ESSAY

Envisioning future strategies for vaccination against tuberculosis

Stefan H. E. Kaufmann

Abstract | The design of tuberculosis vaccines has entered a new era. Although several new vaccine candidates will pass Phase I clinical trials within the next year, I believe that the most effective vaccination strategy will be to combine different vaccine candidates and to use a prime–boost approach. This strategy, however, would require several years of iterative vaccine trials, unless the process is expedited by the identification of reliable biomarkers for assessing vaccine efficacy. In this Essay, I briefly summarize past and present attempts to develop a vaccine against tuberculosis, and I describe, using imagined scenarios, the tuberculosis vaccination schemes that might become available from a large repertoire of candidate schemes in the near and distant future.

"It is tough to make predictions, especially about the future." Yogi Berra

About one-third of the world's population is thought to be infected with *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB). Although TB kills more people worldwide than any other disease caused by a single bacterial pathogen (~2 million individuals annually), only a small proportion (~10%) of those who are infected with *M. tuberculosis* develop active disease (~8–10 million individuals annually)[1]. The disease is particularly prevalent in developing regions, notably in Africa and Southeast Asia, which had 1.0 million and 1.5 million cases of active TB, respectively, in 2004. China, India and Russia add further to the toll, having a combined total of ~2 million cases of active TB in 2004.

Infected individuals mount a cell-mediated immune response, but they do not achieve sterile eradication (see Glossary): the immune response restricts the *M. tuberculosis* bacilli to granulomas (which are structured aggregations of activated macrophages and T cells) in the lungs. Therefore, worldwide, of the 150,000 individuals per day who become infected with *M. tuberculosis*, only 15,000 develop active disease during their lifetime. The risk of developing active TB, however, is increased by several hundredfold in individuals who are infected with both *M. tuberculosis* and HIV[1], of whom there are more than 15 million.

Although drugs that cure TB are available, compliance is poor, owing to the complicated and long-lasting treatment regimen. This has contributed to the steady rise of multidrug-resistant (MDR) strains of *M. tuberculosis*. At present, 50 million individuals are infected with MDR strains of *M. tuberculosis*; in several regions, more than 10% of TB cases are MDR[1].

The current vaccine, *Mycobacterium bovis* bacillus Calmette–Guérin (BCG), has been administered more than 3 billion times and is still widely used. Yet it only protects against severe forms of childhood TB and does not reliably prevent the most prevalent form of the disease — adult pulmonary TB — at least in countries with a high incidence of TB, where a vaccine is most needed. Therefore, it is imperative that we develop more effective TB vaccines. In this Essay, I first describe the efforts made at the turn of the last century that led to the development of the current vaccine, BCG. Second, I summarize the attempts made at the beginning of this century that might lead to the generation of new TB vaccines. Last, I discuss my view of the prospects for these strategies, and for future vaccination strategies, in the amelioration of the pressing health problems caused by TB.

The past: generation of the first vaccine

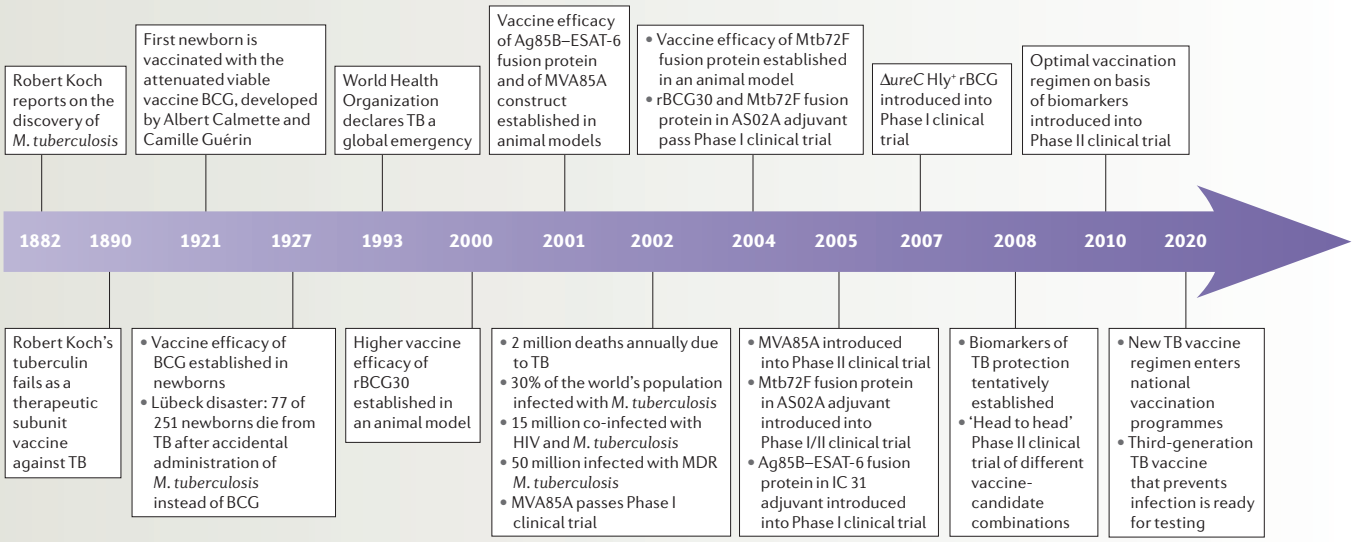
Robert Koch (1843–1910), who elucidated the aetiology of TB in 1882, and Albert Calmette (1863–1933), who together with Camille Guérin (1872–1961) described the first vaccine against TB in 1921, are considered to be the pioneers of TB research (FIG. 1). They probably would have been disappointed by the slow progress of TB research during the second half of the twentieth century. But they would have been pleased to see that, in the twenty-first century, there is renewed interest in the development of new TB vaccine candidates and that these candidates are likely to be in clinical trials soon (TIMELINE). Koch can be viewed as the father of subunit-vaccine design: in 1890, he described a subunit vaccine, called tuberculin, that he claimed protected against TB in guinea pigs[2]. However, this claim was soon contested in a multicentre clinical trial comprising more than 1,700 patients. The trial assessed the therapeutic value of Koch's compound for patients suffering from different forms of the disease, including skin TB and pulmonary TB[3]. The compound was shown to be a complete failure for TB therapy, and because of the great disappointment this caused, tuberculin was never tested as a prophylactic vaccine.

Calmette and Guérin are credited with the development of the first live vaccine against TB. This vaccine was originally known as bacille bilié Calmette–Guérin because it was cultured on potato slices

Robert Koch | Albert Calmette | Camille Guérin

Figure 1 | **The heroes of tuberculosis research.** Robert Koch (1843–1910) described the aetiology of tuberculosis in 1882 but failed to develop a subunit vaccine against tuberculosis in 1890. Albert Calmette (1863–1933) and Camille Guérin (1872–1961) described the first attenuated vaccine against tuberculosis in 1921. Images of Calmette and Guérin courtesy of C. Locht, Institut Pasteur, France.

Timeline | **Tuberculosis vaccination: past, present and future**



Ag85B, antigen 85B; AS02A, an oil-in-water-emulsion-based adjuvant containing monophosphoryl lipid A and QS-21 (GlaxoSmithKline); BCG, *Mycobacterium bovis* bacillus Calmette–Guérin; ESAT-6, early secretory antigen 6; Hly, listeriolysin O; IC 31, adjuvant containing oligodeoxynucleotides and polycationic amino acids (Intercell AG); MDR, multidrug-resistant; Mtb, *Mycobacterium tuberculosis*; *M. tuberculosis*, *Mycobacterium tuberculosis*; MVA85A, recombinant modified vaccinia virus Ankara expressing antigen 85A; r, recombinant; TB, tuberculosis; *ureC*, urease C.

soaked with ox gall[4], and it is now known as *M. bovis* bacillus Calmette–Guérin: that is, BCG. In 1906, Calmette and Guérin started serial passage of a culture of *M. bovis* at 14-day intervals. By the thirtieth passage, which was obtained in 1908, they began testing the bacilli for safety and ability to afford protection in various animal species. By 1919, they felt confident enough to carry out large-scale experiments in different animal species. These experiments showed that the bacilli were completely attenuated — that is, they did not cause disease — and that the bacilli provided protection from infection with virulent *M. bovis* or *M. tuberculosis*.

Consequently, the first human newborn was vaccinated in 1921. Between 1921 and 1924, a large number of babies born in households that included at least one patient with TB were vaccinated. In 1927, the data from more than 20,000 vaccinees were evaluated[5]. In households that included individuals with TB, ~25% of unvaccinated newborns died in the first years of life. By contrast, less than 1% of vaccinated newborns in such households died as a result of TB, and the death rate of these newborns was less than 5% when all deaths from unknown causes were included.

In these studies, BCG was routinely given orally, and Calmette and Guérin were convinced that the oral route of administration was crucial. However, in most countries, the route was subsequently changed to parenteral administration, not the least because of the 'Lübeck disaster', which occurred in Germany in 1927: 251 newborns were accidentally vaccinated with virulent *M. tuberculosis* instead of BCG, resulting in active TB in 212 children, of whom 77 died[6]. It was felt that local injection into the skin was safer than oral administration, which could cause severe side-effects in some cases.

Since then, vaccination with BCG has continued to be part of the World Health Organization Expanded Program on Immunization in numerous countries, and it has an excellent safety record. Until recently, however, no attempts had been made to improve the efficacy of vaccination against TB. Indeed, immunological research into TB has only been revitalized, with a strong emphasis on vaccine development, since the end of the last century. This has taken place with the advent of increased support from governmental, non-governmental and philanthropic organizations for research into the major infectious diseases: AIDS, TB and malaria.

The present: designing vaccines

In the past few years, there have been important breakthroughs in the development of an improved vaccine against TB. Some vaccine candidates have passed Phase I clinical trials, and some will enter Phase II clinical trials in the next few years (TIMELINE; TABLE 1). Therefore, 'head to head' testing (direct comparison) of these candidates might be possible. I consider that this is a good opportunity, because I believe that it is unlikely that a single vaccine will arise as a 'magic bullet'. Instead, it seems that a combination of two or more vaccine candidates is more likely to be successful[7,8]. Novel vaccine candidates include the following: improved recombinant BCG (rBCG) vaccines, which have greater antigenicity or immunogenicity than the parental BCG vaccine and could replace the current BCG vaccine; and subunit vaccines composed of dominant secreted antigens, which could boost the immune response after priming with BCG. The most advanced vaccines are described in detail in TABLE 1. (I categorize the virus-based recombinant vaccine known as MVA85A as a subunit vaccine — despite it being composed of a viral carrier — because it contains a single *M. tuberculosis* antigen, antigen 85A.)

Ultimately, the two types of vaccine candidate could be combined: that is, the strategy could be to prime with an improved rBCG vaccine and boost with a subunit vaccine. This approach would require several years of iterative vaccine trials and ~10 years of Phase III clinical trials before it would become clear whether a vaccine is satisfactory (that is, better than BCG, which is the 'gold standard'). Because of these timescales, finding ways to reduce the duration of vaccine trials has a high priority so that a more successful vaccination plan

can be implemented as soon as possible. To achieve this goal, it is essential to define *in vivo* correlates of protection, which are now frequently termed biomarkers[8]. The biomarkers for protection against TB are ill-defined at present. Interferon-γ produced by peripheral-blood T cells is the best indicator of protective immunity that has been defined so far. However, it is insufficient as a robust biomarker, because protective immunity against TB depends on several factors, including other cytokines and chemokines, several T-cell subsets and specific patterns of T-cell migration. Biomarkers are now being defined with support from Grand Challenges in Global Health, a programme managed and administered by the Bill & Melinda Gates Foundation.

When enough biomarkers have been defined, it should be possible to use a tailored combination of these (known as a biosignature) to characterize the immune response that is induced by different vaccine candidates in Phase II clinical trials and thereby to determine whether the responses differ qualitatively or quantitatively[8]. Despite such promise, I am fully aware that there are several obstacles inherent in the translation of data from well-controlled experimental settings to field trials, and these obstacles might hinder the elucidation of biomarkers. Yet I remain optimistic that a robust test that allows determination of biosignatures in the field can be developed. If vaccine candidates induce quantitatively different responses, only those inducing the most potent biosignature of protective immunity would be selected for Phase III clinical trials. Qualitatively different candidates would be combined to optimize the immune response such that the protective biosignature that has been deemed most likely to result in protection is generated.

The near future: preventing disease

To understand new TB vaccine regimens better, let us imagine the fate of two children in a developing country in Africa that has a high rate of infection with TB. These children were both lucky enough to be born to HIV-negative mothers around the turn of this century. Infant Sipho was vaccinated with BCG soon after birth and therefore has a strong, although not 100%, chance of being protected against the severe forms of childhood TB (FIG. 2). Indeed, when he becomes infected with *M. tuberculosis*, the infection is successfully controlled, although vaccine-induced immunity fails to eradicate the *M. tuberculosis* bacilli. Sipho then grows up and, during adolescence, frequently comes into contact with prevalent environmental *Mycobacterium* spp. Therefore, as a young adult, his immune system has already encountered three types of *Mycobacterium* spp.: BCG, *M. tuberculosis* and environmental *Mycobacterium* spp. By this time, BCG and any environmental *Mycobacterium* spp. encountered have been eradicated by his immune system. Only the *M. tuberculosis* bacilli persist, in a dormant stage in granulomas: that is, Sipho is latently infected with *M. tuberculosis*.

Infant Thandi is also lucky, because although she was not vaccinated with BCG, she does not become infected with *M. tuberculosis*. However, she is frequently infected with environmental *Mycobacterium* spp. while growing up. Her immune system succeeds in eradicating these mycobacteria and remains slightly activated.

In ~2020, the adults Sipho and Thandi marry, and a child, Ayanda, is born. At this time, several TB vaccine candidates have successfully passed Phase III clinical trials and become available for broad-scale application (TIMELINE). I envisage that different types of vaccine will be required by the members of this small family. The parents have already both developed an immune response to mycobacteria. However, conventional tuberculin testing does not distinguish properly between the mycobacteria-specific immune responses generated by Sipho and Thandi (that is, between the immune responses generated by infection with *M. tuberculosis*, contact with environmental *Mycobacterium* spp. and vaccination with BCG). Nonetheless, I hope that, by ~2020, a tailored test kit for biomarkers has become available, allowing individuals vaccinated with BCG to be distinguished from unvaccinated individuals infected with *Mycobacterium* spp. This is an important distinction because the efficacy of a live priming vaccine could be affected by existing immunity to the microorganism and

Table 1 | **The most advanced tuberculosis vaccine candidates**

Candidate	Composition	Status	Reference
Subunit vaccines*			
Mtb72F in AS02A	Fusion protein of Rv0125 and Rv1196, formulated in AS02A (GlaxoSmithKline), an oil-in-water-emulsion-based adjuvant that contains monophosphoryl lipid A and QS-21	• Phase I clinical trial in healthy uninfected volunteers completed • Phase I/II clinical trial started	11
Ag85B–ESAT-6 fusion protein in IC 31	Fusion protein of Ag85B (Rv1886c) and ESAT-6 (Rv3875), formulated in IC 31 (Intercell AG), an adjuvant composed of oligodeoxynucleotides and polycationic amino acids	• Phase I clinical trial in healthy uninfected volunteers started	12
MVA85A‡	Replication-deficient recombinant vaccinia virus that expresses antigen 85A (Rv3804c)	• Phase I clinical trial in healthy uninfected volunteers and BCG-vaccinated volunteers completed • Phase II clinical trial started	13
Recombinant BCG§			
rBCG30	rBCG that expresses Ag85B (Rv1886c); has increased antigenicity	• Phase I clinical trial in healthy uninfected volunteers completed	14
Δ*ureC* Hly⁺ rBCG	rBCG that expresses listeriolysin O from *Listeria monocytogenes* and is deficient in urease; has increased immunogenicity	• GMP production ongoing • Phase I clinical trial in healthy uninfected volunteers to start in 2007	15

*Subunit vaccines contain a single antigen and have only minor side-effects, but they need to be administered with a potent adjuvant. ‡MVA85A (recombinant modified vaccinia virus Ankara expressing antigen 85A) generates an immune response that has few side-effects, is directed against a single antigen, and is mediated by both $CD4^+$ and $CD8^+$ T cells. §Recombinant BCG (rBCG) vaccines have only minor side-effects and have greater antigenicity or immunogenicity than the parental BCG vaccine. Ag85B, antigen 85B; BCG, *Mycobacterium bovis* bacillus Calmette–Guérin; ESAT-6, early secretory antigen 6; GMP, Good Manufacturing Practice; Hly, listeriolysin O; Mtb, *Mycobacterium tuberculosis*; *ureC*, urease C.

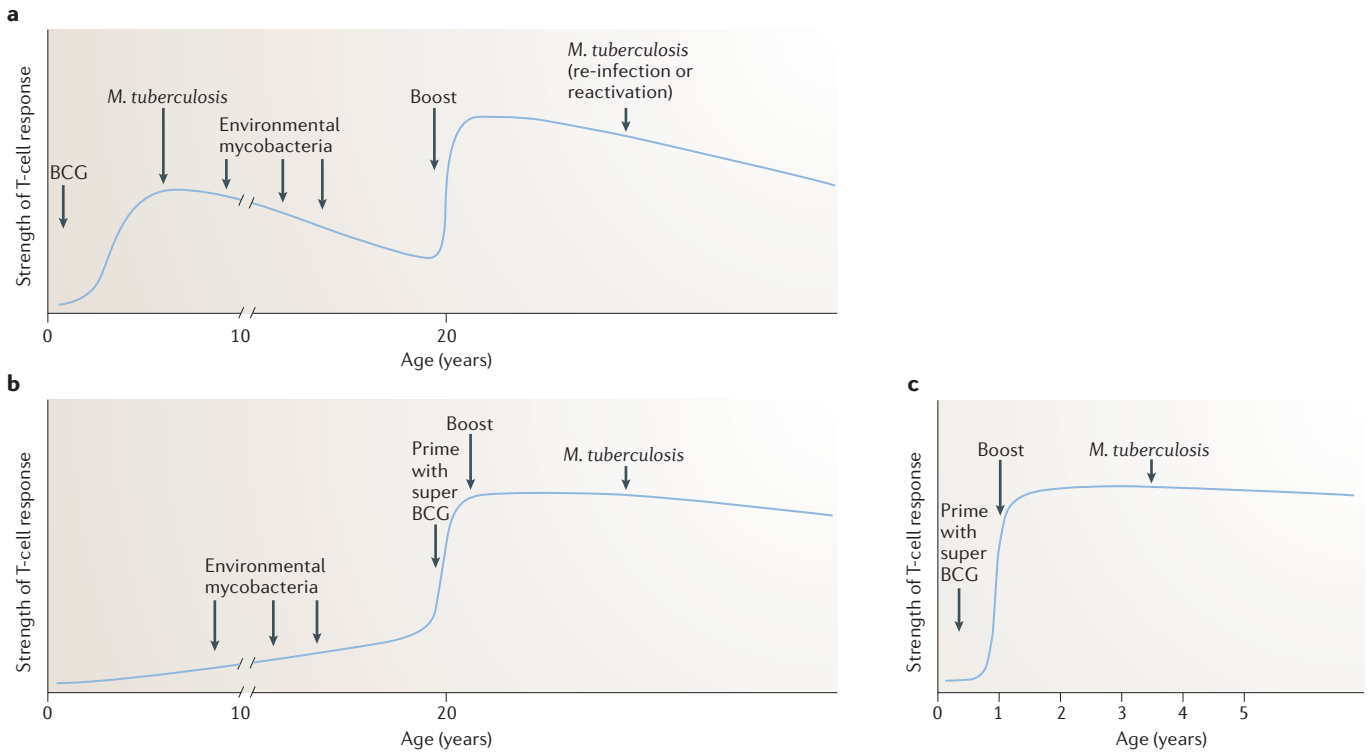


Figure 2 | **A virtual vaccination scheme against tuberculosis.** **a** | Vaccination with *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) induces an immune response that protects against childhood tuberculosis (TB) (indicated by the strength of the T-cell response). Infection with *Mycobacterium tuberculosis* and environmental mycobacteria might then increase this response further. However, during adolescence and adulthood, this immune response weakens (indicated by a decreasing T-cell reponse) and fails to protect against reactivation of latent infection or re-infection. Therefore, the immune response needs to be boosted (indicated by an increased T-cell response) by administration of a subunit vaccine. This vaccination strategy is best suited for Sipho. **b** | In the absence of vaccination with BCG or infection with *M. tuberculosis*, exposure to environmental mycobacteria might stimulate a T-cell response that is too weak to provide protection against TB after infection with *M. tuberculosis* but is strong enough to affect the efficacy of the BCG vaccine. Priming with a superior recombinant BCG (rBCG) vaccine and subsequent boosting with a subunit vaccine should therefore stimulate a marked immune response that can protect against subsequent infection with *M. tuberculosis* (indicated by a markedly increased T-cell response). This vaccination strategy is best suited for Thandi. **c** | For infants, such as Ayanda, the best vaccination strategy would be to prime the immune response with a superior rBCG vaccine, then to follow this with a boost with a subunit vaccine to protect against TB after subsequent infection with *M. tuberculosis* (indicated by a markedly increased T-cell response).

to related species. By contrast, subunit vaccines are designed to boost existing immune responses elicited by previous vaccination. Therefore, because of his childhood vaccination with BCG (which would have a priming effect) and his latent infection with *M. tuberculosis*, Sipho is suited to a booster vaccination with a new post-exposure subunit vaccine[7].

The immune system of young Ayanda has not yet been in contact with any *Mycobacterium* spp. and therefore would benefit most from a new pre-exposure priming vaccine that replaces the conventional BCG vaccine, probably an improved rBCG vaccine. This priming vaccination with rBCG would be followed by a heterologous boost, in childhood, with a pre-exposure subunit vaccine, thereby allowing induction of the most potent protection possible[7]. I envisage this heterologous prime–boost strategy to be the optimal choice for other newborns.

Selection of a vaccine best suited for Thandi is the most difficult choice. On the one hand, she requires a pre-exposure priming vaccination because she was not vaccinated with BCG and she is not latently infected with *M. tuberculosis*. But, on the other hand, her exposure to environmental *Mycobacterium* spp. might have induced mycobacteria-specific immune responses that could impair the effect of vaccination with rBCG. Perhaps a heterologous prime–boost scheme that comprises, first, a priming vaccination with an improved rBCG vaccine and, second, a booster vaccination with a subunit vaccine would be the most appropriate choice[7].

At about the same time that Ayanda is born, an HIV-infected woman in the neighbourhood delivers a baby who, unfortunately, also becomes infected with HIV. For safety reasons, this infant is not eligible for the new live, pre-exposure priming, TB vaccine. Despite its safety profile being better than that of the BCG vaccine, it cannot be excluded that the live vaccine might harm the child, whose immune system is probably weakened by infection with HIV. However, a pre-exposure subunit vaccine will be offered in the hope that the child's immune system is still sufficient to develop an immune response that can combat subsequent infection with *M. tuberculosis*[7]. I hope that using a combination of these vaccine candidates, rather than there being a competition between them, will help to markedly reduce the global TB burden. Yet it is doubtful that any vaccination schedule could result in sterile eradication.

The distant future: preventing infection

All of the potential vaccination regimens discussed here are based on preventing disease reactivation rather than infection, and they are unlikely to eliminate *M. tuberculosis*. In fact, the most successful vaccines in use

— those against measles, mumps, rubella, diphtheria, tetanus and polio — prevent diseases rather than infection. Ultimately, however, they achieve sterile eradication of the causative agents. At present, there is no evidence that gives reason to hope that this can be achieved for TB. T cells, which are the lymphocytes that are active after vaccination with BCG, cannot prevent individuals from becoming infected with *M. tuberculosis* because they do not interact with microbial antigens directly and sense only infected cells[8]. Although T cells can achieve sterile eradication of less robust microorganisms, this seems to be an ambitious goal in the case of TB, in which the pathogen hides from T cells inside macrophages that are concealed in a granulomatous structure surrounded by a fibrotic wall[9].

Accordingly, our current strategies for vaccination against TB are attempts, at best, to control the spread of *M. tuberculosis* by preventing disease reactivation, but this depends on a competent mycobacteria-specific T-cell response being sustained. Even a mycobacteria-specific T-cell response that is remarkably potent initially will fail to control infection after the immune system has weakened, as shown by the enormously increased risk of TB reactivation in individuals infected with both HIV and *M. tuberculosis*. In conclusion, on the positive side, the new generation of vaccines have a good chance of reducing the burden of TB. But, on the negative side, they are not likely to succeed in eradicating the microorganisms from individuals who become infected with *M. tuberculosis*. Therefore, alternative strategies for disease eradication need to be considered.

The main alternative strategy involves the generation of specific antibodies that could prevent individuals becoming infected with *M. tuberculosis*. Therefore, it is conceivable that, during the introduction of the second generation of vaccines into national vaccination programmes (hopefully ~2020), the ambitious scientists who will be embarking on designing a third generation of vaccines against TB are likely to target the site of entry of the *M. tuberculosis* bacilli: that is, the lungs. This third generation of vaccines could be designed to be administered intranasally and to stimulate potent mucosal host defences by inducing local production of specific IgA, which would then target *M. tuberculosis* in the alveolar space. Because *M. tuberculosis* bacilli enter the lungs in small numbers, specific IgA that is present at pulmonary sites could help to attack invading pathogens on first encounter. With the help of complement, such IgA could directly damage *M. tuberculosis* bacilli. In parallel, intracellular killing of *M. tuberculosis* could be strengthened by using a new type of drug that modulates the signalling pathways that are involved in phagocyte activation, which are frequently impaired by *M. tuberculosis*[10]. The advantages are obvious: only small numbers of bacilli need to be eradicated to prevent the infection, dissemination and persistence of *M. tuberculosis* in the host. As a consequence, the need for sustained control of dormant *M. tuberculosis* by the immune system would become obsolete. It is hoped that this third generation of vaccines will ultimately lead to the global eradication of TB.

Concluding remarks

Although the described scenarios are all imaginary and represent my vision, important steps towards a new TB vaccine have already been achieved in terms of the basic research. However, translation of this basic research into applications requires trials that involve tens of thousands of volunteers, and this is the most difficult part of achieving a successful vaccination programme. Conversely, new findings will be made during clinical trials of the current crop of second-generation vaccines, and these findings will need to be verified under precise experimental conditions in the laboratory. This will allow characterization of the immune mechanisms that are elicited by the vaccine candidates and of the biological role of these mechanisms in protection, thereby gradually leading to the production of a tailored test kit of the biomarkers that are needed to identify the best vaccination schedule. Hopefully, iterative vaccine testing, in which clinical and basic research complement each other, will pave the way for current and future strategies to combat TB.

Glossary

Biomarker
A biological molecule that can easily be obtained and quantified and that allows prediction of the presence of a disease, the activity of a drug or the efficacy of a vaccine. Several biomarkers are often required: together, they are known as a biosignature. Frequently used biomarkers include mRNA, proteins and small molecules, all of which are typically obtained from the blood.

Granuloma
A structure that consists of a collection of macrophages at different stages of activation, usually surrounded by a layer of lymphocytes. These macrophages resemble epithelial cells and often include multinucleated giant cells. Granuloma formation is a chronic inflammatory response that can be initiated by various infectious and non-infectious agents.

Heterologous prime–boost strategy
When a single application of a vaccine is insufficient, repeated vaccinations are carried out using different vaccine preparations, allowing the sequential stimulation of a better immune response.

Ox gall
The secretion from the gall bladder of oxen. Previously used to emulsify lipid–water mixtures: for example, to modify the lipid nature of mycobacteria.

Phase I clinical trial
The assessment of the safety of a drug or vaccine in healthy volunteers.

Phase II clinical trial
The determination of the efficacy of a drug or vaccine by analysing cells or other material from treated volunteers.

Phase III clinical trial
The determination of the *in vivo* efficacy of a drug or vaccine in volunteers. In the case of tuberculosis, the efficacy of a vaccine against natural infection is tested in volunteers.

Serial passage
The transfer of microorganisms from one culture to the next (using either liquid or solid culture).

Sterile eradication
The complete elimination of the microbial pathogen from the host.

Subunit vaccine
A vaccine that comprises only a small part of the entire pathogen. Subunit vaccines are typically recombinant proteins.

Tuberculin testing
The intracutaneous administration of proteins from *Mycobacterium tuberculosis* (known as purified protein derivatives) to determine previous interactions of the host with the pathogen. A positive tuberculin test is generally taken as an indication of latent infection with *M. tuberculosis*.

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doi:10.1038/nri1920
Published online 18 August 2006

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Acknowledgements

Studies on TB have been carried out with financial support from the following: the European Union Framework Programme 6 projects Tuberculosis Vaccine (TBVAC) and Mucosal Vaccines for Poverty Related Diseases (MUVAPRED); the Bundesministerium für Bildung und Forschung competence networks Structural Genomics of *M. tuberculosis*, PathoGenoMics, and Genomics of Tuberculosis (Germany); the Deutsche Forschungsgemeinschaft priority programme Novel Vaccination Strategies (Germany); and the Bill & Melinda Gates Foundation Grand Challenges in Global Health initiative (United States). Studies on the history of TB have been generously supported by Fonds der Chemischen Industrie (Germany). I am grateful to T. Walker and A. Ozin for critical reading of the manuscript, as well as D. Schad for graphical work, and S. Sibaei and M. L. Grossman for excellent help. I thank H. McShane, P. Mettens and P. Andersen for sharing unpublished observations.

Competing interests statement

The author declares competing financial interests: see web version for details.

DATABASES

The following terms in this article are linked online to:
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interferon-γ
Infectious Disease Information:
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