Limitations of the BCG vaccine and new prophylaxis strategies against human tuberculosis

Limitações da vacina BCG e novas estratégias profiláticas contra tuberculose humana

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ABSTRACT

BCG (Bacille Calmette Guérin), an attenuated vaccine derived from *Mycobacterium bovis*, is the current vaccine against tuberculosis. Notwithstanding its protection of children, BCG has failed to protect adults against active pulmonary tuberculosis, especially in countries where the disease is endemic. Any new tuberculosis vaccine should protect several categories of people, including children, adults, the elderly and immunodeppressed patients. An important feature is immunization safety for all of these classes. The aim of this review is to describe new vaccination strategies, such as subunit vaccines, DNA vaccines, vaccines with live microorganisms and vectors, and to discuss the application of these new strategies for the control and eradication of tuberculosis.

Keywords: Tuberculosis/prevention & control; BCG/therapeutic use; Vaccines, DNA/therapeutic use; Vaccines, DNA/administration & dosage; Vaccines; subunit/administration & dosage; Tuberculosis vaccines; Recombinant proteins/immunology

RESUMO

A atual vacina contra a tuberculose, o BCG (Bacilo Calmette Guérin), uma vacina atenuada, derivada do *Mycobacterium bovis*, apesar de proteger as crianças contra a enfermidade, falha na proteção contra a tuberculose pulmonar ativa em adultos, principalmente em países onde a doença é endêmica. Uma nova vacina para tuberculose deve proteger várias categorias de indivíduos, como crianças, adultos, idosos e imunocomprometidos. Sendo assim, uma característica importante a se considerar é a seguridade vacinal para todas as classes de imunizados. Esta revisão propõe apresentar as novas estratégias de vacinação, tais como subunidades vacinais, vacinas de DNA, vacinas com micro-organismos e vetores vivos e discutir as aplicações dessas novas estratégias no controle e erradicação da tuberculose.

Descritores: Tuberculose/prevenção & controle; BCG/uso terapêutico; Vacinas de DNA/uso terapêutico; Vacinas de subunidades/administração & dosagem; Vacinas de DNA/administração & dosagem; Vacinas contra a tuberculose; Proteínas recombinantes/imunologia

INTRODUCTION

Tuberculosis (TB) remains as one of the most serious Public Health problems worldwide; it is one of the main causes of death in poor and developing countries, especially in sub-Saharan Africa, where it may be associated with the human immunodeficiency virus (HIV)[1,2]. It has been estimated that one third of the world population is infected by *Mycobacterium tuberculosis*, and that there are about 8 to 9 million new TB cases each year, and about 1 to 2 million yearly deaths due to TB[2].

M. tuberculosis is transmitted from person to person, starting with a bacilliferous respiratory (pulmonary and/or laryngeal) TB carrier through respiratory routes (coughing, sneezing, speaking, singing, and breathing). Infected particles are named Flügge's droplets that, when dry, are named Wells' droplet-nuclei (containing not more than three bacilli). After overcoming the defenses of the respiratory tract (nasal hairs, mucus and ciliary beats), Wells' droplet-nuclei reach the alveoli, where they are phagocytosed by alveolar macrophages. If *M. tuberculosis* survives, the bacilli multiply within the macrophages. The bacilli are released upon cell death and proceed to infect other macrophages and multiply, resulting in a large number of bacteria in the primary

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pulmonary lesions. Bacilli may enter lymphatic vessels and spread to regional lymph nodes, forming what is known as Ranke primary complex, which consists of inoculation site granulomas (Gohn's nodules), lymphangitis, and enlarged regional lymph nodes. From primary complex lymph nodes, bacilli may reach tracheal and vertebral lymph nodes. Bacilli may also enter the bloodstream through the thoracic duct, establishing themselves in the upper portions of the lungs or in various other organs[3-6]. The primary infection remains controlled in about 95% of adults, in which the bacilli remain latent (with no or little metabolic activity); at this point, hypersensitivity to the bacillus develops and the intradermal tuberculin test (PPD) becomes positive. Carriers of latent *M. tuberculosis* infection have a 5% risk of developing active TB throughout life; the risk is higher within the first two years of infection[3-6].

The HIV (or acquired immunodeficiency syndrome, Aids) infection is related to an increased risk of TB (annual risk of 7 to 10% in patients co-infected with HIV/*M. tuberculosis*). It is also responsible for a major proportion of TB cases where both epidemics coexist, particularly in sub-Saharan Africa, thus becoming a barrier against controlling TB[1].

Many countries have been unable to implement a diagnostic network, to provide appropriate anti-TB medication, or even to adopt directly observed therapy (DOT)[2]. Additionally, current control strategies do not deal with the huge reservoir of individuals with latent *M. tuberculosis* infection[2,7].

Thus, novel anti-TB vaccines (for prevention or therapy) would be one of the most significant developments in the global struggle against tuberculosis[8-10].

BCG VACCINE

BCG (Bacille Calmette-Guérin) is the only vaccine available for preventing TB in humans. The BCG vaccine was developed in France between 1908 and 1921 by Albert Calmette (1863-1933) and Camille Guérin (1872-1961), following 230 serial passes of a pathogenic *Mycobacterium bovis* strain in laboratory. This attenuated version, the Bacille Calmette-Guérin (BCG) strain, was non-virulent in cattle, horses, rabbits and guinea pigs. In 1921, this then recently developed vaccine was administered to French children, resulting in a 90% mortality decrease. BCG has since become one of the most widely used vaccines worldwide. It is thought that about 3 billion doses have been given since 1921. About 115 million doses are given annually to around 80% of children in the world[9,8,11].

Many laboratories worldwide have sub-cultivated BCG variants since it was first used in 1921. BCG vaccines may be sub-classified into two main groups: the Tokyo, Moreau, Russia and Sweden BCG group, which secretes a significant amount of the MPB70 gene, contain two copies of the IS6110 insertion sequence and harbors the MPB64 and methoxymycolate genes; and the Pasteur, Copenhagen, Glaxo and Tice BCG group, which secretes little MPB70, contains a single IS6110 insertion copy and does not have the MPB64 and methoxymycolate genes. Comparative genomic analyses between BCG and *M. bovis*, and among other strains, have shown that BCG lost over 100 genes in comparison to *M. bovis*. Such gene diversity has probably resulted in phenotypical and immunological differences that may explain variations in the effectiveness of BCGs[11].

Although BCG is a low-cost and widely used vaccine, its efficacy has widely varied in clinical assays – values ranged from 0 to 80%. Many explanations were given for such discrepancy in the protective effect of BCGs: a) method differences among clinical assays; b) genetic differences among sample populations; c) degrees of malnutrition in vaccinated subjects; d) variation in the virulence of *M. tuberculosis* strains; e) effects of environmental mycobacteria exposure on the immune response to BCG; and f) different protection levels against clinical forms of TB[8-9,11-12].

Notwithstanding such differences in the efficacy of BCGs against pulmonary TB, it is clear that these vaccines protect children from severe clinical forms of TB, such as tuberculous meningitis and miliary TB. A recent metanalysis[13] suggested that the 100.5 million BCG doses given to children in 2002 prevented 29,729 cases of tuberculous meningitis (CI95%: 24,063 - 36,196) during the first five years of life in those children, one case for each 3,435 vaccinations (2,771 - 4,177), and 11,486 cases of miliary TB (7,304 - 16,280), one case for each 9,314 vaccinations (6,172 - 13,729). The majority of studies, however, have reported that the protective effect of BCG lasts for not more than 10 to 20 years[8].

Arlindo de Assis first used BCG in Brazil in 1927; he produced the vaccine at the Liga Brasileira contra a Tuberculose (Brazilian League against Tuberculosis) from the Moreau strain, which was named the Moreau – Rio de Janeiro BCG. Intradermal BCG was started in Brazil only in 1968[12,14]. A study conducted in Manaus and Salvador revealed that neonatal BCG vaccination protected against TB for 15 to 20 years (39%; CI95%: 9 - 58)[15]. A case-control study in the metropolitan area of São Paulo showed that the efficacy of BCG for protection against tuberculous meningitis reached 84.5%[16].

Recommendations for the use of BCG vaccine in Brazil are as follows: a) newborn weighing at least 2 kg, with no clinical events; b) newborn of mothers with the Aids; c) HIV-positive children or children of mothers with the Aids if they are tuberculin-negative

and asymptomatic; d) contacts of leprosy patients; e) tuberculin-negative healthcare professionals (healthcare professionals working with leprosy patients should be treated as contacts of such patients); e) non-reactors to tuberculin admitted to military service; and f) the native Indian population with no vaccine scar[12,17-18]. It is expected that the Ministry of Health will review the recommendations for BCG vaccination of healthcare professionals in the coming years[12].

NEW VACCINES

Because BCG offers poor protection against pulmonary tuberculosis in adolescents and adults, a new TB vaccine is needed, especially in countries with a high prevalence of this disease.

Live microorganism compound vaccines are potential alternatives, since they induce cell and humoral immune responses and require no adjuvants, since the bacterial components fulfill this role. These vaccines, however, are not risk-free; reversion of virulence or possible disease induction in the presence of immunosuppression may occur[19].

The advantage of attenuated *M. tuberculosis* vaccines is that they are more immunogenic, since hundreds of genes are lost in repeated passes of *M. bovis* – BCG bacilli in the laboratory[20]. An example is the RD1 region of *Mycobacterium tuberculosis*, which is probably associated with cytotoxic protein production[21].

Many studies of attenuated *M. tuberculosis* strains have been carried out, such as *M. tuberculosis pho*, built from a single rupture of the *pho* gene. This gene product is part of a protein complex that allows microorganisms to survive when faced with different external stimuli. The mutant *pho*P strain is less able to multiply when cultivated in bone marrow derived macrophages in mice. This mutation does not change the survival of the bacillus within macrophages, although the gene is necessary for intracellular growth of *M. Tuberculosis*[22]. Recently, a mutant *M. tuberculosis* with a defect in a mycobacterial lipid (Mtb *drrC*) was shown to provide more protection in mice compared to BCG[23].

Studies applying recombinant DNA techniques aiming at changing BCG *M. bovis* or *M. tuberculosis* to yield new anti-TB attenuated vaccines have also been carried out[24]. A *M. tuberculosis* SO2 mutant strain was tested in guinea-pigs based on this rationale; it provided satisfactory protection, increasing the survival and reducing disease severity, compared to BCG.

BCG safety is a growing concern because of HIV infection. Auxotrophs, microorganisms that require an external source of growth factors (not being able to synthesize them), were developed to avoid the potentially adverse effects of BCG in immunodeficient individuals. Results have shown that these strains are safe in severely combined immunodeficient mice, also providing similar protection in normal TB-susceptible mice, which suggests that this vaccination method is safer[24]. Derrick[25] worked with a mutant *M. tuberculosis* ($mc^2$6030) with limited replication on normal and immunodeficient mice. Additionally, $TCD8^+$, NK1.1 and TCR$\gamma\delta$ depletion yielded poor vaccine-induced protection, suggesting that double-negative cells were responsible for vaccine-induced protection. A further finding was that these cells are MHC II-dependent and secrete INF-γ, but less than $TCD8^+$. Henao-Tamayo[26] also investigated the virulence of *M. Tuberculosis* by removing the Rv3763 lipoprotein from the TB bacillus. Protection against tuberculosis was similar among animals given the mutant *M. tuberculosis* and BCG; CD4, CD8, and IFN-γ-secreting cell activation was also similar, suggesting that vaccinogenic properties were preserved notwithstanding the attenuation of mutated microorganisms.

Using DNA vaccines is a risky alternative, because these vaccines are made to code many antigens selected not to interfere with skin sensitivity tests. Selection of DNA vaccine antigens is limited by expressed protein immunogenicity. Many DNA vaccines containing plasmids with mycobacterial antigen genes, such as mycolyl transferase (complex 85) members[27] and heat shock proteins (Hsp60, 65, 70)[28-31], have been tested against tuberculosis in animal models.

Okada[32] worked with DAN vaccines by combining the heat shock protein 65 (HSP-65) and IL-12, using the hemagglutinating virus of Japan (HVJ) as a vector, to increase therapeutic efficacy and T CD8+ and CD4+ cell expression in an MDR-TB-boosted murine model. In a simian model, this vaccine yields effective protection (survival and immune response), indicating that it may be used against *M. tuberculosis*, including MDR-TB.

Ag85-85A, 85B and 85C–PstS-1 antigens, the heat shock proteins hsp65 and 70, and ESAT-6 are the main candidates for making a DNA vaccine against tuberculosis[33-34]. These antigens induce a strong immune response by mobilizing the cell system, CD4 - CD8, Th1, Th2, macrophages, and monocytes in general, producing active cytokines such as interferon gamma and the alpha tumor necrosis factor. Mice given DNA vaccines showed evidence of cell mobilization and acquired significant anti-TB protection[34]. Paula[35] improved DNA vaccines by co-encapsulating DNAhsp65 and the trehalose dimycolate (TDM) adjuvant in biodegradable spheres so that the vaccine could be given in a single dose. These authors tested mice and guinea pigs, demonstrating good effectiveness and decreased lung disease in both species.

Proteins CFP-10 (Rv3874), GroES (Rv3418c) and the complex 85 are often used as recombinant antigens due to their T-cell activation inducing ability. These antigens induced similar protective Th1 responses in mice, suggesting that none are immunodominant[36,24]. The ESAT-6 (early secreting antigenic target 6) (Rv3875), a 6-Kda protein coded in the RD-1 (region of difference 1) and characterized by Sorensen[37-38] from various *M. Tuberculosis* complex species – except for *M. bovis* BCG subtypes – has been widely used[37,39-40]. There was a strong T-cell response to ESAT-6 in *M. tuberculosis* infected animals that were treated and reinfected, suggesting that this protein is also immunogenic[24,41-42]. Brandt[43] confirmed this fact by evaluating the potential of ESAT-6 as a vaccine model; the author showed that vaccination with this antigen induces a T-cell response and similar protection as BCG. Rigano[44] studied transgenic plants (*Arabidopsis thaliana*) to express a fusion protein containing the *M. tuberculosis* ESAT-6 antigen and an *Escherichia coli* enterotoxin (LTB-ESAT-6); the author used a mouse ration and immunized these animals orally. Protection was poor following an *M. tuberculosis* boost, notwithstanding an increased CD4+ T-cell IFN-γ production in mesenteric lymph nodes. These results suggest that being immunogenic is not a sufficient condition; the administration route of any vaccine is crucial for protection.

VACCINATION STRATEGIES FOR NEW VACCINES AGAINST TUBERCULOSIS

Any new anti-TB vaccine, regardless of efficacy in animals, necessarily needs to go through many clinical trials before being made available for immunization programs worldwide.

Before starting clinical trials, product (vaccine) profiles need to be clearly defined, such as: target groups – newborn, children, adolescents, adults, non-infected individuals, infected individuals, immunocompetent patients, immunodeficient patients, etc.; protection class – protection against infection, against pulmonary TB, against disseminated disease, etc.; safety – safer or less safe than BCG; dosage and vaccination schemes – one dose after birth with a second dose in childhood and a third dose in adolescence[45-46].

A first hurdle after animal tests is safety testing in human beings (phase I clinical trials). These tests initially require a small sample comprising healthy, tuberculin-negative adults, and are conducted in the country where the vaccine is developed. Additional phase I trials may be undertaken in tuberculin-positive subjects, children, or in other groups for which vaccination is indicated. Phase II trials are done for candidate vaccines approved in phase I clinical trials. Phase II trials consist of gathering clinical samples to measure the immunological response to the vaccine (immunogenicity). Vaccines approved in phase I (safety) and II (immunogenicity) trials are tested in phase III trials to verify whether target populations are in fact protected against TB, as defined in the vaccine profile (efficacy)[45,47].

Phase I and II trials are relatively small and inexpensive and, generally, carried out by those who are responsible for developing the vaccine. Phase III (efficacy) trials, however, are large, complex and expensive, and require partnerships among many international public and private organizations. The following example illustrates the complexity of these trials: testing a new vaccine as a primary immunogenic vaccine (priming vaccine), such as a recombinant BCG or attenuated *M. tuberculosis*, requires this vaccine to be used in non-infected (*naïve*) subjects and compared to a placebo or non-vaccinated group. The protection of adults against pulmonary TB by this preventive vaccine may only be detected decades later, since TB progresses slowly. This type of trials requires at least 100 thousand participants, a discouraging perspective for any healthcare system or vaccine manufacturer[9,45,47].

A faster strategy is to compare the efficacy of priming vaccines with a standard BCG vaccine to study its effect on childhood TB. Although such study requires a large sample, it may be ready within a few years[45].

New anti-TB vaccines may be extremely useful in countries with higher incidence and prevalence of TB and in countries with ample BCG vaccine coverage. Thus, new anti-TB vaccines need to be tested in this context. Because few of the new vaccines used individually have provided superior protection in laboratory animals in comparison to BCG, many research groups have adopted strategies with an initial vaccination (preventive) followed by reinforcement vaccination (boost); in other words, a priming-boost strategy. In this existing model, BCG-induced immunity is boosted by a new vaccine (for example, protein/peptide or attenuated vector)[8,9,45,47].

The abovementioned strategies are examples of preventive or priming vaccination aimed at avoiding *M. tuberculosis* infection. A new therapeutic vaccine (post-exposure) would be interesting for controlling tuberculosis worldwide, a vaccine that could be given to subjects with latent *M. tuberculosis* infection (MTBLI) – which comprises about one third of the world population – to halt tuberculosis. It is not fully clear whether preventive vaccines in current development would be efficient, or even safe, in MTBLI. Safety issues have also been questioned because of the possibility that vaccines could cause Koch's phenomenon in subjects with MTBLI. Robert Koch found that guinea-pigs inoculated with whole bacteria or culture filtrates presented necrosis of the

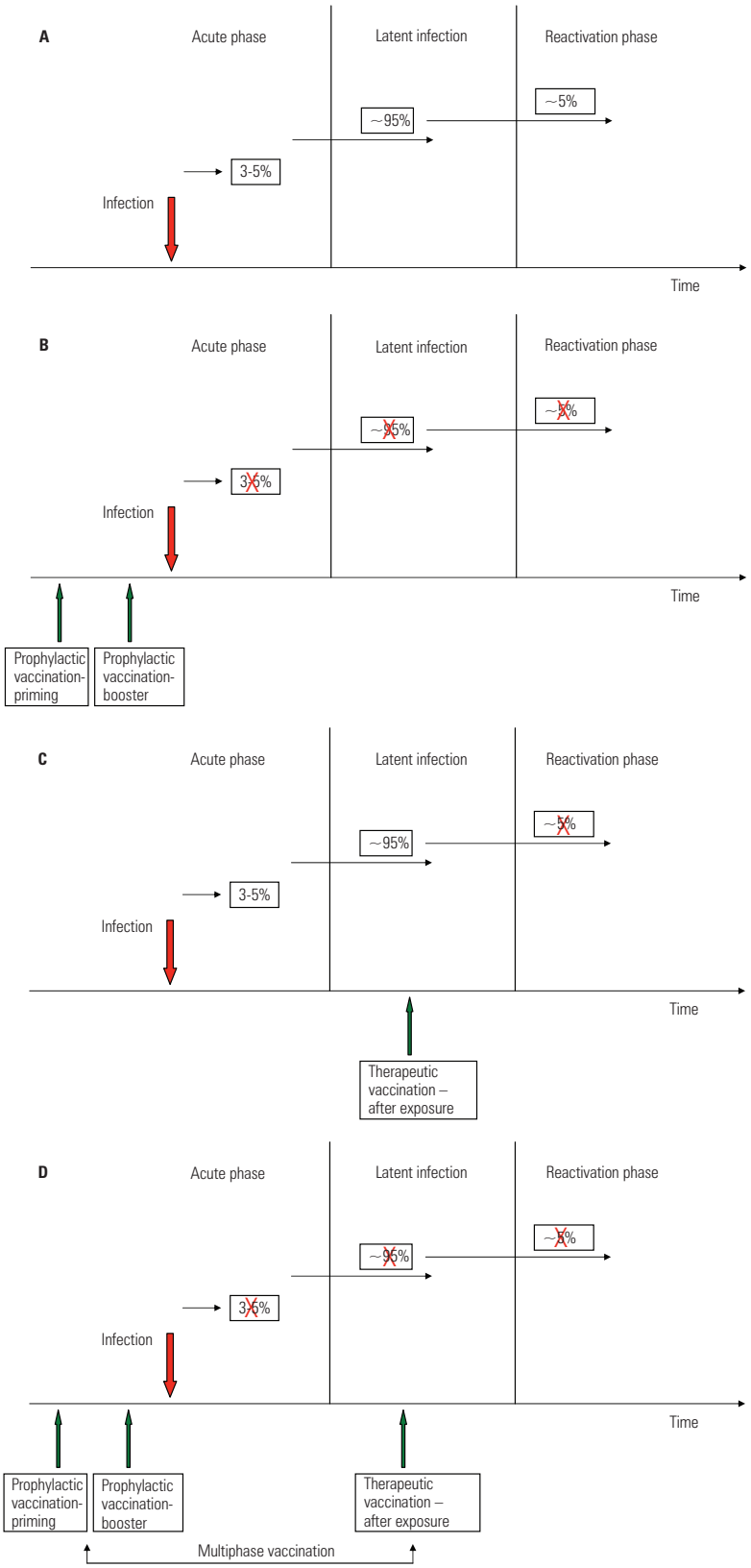

Figure 1. (A) The natural evolution of *M. tuberculosis* infection. Three to five percent of subjects will manifest clinical symptoms of primary TB, and about 95% will remain asymptomatic, harboring the bacilli in their organisms and becoming latent infection carriers of *M. tuberculosis* (MTBLI). Five percent of MTBLI patients will progress to secondary TB. (B) The desired effect of a preventive anti-TB vaccine is to avoid infection by *M. tuberculosis* and the development of tuberculosis in its various clinical forma. These vaccines should be given early – soon after birth – as a single dose; or preferentially as a first single immunizing dose and a subsequent boost. (C) A therapeutic vaccine (post-exposure) could be given after *M. tuberculosis* is established, to avoid MTBLI patients from falling ill with the disease. (D) A potentially useful anti-TB vaccination scheme in endemic countries could include a preventive vaccine to avoid that susceptible individuals become infected; a therapeutic vaccine could be added to avoid that MTBLI patients become diseased (multiphase vaccination).

inoculation site or the original TB site four to six weeks after the onset of infection. PPD inoculation in patients with active TB may result in inoculation site necrosis. The data available from experimental models did not show that the main vaccine candidates could induce Koch's phenomenon, as well as did not show any effect against latent infection. It is known that, during active infection, the gene expression of *M. tuberculosis* differs from that in the latent phase, and that the immune responses in healthy subjects with MTBLI focus on different antigens compared to those expressed in active infection. Thus, a therapeutic vaccine should contain antigens that are over expressed in the latent phase, such as HspX (α-crystalline) or rfp (resuscitation-promoting factor) gene products. A post-exposure (therapeutic) vaccine is a more remote possibility than new preventive anti-TB vaccines [8-10,46]. Figure 1 summarizes possible strategies for new anti-TB vaccines.

Being optimistic, and considering that there are a number of vaccines in phase I and II trials, it is thought that there might be one or more approved and distributed new anti-TB vaccines by around 2015, which will help to effectively control tuberculosis[47].

FINAL CONSIDERATIONS

Although the efficacy of BCG has been widely debated, particularly because it does not protect adults from active pulmonary tuberculosis, this vaccine is still the only one available for protecting children against tuberculosis.

Subunit vaccines are safer and allow for superior quality control in comparison to live mycobacterial vaccines, being, therefore, good candidates for augmenting the effect of BCG.

There are many candidate vaccines undergoing laboratory tests; it is expected, however, that few of these will be used clinically.

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