LEADING ARTICLE

Vaccine Development for Tuberculosis: Current Progress

Ian M. Orme

Abstract Very substantial efforts have been made over the past decade or more to develop vaccines against tuberculosis. Historically, this began with a view to replace the current vaccine, Bacillus Calmette Guérin (BCG), but more recently most candidates are either new forms of this bacillus, or are designed to boost immunity in children given BCG as infants. Good progress is being made, but very few have, as yet, progressed into clinical trials. The leading candidate has advanced to phase IIb efficacy testing, with disappointing results. This article discusses the various types of vaccines, including those designed to be used in a prophylactic setting, either alone or BCG-boosting, true therapeutic (post-exposure) vaccines, and therapeutic vaccines designed to augment chemotherapy. While there is no doubt that progress is still being made, we have a growing awareness of the limitations of our animal model screening processes, further amplified by the fact that we still do not have a clear picture of the immunological responses involved, and the precise type of long-lived immunity that effective new vaccines will need to induce.

1 Introduction

About two decades ago, funding began to become available to stimulate design and development of new vaccine candidates that potentially could boost or completely replace the Bacillus Calmette Guérin (BCG) vaccine. By that time of course, BCG was widely used around the world (about

85 % coverage), but it was well known that its efficacy had tremendous variability from trial to trial, and while it was generally thought that the vaccine did some beneficial things in children, specifically the prevention of disseminated and meningeal tuberculosis, overall its ability to protect adults from pulmonary tuberculosis (the source, obviously, of disease transmission) was poor [1].

As a result, at the time [2–6] a rather impressive collection of, in some cases very innovative, vaccine candidates began to appear. However, most inventors lacked the facilities to test these, resulting in the establishment of the Vaccine Screening Contract at Colorado State University, USA, and soon after by the establishment of a similar facility at the Health Protection Agency in the UK. As one can imagine, most candidates did not work, or least reach the levels of immunity and protection given by BCG in such screening models, but these programs were nevertheless successful because they gave new insights into what could potentially work and what would not.

The spectrum of candidates meeting the initial criteria was very broad, and included multiple types of subunit vaccines, DNA vaccines, live attenuated mutants, vaccines based on viral vehicle delivery, and new forms of recombinant BCG itself. Recently, there has been some inevitable attrition, but as we stand today, there are four subunit-based candidates and two virus-based candidates that have reached the top of the 'pipeline' and are being tested in the clinic.

2 The Aeras Foundation 'Pipeline'

The Aeras Foundation is a not-for-profit organization based in Rockville (MD, USA) dedicated to the development and testing of new tuberculosis (TB) vaccine candidates, and

I. M. Orme (✉)
Department of Microbiology, Immunology and Pathology,
Colorado State University, Fort Collins, CO 80523, USA
e-mail: ian.orme@colostate.edu

the instigation of clinical trials. There are currently six vaccines at the top of the candidate pipeline (see Table 1).

MVA85A is a recombinant strain of the Ankara vaccinia strain developed originally as a smallpox vaccine about 30 years ago, which expresses Ag85A. It does not replicate in humans and has been shown to be very safe. Of all the candidates in the pipeline, it has undergone by far the most extensive testing in multiple animal models, giving consistently positive results. Since about 2002, this vaccine has been tested in at least a dozen early-phase clinical trials, which is a testimony to the care the developers (at the University of Oxford, led by McShane) have taken in its assessment. In a double-blind, phase IIb efficacy trial in Cape Town, South Africa, for which the results have just been announced [7], efficacy in infants could not be demonstrated. This is discussed further below.

Aeras-402 is a replication-deficient adenovirus expressing Ag85 and TB10.4, now undergoing extensive clinical trial testing. The virus is of the serotype-35 variety, reflecting the limitation that the most common adenovirus, serotype-5, cannot be used because of the high level of pre-existing seropositivity in many people [8]. There are data that vaccination with this candidate seems to preferentially drive the sensitization of CD8 T cells, which could be of value in HIV-positive individuals [9].

M72 is a fusion protein first developed a decade ago at Corixa in Seattle, USA, based on recognition by T-cell lines from purified protein derivative (PPD)-positive healthy donors. Mice vaccinated with M72 in AS01B adjuvant were then shown [10] to be highly protected against aerosol challenge, and further studies in guinea pigs were also protective. It was then found [11] that M72 given in AS02A adjuvant in conjunction with BCG significantly prolonged the survival of these animals over BCG alone. In these animals, lung lesions could still be seen, but there was considerable evidence of lesion healing and airway remodeling and reestablishment.

Recently, safety and immunogenicity studies of M72 in AS02 adjuvant were performed in BCG-vaccinated adults, and adults previously given chemotherapy for TB [12]. CD4 responses were seen in both groups, and while some adverse reactions to the vaccine were observed, none were considered serious.

The M72 candidate has now reached phase IIa trials in adults who were BCG-vaccinated, HIV-negative, and were either infected or uninfected with *Mycobacterium tuberculosis* [13]. These individuals were given two intramuscular doses of M72 in the liposomal adjuvant AS01 and then monitored over the next 7 months. The vaccine was safe, with only minor adverse reactions (the most prominent being brief flu-like symptoms). The vaccine gave robust long-lived T-cell responses, which, if broken down by secretion of interleukin (IL)-2, tumour necrosis factor (TNF), interferon (IFN)-γ, and IL-17, was produced by as many as eight different subsets of CD4 cells. CD8 T-cell responses were also seen, but to a lesser degree, and only increased to any extent in the infected individuals, suggesting they were boosting pre-existing CD8 responses. This is a little curious, since M72 is known to have a CD8 epitope.

H56 is a fusion protein consisting of Ag85B, ESAT-6, and Rv2660. The latter antigen is expressed throughout the immune response (both early and during the chronic stages) and for this reason is identified as a 'latency antigen'. The author finds this difficult to explain to the reader, but it seems to depend on the fact that this antigen is still expressed in a model of 'incomplete chemotherapy' (the underlying concept equally unexplainable), where bacilli surviving are latent. This is 100 % wrong; these bacilli are in fact adapting and quite the reverse, as discussed elsewhere [3].

This is an issue for another time. The reality is that a vaccine that could target bacteria that persist after apparently successful chemotherapy and thus reduce the risk of reactivation of disease could be highly useful. The data regarding the ability of H56 to achieve this are based in two sets of studies, in mice [14] and in macaques [15]. In a first experiment, H56 protected as well as BCG, but Rv2660 by itself had no protective effect, indicating that the protection observed was all due to Ag85 and ESAT. In this experiment, an assay at 4 weeks after challenge with H37Rv showed that both H56 and BCG gave about 1-log

Candidate	Description
MVA85A	Ag85A expressed by modified Vaccinia Ankara virus
Aeras-402 (Crucell adenovirus-35)	Adenovirus expressing Ag85B and TB10.4
M72F	Fusion of Rv1196 and Rv0125 delivered in AS01 adjuvant
H56	Fusion of Ag85B, ESAT-6, and Rv2660 delivered in IC31 adjuvant
ID93	Fusion of Rv1813, Rv2608, Rv3619, and Rv3620 delivered in GLA-SE adjuvant
Hybrid-4 [H4]	Fusion of Ag85B and TB10.4 delivered in IC31 adjuvant

Table 1 The Aeras Foundation candidate pipeline

At the current time, all these candidates are primarily designed to boost neonatal Bacillus Calmette Guérin (BCG) vaccination. In addition, ID93 and H56 have been tested in therapeutic (post-exposure) vaccination models

protection, as expected, but, at 12 and 24 weeks BCG protection had been essentially lost, whereas colony-forming units (CFUs) in animals given H56 were still slightly lower and significant. However, this study contained no Ag85/ESAT-6 fusion control, so the contribution of Rv2660 could not be determined. This was then addressed in a further experiment that then included H1 (Ag85 and ESAT only). In this study, the effects of BCG were more pronounced and sustained over 24 weeks. Over the first 6 weeks, BCG, H1, and H56 were equally protective, whereas this protection was lost in the case of H1 but retained by H56 and BCG. This suggests that Rv2660 does contribute to retention of resistance in this model. Prime boosting studies suggested similarly, with no essential improvement over BCG by the two fusions measured after 4 weeks, but with a ~ 0.7-log improvement using H56 when measured at 24 weeks.

Unfortunately, attempts to reproduce these findings in the definitive macaque model were less compelling [15]. In survival studies, in a high-dose study, BCG did provide some protection, but boosting with H56 did not seem to improve this, whereas, in a low-dose study, the BCG control was not protective, invalidating the experiment.

ID93 is a fusion of Rv2608 (a member of the PE/PPE family), Rv3619, Rv3620 (EsX family), and Rv1813 (a latency associated protein), which follows the philosophy of the Infectious Disease Research Institute (IDRI) group to base vaccines on recognition by human T-cell lines of specific antigens. The potency of this vaccine was further augmented by parallel studies that have led to the identification of a potent Toll-like receptor (TLR)-4 stimulating adjuvant that is a synthetic hexa-acylated molecule based on the structure of lipid-A, which was initially developed at the Ribi Company. This adjuvant, GLA-SE is a glucopyranosyl lipid stable emulsion, and, in this vehicle, ID93 has been extensively tested in animal models [16, 17]. In addition to prophylactic use, ID93 has also been tested as a post-exposure vaccine in augmenting chemotherapy (see below).

Hybrid-4 (H4) is a fusion of Ag85B and TB10.4, similar therefore to Aeras-402, but in this case delivered by the IC31 adjuvant. In this form [18], the vaccine is protective and generates multifunctional T cells.

3 The MVA85A Phase IIb Vaccine Trial

MVA85A is a recombinant strain of the modified Vaccinia Ankara virus engineered to express the immunodominant antigen Ag85A, a protein made in large quantities during cell wall elongation and fission as the bacterium divides. The vaccine has been tested in a large number of careful and extensive animal studies, described elsewhere [19–23].

This work led to a recent study in rhesus macaques [24], which tested the ability of MVA85A to vaccinate rhesus macaques when delivered by aerosol, thus targeting mucosal immunity in the upper respiratory tract. This approach generated strong antigen-specific responses in the lungs and was well tolerated. Moreover, if given by this route, antibodies to the viral vehicle were not generated to any extent, which is of practical usefulness.

Safety is of course paramount, and in a randomized phase I trial [25] MVA85 administration was tested by the intramuscular and intradermal routes in 24 healthy human volunteers. There were no serious adverse reactions, and both routes generated strong and sustained Ag85-specific CD4 T-cell responses.

All this was the prelude to the first phase IIb trial of MVA85A, indeed the first infant efficacy trial of any tuberculosis vaccine for nearly a half century [7]. Between 2009 and 2011, over 2,700 infants (given BCG as neonates) were enrolled, with 1,399 infants given a boosting inoculation with MVA85A within 6 months of being given BCG. At the primary efficacy endpoint, 39 infants out of 1,395 developed tuberculosis, whereas 32 out of 1,399 infants given the MVA85A boost developed the disease. While this indicated a decrease, it was unfortunately well within the statistical confidence limits of the study.

At one level, this study could be regarded as a failure, on paper it was, but this is not the case. It is historic, and demonstrates that, with good planning (the Oxford Emergent Tuberculosis Consortium), a solid source of funding (Aeras Foundation and the Wellcome Trust), and an outstanding expert local team (Universities of Cape Town and Stellenbosch), a trial of this magnitude can be conducted. Above all, it was completely safe (if it had not been, this could have been a disaster for the field in general).

Given the excellent data to that point, why did MVA85A fail? One possibility is that the BCG vaccine, at least in the trial area, protected neonates well by itself, so adding something extra with a boosting vaccine was a statistical impossibility. For obvious reasons, there was no 'no-BCG' arm of the trial, but if this was indeed the case it would stack the deck against MVA85A improving matters.

In my opinion, this reflects the fact that we know nothing about the biology of the predominant clinical isolates in the Western Cape trial area. We tried to address this recently, and we collected a panel of representative strains from this area. They grew well in guinea pigs and caused lung damage as expected, but they seemed to be a little less virulent than a similar panel of isolates collected in the Bay Area of San Francisco [26]. Our experience to date with these clinical strains, the majority of which are high virulence Beijing strains, is that they are rather impervious to BCG vaccination in animal models, something we have attributed [27, 28] to the ability of these strains to induce

regulatory T cells that counteract the protective immunity induced by the BCG vaccine.

We chose two Cape Town strains at random and infected guinea pigs that had been BCG vaccinated, expecting protection to be poor and transient, based on our studies to date [27]. Instead, we measured some of the best protection we have ever seen (Shanley et al, unpublished data). Not only was this totally unexpected, but if it represents a general property of this panel of Cape Town isolates, then it predicts that BCG efficacy against these will be very high, and it would be statistically impossible to demonstrate boosting. It is quite possible the same thing happened in the MVA85A trial.

4 Other Leading Vaccine Candidates

Several other vaccines are currently well into development (Table 2). This includes VPM1002, which is a recombinant BCG (rBCG) Prague urease-deleted vaccine that expresses the listeriolysin molecule, being developed independently of Aeras. The concept behind this candidate is highly innovative, based on the idea that since BCG is a relatively poor inducer of CD8 T cells, if a recombinant could be made expressing a lysin that allows escape of antigens from the endosomal pathway into the cytoplasm, this could promote Class-I presentation, and in addition cause cell apoptosis and cross presentation. In a first study [29], mice were vaccinated by the (rather unusual) intra-peritoneal route with BCG or the rBCG, then challenged by aerosol 120 days later with a moderately high dose of H37Rv. In this study, there were no differences between the two vaccines from day 15 through day 120, but at day 150 the colony-forming units (CFUs) in the lungs had dropped from ∼3.5-log in the BCG controls to ∼2.25-log in mice given rBCG. In two further studies, the BCG vaccines were given intravenously (an assumption on my part, the figure legends never state) and then 120 days later challenged with either 30 or 200 bacilli. In the low-dose study, the CFUs in the lungs were lower in the rBCG group at day 90

but not day 120. A similar result was seen on day 90 in the high-dose study. In a further study, a Beijing strain was used instead, but it should be noted that this infection did not grow any better than H37Rv despite the rather high aerosol dose, and thus was obviously not of any higher virulence. Here, the rBCG was highly protective straight away, whereas the BCG controls showed early evidence of control but this was then lost (our experience has been similar [27]).

Recently [30], the results of a phase I safety and immunogenicity trial of VPM1002 were described. Healthy volunteers were given the vaccine intradermally, and adverse injection site reactions were very minor. Immunogenicity was determined by T-cell secretion of IFNγ, and cells from vaccinated individuals produced this cytokine, as well as TNF and IL-2. However, one might note that the frequency of responsive T cells, despite stimulation with PPD, was no more than about 0.2 %, which seems to be low.

SO2 is a mutant of *M. tuberculosis* in which the *phoP* gene has been disrupted, an event [31] that has been shown to have a profound effect on *M. tuberculosis* gene expression and virulence. This mutant cannot grow well in murine macrophages or infected mice, but can vaccinate mice against virulent challenge. In addition, protection can be demonstrated in both guinea pig and macaque models [32]. These studies suggested that the superior activity of the vaccine might be due to its ability to induce central memory T cells, much in keeping with our own ideas on this subject [33].

The idea that attenuated live mutants of *M. tuberculosis* could be used in vaccines is of course not new, much of it pioneered by Jacobs and colleagues, and its attraction lies in the possibility that to combat the newly emerging high-virulence clinical isolates, one must vaccinate with candidates that are by themselves of high potency. To date, nobody seems to want to try this, but one could argue that these types of vaccines are probably safe enough to at least give to adults, and this could be done after any protective effects of neonatal BCG vaccination had been lost (which

Table 2 Other leading candidates

Candidate	Description
VPM1002	Recombinant BCG expressing the listeriolysin molecule
AdAg85A	Adenovirus expressing Ag85A
ΔleuCD, ΔpanCD, ΔsecA2 (etc)	Multiple auxotrophs/mutants with vaccine potential
ΔIKEPLUS	*Mycobacterium smegmatis* mutant expressing *Mycobacterium Tuberculosis esx-3* genes, possible central memory T-cell inducer
SO2	Mutant vaccine based on phoP deletion in *M. tuberculosis*

BCG Bacillus Calmette Guérin

we know happens). It is firmly established by animal studies that several mutants, including ΔleuCD, ΔpanCD, ΔsecA2 as examples [34–40], are potent vaccines.

An even more intriguing example is a mutant of *Mycobacterium smegmatis* in which the *esx*-3 genes have been replaced by the orthologous genes from *M. tuberculosis*. This candidate [41], Δikeplus, not only protected mice against a very high intravenous inoculum of *M. tuberculosis*, but dropped the bacterial load in the lungs quite precipitously. How it does this is still unclear, but it may reflect the fact that the vaccine can be safely given in high doses and is rapidly destroyed by the host, resulting in strong central memory T-cell generation.

5 Therapeutic Vaccines

Therapeutic vaccines come in three varieties (Table 3). The first are those that could be given soon after exposure, either naturally or deliberately (biodefense situation), to try to halt the infection. The second type would be those that could be given to facilitate or augment chemotherapy, resulting in quicker clearance of the infection. A third type would be those that could be given after chemotherapy had ceased, or to patients diagnosed with latent TB, to prevent the reappearance of the infection and reactivation of disease.

Very early studies [42] showed that post-exposure vaccination was not effective once the infection had become contained (chronic), and in the guinea pig model this had to be performed before the (essentially irreversible) necrosis had developed. In the latter model, one new candidate and potent TLR2 agonist, F36 (Rv1411 plus ESAT-6), had a mild protective effect but this did not translate into prolonged survival in this study [42].

At this point, two candidates have shown some reasonable protection in augmenting chemotherapy when given in tandem, or afterwards. These are RUTI, and the pipeline vaccine ID93.

RUTI was developed by Cardona, who has long been a proponent of using vaccination to augment chemotherapy [43]. RUTI consists of bacterial fragments of *M. tuberculosis* that are detoxified and delivered in a liposome formulation. Although Cardona has a more conventional viewpoint [43] regarding latency and pathogenesis that differs significantly from our own [3], it is very possible that RUTI can prime or expand immunity to antigens that preferentially become expressed as the bacilli become stressed (which is how RUTI is made from bacilli in vitro). Many of these are within the DosR regulon, which is usually interpreted as a primary mechanism preceding bacterial latency, although we have recently suggested that what this actually represents is a rapid adaptation by the bacillus, not only to adapt to an extracellular environment but also potential pending escape from the necrotizing lung tissues and transmission (Orme, unpublished data). RUTI given straight after chemotherapy can not only reduce the numbers of residual bacteria (based on CFU determinations—although there are caveats to these measurements [3]) but also enhances the CD4 response and significantly expands the CD8 response. Furthermore, in one of the only demonstrations of its kind, even serum from RUTI-treated mice seemed to protect.

More recently, Gil et al. [44] studied RUTI in mice infected via the intra-peritoneal route with H37Rv (a rather unusual route). Three weeks later, mice were treated with isoniazid and rifapentine until week 9, then vaccinated at that point and then again 2 weeks later with RUTI given subcutaneously. Protection was based on spleen CFUs. There was a slow increase in CFU in controls, and 6 weeks of drug therapy reduced this bacterial load by 2-log. Once drug therapy was halted, the bacterial load re-grew about 1.5-log, but this was essentially prevented in mice given RUTI.

This was followed by a phase I trial [45] of RUTI in 24 healthy participants. Mild adverse reactions to vaccination were observed, but none serious. After vaccination, ELISPOT analysis showed immunity to several major *M. tuberculosis* antigens and to PPD, although some individuals did not give responses above a placebo background.

ID93/GLE-SE, described above, has also been tested as a therapeutic vaccine in augmenting the effects of

Table 3 Therapeutic vaccines

Candidate	Description
RUTI	Liposome encapsulated detoxified mycobacterial fragments
H56	See above; reduce growth of latent bacteria after chemotherapy
Mycobacterium indicus pranii	Saprophytic mycobacterium; augment chemotherapy
Mycobacterium vaccae	Dead mycobacterium with apparent immunomodulator activity
ID93	See above; augment chemotherapy
F36	Fusion of Rv1411 and ESAT-6; early-use vaccine
CSU/ID57	'Three-iron' fusion of Rv1909, Rv2711, and Rv2359; early-use vaccine delivered in GLA-SE adjuvant

chemotherapy [46]. These studies used the SWR/J mouse, which my laboratory showed several years ago [47] are exquisitely sensitive to tuberculosis infection, for reasons that are still unclear. Moreover, if infected and given rifampacin and isoniazid chemotherapy, the SWR strain lives much longer than controls, but the infection grows back and the mice die about 200 days after initial infection. The ID93 vaccine, when given either in conjunction with or after drug therapy, significantly increased the survival of these animals, with only about 50 % mortality in these mice even 400 days after infection. Equally importantly, ID93 vaccination improved survival even if the duration of chemotherapy was reduced to just 60 days.

Less impressive results were seen in studies in cynomolgus macaques, but the vaccine was perfectly safe and there was a trend towards a therapeutic effect. Animals given both vaccine and drug therapy appeared more healthy (by chest x-ray) and, in two out of five animals, no CFUs could be detected in the lungs (in the other three, there were similar numbers to monkeys just receiving chemotherapy).

While on this topic, we should also briefly review another immunotherapeutic vaccine candidate. *Mycobacterium w*, now renamed *M. indicus pranii* (Mip), is a saprophytic mycobacterium that was first described in the 1970s as having a (presumably non-specific) effect in people with multi-bacilliary leprosy, and several studies on this potential application were performed in the early 1990s. Recently, several studies have appeared addressing its use against tuberculosis.

A 2011 study [48] looked at combined chemotherapy and Mip immunotherapy in mice against both H37Rv, an isoniazid (INH)-resistant strain, and two multidrug resistant (MDR) strains. While barely protective by itself, Mip augmented chemotherapy when the effects were measured after 4 weeks, but this effect was lost at the 6-week point. Immunotherapy seemed to marginally improve treatment of an INH-resistant strain, but not against the MDR strains. This implies that Mip could potentially augment chemotherapy but would not be effective where the efficacy of chemotherapy was itself diminished.

Gupta et al. [49] investigated whether Mip could augment chemotherapy, when given subcutaneously or by aerosol. When started on day 30 of the infection, drug treatment alone dropped the bacterial load in the lungs by ∼3.5-log, and co-treatment with killed Mip added about 0.7–1.0-log depending on the length of treatment. As would be anticipated, organ gross pathology scores were significantly reduced, although most of this could probably be attributed to the chemotherapy. Immune parameters were improved by Mip immunotherapy, including an expansion of CT-4+ cells, thus confirming work by Ordway et al. [50], who first suggested the use of this marker in

this type of study. This was interpreted by Gupta et al. as indicating T-cell activation, which may be the case, although it also might herald disease reactivation [50].

A further study [51] looked at expression of CXCL10 (IP-10, a potential diagnostic marker) and CXCL11 (a ligand for CXCR3, important in T-cell migration) in guinea pigs infected with H37Rv. Message for these markers fluctuated in animals given Mip, chemotherapy, or both sets of treatments. The authors concluded that Mip substantially enhanced the effects of chemotherapy, but this may be an element of the study design. First, the challenge dose was huge (250–300 bacilli) but, despite this, the infectious load in the lungs barely reached log-6, and then declined slightly. Chemotherapy was given just 2 days after infection, a model that does not reflect reality. As a result, this by itself was very effective (a better model would be to set it up so chemotherapy took more time to work so that enhancement effects would be more obvious), with the infection growing 2-log over the first 4 weeks, with total clearance by 6 weeks. Mip, given by itself, had only very minor effects on the growth of the infection, but, in animals given both treatments, no growth of the infection was observed at the 4-week time point. This was followed by a similar study in guinea pigs looking at the immunogenicity of Mip [52]. Animals were vaccinated with live or heat killed preparations of Mip or BCG. After exposure by aerosol to ∼50 bacilli 3 weeks after vaccination, the numbers of bacilli recovered from the lungs of control animals 4 weeks later was below log-4 (in most other models, ∼20 H37Rv grow to between 5 and 6-log in 30 days). BCG was highly protective, and Mip even more so. Histological data showed the lungs of the BCG-vaccinated animals to be severely consolidated, despite the extremely low CFU levels recorded (2-log in the lungs), a very strange result. Similar protection was seen if the challenge was delayed for 7 months.

Finally, a candidate that has had a rather contentious past (its abilities early on were perhaps over-stated) is heat-killed *M. vaccae*. Recently, this was tested in HIV-positive individuals in Africa with very low CD4 counts, and it was found that multiple doses reduced the incidence of pulmonary disease based on the secondary endpoint, although not against the primary [53].

6 Current Limitations to Vaccine Testing and Design

New candidates undergo a series of studies in various animal models to determine the type of immunity they induce (cell-mediated immunity rather than antibody-mediated) and then whether vaccination enables the animal to express specific acquired resistance to *M. tuberculosis*, almost always to one of the two laboratory-adapted strains

(H37Rv, Erdman), and now at least, usually after low-dose aerosol challenge.

A variety of models are used, but studies invariably start with the mouse, given the relatively low cost and the huge number of immunological reagents at our disposal [54]. This animal develops a strong TH1 response if the vaccine is of any value, slows the course of the infection down (usually by about 1-log), and develops lymphocytic granulomas. (The author must express his exasperation that, even in 2013, reviews still make the totally false statement that mice do not develop granulomas, despite their description in great detail [55, 56]). The same can be said for the mythology that 'mice are resistant, guinea pigs are susceptible', which is continuously repeated. There are mouse strains that are in fact extremely susceptible (SWR, C3HeB/FeJ, etc.) [47, 57–59], and the idea that guinea pigs are more susceptible only applies to H37Rv infection and falls down once clinical strains are used. We now have plenty of examples where guinea pigs (which are actually quite tough little animals) can resist certain Beijing strains longer than 'resistant' C57BL/6 mice.

The guinea pig is considered the gold standard for vaccine testing, mainly due to the classical studies of Smith and then his wonderful protégé McMurray, plus the recognition that the pathology of the disease process in this animal has multiple similarities to that seen in humans [60, 61]. An earlier limitation reflecting the lack of reagents is gradually being solved, and the field can now get decent information, including flow cytometry and rRT-PCR data [62]. Another advantage is that the animal develops lesion necrosis and calcification, and we now hypothesize [3] that bacilli persisting in necrosis do so by forming biofilm-like clusters or communities (necrosis-associated extracellular clusters [NECs]), which we can now detect using advanced staining methods [63, 64], findings that directly challenge the current concept [65] that intact macrophages harbor latent bacteria. Based on this, this author has suggested that latency does not actually happen at all [3, 66].

A final testing stage involves non-human primates, usually macaques. While very expensive, this model has the advantage that, when infected, it can exhibit both active and latent/persistent forms of disease [67]. However, very recently, different results in terms of the ability to protect macaques by BCG vaccination have been observed [68] and this needs further evaluation.

Animal models have certain limitations, and even those of us who have devoted our careers to developing these models are fully willing to concede these [69]. In our conventional study designs, the vaccine-to-challenge interval is probably far too short and the challenge is given at the time where effector immunity to the positive control BCG vaccine is peaking. Hence, not only are we measuring effector T cells and not *memory* immunity, but if a candidate induces immunity with different kinetics, this property would be missed. Another problem is that even mouse experiments cost money, so it is conventional to use '$n = 5$' when, in reality, this means our studies are statistically under-powered (this becomes even more difficult when larger animals are used [69]).

What do we want our vaccine to actually do? Ideally, a vaccine should be given to infants before there is much risk of exposure, but the immunity engendered should either be long lived by itself or amenable to boosting so that disease is prevented 10–15 years later when TB disease currently peaks, in early adulthood. Longevity is indeed the key; we want to vaccinate young children but the primary cause of transmission is years later, involving adult pulmonary disease. In addition, an ideal vaccine would be safe in people harboring HIV, or (if a vaccine is given in adulthood) safe in people already carrying the TB bacillus [70].

As noted above, when the field first started to grow, very few places had the level-III facilities to test new candidates, and so vaccine screening programs were established at Colorado State University (by the author) and at the Health Protection Agency (by Williams and colleagues). However, within a decade or so, those with a vested interest had developed their own testing facilities (purchased a Glas-Col, or Henderson device, or Madison chamber) and so efficacy testing could be carried out in-house. This created a competitive edge that did not initially exist, and so, at a time when comparison of data and multiple laboratory validation of potential candidates would have been of great value to the field, and would have led to consensus, the opposite happened.

7 Conclusions

The TB vaccine field has come a long way. We have some potentially effective candidates (and I would include MVA85A here, despite recent events). However, there is a concern that a large number of candidates are based on the Ag85 proteins; these are certainly very immunogenic, but a recent study also found that immunodominant epitopes are highly conserved [71]. As I have noted [3], there may be a sinister reason for this. Even if Ag85 appears to work, we have no read-out of 'vaccine take', i.e. no adequate correlate of protection. The bottom line here is that our approaches to date are clearly based on a very limited number of antigens, which may come back to haunt us.

This also reflects the issue (and the author has spent much of his career on this) that we still know so little about the immune response. As noted recently [72], we still do not even know exactly what BCG actually *does*. We thought we understood the basic nuts and bolts of

protective immunity, but we know *nothing* about the nature of the memory immune response we expect good new vaccines to generate (there are two known subsets, and now possibly three), plus a decade ago we had no clue about the role in TB played by TH17 cells, Foxp3 regulatory T cells, T cell plasticity issues (TH17 suddenly expressing IFNγ, or turning into regulatory T cells), TH22, Tc9, or cells that are potentially detrimental such as Gr-1^{int} cells that arrive during necrosis (Obregon-Henao, unpublished data).

Finally, we have a reasonable (but certainly not large) number of candidates, but the reality is that the number of trials that can be performed is very limited, because of both cost and capacity. While we continue to make efforts to improve the ways we do things at the pre-clinical level (despite the current funding climate), the fact is that once vaccines reach clinical testing, the costs become astronomic, even prohibitive.

References

1. Smith KC, Orme IM, Starke J. The BCG Vaccine. In: Plotkin S, Orenstein W, Offit P, editors. Vaccines. 6th ed. London: WB Saunders; 2012.
2. Andersen P, Doherty TM. The success and failure of BCG: implications for a novel tuberculosis vaccine. Nat Rev Microbiol. 2005;3:656–62.
3. Orme IM. Development of new vaccines and drugs for TB: limitations and potential strategic errors. Future Microbiol. 2011; 6:161–77.
4. Orme IM. New vaccines against tuberculosis: the status of current research. Infect Dis Clin North Am. 1999;13:169–85.
5. Andersen P. TB vaccines: progress and problems. Trends Immunol. 2001;22:160–8.
6. Kaufmann SH. Is the development of a new tuberculosis vaccine possible? Nat Med. 2000;6:955–60.
7. Tameris MD, Hatherill M, Landry BS, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet. 1 Feb 2013 (Epub ahead of print).
8. McShane H. Tuberculosis vaccines: beyond bacille Calmette-Guerin. Philos Trans R Soc Lond B Biol Sci. 2013;366:2782–9.
9. Abel B, Tameris M, Mansoor N, et al. The novel tuberculosis vaccine, AERAS-402, induces robust and polyfunctional CD4+ and CD8+ T cells in adults. Am J Respir Crit Care Med. 2010; 181:1407–17.
10. Skeiky YA, Alderson MR, Ovendale PJ, et al. Differential immune responses and protective efficacy induced by components of a tuberculosis polyprotein vaccine, Mtb72F, delivered as naked DNA or recombinant protein. J Immunol. 2004;172:7618–28.
11. Brandt L, Skeiky YA, Alderson MR, et al. The protective effect of the Mycobacterium bovis BCG vaccine is increased by coadministration with the Mycobacterium tuberculosis 72-kilodalton fusion polyprotein Mtb72F in M. tuberculosis-infected guinea pigs. Infect Immun. 2004;72:6622–32.
12. Spertini F, Audran R, Lurati F, et al. The candidate tuberculosis vaccine Mtb72F/AS02 in PPD positive adults: a randomized controlled phase I/II study. Tuberculosis (Edinb). 2012;93: 179–88.
13. Day CL, Tameris M, Mansoor N, et al. Induction and Regulation of T Cell Immunity by the Novel TB Vaccine M72/AS01 in South African Adults. Am J Respir Crit Care Med. 10 Jan 2013 (Epub ahead of print).
14. Aagaard C, Hoang T, Dietrich J, et al. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. Nat Med. 2011;17:189–94.
15. Lin PL, Dietrich J, Tan E, et al. The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent *Mycobacterium tuberculosis* infection. J Clin Invest. 2012;122:303–14.
16. Bertholet S, Ireton GC, Ordway DJ, et al. A defined tuberculosis vaccine candidate boosts BCG and protects against multidrug resistant *Mycobacterium tuberculosis*. Sci Transl Med. 2010; 2(53):53ra74.
17. Baldwin SL, Bertholet S, Reese VA, Ching LK, Reed SG, Coler RN. The importance of adjuvant formulation in the development of a tuberculosis vaccine. J Immunol. 2012;188:2189–97.
18. Billeskov R, Elvang TT, Andersen PL, Dietrich J. The HyVac4 subunit vaccine efficiently boosts BCG-primed anti-mycobacterial protective immunity. PLoS One. 2012;7:e39909.
19. Goonetilleke NP, McShane H, Hannan CM, Anderson RJ, Brookes RH, Hill AV. Enhanced immunogenicity and protective efficacy against *Mycobacterium tuberculosis* of bacille Calmette-Guerin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara. J Immunol. 2003; 171:1602–9.
20. McShane H. Developing an improved vaccine against tuberculosis. Expert Rev Vaccines. 2004;3:299–306.
21. McShane H, Brookes R, Gilbert SC, Hill AV. Enhanced immunogenicity of CD4(+) T-cell responses and protective efficacy of a DNA-modified vaccinia virus Ankara prime-boost vaccination regimen for murine tuberculosis. Infect Immun. 2001;69:681–6.
22. McShane H, Hill A. Prime-boost immunisation strategies for tuberculosis. Microbes Infect. 2005;7:962–7.
23. Williams A, Goonetilleke NP, McShane H, et al. Boosting with poxviruses enhances *Mycobacterium bovis* BCG efficacy against tuberculosis in guinea pigs. Infect Immun. 2005;73:3814–6.
24. White AD, Sibley L, Dennis MJ, et al. An evaluation of the safety and immunogenicity of a candidate TB vaccine, MVA85A, delivered by aerosol to the lungs of macaques. Clin Vaccine Immunol. 2013;20(5):663-72.
25. Meyer J, Harris SA, Satti I, et al. Comparing the safety and immunogenicity of a candidate TB vaccine MVA85A administered by intramuscular and intradermal delivery. Vaccine. 2013; 31:1026–33.
26. Kato-Maeda M, Shanley CA, Ackart D, et al. Beijing sublineages of *Mycobacterium tuberculosis* differ in pathogenicity in the guinea pig. Clin Vacc Immunol. 2012;19:1–10.
27. Ordway DJ, Shang S, Henao-Tamayo M, et al. Mycobacterium bovis BCG-mediated protection against W-Beijing strains of *Mycobacterium tuberculosis* is diminished concomitant with the emergence of regulatory T cells. Clin Vaccine Immunol. 2011; 18:1527–35.
28. Shang S, Harton M, Tamayo MH, et al. Increased Foxp3 expression in guinea pigs infected with W-Beijing strains of *M. tuberculosis*. Tuberculosis (Edinb). 2011;91:378–85.
29. Grode L, Seiler P, Baumann S, et al. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille

Calmette-Guerin mutants that secrete listeriolysin. J Clin Invest. 2005;115:2472–9.

30. Grode L, Ganoza CA, Brohm C, Weiner J 3rd, Eisele B, Kaufmann SH. Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. Vaccine. 2013;31:1340–8.

31. Nambiar JK, Pinto R, Aguilo JI, et al. Protective immunity afforded by attenuated, PhoP-deficient *Mycobacterium tuberculosis* is associated with sustained generation of CD4+ T-cell memory. Eur J Immunol. 2012;42:385–92.

32. Verreck FA, Vervenne RA, Kondova I, et al. MVA.85A boosting of BCG and an attenuated, phoP deficient *M. tuberculosis* vaccine both show protective efficacy against tuberculosis in rhesus macaques. PLoS One. 2009;4:e5264.

33. Orme IM. The Achilles heel of BCG. Tuberculosis (Edinb). 2010;90:329–32.

34. Hinchey J, Jeon BY, Alley H, et al. Lysine auxotrophy combined with deletion of the SecA2 gene results in a safe and highly immunogenic candidate live attenuated vaccine for tuberculosis. PLoS One. 2011;6:e15857.

35. Sambandamurthy VK, Derrick SC, Hsu T, et al. *Mycobacterium tuberculosis* DeltaRD1 DeltapanCD: a safe and limited replicating mutant strain that protects immunocompetent and immunocompromised mice against experimental tuberculosis. Vaccine. 2006;24:6309–20.

36. Sambandamurthy VK, Derrick SC, Jalapathy KV, et al. Long-term protection against tuberculosis following vaccination with a severely attenuated double lysine and pantothenate auxotroph of *Mycobacterium tuberculosis*. Infect Immun. 2005;73:1196–203.

37. Sambandamurthy VK, Jacobs WR Jr. Live attenuated mutants of *Mycobacterium tuberculosis* as candidate vaccines against tuberculosis. Microbes Infect. 2005;7:955–61.

38. Sampson SL, Dascher CC, Sambandamurthy VK, et al. Protection elicited by a double leucine and pantothenate auxotroph of *Mycobacterium tuberculosis* in guinea pigs. Infect Immun. 2004;72:3031–7.

39. Zimmerman DM, Waters WR, Lyashchenko KP, et al. Safety and immunogenicity of the *Mycobacterium tuberculosis* DeltalysA DeltapanCD vaccine in domestic cats infected with feline immunodeficiency virus. Clin Vaccine Immunol. 2009;16:427–9.

40. Hinchey J, Lee S, Jeon BY, et al. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. J Clin Invest. 2007;117:2279–88.

41. Sweeney KA, Dao DN, Goldberg MF, et al. A recombinant *Mycobacterium smegmatis* induces potent bactericidal immunity against *Mycobacterium tuberculosis*. Nat Med. 2011;17:1261–8.

42. Turner J, Rhoades ER, Keen M, Belisle JT, Frank AA, Orme IM. Effective preexposure tuberculosis vaccines fail to protect when they are given in an immunotherapeutic mode. Infect Immun. 2000;68:1706–9.

43. Cardona PJ. RUTI: a new chance to shorten the treatment of latent tuberculosis infection. Tuberculosis (Edinb). 2006;86:273–89.

44. Gil O, Vilaplana C, Guirado E, et al. Enhanced gamma interferon responses of mouse spleen cells following immunotherapy for tuberculosis relapse. Clin Vaccine Immunol. 2008;15:1742–4.

45. Vilaplana C, Montane E, Pinto S, et al. Double-blind, randomized, placebo-controlled phase I clinical trial of the therapeutical antituberculous vaccine RUTI. Vaccine. 2010;28:1106–16.

46. Coler RN, Bertholet S, Pine SO, et al. Therapeutic immunization against *Mycobacterium tuberculosis* is an effective adjunct to antibiotic treatment. J Infect Dis. 2013;207(8):1242–52.

47. Turner OC, Keefe RG, Sugawara I, Yamada H, Orme IM. SWR mice are highly susceptible to pulmonary infection with *Mycobacterium tuberculosis*. Infect Immun. 2003;71:5266–72.

48. Faujdar J, Gupta P, Natrajan M, et al. *Mycobacterium indicus pranii* as stand-alone or adjunct immunotherapeutic in treatment of experimental animal tuberculosis. Indian J Med Res. 2012;134:696–703.

49. Gupta A, Ahmad FJ, Ahmad F, et al. Efficacy of *Mycobacterium indicus pranii* immunotherapy as an adjunct to chemotherapy for tuberculosis and underlying immune responses in the lung. PLoS One. 2012;7:e39215.

50. Ordway DJ, Shanley CA, Caraway ML, et al. Evaluation of standard chemotherapy in the guinea pig model of tuberculosis. Antimicrob Agents Chemother. 2010;54:1820–33.

51. Rawat KD, Chahar M, Reddy PV, et al. Expression of CXCL10 (IP-10) and CXCL11 (I-TAC) chemokines during *Mycobacterium tuberculosis* infection and immunoprophylaxis with *Mycobacterium indicus pranii* (Mw) in guinea pig. Infect Genet Evol. 2012;13:11–7.

52. Gupta A, Ahmad FJ, Ahmad F, et al. Protective efficacy of *Mycobacterium indicus pranii* against tuberculosis and underlying local lung immune responses in guinea pig model. Vaccine. 2012;30:6198–209.

53. von Reyn CF, Mtei L, Arbeit RD, et al. Prevention of tuberculosis in Bacille Calmette-Guerin-primed, HIV-infected adults boosted with an inactivated whole-cell mycobacterial vaccine. AIDS. 2012;24:675–85.

54. Orme IM. The mouse as a useful model of tuberculosis. Tuberculosis (Edinb). 2003;83:112–5.

55. Rhoades ER, Frank AA, Orme IM. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent *Mycobacterium tuberculosis*. Tuber Lung Dis. 1997;78:57–66.

56. Turner OC, Basaraba RJ, Frank AA, Orme IM. Granuloma formation in mouse and guinea pig models of experimental tuberculosis. In: Boros DL, editor. Granulomatous infections and inflammation: cellular and molecular mechanisms. Washington DC: ASM Press; 2003. p. 65–84.

57. Driver ER, Ryan GJ, Hoff DR, et al. Evaluation of a mouse model of necrotic granuloma formation using C3HeB/FeJ mice for testing of drugs against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2012;56:3181–95.

58. Kramnik I. Genetic dissection of host resistance to *Mycobacterium tuberculosis*: the sst1 locus and the Ipr1 gene. Curr Top Microbiol Immunol. 2008;321:123–48.

59. Pichugin AV, Yan BS, Sloutsky A, Kobzik L, Kramnik I. Dominant role of the sst1 locus in pathogenesis of necrotizing lung granulomas during chronic tuberculosis infection and reactivation in genetically resistant hosts. Am J Pathol. 2009;174:2190–201.

60. Basaraba RJ, Orme IM. Pulmonary tuberculosis in the guinea pig. In: Leong FY, Dartois V, Dick T, editors. A color Atlas of comparative pathology of pulmonary tuberculosis. Baton Rouge: CRC Press; 2010.

61. Basaraba RJ. Experimental tuberculosis: the role of comparative pathology in the discovery of improved tuberculosis treatment strategies. Tuberculosis (Edinb). 2008;88(Suppl 1):S35–47.

62. Ordway DJ, Orme IM. Animal models of mycobacteria infection. Curr Protoc Immunol. Chapter 19: Unit 19 5.

63. Hoff DR, Ryan GJ, Driver ER, et al. Location of intra- and extracellular *M. tuberculosis* populations in lungs of mice and guinea pigs during disease progression and after drug treatment. PLoS One. 2011;6:e17550.

64. Ryan GJ, Hoff DR, Driver ER, et al. Multiple *M. tuberculosis* phenotypes in mouse and guinea pig lung tissue revealed by a dual-staining approach. PLoS One. 2010;5:e11108.

65. Barry CE 3rd, Boshoff HI, Dartois V, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol. 2009;7:845–55.

66. Orme M. The latent tuberculosis bacillus (I'll let you know if I ever meet one). Int J Tuberc Lung Dis. 2001;5:589–93.

67. Lin PL, Rodgers M, Smith L, et al. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. Infect Immun. 2009;77:4631–42.

68. Sharpe SA, McShane H, Dennis MJ, et al. Establishment of an aerosol challenge model of tuberculosis in rhesus macaques and an evaluation of endpoints for vaccine testing. Clin Vaccine Immunol. 2010;17:1170–82.

69. Williams A, Hall Y, Orme IM. Evaluation of new vaccines for tuberculosis in the guinea pig model. Tuberculosis (Edinb). 2009; 89:389–97.

70. Checkley AM, McShane H. Tuberculosis vaccines: progress and challenges. Trends Pharmacol Sci. 2011;32:601–6.

71. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of *Mycobacterium tuberculosis* are evolutionarily hyperconserved. Nat Genet. 2010;42:498–503.

72. McShane H, Jacobs WR, Fine PE, et al. BCG: myths, realities, and the need for alternative vaccine strategies. Tuberculosis (Edinb). 2012;92:283–8.