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Mycobacterium Tuberculosis-Specific Antigens As Subunit Vaccines Against Tuberculosis

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Tuberculosis (TB) is a chronic infectious disease caused by the bacterium *Mycobacterium tuberculosis*. TB is present globally and ranks among the top 10 causes of all deaths and it is the leading cause of death from a single infectious agent, with a ranking above HIV/AIDS [1]. According to the world-wide estimates from the World Health Organization (WHO), about 10 million people became ill with TB and 1.5 million people died of it in 2018 [1]. The situation of TB is further complicated as a public health problem due to drug-resistance and increased susceptibility of HIV/AIDS patients. In 2018, there were about 0.5 million new cases of TB resistant to rifampicin (the most effective first-line anti-TB drug) and 0.4 million of them had multidrug resistant TB Moreover, about 25% of the global population is estimated to be latently infected with *M. tuberculosis*, and 5 – 15% of them have the lifetime risk of developing clinically active TB disease. An efficient and effective TB control program requires development of effective drugs against resistance strains, cost-effective technologies for the specific diagnosis of latent and active disease and effective, safe and affordable vaccines. Among the various possibilities, the availability of new TB vaccines deserves priority because cost-effective and safer vaccines are the best option as such vaccines have been effective in controlling and eradicating many infectious diseases.

The currently available vaccine against TB is a live attenuated strain of pathogenic *Mycobacterium bovis* known as Bacillus Calmette-Guérin (BCG). Although BCG has been extensively used to vaccinate against TB since 1921 and it is the most widely used vaccine in the world, it is the most controversial vaccine in current use. This is because BCG has failed to consistently protect against the major manifestation of TB in adults, i.e. pulmonary TB [4]. The variations in protection have ranged from nil (e.g. in India and Malawi) to 80% (e.g. in the United Kingdom). Furthermore, as BCG is a live mycobacterium, it is not suitable for vaccinating immuno compromised individuals, particularly patients suffering from HIV/AIDS, due to the possibility of causing disease in such individuals. Since BCG vaccination induces positivity to the commonly used tuberculin skin test for the diagnosis of TB, it becomes difficult to use this test for diagnostic or epidemiological investigations in populations vaccinated with BCG. In addition, BCG has antigens cross-reactive with environmental mycobacteria leading to masking or blocking effects. According to masking hypothesis, early sensitization with environmental mycobacteria confers some level of protection against TB that masks the effect of a vaccine given later in life due to presence of cross-reactive antigens. Whereas, the blocking hypothesis postulates that previous immune response to cross-reactive antigens, because of environmental mycobacteria sensitization, prevents vaccine taking of a new TB vaccine [5]. The use of *M. tuberculosis*-specific antigens may overcome these effects. Therefore, to have vaccines better than BCG, development of new subunit vaccines based on *M. tuberculosis*-specific antigens is needed.

The identification of *M. tuberculosis*-specific antigens has been facilitated by advances in mycobacterial genome sequencing and the comparative genomics to identify *M. tuberculosis*-specific genomic regions. Such studies have identified 11 *M. tuberculosis*-specific genomic regions known as regions of differences (RDs), which are deleted/absent in all BCG substrains currently used in different parts of the world to vaccinate against TB [7]. In silico analysis has suggested that these RDs can potentially encode 89 proteins. To identify the candidate proteins suitable for vaccine development, it is essential to identify the immunodominant proteins from the RDs that can mediate protection against TB.

Protection in TB is primarily mediated by cellular immunity involving the interaction of antigen-specific T cells and macrophages. This interaction is often indicated by antigen-induced proliferation of T cells and is

dependent on the interplay of cytokines secreted by these cells. Although a broad spectrum of cytokines contribute to protection, the T-helper type 1 (Th1) cytokines, dominated by secretion of IL-2 (responsible for proliferation of antigen-reactive T cells) and IFN-γ (responsible for activation of macrophages to kill ingested bacilli), are considered principal mediators of protective immunity against TB. Therefore, Th1 cell reactivity, indicated by antigen-induced cell proliferation and secretion of IFN-γ, has been used as a marker to detect antigens involved in protective immunity and thus suitable for the identification of candidate vaccines.

The screening of *M. tuberculosis*-specific antigens with human peripheral blood cells and naturally infected cattle identified a total of six antigens inducing strong Th1-responses, i.e. PE35, PPE68, ESAT-6, CFP10, Rv3619c and Rv2346c. Testing of these antigens in animal models of TB have shown that all of these antigens are capable of inducing Th1 responses when used along with appropriate adjuvants and delivery systems like DNA vaccine vectors and non-pathogenic mycobacteria, including BCG. In mice and guinea pigs, immunizations with preparations containing ESAT-6, CFP-10 and Rv3619c have provided protection against challenges with virulent *M. tuberculosis*.

In humans, a recombinant subunit tuberculosis vaccine candidate containing dextran-binding domain modified Ag85a and ESAT6-CFP10 *M. tuberculosis* antigens with an adjuvant has been tested in phase I clinical trial. Safety and immunogenicity of this vaccine were determined in an open-label clinical trial on healthy volunteers previously vaccinated with BCG. The candidate vaccine had an acceptable safety profile and was well-tolerated. Furthermore, the immunization protocols lead to a significant increase in the concentration of the Th1-cytokine IFN-γ and IgG antibodies. The immune responses were induced to all antigens included in the vaccine preparation.

In conclusion, several *M. tuberculosis*-specific antigens induce protective Th1-type immune responses with human cells *in vitro*. The immunogenicity and protective efficacy studies in animals have shown that these antigens may be useful as subunit vaccine candidates against TB. In a human phase I trial, a preparation containing *M. tuberculosis*-specific antigens has been found immunogenic to induce both cellular and humoral immunity. Further studies are required to determine the protective efficacy in phase II and Phase III clinical trials in humans to determine the protective efficacy of the candidate vaccine.

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