Vaccination strategies for tuberculosis

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The widespread plague of tuberculosis (TB) has continued despite the availability of licensed TB vaccine (BCG) and directly observed chemotherapy (DOTS) programmes. Also, emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* presents serious challenges for the DOTS strategy. Today, TB still remains a deadly disease and leading infectious killer of youth and adults. Clearly, there is a need for an improved TB vaccine. Various efforts have been made in this direction, and a heterologous prime-boost regimen probably represents the best hope for an improved vaccine regimen to prevent TB. The first generation of new vaccines might also complement drug-treatment regimens and be effective against reactivation of TB from the latent state, which would significantly enhance their usefulness.

Keywords: HIV infection, strategies, tuberculosis, vaccination.

TUBERCULOSIS (TB) remains one of the oldest and deadliest diseases in the current scenario. It is more prevalent in individuals who are infected with HIV/AIDS, which also results in the high incidence of dual infection and subsequent suppression of immune response. The deadly nature of this disease can be comprehended from the fact that out of 15 million people who were co-infected with both HIV and *Mycobacterium tuberculosis* in the year 2003, nearly 6 lakhs died of TB. This represents the major risk factor for mortality amongst individuals with HIV infection. Incidence of TB is increasing the fastest in African countries that are affected by HIV, followed by eastern Europe and the former Soviet Union, both of which are plagued by multi-drug resistant (MDR) strains of TB that present an ominous global threat. In developing nations like India, poverty, malnutrition and over-population have helped in the spread of TB.

Antibiotics that have been widely available throughout the country since a long time[1], have failed to be of any significance, as still an estimated 8–10 million new infections occur every year[2]. Most of the pathogenic strains are now resistant to first-line drugs being used for the treatment of the disease. This has resulted in MDR strains of TB. Today, almost 80% of all new cases of MDR TB is due to resistance to three or more drugs. Although the BCG vaccine protects against childhood TB, it fails to protect against the most common form of the disease, pulmonary TB in adults[3].

It is therefore necessary not only to develop a vaccine that could boost BCG, but also to develop a post-exposure vaccine. Various vaccination strategies have been postulated for the development of the TB vaccine, such as improving the current BCG vaccine, over-expression of the immunodominant antigens, endosomal escape, recombinant fusion protein and heterologous prime-boost strategy. However a practical approach has been a hybrid one, which can be termed as a multiphase vaccine that can be administered regardless of the infection status of the individual and with activity both in naïve and already infected individuals. However, an alarming challenge facing the development of these new vaccines is their safety, especially in populations affected with both HIV and TB.

Immunological basis for rational design of TB vaccines

Early attempts on vaccination against infectious disease dates back to the 19th century, when Edward Jenner made his first attempt to develop a vaccine against cowpox, which was widely used to protect both animals and humans. It is now known that in case of *M. tuberculosis* infection, it is the acquired immunity that plays a major role in host defence. In contrast, protective humoral immunity to *M. tuberculosis* infection has not been identified so far, although there is some evidence suggesting that antibodies might be involved in protective immunity against TB[4]. It is generally believed that antibodies have little or no role in host resistance against TB[5,6]. Therefore, now the aim of the TB vaccine is to stimulate and maintain long-term mycobacterial antigen-specific memory T-cells in the host. It is believed that memory T-cells can respond rapidly to *M. tuberculosis* infection and accelerate the formation of high quality of granuloma at the infection site, hence effectively aborting or controlling the infection before the bacteria can undergo significant rounds of replication[6–8].

It has been shown that T-lymphocytes play a central role in *M. tuberculosis* infection[9,10]. Different T-cell populations contribute to protection, and some of them develop as a specific counter-measure against the mycobacterium. Though the antigenic ligands presented by major histocompatibility complex (MHC) II molecules are the main

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players in the field of TB control, CD8 T-cells which recognize antigenic ligands presented by MHC I molecules, also a play a role in the protective immunity against *M. tuberculosis* infection. Additionally, the role of the unconventional T-cells is not fully understood. However, from one of the recent findings in non-human primates, it has been showed that $\gamma\delta$ T-cells contribute to protection against TB. It is thus clear from this fact that $\gamma\delta$ T-cells do play a protective role in the host defence against infection. The $\gamma\delta$ T-cells are specific for low molecular weight metabolites comprising phosphate, whereas CD1-restricted T-cells recognize the glycolipids abundant in the mycobacterium cell wall.

Mode of action

M. tuberculosis infection is generally revealed by the development of a delayed-type hypersensitivity response to tuberculin antigen (TST). The disease spreads through the aerosol route. Induction of host immune responses starts with the recognition of *M. tuberculosis* by macrophages through Fc receptors, complement receptors, mannose receptors and Toll-like receptors (TLRs) expressed on their surface[11,12]. After phagocytosis, the organism successfully resides in the endosome compartment of the macrophage and hides from the immune-mediated destruction using various mechanisms, including changing the endosomal pH, inhibiting apoptosis and destroying toxic superoxides that are secreted by immune cells. Later, infected alveolar macrophages and dendritic cells migrate to adjacent lymph nodes where mycobacterial antigens are presented and a T-helper-1 (T_H1)-type response is initiated. The granuloma initially consists of mainly the $CD4^+$ and $CD8^+$ T-cells, but a complex array of T-cells, including $CD4^+$, $CD8^+$, $\gamma\delta$ T-cells and CD1-restricted $\alpha\beta$ T-cells is also involved to orchestrate immune responses and to contain infection. At later stages, a fibrotic wall and lymphoid follicular structures surround the granuloma.

This complex immune response subsequently results in the formation of the granuloma around infected macrophages. The granuloma can persist for decades and can contain the infection in a state of dormancy by depriving mycobacteria of oxygen and nutrients. However, failure to contain the infection may result in the release of organisms, and cessation and necrosis with active clinical disease and transmission.

It should be noted at this point that IFN-γ, which synergizes with tumour-necrosis factor-α (TNF-α), is central to the activation of macrophages.

Antigen processing and presentation

It is the cellular immune response which plays a dominant role in the host defence against the disease. The antigen is processed and presented by antigen presenting cells (APC), which are processed through the exogenous pathway and presented on major histocompatibility complex (MHC) class II molecules to $CD4^+$ T-cells, whereas *de novo* synthesized proteins by APC are processed through the endogenous pathway and presented on MHC class I molecules to $CD8^+$ T-cells, a process referred to as cross-presentation[13]. Subsequently, this phenomenon has also been demonstrated in dendritic cells (DCs) upon engulfing an apoptotic body such as virus-infected or dead tumour cells[14]. Recent studies indicate that this process is mediated through a phagosome–endoplasmic reticulum (ER) fusion that results in a specialized, self-sufficient, ER–phagosome mix compartment that regulates the loading of exogenous antigen peptides onto phagosomal MHC class I molecules[15,16]. It is strongly believed that the selection of the vaccine platform largely influences the antigen presentation process and the subsequent immune response[17].

Vaccination strategies

The first persons to succeed in developing a vaccine for TB were Albert Calmette and Calmitte Guerin of the Pasteur Institute, who attenuated a mycobacterium related to *M. tuberculosis (Mycobacterium bovis* bacillus Calmette–Guerin [BCG]) by growing it in on a culture medium for 13 years, and monitoring its decrease in virulence in animals throughout this period[18]. The present scenario gives two potential vaccine strategies against TB: before (pre-exposure, prophylactic) or after (post-exposure, therapeutic) exposure to the pathogen.

The current BCG vaccine

BCG is the attenuated strain of *M. bovis*, and was derived from the parental strain by passage on potato-derived media. Replacing BCG will not be an easy task; it is perhaps the most used vaccine in the world and despite its limitations, it is cheap, safe and well-established. Neonatal vaccination with BCG seem to consistently provide significant protection against the most severe childhood symptoms of the disease, such as TB meningitis. However, most studies have reported that the efficacy of BCG lasts for no more than 10 to 20 years[19–21]. However, in contrast to its record in human trials, BCG has generally provided high levels of protection in animal models (particularly the guinea pig model) of TB and although considerable progress has been made in the development of efficient subunit vaccines, there is no report for efficacies exceeding that of BCG in naïve individuals. Therefore, by this measure, the only vaccines that show promise so far as a replacement for BCG is concerned, are to be found among the live mycobacterial vaccines. The first of these to enter the clinic, the rBCG30 vaccine, has already passed the phase I clinical trials[22]. This vaccine was derived from a strain of BCG[22]; which has been genetically

modified to over express the immunodominant antigen Ag85B. The idea behind this vaccine was to improve BCG by expressing higher levels of an antigen that had already been shown to be protective. The improved efficacy of rBCG30 came as something of a surprise, since BCG possesses a immune response to the antigens it encodes[23], reminding us that the mere presence of a gene tells us little about its expression *in vivo* and that the effect of a gene may be modulated by the presence or absence of other genes.

Over-expression of immunodominant antigens

One of the potential vaccine candidates has been developed by M. Horwitz and his group (UCLA), which over expresses the immunogenic antigens and has been named as rBCG30. This has been found to over express the immunodominant antigen Ag85B and induce an increased protection compared with its parental strain[22,24]. Ag85B is a secretary and immunogenic protein of *M. tuberculosis* and BCG with mycolyl-transferase activity, which is required for mycobacterial cell-wall synthesis. This antigen has a molecular weight of about 30 kDa. Phase I clinical trials were conducted on over 30 individuals for this vaccine, and there were no significant safety issues. It was observed that it induced an increased level of protection compared with its parental BCG strain[25], although the study did not meet its predefined immunogenic end-points.

Protective mechanism (endosomal escape)

It has been established that both MHC I and MHC II pathways play a crucial role in the protective immune response against TB. Thus the protective antigens must also be presented to the MHC I pathway for eliciting effective immune response. In order to achieve this, an additional agent was incorporated in the vaccine which could punch holes in the phagosome and thus facilitate its release from the phagosome to the cytosol in order to be presented to the MHC I pathway. The agent used for this purpose was Listeria monocytogenes (listeriolysin). This is a sulphydryl-activated cytolysin that forms pores in the membrane of the early phagosome and allows some leakage of BCG from the phagosome to the cytosol[26].

Since the activity of Hly (lysteriolysin) is optimal in an acidic environment, the BCG urease gene (*UreC*) was deleted. *UreC* is known to block the acidification of the phagosome and thus inhibits its fusion to the lysosome. These vaccines (rBCGΔUreC:Hly+ vaccines) showed better protective efficacy against the virulent *M. tuberculosis* strain, Beijing/W. Following the entry of BCG into the cytoplasm, a process known as apoptosis (programmed cell death) occurs. This in turn kills the bacilli and sheds antigen as apoptotic bodies/blebs to be presented to the dendritic cells, which in turn activate T-cells by a mechanism known as cross-priming[27]. An rBCG vaccine has been constructed that escapes from the endosome using the pH-independent perfringolysin (rBCG-pfo), instead of Hly[28], which over express immunodominant antigens, including Ag85A, Ag85B and TB10.4. This vaccine is in phase I clinical trials[29].

Fusion proteins (recombinant proteins) for immunoprotection

Recombinant fusion protein-based vaccine candidates have been developed considering that the fusion protein would be more helpful in inducing protection than any other single protein. It would also be both cost-effective and simple in production, and induce both strong cellular and humoral immunity against the pathogen. Various workers have developed different candidates in this direction, first by identifying the immunogenic antigens and developing the vaccine candidate from them. Using the Hybrid 1 approach, which consisted of immunogenic antigens such as Ag85B and ESAT6, the first attempt was made. A genetic fusion approach was also to generate hybrid protein vaccines consisting of Mtb 39 and Mtb 32 (Mtb72F)[30]. These candidate vaccines were selected on the basis of their strong recognition by immune cells in infected individuals and experimentally vaccinated or infected animals. The generation of recombinant protein subunit vaccines comprising multiple open reading frames is both cost-effective and simple, and the fusion vaccines can be more immunogenic than the individual components. It has been observed that both these vaccines protected against infection in the mice and guinea pigs model of tuberculosis[30–33].

Prime-boost vaccine strategies

The main advantage of using a heterologous prime-boost approach is that it preferentially expands TB-specific pre-existing memory T-cells against antigenic epitopes shared by both the prime and booster vaccines. This approach also minimizes the potential of generating antibody and T-cell neutralizing effects that would impact on the 'take' and effectiveness of a non-replicating viral vector boost.

Early attempts made in this direction consisted of using a BCG prime-boost approach in which the mice model was used to boost the BCG primed mice with Ag85A. It was observed that mice boosted with an adjuvanted Ag85A protein when they had lost their BCG-induced capacity, resisted an aerosol TB challenge compared to non-BCG-primed animals given adjuvanted protein[34]. This suggests that adjuvanted proteins, including Mtb72F, Hybrid-1 and HyVac-4 could induce superior protection when given as boosters in primary regimens, or in adolescence. Such recombinant protein regimens, however, might induce primarily antigen-specific $CD4^+$ T-cells instead of the de-

sired balanced $CD4^+$ and $CD8^+$ T-cell responses. A recent study in mice of a BCG prime followed by a booster with an *M. tuberculosis* DNA vaccine encoding the gene product Rv3407 (a 10 kDa protein of unknown function) also showed superior protection compared with BCG alone[35]. In a phase I clinical trial recently reported[36], immunization of volunteers from 6 months to 38 years after BCG vaccination with MVA resulted in persistent, high levels of IFN-γ-secreting $CD4^+$ T-cells, which only induced transient responses. BCG prime followed by a booster with a single dose of these adenovirus recombinant vaccines in mice have yielded significantly increased antigen-specific $CD4^+$ and $CD8^+$ T-cell responses compared with adenovectored TB vaccines or BCG administered alone. rBCG vaccines have been constructed with over 50% escape from the endosome that over express the immunodominant antigens to function as a prime for this adenovector boost[29]. Double stranded RNA capsids have also been constructed, which can be orally delivered using engineered *Shigella* spp. as vectors. *Shigella* delivers these capsids into the cytoplasm, where they produce mRNA that directs cellular production of *M. tuberculosis* antigens[37] and induce cellular immunity. Such an oral vaccine boost to an rBCG prime might have low cost and ease of deliverability desirable in a TB vaccine for the developing world.

Conclusion

A safe and effective heterologous prime-boost regimen, which boosts or augments BCG or rBCG, is perhaps the most realistic strategy for future TB control through immunization. Ideally the rBCG prime in such a regimen would include the over expression of important antigens from different stages of the life cycle of a pathogen and would induce cross-priming and increased $CD4^+$ and $CD8^+$ T-cell responses, as well as increased safety in immunocompromised individuals. The ideal booster would comprise recombinant proteins delivered with adjuvants, or delivered by non-replicating viral or capsid vectors. Because of the great promise and practical usefulness, rBCG prime-heterologous boost strategies for malaria and HIV are also currently being explored.

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