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Progress in Tuberculosis Vaccines Development

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Abstract: Tuberculosis (TB) caused by Mycobacterium tuberculosis (M. tb) continues to be one of the major public health problems in the world. Failure of the BCG vaccine to protect in endemic regions and increasing problems with multi-drug-resistant TB calls for development of better vaccines to prevent reactivation of tuberculosis. The eventual control of this disease will require innovation in scientific research and development of a safe and effective vaccine. Furthermore, new vaccines should also prevent development of TB in HIV-infected individuals.

Key words: BCG; DNA vaccines; Subunit vaccine, Live recombinant vaccines, Salmonella typhi Ty21A.

General View of Tb Vaccines: Among all infectious diseases that afflict humans, tuberculosis remains the number one killer in adults. At present, epidemiologists estimate that one-third of the world population is infected with tubercle bacilli, which is responsible for 9 million new cases of TB and up to 2 million deaths annually throughout the world. Tuberculosis gained importance as a public health problem in the recent years[1, 2]. For many reasons including the rise of multi-drug resistant strains of M. tuberculosis (MDR-TB) which cannot be treated by first-line drugs, the fails BCG to protect against the most frequent form of disease, pulmonary tuberculosis in adults and the growing TB/HIV co-epidemic in large areas of the world. A vaccine is urgently needed for control. For the above mentioned reasons the development of more efficient anti-TB vaccines has become a necessity for adequate control and elimination of this global threat [3].

Although the M. bovis bacillus Calmette-Guerin (BCG) vaccine is the commonly used anti-tuberculosis vaccine in the world, tuberculosis is still a major problem worldwide. The current vaccine candidates are however developed to protect against primary tuberculosis and not against reactivated latent tuberculosis[4].

To improve the BCG vaccine, recombinant BCG strains were constructed which express cytokines such as IFN-g or IL-2, IL-12 and GM-CSF in attempts to evoke more potent immune responses against M. tuberculosis[5, 6].

The Bcg Vaccine and its Efficacy: BCG is a live vaccine used to protect against TB. BCG was derived from a strain of M. bovis, which is closely related to M. tuberculosis and is a member of the M. tuberculosis complex. Clinical trials of the BCG vaccine have been undertaken since it was first developed in the 1920s. In 1994, a review of the literature, including meta-analyses of BCG vaccination studies was published which demonstrates varying efficacy for BCG in providing protection against miliary and meningitis TB in children. The efficacy of the conventional bacille Calmette-Guerin (BCG) vaccine is often questioned because of conflicting trial results that ranges from 0% in South India to 80% in the UK[7-9]. Recently, Roth et al.,[10,11] studies showed that BCG has valuable non-targeted effects on general child survival in low-income countries, often with the most obvious effect among girls. Indeed genotypic and phenotypic differences have been confirmed in these BCG strains. Free living nonpathogenic mycobacteria, which resemble M. tuberculosis may be responsible for infecting individuals, thereby providing partial immunity against M. tuberculosis[8, 9, 12]. The BCG vaccine is the only widely used vaccine against childhood TB. One assumption is that immunological memory generated by BCG disappears and the individual becomes equivalent to a naïve host. BCG antigens must be presented to major histocompatibility complex (MHC) class II molecules to activate CD4+ cells and to MHC class I molecules to activate CD8+ cells. However, BCG fail to activate CD8+ cells sufficiently. Grode et al.,[13] have engineered a BCG strain which overcomes this problem and allows release of antigen into the cell and thereby stimulates a CD8+ cell response in addition to the CD4 response seen with conventional BCG.
Development of Candidate Vaccines Other than BCG: Since BCG vaccination is not likely to have a significant impact on an adult TB epidemic, numerous new approaches to vaccine development are being pursued and tested in small animal models. The new techniques for preparing vaccines include development of modified BCGs, live-attenuated strains of *M. tuberculosis*, subunit vaccines, as well as naked DNA vaccines. New vaccines being developed against TB focuses on T lymphocyte populations because T-cells are central to protection against the disease.

An effective antituberculous vaccine involves the identification and isolation of key components of the pathogen that are competent of inducing a protective immune response. Clues to categorize promising subunit vaccine candidates may be found in their physicochemical and immunobiological properties. The majority of the antigens used to date for vaccine purposes have been selected after they subjected to challenge studies in different animal models to evaluate the protective efficacy[14, 15].

From several studies mentioned in this review a number of *M. tuberculosis* antigens recognized by T-cells secreting IFN-γ can be considered as promising candidates for development as new vaccines against TB. The first data showing realistic protection against *M. tuberculosis* using selected antigens came from using plasmid DNA as a system to deliver *M. tuberculosis* antigens such as Ag85[16-19], ESAT-6[17, 19-21], MTP40[22], 38 kDa[23] and MPT64[24,25]. Further, as a vaccine based on a single antigen cannot be consistently protective in a genetically diverse population, experimental vaccines based on the combination of protective antigens also need to be evaluated.

A good candidate antigen to create a therapeutic vaccine against TB is the ESAT-6 protein. ESAT-6, a secretory protein from *M. tuberculosis*, is a dominant target for cell mediated immunity in the early phase of infection in TB patients as well as in various animal models, causing T-cell proliferation and gamma interferon production[20, 21]. Delogu et al.[26] constructed DNA vectors with the genes encoding the secreted protein MPT64 (23 kDa), expressed as a chimeric protein fused with one of three variants of the ubiquitin protein (UbG, UbA and UbGR) known to differentially affect the intracellular processing of the co-expressed antigens. Protection in animal models has also been reported with plasmid DNA-based vaccines (DNA vaccines) with genes for other secretory proteins like ESAT-6 and MPT64. However, in a comparison study, DNA-Ag85B provided better protection than DNA-ESAT-6 and DNA-MPT64[28]. Moreover, co-immunization with DNA-Ag85B, DNA-ESAT-6 and DNA-MPT64 provided better protection than the protection induced by any single DNA vaccine[27]. Furthermore, a primary vaccination in mice with Ag85B-encoding plasmid DNA (DNA-85B) was protective against *Mycobacterium tuberculosis* infection and associated with Ag85B-specific CD4(+) T cells producing IFN-g[28].

In another study, immunization with multivalent combination DNA vaccine (plasmid containing the genes encoding ESAT-6, MPT64, MPT63 and KatG) generated immune responses that indicated a lack of antigenic competition and the ability to induce a strong protective effect[29].

The 38 kDa antigen is a non-secretory lipoprotein. This protein has been found to induce strong antibody and T-cell responses and provide partial protection against *M. tuberculosis* infection in mice[23]. Parra and his colleagues[30], isolated and characterized a gene, designated as MTP40, that appears to be highly species specific, present only in *M. tuberculosis* strains and absent in *M. bovis* strains and *M. bovis* BCG strain. This gene encodes a 13.8 kDa protein.

In peptide mapping of the MTP40 antigen, Falla et al.[22] demonstrated the presence of several human B cell and T-cell epitopes within MTP 40 antigen, which can be used as a possible candidate for the development of a subunit vaccine.

Recombinant BCG: For the development of new tuberculosis vaccine candidates, genetically modified variants of the live BCG vaccine are on progress. Since BCG has a proven safety record for many decades, but unfortunately it lacks effectiveness the use of recombinant DNA technology has made the development of recombinant live attenuated vaccine such as BCG more efficient. Recombinant DNA methodologies have been used in the expression of assumed protective antigen(s) or cytokine(s) such as IL-2, IFN-γ in BCG to boost its protective effect[31]. Bao et al.[32] constructed two rBCG strains expressing ESAT-6. To date, several candidates of this type have shown protective efficacy in mouse or guinea pig models similar to, but not significantly better than, BCG.

When Dhar et al., (2004) immunized BALB/c mice with recombinant BCG that over expressed Ag85A an increased humoral response was observed compared to mice immunized with wild type BCG.

Sugawara et al.[33] showed that a recombinant form of BCG Tokyo with an Ag85A was better than Ag85A DNA in terms of protective efficacy against *M. tuberculosis*. Besides that they concluded that peptide boosting is important for the induction of higher
protective efficacy by recombinant BCG Tokyo or a tuberculosis DNA vaccine and both recombinant BCG Tokyo (Ag85A) and Ag85A DNA vaccine induce Th2 cytokine mRNA expression significantly\textsuperscript{33}.

**Attenuated Strains of M. Tuberculosis:** To overcome this, BCG has also been modified by developing auxotrophic mutants. Guleria et al.\textsuperscript{34} showed in his study of five auxotrophic strains of BCG produced by insertion mutagenesis that auxotrophic strains of BCG represent a potentially safe and useful vaccine against TB for populations at risk for HIV. However, animal studies of the auxotrophic mutants tested so far have provided less protection than parental BCG. It is hoped that vaccines based on auxotrophic strains of BCG or *M. tuberculosis* would be safe even for immunocompromised individuals.

**Subunit Vaccines:** Instead of using whole protein preparations as vaccine candidates, researchers found that T-cell epitopes which were recognized directly by the receptors on T-lymphocytes may be used in the development of vaccines. Some investigators are trying to identify peptide antigens that could evoke a protective immune response. Subunit vaccines can be used to deliver *M. tuberculosis* antigens that have been identified to potentially induce a strong protective immune response. Since the publication of the genome sequence of *M. tuberculosis*, several studies have focused on the development of TB vaccine candidates of this type based on secreted proteins found in the culture filtrate protein (CFP), that are visible to the T-cell immune system \textsuperscript{35}.

**DNA Vaccines:** The great challenges for researchers working in the field of vaccinology are optimizing DNA vaccines for use in humans or large animals and creating effective single-dose vaccines using appropriated controlled delivery systems. The principle of DNA vaccines is based on the fact that instead of exposing the antigen-presenting cell to the antigen in form of exogenous protein, the gene (or genes) encoding the antigen (or antigens) of interest is placed under the control of a strong viral promoter of an eukaryotic expression plasmid such as cytomegalovirus (CMV). This ensures strong expression of the inserted DNA once the plasmid enters a host cell. The plasmid is taken up by cells and then the gene encoding the antigen is transcribed and translated. The use of DNA vaccine for the prophylaxis of mycobacterial diseases is reviewed by Huygen\textsuperscript{36}, DNA encoding specific antigens of *M. tuberculosis* has a number of advantages over other vaccination strategies, including stability, ease of preparation and storage and safety for immunocompromised hosts. DNA vaccines do not induce vector immunity and can be used for boosting studies\textsuperscript{37}, first data showing significant protection against *M. tuberculosis* came from studies using naked DNA immunization.

The first demonstration of the possible efficiency of DNA vaccine against mycobacteria was in 1994, after immunization of mice with the J774 macrophage cell line transfected with the *M. leprae* Hsp65 gene and showed protection against intravenous challenge with virulent *M. tuberculosis* and BCG. Other studies by Huygen et al.\textsuperscript{38} and Tascon, et al.\textsuperscript{39} on DNA vaccination of mice, using DNA encoding the major secreted protein, Ag85A, Hsp65, or the 32 kDa mycolyl transferase from *M. tuberculosis* confirmed that DNA vaccination provided some protection against challenge with *M. tuberculosis*.. In mice different studies, using several mycobacterial antigens delivered as naked DNA have elicited protection against virulent infection, including *M. tb* 8.4\textsuperscript{40}, Ag85\textsuperscript{24, 38, 41, 42}, Mtb 41\textsuperscript{43, 44}, MPT 39\textsuperscript{45}, MPT 63 and MPT 83\textsuperscript{29}, PstS-3\textsuperscript{46}, ESAT-6\textsuperscript{20, 21, 24}, MPT64\textsuperscript{24} and the 38 kDa lipoprotein\textsuperscript{21}.

The protective effect of immunization with a DNA vaccine expressing Ag85B followed by BCG was found to be higher than BCG alone, in C57BL/6 mice, upon subsequent challenge with aerosolized *M. tuberculosis*\textsuperscript{47}.

A rBCG construct that overexpresses the 30 kDa Ag85B major secretory protein has been constructed and its use as a vaccine was shown to enhance survival compared to vaccination with conventional BCG in the guinea pig model of TB\textsuperscript{48}.

Britton & Palendira\textsuperscript{49} demonstrated that immunization of mice with a DNA vaccine expressing Ag85B (DNA-85) combined with a plasmid expressing IL-12 increases the protective effect of the DNA vaccine expressing Ag85B. This study showed that the protective effect was as protective as BCG. A DNA vaccine was previously constructed in our laboratory which contained a synthetic gene called VacII encoding T-cell epitopes from four *M. tuberculosis* genes namely ESAT-6, MTP40, 38 kDa and MPT64. DNA vaccination may or may not ultimately prove to be an alternative to BCG. However, these studies indicate that vaccines that are superior to BCG may not be far away.

**Live Recombinant Vaccines:** Live recombinant vaccines other than rBCG have been used as carriers such as *M. vaccae*. They are safe and stimulate the proliferation of lymphocytes and the secretion of IFN-g
in both healthy and HIV-Infected individuals, in addition to BCG-primed subjects\cite{50,51}.

Vaccinia virus has been used as a potential vector for overexpressing mycobacterial antigens and has been tested experimentally using the 38 kDa antigen of M. tuberculosis. Salmonella spp. which enters the body through the mucosal surfaces, are not only capable of gene transfer to antigen presenting cells, but they are also natural adjuvants and induce cytotoxic immune responses\cite{23,52}.

Salmonella antigens have been successfully used to immunize mice against intracellular pathogens\cite{53,54}. Thus, Salmonellae may be engineered to serve as an oral TB vaccine by expressing protective antigens of M. tuberculosis.

**Live Attenuated Typhoid Vaccine:** The live vaccines offer great promise but the obstacle of developing such vaccines is the difficulty in accomplishing a logical level of attenuation without changing their immunogenicity.

Sarhan,\cite{55} explore the use of the live attenuated typhoid vaccine, S. typhi Ty21a, for development a candidate vaccines against TB. S. typhi Ty21a was utilized in a surface display system as well as a carrier of a DNA vaccine. In the surface display approach, a surface display expression system was developed by the construction of a synthetic gene coding for the N-terminal of the ice nucleation protein from *Pseudomonas syringae* using a method called assembly polymerase chain reaction (PCR)\cite{56}. In the second approach, S. typhi Ty21a was utilized as a carrier of DNA vaccine. In this study, S. typhi Ty21a was transformed with a previously constructed DNA vaccine called pJWVacII to create a carrier strain\cite{55}. Both newly constructed vaccine candidates, r-STVII and STVII-c, were shown to be safe when tested in C57BL/6 mice.

**Conclusion:** The current TB vaccine varies in effectiveness depending on where it is used. Scientists have suggested this is due to environmental factors or using different bacterial strains to create the vaccine. In this brief review 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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