YALE JOURNAL OF BIOLOGY AND MEDICINE 83 (2010), pp.209-215.
Copyright © 2010.

REVIEW

Toward Novel Vaccines Against Tuberculosis: Current Hopes and Obstacles

Christoph A. Thaiss[a*] and Stefan H.E. Kaufmann[b*]

[a]Department of Immunobiology, Yale University School of Medicine, New Haven, Connecticut; [b]Department of Immunology, Max Planck Institute for Infection Biology, Berlin, Germany

Approximately 2 million people die of tuberculosis (TB†) each year. The current vaccine, Bacille Calmette-Guérin (BCG), albeit widely employed, does not protect against adult pulmonary disease, and new vaccines are urgently needed to reduce the incidence of TB worldwide. New insights into the cellular and molecular mechanisms that underlie the interactions between *Mycobacterium tuberculosis* and its host have been exploited to develop novel vaccine candidates that recently have entered clinical trials. This review provides a brief overview of different approaches toward a new vaccination strategy and summarizes major challenges for the next decade.

INTRODUCTION

Tuberculosis (TB) is an ancient human scourge that causes approximately 2 million deaths each year [1]. Two billion individuals worldwide, about one-third of the human population, are infected with the causative agent of TB, *Mycobacterium tuberculosis* (*M. tuberculosis*). Ninety percent of infected individuals are latently infected, i.e., they harbor the pathogen in its dormant form, whereas the remaining 10 percent suffer from active disease [2]. In the Western world, the infection is held in check by an efficient health care system, while in many regions of the developing world, resources that are available to prevent, identify, and treat active TB are limited and in many cases overwhelmed by the high number of infected patients. In these regions, TB is a serious obstacle to economic development, and new vaccines are desperately needed to reduce the incidence of TB in the long term [3].

*To whom all correspondence should be addressed: C. Thaiss, 300 Cedar Street, PO Box 208011, New Haven, CT 06520; E-mail: christoph.thaiss@yale.edu; or S.H.E. Kaufmann, Charitéplatz 1, 10117 Berlin, Germany; E-mail: Kaufmann@mpiib-berlin.mpg.de.

†Abbreviations: TB, tuberculosis; BCG, Bacille Calmette-Guérin; APCs, antigen-presenting cells; TLR2, toll-like receptor.

Keywords: tuberculosis, vaccines, BCG, global open access

Conflict of interest: S.H.E. Kaufmann is co-inventor of the rBCGΔUreC:Hly vaccine candidate.

Here, we will summarize current efforts to use our understanding of the immune response against *M. tuberculosis* for the rational design of new vaccines and present an overview of the major challenges that remain to be solved.

THE IMMUNE RESPONSE AGAINST *M. TUBERCULOSIS* AND EVASION STRATEGIES

After *M. tuberculosis* is inhaled, it is phagocytosed by antigen-presenting cells (APCs) in the lung, such as alveolar macrophages, lung parenchyma macrophages, and dendritic cells. Subsequently, these cells elicit local inflammatory responses, leading to the recruitment of mononuclear cells from the blood, which in turn become potential targets for infection [4]. Inside the phagosomal compartment, the mycobacteria employ their first immune evasion strategy as they prevent phagosome acidification and thus survive within this compartment [5]. Second, *M. tuberculosis* apparently can escape into the cytosol and thus evade phagosomal effector mechanisms [6].

The pathogen is eventually controlled by granuloma formation, which is the defining histopathologic hallmark of the disease. The granuloma, first being an amorphous aggregate of macrophages, neutrophils, and monocytes, develops into a more organized structure with the initiation of an adaptive immune response. Immune cells and a fibrotic wall surround the granulomas in order to prevent bacterial spreading [7]. In this form, disease outbreak can be prevented over long periods of time unless the immune response weakens. Massive cell death leads to caseation of the granuloma, and *M. tuberculosis* can no longer be enclosed. *M. tuberculosis* exploits cell necrosis to leave its host cells and spread, whereas apoptotic cell death sustains plasma membrane integrity and thus impedes *M. tuberculosis* exit. Here again, the bacteria apparently have developed an evasion strategy, since a recent report found that virulent *M. tuberculosis* blocks apoptosis by inhibiting prostaglandin E_2 (PGE_2) production [8].

The preponderance of evidence indicates a crucial role for T cells in the containment of *M. tuberculosis* [9]. $CD4^+$ T cells, predominantly T helper (T_H) 1 and T_H17 cells, exert their protective function by the production of cytokines, including IFNγ and IL-17, respectively [10,11]. *M. tuberculosis*-specific $CD4^+$ T lymphocytes are activated by APCs that have taken up and processed *M. tuberculosis*-derived antigens that are presented by MHC class II molecules. Importantly, the bacteria have developed a further immune evasion strategy to interfere with this process, since they are capable of inhibiting MHC class II molecule expression and antigen presentation. This evasion strategy is based on innate immune recognition of the bacteria via Toll-like receptor 2 (TLR2), indicating that, during the course of evolution, *M. tuberculosis* has found a way to turn the spear and exploit the host's innate defense mechanisms to its own advantage [12].

Notably, $CD8^+$ T cells contribute to host defense, not only by cytokine production, but also by perforin- and granzyme-mediated cytotoxic activity against the pathogen and infected phagocytes. In contrast to $CD4^+$ T cells, it is required for the priming of $CD8^+$ T cells that APCs take up exogenous antigen and present it in complex with MHC class I molecules, a process called cross-presentation [13]. Remarkably, cross-presentation also is subject to inhibition through bacterial evasion strategies that utilize eicosanoid pathways [8].

Furthermore, the involvement of lymphocytes in host defense against an infection leads to the development of a memory response that normally rapidly elicits a secondary response after re-encounter of the pathogen [14]. In the case of chronic TB, however, the memory response must be tightly controlled in order to master the delicate tightrope walk between immunopathology and host integrity.

Taken together, this brief summary of the immune response against *M. tuberculosis* and the evasion strategies employed by the pathogen already suggests that a successful vaccination will be based on efficient antigen presentation and activation of T cells, as well as the induction of appropriate memory responses. How new vaccines try to address these important hallmarks of a

successful immune response will be detailed below.

THE CURRENT VACCINE

Bacille Calmette-Guérin (BCG), the current vaccine for TB, has been used for decades and about 4 billion individuals have received the vaccination so far [15]. BCG is impressive with respect to its low cost and its high safety [16]. Nonetheless, the vaccine has several limitations [17]. BCG, albeit protective against severe childhood TB, does not satisfactorily prevent adult pulmonary disease. The potential reasons for this failure are multifaceted.

First, exposure to environmental mycobacteria, which is common in developing countries, has been reported to weaken and shorten the immune response elicited by BCG and could, therefore, affect the outcome of BCG vaccination [18]. Second, helminth infection is believed to diminish the efficacy of BCG vaccination because it favors the development of a T_H2 response and thereby weakens T_H1 polarization, which is induced by the vaccine [19]. While a T_H2 response is an immune response directed against extracellular microbes and mainly driven by interleukin-4, T_H1 responses are IFNγ-mediated responses against intracellular pathogens and thus beneficial for host defense against *M. tuberculosis*. Third, recent discussions suggest that some individuals might clear BCG before a protective and sustained immune response can develop.

Moreover, we are only beginning to understand how numerous human genetic polymorphisms are linked with susceptibility to TB and with different outcomes of BCG vaccinations between individuals [20]. Different *M. tuberculosis* lineages seem to have adapted to distinct host populations during evolution, and, hence, the degree of virulence/persistence of *M. tuberculosis* depends in part on the genetic background of the host [21].

It has been known for some time that repeated BCG vaccination can have detrimental effects ("Koch phenomenon"). Recently, it has been revealed that repeated exposure to mycobacterial antigen, especially in the form of BCG vaccination after *M. tuberculosis* infection, promotes IL-17-dependent immunopathologies of the lung [22]. In this study, repetitive vaccination was found to cause abundant cytokine expression and recruitment of granulocytes to infected tissue. Apparently, repeated exposure to antigen disturbs the homeostatic balance between disease containment and immunopathology.

Many of these problems, however, are not BCG-specific and have important implications for the design of effective vaccines against *M. tuberculosis*.

NEW VACCINE CANDIDATES

Considering that about 2 billion humans are presumably infected with *M. tuberculosis*, with only 10 percent developing active disease, it is obvious that vaccination strategies follow two different approaches: pre-exposure vaccination in order to prevent disease in individuals that have so far not encountered *M. tuberculosis* versus post-exposure vaccination that aims at inhibiting disease outbreak in individuals that are already infected. Up to now, the majority of novel candidates belongs to the first group. According to their strategies of how to support the immune system, four categories can be distinguished.

The first category follows the approach to improve the current BCG vaccine through recombinant (r)BCG strains. The two major representatives of this group are rBCG30, which is a BCG strain overexpressing the immunodominant *M. tuberculosis* antigen 85B, and rBCGΔUreC:Hly, which is deficient in urease (with the consequence of an acidic pH in vaccine-containing phagosomes) and expresses listeriolysin (which enables it to perforate the phagosomal membrane) [23]. Both candidates have successfully completed Phase I clinical trials. The mechanism underlying their function is improved antigen presentation, which in turn leads to a stronger T cell response. Most importantly, their safety in preclinical tests was

improved in comparison to BCG, especially in immunocompromised mice. As potential BCG replacements, these candidates will function as pre-exposure priming vaccines.

In contrast, the second category of vaccine candidates is considered more for heterologous prime-boost strategies, with BCG or rBCG as the prime. The first subgroup includes viral vectors that express immunodominant *M. tuberculosis* antigens for the initiation of strong lymphocyte responses. MVA85A is a modified vaccinia strain [24], while AERAS-402 and AdAg85A make use of adenoviruses that are incapable of replication, with the advantage of a strong lung tropism that leads to an increased expression of immunodominant antigen at the site of mycobacterial entry [25,26]. It may be problematic, however, for their application in humans, if neutralizing antibodies against the viral vectors in the recipient clear the viral particles before they have had a chance to exert an immunostimulatory effect.

The second subgroup for heterologous prime-boost comprises fusion proteins of immunodominant antigens, again with the aim of mounting strong immune responses against immunologically important *M. tuberculosis* antigens. To ensure immunogenicity, antigenicity has to be combined with adjuvanticity, and, hence, these vaccine candidates are administered as protein adjuvant formulations. Hybrid-1 includes the antigens 85B and ESAT-6 and has been combined with adjuvants IC-31 (a TLR9 agonist plus polycationic peptide) and CAF01 (a mycobacterial cell wall component delivered in cationic liposomes). IC-31 is also used in combination with HyVac4/AERAS-404, which also includes the antigen 85B, but together with TB10.4 instead of ESAT-6 [27]. In addition, the antigens Rv1196 and Rv0125, whose function is poorly understood, are combined in the M72 vaccine that is supplemented with adjuvants AS01 or AS02 (which exert a TLR4-agonistic effect) [28].

Finally, the inactivated mycobacteria *M. vaccae* and the semi-purified *M. tuberculosis* fragments RUTI are considered for application after infection, more precisely as therapeutic vaccinations that could potentially synergize with chemotherapy [29,30]. *M. vaccae* is a whole-cell vaccine that consists of heat-inactivated environmental mycobacterial saprophytes. This vaccine is thought to mount a protective immune response by providing cross-reactive antigens. RUTI comprises detoxified and fragmented *M. tuberculosis* components carried in liposomes. The rationale behind both strategies takes advantage of the bactericidal effects of chemotherapy in order to eliminate growing bacteria and subsequently reduce the likelihood of regrowth of remaining pathogens through the elicitation of a strong cellular immune response.

NEW HOPES AND MAJOR OBSTACLES

With the vaccine candidates described above, the end of the pipeline is still far from being reached. Instead, ongoing basic research that aims at further vaccine improvement has revealed mechanisms that potentially can be exploited for vaccine constructs. For instance, efforts to improve the efficacy and safety of live vaccine strains include the deletion of anti-apoptotic genes, the modification of DNA repair molecules, and the generation of auxotrophic strains [31]. These strategies still allow the vaccine to replicate and survive for a limited time period (as long as necessary for initiating sufficient immunity), but decrease the risk of dissemination by restricting its life time to a minimum.

All of the vaccine candidates described above aim at preventing primary TB or reactivation of latent TB. However, the ultimate goal is to achieve sterile eradication, i.e., the complete elimination of *M. tuberculosis* from the host after infection. The optimal future vaccination scenario could conceivably consist of the following steps:

First, soon after birth, a highly potent BCG replacement will be given. The two currently evaluated candidates are promising, and new approaches are on their way, including AERAS-rBCG, which combines the strategies of rBCG30 and rBCGΔUreC:Hly

[32], and an *M. tuberculosis* mutant that lacks the virulence factors phoP and fadD26 [33].

Booster vaccines — either in the form of viral vectors or protein/adjuvant formulations — will then be given repeatedly, ideally comprising a whole array of antigens from different stages of the *M. tuberculosis* life cycle. Thus, boosters given during infancy should reflect the profile of antigens associated with the metabolically active state, while for adults, it might be an advantage to include dormancy antigens. Such an optimally tailored vaccination, however, remains a major aim of current and future research.

Another vision for further development aims at improving the antibody response against *M. tuberculosis*, with a special focus on pre-existing antibodies that are available quickly enough to opsonize the mycobacteria briefly after their entry into the lung. Tremendous progress toward prevention of the disease would be achieved if antibodies could target *M. tuberculosis* to phagocytic Fc receptors and thereby strongly facilitate uptake and subsequent killing of the pathogen by activated macrophages. Moreover, efficient phagocytosis of mycobacteria that enter the alveolar system of the lung would prevent the infection of "bystander" cells, such as epithelial cells or freshly recruited non-professional phagocytes, which are less efficient in clearing the pathogen and can be used by *M. tuberculosis* as a niche to escape elimination.

What are the main roadblocks for further development and assessment of current vaccine candidates? First, the time needed for clinical evaluation is critically dependent on reliable biomarkers that allow for the distinction between non-infected individuals, latently infected subjects, and patients with active disease [34]. Optimally, a biosignature — based on data from transcriptome, proteome, and metabolome analysis — will be able to predict the clinical endpoint of disease outbreak [35]. Current diagnostic tests mainly focus on IFNγ production by peripheral lymphocytes, but new indicators are on their way, potentially including different cytokines and antigens associated with dormant and active *M. tuberculosis* [36].

Second, suitable animal models are crucial for determining the effectiveness of new vaccine candidates and evaluating their function in various circumstances, including immunodeficient recipients. The mouse as the most widely used model to study disease has a certain limitation insofar as it does not reflect the full spectrum of granuloma formation upon *M. tuberculosis* infection. However, various genetically modified mouse strains allow the analysis of mediators that participate in the immune response and the determination of factors that are critical for vaccine-induced immunity. Very recently, mice deficient in nitric oxide synthase 2 have been reported to manifest human-like granumolas after dermal infection with *M. tuberculosis* [37]. The spectrum of granulomas is found in Guinea pigs, where they show a similar composition of cells to humans. TB in nonhuman primates resembles human disease, but cost and ethical considerations are major hurdles for broad application. Nonetheless, the lack of an optimal animal model still remains a barrier for current efforts to test vaccine efficacies.

CONCLUSION

After decades of stagnancy in TB research, the past 10 years have shown considerable progress in our understanding of the interaction between *M. tuberculosis* and its host, and this understanding has lead to the development of about a dozen vaccine candidates that are currently evaluated in clinical trials. There are not many fields of biomedical research in which science potentially can contribute to such a high extent to socioeconomic development in numerous countries as is the case in the ongoing quest against current pandemics. Incentives must be created for researchers to tackle the most urgent roadblocks that still thwart an efficient prevention and treatment of the major infectious diseases, many of which are generally considered both preventable and cur-

able [38]. By accepting the principle of global open access, partnerships between public, philanthropic, and private institutions can lay the foundation for improved development and clinical evaluation of new diagnostics, drugs, and vaccines.

In the development of a new vaccine against TB, the first steps have been taken, as outlined in this article. Nonetheless, major challenges remain, and both scientific and financial investments will prove pivotal for further substantial progress in the coming years, with the ultimate goal to create a strategy that allows for prevention of *M. tuberculosis* infection or sterile eradication of *M. tuberculosis*.

Acknowledgments: We thank the members of the Kaufmann laboratory for helpful discussions and comments. CAT receives a scholarship from Yale University and the German Academic Exchange Service.

REFERENCES

1. Lönnroth K, Castro KG, Chakaya JM, et al. Tuberculosis control and elimination 2010-50: cure, care, and social development. Lancet. 2010;375(9728):1814-29.
2. Kaufmann SHE, Hussey G, Lambert P. New vaccines for tuberculosis. Lancet. 2010;375(9731):2110-9.
3. Russell DG, Barry CE, Flynn JL. Tuberculosis: what we don't know can, and does, hurt us. Science. 2010;328(5980):852-6.
4. Cooper AM. Cell-mediated immune responses in tuberculosis. Annu Rev Immunol. 2009;27:393-422.
5. Russell DG. Who puts the tubercle in tuberculosis? Nat Rev Microbiol. 2007;5(1):39-47.
6. van der Wel N, Hava D, Houben D, et al. M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. Cell. 2007;129(7):1287-98.
7. Russell DG, Cardona P, Kim M, et al. Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol. 2009;10(9):943-8.
8. Divangahi M, Desjardins D, Nunes-Alves C, et al. Eicosanoid pathways regulate adaptive immunity to Mycobacterium tuberculosis. Nat Immunol. 2010;11(8):751-8.
9. Cooper AM. T cells in mycobacterial infection and disease. Curr Opin Immunol. 2009;21(4):378-84.
10. Quesniaux VFJ, Jacobs M, Allie N, et al. TNF in host resistance to tuberculosis infection. Curr Dir Autoimmun. 2010;11:157-79.
11. Khader SA, Cooper AM. IL-23 and IL-17 in tuberculosis. Cytokine. 2008;41(2):79-83.
12. Harding CV, Boom WH. Regulation of antigen presentation by Mycobacterium tuberculosis: a role for Toll-like receptors. Nat Rev Microbiol. 2010;8(4):296-307.
13. Kurts C, Robinson BWS, Knolle PA. Cross-priming in health and disease. Nat Rev Immunol. 2010;10(6):403-14.
14. Pichichero ME. Booster vaccinations: can immunologic memory outpace disease pathogenesis? Pediatrics. 2009;124(6):1633-41.
15. Parida SK, Kaufmann SHE. Novel tuberculosis vaccines on the horizon. Curr Opin Immunol. 2010;22(3):374-84.
16. Ho MM, Southern J, Kang H, et al. WHO Informal Consultation on standardization and evaluation of BCG vaccines Geneva, Switzerland 22-23 September 2009. Vaccine. 2010;28(43):6945-50.
17. Franco-Paredes C, Rouphael N, del Rio C, et al. Vaccination strategies to prevent tuberculosis in the new millennium: from BCG to new vaccine candidates. Int J Infect Dis. 2006;10(2):93-102.
18. Weir RE, Black GF, Nazareth B, et al. The influence of previous exposure to environmental mycobacteria on the interferon-gamma response to bacille Calmette-Guérin vaccination in southern England and northern Malawi. Clin Exp Immunol. 2006;146(3):390-9.
19. Hatherill M, Adams V, Hughes J, et al. The potential impact of helminth infection on trials of novel tuberculosis vaccines. Vaccine. 2009;27(35):4743-4.
20. Dye C, Williams BG. The population dynamics and control of tuberculosis. Science. 2010;328(5980):856-61.
21. Caws M, Thwaites G, Dunstan S, et al. The influence of host and bacterial genotype on the development of disseminated disease with Mycobacterium tuberculosis. PLoS Pathog. 2008;4(3):e1000034.
22. Cruz A, Fraga AG, Fountain JJ, et al. Pathological role of interleukin 17 in mice subjected to repeated BCG vaccination after infection with Mycobacterium tuberculosis. J Exp Med. 2010;207(8):1609-16.
23. Grode L, Seiler P, Baumann S, et al. Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guérin mutants that secrete listeriolysin. J Clin Invest. 2005;115(9):2472-9.
24. McShane H, Pathan AA, Sander CR, et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat Med. 2004;10(11):1240-4.
25. Radosevic K, Wieland CW, Rodriguez A, et al. Protective immune responses to a recombinant adenovirus type 35 tuberculosis vaccine in two mouse strains: CD4 and CD8 T-cell epitope mapping and role of gamma interferon. Infect Immun. 2007;75(8):4105-15.
26. Santosuosso M, McCormick S, Zhang X, et al. Intranasal boosting with an adenovirus-vectored vaccine markedly enhances protec-

tion by parenteral Mycobacterium bovis BCG immunization against pulmonary tuberculosis. Infect Immun. 2006;74(8):4634-43.
27. Dietrich J, Andersen C, Rappuoli R, et al. Mucosal administration of Ag85B-ESAT-6 protects against infection with Mycobacterium tuberculosis and boosts prior bacillus Calmette-Guerin immunity. J Immunol. 2006;177(9):6353-60.
28. Von Eschen K, Morrison R, Braun M, et al. The candidate tuberculosis vaccine Mtb72F/AS02A: Tolerability and immunogenicity in humans. Hum Vaccin. 2009;5(7):475-82.
29. Vilaplana C, Montané E, Pinto S, et al. Double-blind, randomized, placebo-controlled Phase I Clinical Trial of the therapeutical antituberculous vaccine RUTI. Vaccine. 2010;28(4):1106-16.
30. von Reyn CF, Mtei L, Arbeit RD, et al. Prevention of tuberculosis in Bacille Calmette-Guérin-primed, HIV-infected adults boosted with an inactivated whole-cell mycobacterial vaccine. AIDS. 2010;24(5):675-85.
31. Kaufmann SHE. Novel tuberculosis vaccination strategies based on understanding the immune response. J Intern Med. 2010;267(4):337-53.
32. Sun R, Skeiky YAW, Izzo A, et al. Novel recombinant BCG expressing perfringolysin O and the over-expression of key immunodominant antigens; pre-clinical characterization, safety and protection against challenge with Mycobacterium tuberculosis. Vaccine. 2009;27(33):4412-23.
33. Verreck FAW, Vervenne RAW, 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(4):e5264.
34. Wallis RS, Pai M, Menzies D, et al. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet. 2010;375(9729):1920-37.
35. Kaufmann SHE, Parida SK. Tuberculosis in Africa: learning from pathogenesis for biomarker identification. Cell Host Microbe. 2008;4(3):219-28.
36. Parida SK, Kaufmann SHE. The quest for biomarkers in tuberculosis. Drug Discov Today. 2010;15(3-4):148-57.
37. Reece ST, Loddenkemper C, Askew DJ, et al. Serine protease activity contributes to control of Mycobacterium tuberculosis in hypoxic lung granulomas in mice. J Clin Invest. 2010;120(9):3365-76.
38. Ottenhoff THM. Overcoming the global crisis: "yes, we can", but also for TB ... ? Eur J Immunol. 2009;39(8):2014-20.