon BCG entirely, the strategy can include priming with BCG, followed by boosting with an antigen [18]. Even here there are complications, or perhaps rather, over-abundant choices, in deciding what the timing should be and what adjuvant to use. In this context, very little work on the effect of adjuvants on TB vaccination has been done, but this may be extremely important [42,43]. The results of this work suggest equivalence of non-live candidates or improved protection to live BCG under the conditions tested. This is extremely encouraging, since it offers a route for non-live vaccines which makes manufacture, quality control and logistics of delivery simpler, as well as posing far less risk for immunocompromised individuals (e.g. HIV-positive humans and FIV-positive felines), as well as any potentially highly vulnerable animal species. In addition, simultaneous vaccination may be an option. Although no enhancement of protection was obtained in cattle [17], in humans enhanced immunization has been reported [53].

8. Clinical trials, evaluation and end-points

There are currently at least 12 candidate vaccines in trial [54]. Some are subunit vaccines, using fusion or recombinant proteins. Some have been shown to boost BCG "immunity" in preclinical studies. One is a recombinant BCG that produces a better CD4 or CD8 response. A quite different approach is to provide a vaccine that will affect macrophage function (such as by enzymatic membrane-perforation) [9,35], lead to apoptosis, cell death, and thereby "immunity", since evidence suggests that apoptosis may be an important step in resistance to TB.

A major problem for research in a slow onset disease such as TB is the time needed for any trial. This suggests the question:

How do we evaluate vaccine efficacy in candidate vaccines without a huge trial taking years to measure outcomes? We know very little about immunity to TB. For example, why/how does BCG protect, if and when it does? There are difficult technical issues with trials, e.g. how long do we wait to see if disease results? Can we use a surrogate marker [55], and if so how do we find one or more?

Furthermore, if certain individuals are largely immune, vaccination is unnecessary in them. For example, the 90% of humans who may be infected but never develop disease. To measure efficacy in a trial in humans or any animal species that is similar, the numbers that will need to be tested may be massive, particularly so if we are trying to measure and improvement over current BCG. If we cannot achieve 100% coverage because of logistical difficulties for example, how do we identify vulnerable individuals to vaccinate, although this will not be relevant to all species, particularly perhaps in free living animals. Finally, if individuals (or species) are innately vulnerable, will vaccination ever work?

9. Will vaccination make a significant impact?

What do we observe to give us hope or conversely suggest that a vaccination strategy may not work? In the absence of immunosuppression, about 90% of infected or exposed humans will not develop active TB. This suggests that the immune system of these individuals copes adequately with this infection. The key question therefore is: is this innate and inviolate, or is it adaptive? If the latter situation exists, then we can move forward. If the former, then the future looks less promising unless we can mimic this in susceptible individuals. At present, our knowledge suggests that susceptibility or resistance to TB is a function of genetics, maternal exposure, exposure to other agents such as helminths or non-tuberculous environmental mycobacteria [56], or other diseases which affect the immune system, such as diabetes. Therefore, there must be some form of protective immunity in by far the majority of humans, which is most likely largely innate and may include some acquired characteristics. We note that most humans who experience an active episode of TB disease can be cured by antibiotic treatment. However, we observe that these individuals are not protected by this prior episode and remain highly susceptible and likely to develop a subsequent episode [57]. We may hypothesize that this is a subset of inherently highly susceptible individuals, who remain so. Given that no protection is gained after an episode of disease caused by the pathogen itself, there may be reason to doubt that we may be able to protect such highly vulnerable individuals by a vaccine. However, there is likely to be a further subset of individuals who are susceptible but can be protected by acquiring immunity. The relative proportion of such subsets may differ across geographic regions or ethnic groups [30,58], which may explain the known differences in BCG efficacy and will confound vaccine studies in different regions and groups

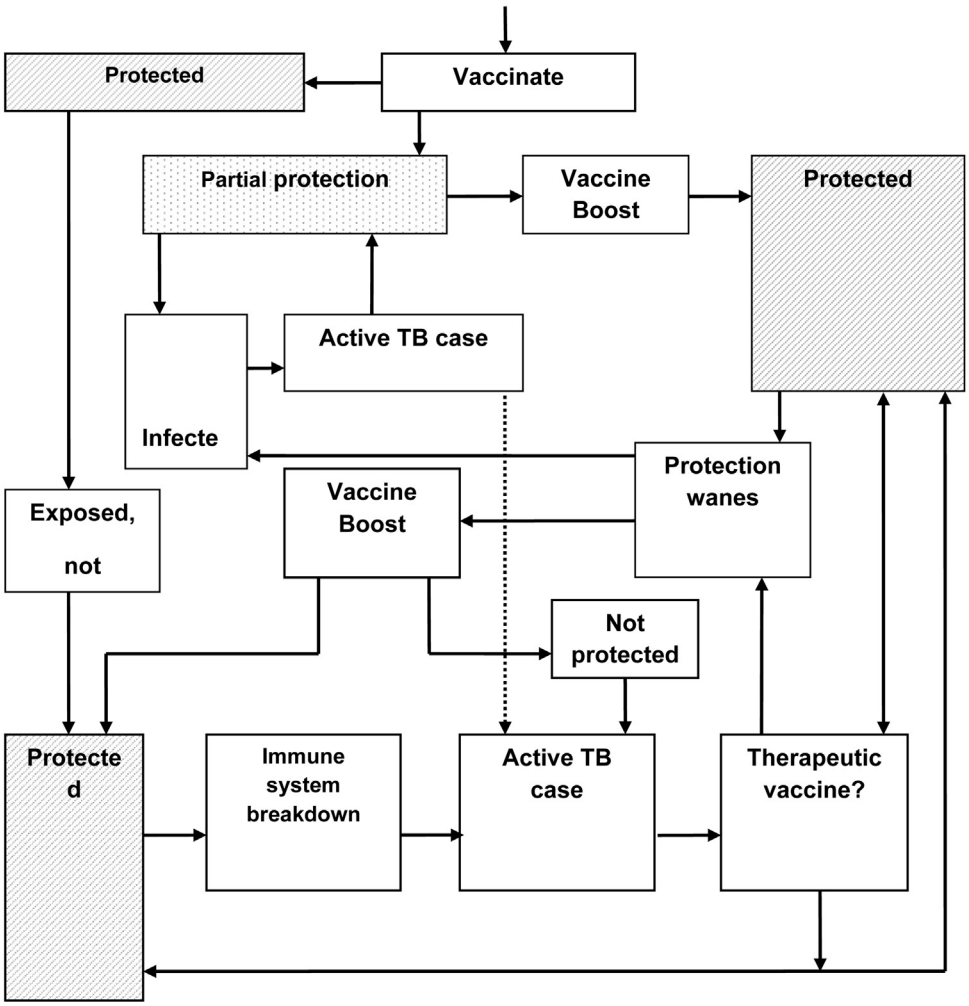

Fig. 1. A simplified scheme illustrating key points to consider for vaccination against TB. It is assumed here that at least some protection is conferred by vaccination. Additional factors not shown here are the type of vaccine used at any given administration, the timing involved, or the dose. The figure also does not illustrate the complication of a post-exposure vaccination event, where there is no active disease. These matters are discussed in the text.

of people. This variability may apply similarly to different animal species.

Once we have some idea of vaccine protection, we can ask the next question: what coverage will be needed for the vaccination to work under the different scenarios? This will also depend on the prevalence of disease and ultimate goal of vaccination, which in turn will depend on its efficacy for a given individual, herd or species. It is highly unlikely that we will achieve 100% coverage or protection, but at a certain point we will probably be able to attain adequate herd immunity to reduce prevalence incidence and achieve control or perhaps even eradication.

10. Goal of vaccination

The goal or aim of vaccination may be quite different for different species. For humans, a highly mobile species, we would optimally want a vaccine that offers individuals full protection against disease and preferably infection as well as disease. However, in the case of possums, an alien species in New Zealand for example, we may want a vaccine that presents transmission and not be concerned about the individual animal and its health status. Our need for protection in wildlife and/or domestic stock may differ, as might our approach to maintenance hosts or spill-over species. Thus, we may aim at eradication under some scenarios should a vaccine confer 100% protection, or control, which would be an improvement over the current situation. The cost of vaccination would have to be considered against potential gain in the DACYs in humans, agricultural losses in livestock or ecosystem damage in wildlife.

11. Will a new vaccine merely shift the population structure of mycobacteria?

We know that the relative proportion of certain strains of *M. tuberculosis* are waxing or waning in different regions of the globe [59]. Furthermore, there is good evidence to

enable us to conclude that various species and strains of mycobacteria cause variable responses in hosts [4,30]. We do not know for certain whether vaccination with BCG is a driver of this change. This is partly as a result of changing vaccination policies, incomplete coverage of vaccination and strain analysis and the use of different BCG strains, all of which makes any analysis or comparison difficult or invalid. However, it is reasonable to suppose that vaccination may offer variable protection against different species or strains of mycobacteria. Should this be the case, we may anticipate initial success with vaccination, followed by emergence or re-emergence of new dominant strains to replace those out-competed by the vaccine. Thus, good surveillance after introduction of new vaccination strategies will be important.

12. Future prospects

The "omics" driven investigation of TB, the host and host–pathogen interactions will improve our understanding of the disease dynamics, which is still poorly understood. Many of the newly elucidated, perhaps fulcrum points of the disease process may present targets for vaccine intervention, also of the therapeutic type. Huge data sets may be amenable to a systems biology approach [60], to study relevant pathways. Responses to TB infection are highly complex and it is unlikely that a simple solution will be found.

Even if no dramatically improved new vaccine candidate is developed, in our opinion the efficacy of current vaccines is not yet fully exploited for humans and animals. We have not exhaustively explored options in terms of timing [6,10,48,52,53], dosage [19,23], adjuvants [22,42,43], prime-boost or simultaneous vaccination for example. The problem with newer candidates is the same: we should not stop exploring delivery options too soon if we find something that shows improvement over current BCG. Even if we use only one candidate, we need to fully explore parameters to optimize it. A major problem will be funding, however.

It is encouraging that BCG has some protective efficacy. On the other hand, recent work in a mouse model suggests that there is initial protection over the short term, then later, T-cells show markers characteristic of exhaustion, followed by a bacterial load increase (I. Orme, personal comm.). This may help to explain our findings which showed increased vulnerability in humans with prior disease [57]. This is a potentially serious flaw in the idea that vaccination in humans can work over the long term. Furthermore, a typical statement from the literature may read "this new candidate offers more efficient containment of late stage infection". In other words, there is a lower bacterial load compared to controls at that same time point. Thus far, the best candidates offer a reduced bacterial load initially, but not protection [8]. Part of the problem with vaccine candidate testing is that the bacterial load in the experimental animal is evaluated at some arbitrary time point, which also means that the animal is euthanized and no subsequent measurements can take place. We have no way of knowing whether the bacterial load will reach the same as the controls, but simply take longer. If this should be the case, then is there any motivation to continue this line of research? On the other hand, if the bacterial load is drastically reduced, maybe mortality will be reduced if the animal can eventually overcome the infection itself and perhaps transmission can be halted. Either way, whilst the individual may not benefit much, from a public health perspective, it may be a justifiable intervention. Overall, whilst there is no clear winner in sight and the field is complex, there is reason to be opportunistic and continue to invest in TB vaccines.

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