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REVIEW

A review of preclinical animal models utilised for TB vaccine evaluation in the context of recent human efficacy data

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SUMMARY

There is an urgent need for an improved TB vaccine. Vaccine development is hindered by the lack of immune correlates and uncertain predictive value of preclinical animal models. As data become available from human efficacy trials, there is an opportunity to evaluate the predictive value of the criteria used to select candidate vaccines. Here we review the efficacy in animal models of the MVA85A candidate vaccine in light of recent human efficacy data and propose refinements to the preclinical models with the aim of increasing their predictive value for human efficacy.

1. Introduction

There is a clear and urgent need for an effective TB vaccine. Although recent progress in drug and diagnostic test development is to be celebrated [1,2] effective vaccination is the only cost-effective measure to achieve long-term control of any infectious disease epidemic. There are two main TB vaccine development strategies currently being pursued, both of which aim to retain the protective benefits of the currently licensed vaccine, BCG, against severe disease in childhood. These are the replacement of BCG with an alternative (more potent and safer) priming vaccine and/or by delivering a booster vaccine months or even years after BCG [3].

The first step in TB vaccine development involves preclinical animal testing in a variety of different species and models. These models are used to evaluate safety, immunogenicity and efficacy. The demonstration of safety in a toxicology study conducted in a relevant animal model is an essential pre-requisite for progressing to clinical testing. The immunological evaluation of new vaccine candidates is typically conducted in mice, but with increasing availability of immunological reagents, can also be conducted in other species [4–7]. These immunogenicity data are used to optimise vaccine regimes and doses prior to the evaluation of protection in *Mycobacterium tuberculosis* challenge experiments. It is the demonstration of protection in these challenge experiments that determines which vaccines progress to clinical evaluation. What protection actually means in these models is an improvement in a disease – related readout, be it bacterial load (expressed as colony forming units or CFU) at a fixed time point post challenge, long-term survival, or pathology score, compared with BCG. It is usual and preferable for protection in more than one animal species to be demonstrated before vaccines advance to clinical testing. The first step in clinical testing involves phase I safety studies, which progress to larger safety and immunogenicity testing in the target population, within TB endemic countries. If successful, these studies then lead into efficacy testing in significantly larger numbers of subjects. Such efficacy trials are huge and costly due to TB incidence rates not being very high, even in the highest burden settings. There is a finite capacity to conduct and to fund such efficacy trials and it is important that data from efficacy trials, when available, are used to review the predictive value of the criteria used to progress candidate vaccines. We can then refine, where needed, the models we use to select which candidate vaccines progress.

Protection in preclinical animal models, together with safety and immunogenicity in phase I/IIa clinical trials, are the three main criteria used to select which candidate vaccines should progress to

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human efficacy testing. Other important criteria used in this selection process include feasibility, standardisation and cost of manufacturing, potential cost of final product, product characterisation, target product profile, regulatory, business and potential health impact [8]. In the animal models, BCG is used as a 'gold standard' control vaccine, which confers a 1-3 log reduction in CFU counts (variable between models), or a significant degree of improvement in survival or pathology score. A candidate vaccine, whether it is a booster vaccine designed to enhance the effects of BCG, or a new whole mycobacterial vaccine, designed to replace BCG, should confer an improvement in 'protection', i.e an improvement in the relevant readout for the study, compared with this BCG alone control group. With almost all of the candidate vaccines currently being evaluated in clinical trials, this improvement is an additional 0.5–1 log reduction in CFU counts. To date, there has only been one candidate vaccine that has demonstrated sterilising immunity in any animal model; this is a recombinant strain of *Mycobacterium smegmatis* expressing the ESX secretion system from *M. tuberculosis* [9]. This sterilising immunity was demonstrated in the murine model, although not in all organs nor in all of the mice. To date, testing of this vaccine has not been reported in any other species but a reduction of bacterial load to undetectable levels is extremely rare in the mouse model. These data suggest that it is at least possible to develop vaccines that are much more effective at reducing bacterial load than the current generation of vaccine candidates. We do not yet know what the effect of such enhanced protection in preclinical models will be on clinical efficacy, but evidence that sterilising immunity is achievable, suggests that the bar for what is considered 'effective' in animal models should perhaps be raised.

Human efficacy trials are currently powered to demonstrate at least a 60% improvement in efficacy over BCG alone. This is a high bar, which is driven mainly by logistics and the cost of efficacy testing. We now have the results of the first phase IIb trial in infants with the most clinically advanced candidate TB vaccine, MVA85A, which did not demonstrate any significant improvement in protection over BCG alone in BCG-vaccinated infants in South Africa [10]. This trial was powered to show a 60% improvement over BCG alone and vaccine efficacy was not statistically significant at 17.3% (95% CI-31.9–48.2). In this article, we review the preclinical efficacy data for MVA85A in the context of this human efficacy study, and consider the implications for preclinical animal testing. It is essential that as a field, we maximise the value of this and subsequent efficacy trials with other candidate vaccines by considering whether refinements to our vaccine selection criteria are now merited. It is hoped that in this iterative way, we will gradually understand more about what these models can tell us and how best to utilise them.

2. Review of preclinical data with MVA85A

MVA85A is a recombinant strain of Modified Vaccinia virus Ankara expressing the conserved mycobacterial antigen 85A [11] and is designed to boost the effects of BCG. The protective efficacy of a BCG prime – MVA85A boost regime has been evaluated in four animal models: mice, guinea pigs, non-human primates and cattle. In mice, boosting BCG administered intranasally (i.n.) with MVA85A, also administered intranasally, increased the level of protection seen compared to i.n BCG alone [12]. Interestingly, in this study, boosting i.n BCG with i.n BCG also improved protection compared to a single BCG i.n. The level of reduction in bacterial load seen when boosting with MVA85A or BCG was comparable, and was approximately a 2 $\log_{10}$ CFU reduction in the lungs. Boosting BCG with MVA85A administered systemically did improve the level of protection seen in the spleen, but not in the lungs. In a guinea pig high dose challenge experiment, BCG boosted with MVA85A and then a second viral vector, a recombinant fowlpox also expressing antigen 85A, led to a statistically significant improvement in survival compared to BCG alone [13]. A previous study with a fixed end point and a lower challenge dose did not distinguish between BCG and BCG – MVA85A [14] and interestingly, BCG boosted with BCG did not enhance protection over a single BCG immunisation. Two separate studies have been conducted evaluating the protective efficacy of a BCG prime – MVA85A boost regime in non-human primates. The first demonstrated a trend towards improved control in comparison to BCG alone, but none of the parameters reached statistical significance [15]. There was an approximately 0.5 $\log_{10}$ reduction in CFU counts in the lungs in the BCG – MVA85A group compared to the BCG group. The second non-human primate experiment, which utilised for the first time an aerosol route of *M. tuberculosis* challenge, did not demonstrate either a significant effect of BCG or any difference between BCG and BCG – MVA85A in terms of survival, a much more stringent read-out with low statistical power [16]. The only read-out which demonstrated a protective effect of vaccination in this study was a significant reduction in overall pulmonary disease burden, and an equivalent effect was seen in both BCG and BCG-MVA85A vaccinated animals. In cattle, challenged with *Mycobacterium bovis*, there were more disease-free animals in a group vaccinated with BCG and then boosted with MVA85A ($n = 4/10$), than there were in a BCG alone group ($n = 1/10$), or in a naïve group ($n = 0/10$) [17]. Only the BCG – MVA85A v naïve group comparison was statistically significant in this experiment. Overall, the BCG-MVA85A vaccination resulted in statistically significant reduction in pathology in four/eight parameters, an improvement over BCG which reduced pathology significantly in only one parameter.

This preclinical work demonstrated that MVA85A can improve BCG induced protection in these animal models. However, this enhanced protection has not been seen in every challenge experiment conducted. Furthermore the level of improvement seen in most of these experiments was relatively modest, and was usually a reduction in bacterial burden rather than sterilising immunity. Prior to obtaining human efficacy data, it was not clear what this modest level of improvement or efficacy meant in terms of predicting efficacy in humans. It is now clear that this variable and modest level of preclinical efficacy does not predict efficacy in BCG-vaccinated infants, at least at the substantial level required in humans for vaccine development to be progressed. What is still unclear is the magnitude of improvement in animal models which is needed in order to predict the substantial level of improvement in protection required in humans and further clinical efficacy data on vaccine candidates with different pre-clinical packages are needed. Vaccine candidates demonstrating consistent and robust high level efficacy across a range of animal models might be more likely to succeed in human efficacy testing. A key issue is how we can use the information derived from the preclinical and clinical efficacy with MVA85A to review and refine where necessary the models used for the selection of candidate vaccines for subsequent efficacy testing.

3. Review of design of preclinical animal model experiments

Over the past decade, progress in TB vaccine R&D has resulted in large numbers of antigens, adjuvants, antigen delivery systems, etc being proposed as potential candidates for an improved TB vaccine. In the absence of a surrogate marker of protection, all of these candidates have needed to be tested for efficacy in animals. This high demand has driven animal study designs which can provide data over a relatively short time scale and, for ethical reasons, to use the minimum number of animals to achieve a clear result. There are

several fundamental differences between efficacy trials in humans and animal models which are important to consider. The animal models *per se* are different compared with humans, in terms of disease manifestation and the immune response to vaccination or infection, with the responses in humans being considerably more variable than experimental animals [18,19]. Many clinically relevant factors are not taken into account in the laboratory setting, such as the strains of *M. tuberculosis* used in challenge experiments which are typically standardised laboratory strains rather than clinical isolates; and the nature of exposure to infection, in terms of frequency and dose. Furthermore, the definition of 'protection' is different in the animal models and human efficacy trials.

3.1. Species differences

There are many species differences which could influence the ability of animal models to predict outcomes in humans. There is an important role in vaccine development for animal species which more closely resemble humans e.g. macaques because they most closely resemble the human response to vaccination and infection. Ethical and financial constraints prevent these more complex models being used as a routine selection tool to narrow down the multiple choices for vaccine development. It is therefore more useful to reserve macaque experiments for vaccines which have already been shown to be effective in smaller animal models. These experiments allow investigations which bridge between animal and human studies, such as identification or verification of correlates of protective immunity which can then be used in the more tractable animal species to make them more relevant to humans.

In the MVA85A trial, one key difference between the animal models and the humans was the age of the subjects. Whereas all of the animal models were conducted in adult animals, the efficacy trial was conducted in infants. Studies of immunogenicity in neonatal animal models are possible and are being conducted [20]. Immunogenicity studies have the potential to identify fundamental differences between the adult and infant immune responses, particularly if conducted in non-human primates. Without an immune correlate of protection, the immunogenicity data needs to be supported by protection studies, but infection of very young animals with *M. tuberculosis* is difficult from both a technical and ethical perspective. Studies where the *M. tuberculosis* challenge is delayed until the animal is more mature are valuable and have been used, for example to study the duration of BCG-induced protection in cattle [21].

Infants are not the only target population for many of the candidate vaccines in development and data from efficacy trials in adults may yet provide important clues about the most predictive animal species for humans. In recent years, there has been increasing recognition of the importance of adolescents and young adults as a target population, in part because this age group is responsible for most *M. tuberculosis* transmission. The next planned efficacy trial with a candidate TB vaccine will be conducted in adults [22]. It may therefore be premature to place too much emphasis on neonates at the pre-clinical stage.

3.2. Clinical trial settings

There are many variables in a clinical trial setting that are controlled for in the laboratory, including diet, exposure to other pathogens including environmental mycobacteria and helminths, and inherent genetic susceptibility. Although it may be possible to incorporate some of these variables into the animal models, in each clinical trial setting, a different set of parameters will apply and the value of doing this may be questionable. Furthermore, increasing the complexity of the preclinical models may impact on the reproducibility of the results and reduce the value of efficacy testing in animals. However, by understanding the key differences, there is an opportunity to improve the models.

3.3. M. tuberculosis strain and exposure

One clear difference between the animal challenge experiments and a human efficacy trial is the means by which individuals become infected. In the animal models, animals are exposed to a relatively high dose, single exposure to a laboratory-adapted strain of *M. tuberculosis*. There are few data on the impact of novel vaccines against clinically relevant strains of *M. tuberculosis* [23–26]. One important question is whether challenging animals with relevant clinical isolates would improve the predictive value of these models, and more data on comparative efficacy against laboratory and clinical strains are needed. A systematic evaluation of the efficacy of different vaccine types against *M. tuberculosis* strains from endemic regions would be extremely useful. Such information would enable a selection of *M. tuberculosis* strains to be used alongside, or potentially in place of the laboratory strains.

Once a more appropriate challenge strain(s) has been selected, consideration should also be given to the means by which animals are infected. A variety of challenge routes are used across the animal models, with inoculation via the respiratory mucosa being widely regarded as the most relevant for human vaccine development. A fundamental difference between field efficacy trials and laboratory animal exposure is the number of exposures. It is highly likely that a human infection develops after multiple exposures, probably containing only a few organisms [27]. Infection may be established after one or several exposures and may not occur even after multiple exposures. In contrast, inoculation of experimental animals is a single high dose exposure (via aerosol, nasal or tracheabronchial delivery) because the study design requires all animals to be infected, in order to keep the number of animals per-group to a minimum. This results in an unnatural 'challenge' to the vaccine-induced immune response and it is likely that the initial host–pathogen interactions are quite different in humans and experimental animals. The natural exposure system developed by Nardell and colleagues addresses several of these issues [28]. In this model, guinea pigs breathe the extracted air from a ward of TB patients and thereby receive multiple, low-dose aerosol exposures to clinical strains. Vaccine efficacy studies in these naturally exposed guinea pigs are planned and will allow a comparative assessment of the response of a vaccinated animal to natural vs experimental infection and this information can then be used to improve the experimental infection systems.

3.4. Magnitude of vaccine efficacy

There is a further discrepancy in terms of the magnitude of vaccine efficacy which clinical and preclinical studies are powered to detect. The MVA85A efficacy trial was powered to detect a 60% improvement over BCG alone [10]. In animal models, the magnitude of improvement upon BCG by candidate vaccines evaluated to date is generally modest and such small increases in efficacy would not be detected in a clinical trial. If we consider a 60% improvement in clinical efficacy a minimum level for progressing a vaccine through to licensure, we should expect the endpoints in the preclinical animal studies to be capable of detecting a similar magnitude of improvement and to be suitably powered.

3.5. Definition of protection

It is important to recognise the different definitions of protection in humans and animal models. In animal models, efficacy is

defined as a statistically significant improvement in disease compared to control groups as measured by disease-related parameters e.g. bacterial load in lungs and or spleen, severity of pathology, time to death. Often in these studies, a vaccine is regarded as providing protection even if there are measurable bacteria/pathology in the organs, and even if some animals do not survive. The equivalent in a human efficacy trial would be a reduction in severe disease but, for ethical reasons there is never an opportunity for severe disease to develop because of active follow-up. Therefore, in humans, efficacy is defined as the prevention of TB disease using clinical endpoints and any individual who meets this disease endpoint definition is not protected. As mentioned above, some vaccination protocols (including MVA85A [17],) are capable of inducing a sterilising or disease-free effect in animals but this level of protection is rare, and is not considered essential for vaccine selection. Thus, the majority of the pre-clinical evidence for protection is based upon a relative reduction in organ CFU or pathology.

There has been much debate over the merits of measuring efficacy at early or late time points post-challenge because some vaccine candidates have a more pronounced effect at the later stages [24,29]. However, whether it is soon after infection, or in the later stages, the effect being demonstrated is usually a partial clearance of bacteria or reduced inflammation/tissue damage. Even though this indicates the potential of the vaccine to induce an effective anti-mycobacterial immune response, we do not understand the meaning of e.g. a 0.5–1.0 log reduction in bacterial load in animals in terms of human disease prevention. Survival studies, where vaccines are compared in terms of preventing animals reaching severe disease end-points, have the potential to measure disease prevention. However, in all reported survival studies to date, the effect of the candidate vaccine has not been prevention of disease but merely a slowing of disease progression such that survival of vaccinated animals is prolonged compared to controls. Until there are sufficient human efficacy data with which to correlate a particular parameter in animal models, it might be more useful to design the animal efficacy studies with a greater emphasis on the efficacy end point of the clinical trial, which is likely to be absence of disease, not simply a reduction in disease burden.

3.6. *M. tuberculosis* infection as an endpoint

Now is the time to consider a fundamental change from *reduction* of disease to *prevention* of disease as the end-point. A potential step even further is to consider establishing animal models to demonstrate protection against *M. tuberculosis* infection, rather than TB disease. This is of relevance to human vaccine testing as prevention of *M. tuberculosis* infection has been suggested as a more feasible endpoint in a human efficacy trial, allowing smaller and shorter efficacy trials. However such a preclinical model would be very difficult to develop as the currently available tools to define infection in humans are not directly transferable to most of the animal models.

Study designs which measure prevention of disease would require large group sizes because presence or absence of infection is a binary endpoint. A time to event analysis would increase the power but the group sizes would still be significantly larger than those currently used in animal challenge experiments. A clear and reliable biomarker which reflects exposure is also needed. Current immune markers measure the induction of an adaptive immune response but do not predict outcome. Biomarkers of an innate immune response to infection would be extremely useful and much progress has been made in recent years with several potential biomarkers being identified [30–32]. Validation of these in the animal species is feasible and quantitative, and thus robust and reproducible assays could be developed. The assay would ideally be performed repeatedly on the same animal to permit time-course analysis but, because the site of infection is the lung, these assays might be more accurate and reliable when performed on lung tissue, at a single time point. Clinical chemistry markers, lung imaging and metabolic markers might also help because they would improve the accuracy with which to define disease or not. Non-human primates (NHPs) are the most suited animal models to perform such analyses because of a direct translation to the clinic but it will never be feasible to perform clinical trial scale studies in NHP. However NHP are invaluable for the validation of biomarkers and to establish assays which bridge pre-clinical and clinical studies, enabling development of improved small animal models. BCG remains the benchmark against which the new endpoints would need to be set.

These novel pre-clinical study designs will involve larger numbers of animals and perhaps a tiered approach to efficacy testing is required, with this level of efficacy screening reserved only for the most promising vaccine candidates. Immunological evaluation will become an increasingly important criterion in vaccine selection, and the need for a correlate of protection becomes even more pressing. Attempts to associate various measures of cell-mediated immunity with risk of disease have to date failed to reveal a clear correlate [33]. In other diseases, functional immune responses such as virus neutralisation or serum bactericidal assays serve as reliable correlates of protection and recent reports of a more functional mycobacterial growth inhibition assay suggests that such assays may be a more useful *in-vitro* tool than more conventional measures of cellular immunity [34]. We will only be able to identify vaccine-induced correlates of protection once we have an effective vaccine. However in the meantime, work on identifying correlates of risk will generate potential correlates for validation in efficacy trials.

The above discussion only addresses efficacy screening in the context of primary infection. Post-exposure vaccines are being developed and these have been evaluated in animal models for their ability to prevent disease reactivation [29]. Whether any of these complex models predict efficacy in humans remains to be proven. The factors and variables which trigger reactivation of latent *M. tuberculosis* infection in humans are not possible to reproduce in an animal model, but mouse models involving relapse following incomplete chemotherapy have proven useful as a screening tool to prioritise candidates. The clinical distinction between latent infection and disease does not exist in animals, other than NHPs, but again the value of NHPs is the power to perform highly relevant in-depth analyses of advanced vaccine candidates with the information used to improve first-line screening models. A clear and accepted (re-)definition of protection in the context of primary infection will certainly help to improve animal models of latency.

4. Conclusions

Eleven years ago, there were no new TB vaccine candidates being tested in clinical trials. Animal studies have enabled prioritisation of candidate TB vaccines in the absence of a correlate of protection and substantial progress has been made on the strength of animal data. However, there was no benchmark for improved protection over BCG in humans, with which to validate the animal models. Recent human efficacy data on MVA85A demonstrate that the modest ‘protection’ observed in preclinical animal models did not predict the substantial efficacy required in humans. The increasing number of vaccines reaching clinical testing is to be welcomed, as it is only by moving different candidate vaccines into clinical testing that we will achieve the goal of developing an

Table 1
Recommendations for improving preclinical evaluation.

- Testing with clinical *Mtb* isolates
- Powering preclinical studies for the same level of efficacy as desired in human clinical trials
- Preclinical studies conducted in same age group, neonates or adults, as target for human vaccine trials
- Robust models of low dose, repeated exposure are developed
- Markers of infection are developed with which infection models can be established
- Better models for BCG boosting vaccines and latency

effective TB vaccine strategy. To date, no candidate vaccine currently in the clinical pipeline has a pre-clinical data package that is markedly different from MVA85A. It is therefore important to consider the implications of the MVA85A efficacy trial for these other candidates and more broadly for preclinical models of TB vaccine development. A comparison of the animal and human data for MVA85A suggests that the animal studies will be more predictive if the study designs are optimised to more closely reflect the targeted effect of the vaccine in the clinical trial. Parameters to be considered include the subjects (species, age), the strains and dose of *M. tuberculosis* and most importantly, the end-point which will be used to define efficacy, which then allows the study to be powered appropriately. Subsequent trials with other candidates in other target populations will provide valuable new information with which we can further refine the animal models. We can only 'validate' these preclinical models once we have a new vaccine which is effective in humans. Until then we should continue to iteratively refine the models, as clinical efficacy data becomes available, the predictive value of which will then be evaluated again in human efficacy trials.

Standardised, head-to-head screening of vaccine candidates in independent laboratories has evolved as an important feature of the global TB vaccine effort, and this must be maintained [14,15]. Furthermore, key preclinical challenge experiments should be repeated to ensure reproducibility, before committing to large, expensive efficacy trials. Animal data are pivotal in the understanding of vaccine induced immune responses and identification of correlates of protection. As refined algorithms for vaccine identification and selection emerge, it is imperative that these are shared and incorporated in a consistent manner.

Table 1 summarises some of the most important elements of an improved strategy for vaccine selection. The current TB vaccine portfolio contains more vaccines than it is possible to test in human efficacy trials and financial and ethical drivers will dictate the number of vaccines we can progress. It is imperative that as a field, we agree upon objective criteria based on accurate, predictive preclinical models so that we have the confidence to select candidate vaccines for efficacy testing and only advance the vaccines which are most likely to succeed.

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