Tuberculosis vaccine

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ABSTRACT

لا يزال مرض السل (TB) واحد من الأسباب الرئيسية للوفاة من عامل التهاب حيث أنه يؤدي إلى مقتل ما يقارب 1.6 مليون شخص سنوياً، معظمهم في البلدان النامية. وتتسم فاعلية اللقاحات الموجودة (BCG) بالكفاءة في مجال منع انتشار هذا المرض لدى الأطفال وحديثي الولادة، ولكن فعاليته ضد السل TB النشط في البالغين مشكوك فيه من قبل عدد من الدراسات السريرية. وهناك تصور عام أن تطوير لقاح جديد أكثر فعالية ضد مرض السل TB من شأنه التخفيف من هذا المرض الفتاك. لقد أصبح توفير لقاح جديد أو تطوير لقاح السل TB من الاحتياجات العاجلة وذلك في السنوات الحديثة. تشمل مثل هذه اللقاحات الجديدة كائنات جديدة و تضعف سلالات بكتيريا السل الجديدة، و تطوير ارتباط سلالات (BCG)، والوحدة الفرعية، و لقاحاتDNA.

Tuberculosis (TB) is still one of the leading causes of death from a single infectious agent, killing 1.6 million people each year, mostly in developing countries. The existing vaccines, Bacille Calmette and Guerin (BCG), are efficient in preventing the most severe disseminated forms of disease in children and newborns, but its efficacy against active TB in adults has been challenged by several clinical studies. It is a common opinion that only the development of a new and more effective vaccine against TB would significantly ease the deadly disease. In recent years, looking for a new vaccine or an improved TB vaccine is urgently needed. Such vaccines include new live and attenuated strains of *Mycobacterium tuberculosis*, improved recombinant BCG strains, subunit and DNA vaccines.

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Mycobacterium tuberculosis (*M. tuberculosis*), a gram-positive bacterium that causes tuberculosis, is the most common infectious agent in the world. Tuberculosis (TB) is a disease that is spreading from one person to another through the air. Once an infected person with pulmonary TB coughs; an infectious aerosol containing small droplet nuclei generated. These small droplet nuclei hold tubercle bacilli and reside floating in the air for hours. If an individual inhale these bacilli, an infection occurs.[1] However, despite intensive research, TB is a serious, debilitating and highly infectious disease affecting millions of people worldwide. If not properly treated, it is often fatal. The importance of development of new TB vaccines has improved in recent years as the disease continues to be a major, global public health problem. Major challenges and concerns for TB vaccine development including: 1) It is estimated that close to 2 billion people (nearly 1/3 of the world population) is infected with *M. tuberculosis*. However, the majority (approximately 90%) does not develop the disease or show clinical symptoms over a lifetime. 2) The World Health Organization (WHO) report on Global Tuberculosis Control 2009 (World Health Organization. Global Tuberculosis Control epidemiology, strategy, financing: WHO report 2009) shows that in 2007 more than one and a half million people died of TB, and the incidence of TB is estimated to be close to 9.27 million new cases annually compared with 9.24 million new cases (140 per 100 000 population) in 2006.[2] An estimated 44% or 4.1 million (61 per 100 000 population) were new smear positive cases and approximately 2 million deaths per year. 3) The vaccine currently used, Bacille Calmette and Guerin (BCG), is efficient in preventing the most severe disseminated forms of disease in children and newborns, but its efficacy against active TB in adults has been challenged by several clinical studies. 4) Although effective antituberculous drugs are available, the long treatment regimen (6-12 months) is not conductive to patient compliance which can lead to the development of drug resistance that made control of this pathogen even more challenging. 5) TB and HIV/AIDS form a lethal combination. Globally, there were

an estimated 1.37 million HIV positive TB patients in 2007. Co-infection with HIV is the most common cause of immunosuppression, and infection with HIV increases the risk of reactivation of latent *M. tuberculosis* infection.[2] 6) There are 3,955 TB cases in Saudi Arabia (15.6 cases out of 100,000 persons) according to a report from WHO, Global Tuberculosis Control, 2009. To achieve the WHO Development Goal of having TB prevalence and incidence by 2015, with the ultimate goal of eliminating TB worldwide by 2050,[3] the importance on the production of an effective vaccine against TB would have numerous global impacts.

Tuberculosis is a main health risk in Saudi Arabia, as well as anywhere in the world. Saudi Arabia is a unique place as it has 6 million expatriates for work purposes. In addition, 2-3 million visitors annually visit the country for religious practices. According to the WHO report on Saudi Arabia, 3,955 cases were notified to be affected with TB in 2007.[3] The Information Center of the Ministry of Health, shows that TB is the most widely spread disease in the country affecting Saudis more than non-Saudis. Infections occur primarily in people between the ages of 15 and 44. Jeddah is at the top of the list of infected cities, followed by Riyadh with Qurayyat having the least number of people suffering from TB. In Saudi Arabia, the disease is in control, but there are chances of it increasing, before it was just in poor areas, but now it has become more prominent and is spreading everywhere, so we have become more concerned. Here, we review the development of new vaccines, the main target of which is to prevent infection itself or disease that occurs after infection.

Pathogenesis of TB. The transmission cycle of *M. tuberculosis* starts with an airborne infectious droplet containing bacilli from an individual with pulmonary TB, in which the bacteria are inhaled by a healthy person and taken up by alveolar macrophages.[4] Once macrophage engulfs the bacteria, this phagocyte begins to release cytokines such as TNF-alpha, which provokes a restricted pro-inflammatory response that leads to the enrollment of other immune effectors cells from blood vessels. Thus, a granuloma is formed; a well-organized structure that holds infected macrophages in the center, surrounded by CD4+ and CD8+ T cells (**Figure 1**). This represents the restraint (self-control) phase of the infection, when there are no obvious signs of disease and host transmission is inhibited.[5] *Mycobacterium tuberculosis* is able to continue in this hostile environment of the granuloma, characterized by nutrient starvation, and depleted oxygen, by using several immune evasion strategies such as arresting the phagosome at an early stage of maturation and preventing fusion with lysosomes.[6,7] As well, *M. tuberculosis* can secrete antigens that inhibit the immune response of functional helper T cells.[8,9] This granuloma can persist for decades as *M. tuberculosis* lies in a state of non-replicating persistence,[10] during which this pathogen differentially expresses certain genes. Thus, an effective balance is established between *M. tuberculosis* and the human immune system in which some immunosuppressive event, such as acquiring HIV/AIDS, disturbs this balance in favor of the pathogen, leading to the breakdown of the granuloma causing viable infectious bacilli to be released into the airways, thus leading to the development of pulmonary TB.[3] The unique characteristic of TB has provided many scientists with a new means of vaccine development: ideally, a vaccine should induce a host immune response that would rapidly contain bacterial multiplication, limit tissue damage, and block the development of the disease.

Tuberculosis vaccines. The most efficient way to fight infectious diseases is the use of effective vaccines. Such vaccines have successfully eliminated one of the most lethal human diseases (smallpox). In the case of TB, the BCG vaccine, which has been used for more than 80 years, is inefficient in controlling the disease. The BCG vaccine protects against childhood TB, but this immunity diminishes with age, is not perfect for its limited ability to protect against the adult form of TB. Therefore, TB still represents a main and yet increasing global dilemma. For this reason, the development of a new more efficient TB vaccine(s) than the current BCG vaccine is one of the main concerns in TB research.[11]

The Bacille Calmette and Guerin vaccine. The currently licensed vaccine against TB, BCG is an attenuated strain of *Mycobacterium bovis* a mycobacterium that infects cattle. Since the first report of successful BCG vaccination in 1921, this vaccine became the most widely distributed vaccine to impede

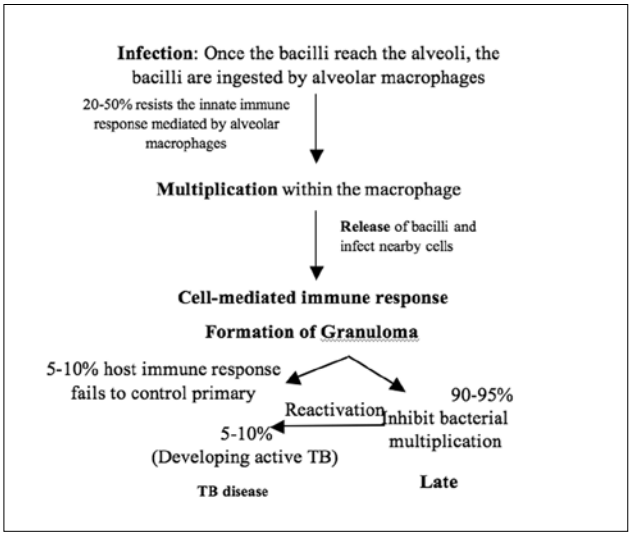

Figure 1 - Natural cycle of tuberculosis development.

global TB progression.[12] Bacilli Calmette-Guerin demonstrated variable protective efficacies ranging from 0-85% in different field trials.[13] This issue of varying efficacy and the estimate that BCG prevents only 5% of all the potentially vaccine-preventable deaths due to TB has caused increased interest in vaccine research.[14]

New TB vaccines. The failure of the BCG vaccine to efficiently prevent pulmonary TB in adults has encouraged the search for a new TB vaccine(s). Today, using modern techniques, several research groups have developed close to 200 new vaccine candidates. Typical vaccine approaches for producing immunity against *M. tuberculosis* have relied upon three main methods: live, attenuated or recombinant (recombinant virus- and bacteria-vectored) vaccines, DNA vaccines, or sub-unit vaccines.

Recombinant BCG (rBCG). Improvements of TB vaccines relied to strengthening the immunogenicity and/or persistence of genetically manipulated recombinant BCG (rBCG) strain. Recombinant BCG may be more effective than parental BCG by introducing extra copies of existing genes or by reintroducing some of the genes that were lost during in vitro attenuation process. Horwitz et al,[15] construct a live recombinant BCG (rBCG30) vaccine, consisting of BCG genetically modified to over express a major secretory protein of *M. tuberculosis* that has been shown to create a strong immune response in animals and humans (30kDa). When Guinea pigs immunized with rBCG30 and challenged with aerosolized *M. tuberculosis* few bacilli were found in their lungs and spleens compared with animals immunized with the parental, conventional BCG vaccine.[15] Other rBCG vaccine candidates reported by Pym et al[16] were constructed by complement BCG with ESAT-6, which is missing in BCG showed an enhanced protection in mice. A novel recombinant BCG expressing fusion protein (Ag85B)- (ESAT-6)-IFN-γ (rBCG-AEI) protective efficacy was evaluated against *M. tuberculosis* H37Rv in mice. The immunogenicity studied showed higher specific antibody titers and significantly increase cellular immune response than BCG.[17]

Qie et al[18] construct a rBCG [rBCG-Ag85B-Mpt64(190-198)-Mtb8.4] which induce an increased Th1-type immune response in mice, characterized by an elevated level of IFN-γ in antigen-stimulated splenocyte culture and a strong IgG2a antibody response. Also, it can elicit longer immune responses than BCG. Later, they showed that the recombinant BCG: rBCG-Ag85B-Mpt64(190-198)-Mtb8.4 is a potential vaccine candidate for further study.[19]

Wang et al,[20] constructed a new rBCG which included Antigen 85B (an important immunodominant antigen of *M. tuberculosis* , and is a promising vaccine candidate molecule) and Rv3425 (a member of the subgroup 3 of the family proteins (PPE), which does not exist in all BCG strains). This construct showed the IFN-γ was significantly higher in the C57BL/6 mice vaccinated with rBCG:Ag85B-Rv3425 than with BCG. The recombinant BCG Tokyo (Ag85A) shows promise as a TB vaccine, induced higher protective efficacy in Cynomolgus monkeys than BCG Tokyo.[21]

The subunit vaccines. One of the possible approaches for the improvement of TB vaccine involves the use of proteins secreted by *M. tuberculosis* during in vitro growth; some of these antigens are highly immunogenic. These proteins or corresponding genes might represent major components of either subunit or DNA-based vaccine preparations. However, it now appears that the development of protective immune responses requires the recognition of a large number of antigens present in the culture fluid. Therefore, it is reasonable to anticipate the development of a multivalent secreted protein-based TB vaccine.[12]

Deoxyribonucleic acid vaccines. Deoxyribonucleic acid vaccination is a novel method to produce antigen-specific antibody and cell-mediated immunity appeared more than 10 years ago as a promising approach to develop new vaccines against a number of infectious agents. Compared to traditional vaccines, DNA vaccines are simple to develop since only the DNA from infectious organisms is used, which avoid the risk of using actual infectious organism. Deoxyribonucleic acid vaccines also provide both humoral and cell mediated immunity and are in general less expensive. For these reasons DNA vaccines have been used as models to test the immunogenicity and protective activity of single *M. tuberculosis* antigens.[22] Mostly secreted and immunogenic proteins such as ESAT6, MPT64, Ag85A/B, MPT83, hsp65, KATG and *M. tuberculosis* 39A have been tested. Deoxyribonucleic acid vaccination in mice elicited significant levels of cell-mediated immune responses. Immunization with constructs expressing 2 or more *M. tuberculosis* antigens as fusion proteins antigens improves levels of protection compared to monovalent DNA vaccines.[23] A novel DNA vaccines expressing mycobacterial HSP65 and IL-12 showed significant protective efficacy via CD8+ and CD4+ T cells in murine models.[24] A plasmid DNA vaccine expressing HSP65 and the human IL-2 fusion gene (HSP65-IL-2-DNA) was constructed by Changhong et al.[25] This construct enhanced Th1-type cellular responses by generating great amounts of interferon-gamma (IFN-γ) and IL-2 compared with the parental BCG vaccine. HSP65-IL-2-DNA vaccine was able to induce both $CD4^+$ and $CD8^+$ T-cell responses. The above studies suggested that DNA vaccines are not less effective than protein subunit vaccination, at least in this animal model. A review of the recent literature

on novel TB vaccines reveals that DNA vaccination is one of the most commonly published approaches to protect against virulent challenge in animal models.[26]

Li et al[27] vaccinated C57BL/6 mice with a DNA vaccine composed of *Mtb8.4* with or without the human *IL-12* gene.[27] The authors reported that the DNA vaccine reduced the bacillary load by more than 2 $\log_{10}$ in both the spleen and lungs 4 weeks postchallenge with a very high dose (10^6 CFU) of virulent *M. tuberculosis* by the intravenous route compared with the placebo group. The degree of protection induced by the DNA vaccine was comparable to that observed in a group of mice vaccinated once with BCG. Zhang et al[28] immunized BALB/c mice with a DNA vaccine consisting of the genes for Ag85 with or without granulocyte-macrophage colony stimulating factor (GM-CSF). Following intranasal challenge with 10^4 CFU of virulent mycobacteria, the authors observed modest reductions in viable bacilli recovered from the lungs and spleens of animals receiving the cytokine-adjuvanted vaccine, but the protection was not as good as that seen in BCG-immunized mice. Another DNA vaccine based upon Ag85A was tested in guinea pigs by Sugawara et al.[29,30] This study showed that the rBCG protected better than the DNA vaccine, and that boosting with peptides enhanced the protection afforded by the DNA vaccine and the level of protection observed was comparable to wild-type BCG alone. Maue et al,[31] tested a DNA vaccine that consisted of the genes for antigens ESAT-6 and CFP-10, combined with GM-CSF and CD80/86, in cattle. Two intramuscular doses in incomplete Freund's adjuvant were given 3 weeks apart. The cattle were challenged by the intratracheal route with 10^3 CFU of virulent M. bovis. The authors reported that the DNA vaccine resulted in reduced lung and pulmonary lymph node pathology and that the level of protection exceeded that of BCG. A novel DNA vaccine adjuvanted with the IL-12 gene and consisting Ag85B, MPT64 and MPT83 genes encoding immunodominant antigens from both *M. tuberculosis* and *Brucella abortus* was developed by Yu et al.[32,33] A significant reduction in lung CFU at 6 weeks postchallenge in the DNA-vaccinated group, compared with the BCG-vaccinated group.[32]

McMurry et al[34] used the sequences of 18 mycobacterial proteins reported to be upregulated within human phagocytes to select T-cell epitopes based upon a computer algorithm, EpiMatrix. A DNA vaccine based upon 17 of these predicted epitopes combined with IL-15 was given to C57BL/6 mice in 3 intramuscular doses at 2-week intervals, followed by 3 intranasal boosts with the peptides in liposomes and CpG oligo-DNA. In total, 2 weeks following the last vaccination, the mice were challenged in an aerosol chamber with 100 CFU of *M. tuberculosis* strain Erdman.

The protective efficacy of a pE6/85-DNA vaccine expressing an ESAT-6-Ag85B fusion protein was evaluated by Derrick et al.[35] A significant reduction was observed in bacillary loads in the lungs and spleens of the mice given BCG alone, but no significant difference was observed with mice given pE6/85.

In conclusion, this brief review showed various types of new vaccines that have been developed, especially in the area of rBCG and DNA vaccine that show considerable promise. More efforts are required to accelerate vaccine development even further in the next decade.

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Related topics

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