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Mycobacterium tuberculosis & its recent vaccine approach: A review

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Abstract
In this review, we argue current advancement in the improvement, recent researches, general manifestation, epidemiology, global TB control program, pathogenesis, clinical features, diagnosis, treatment, risk factors and prevention. In excess of the last 24 years, incredible evolution has been made in TB vaccine research and expansion: In adding up inventive approaches are being pursued to more develop accessible vaccines, as well as discover fresh ones. Thus, there is superior reason for confidence in the field of TB vaccines that it will be probable to build up better vaccines than BCG. Because this BCG vaccine has certain issues which need to be addressed. BCG vaccine has a little impact on the pulmonary TB, means the disease is still transmissible and also the protective ability of BCG against pulmonary TB in adults is incomplete and inconsistent. Another issue of concern that compromises BCG's utility is that infants with HIV have an increased risk of developing disseminated BCG-Osis. A very hopeful TB vaccine, MVA85A, is presently in time II trials and is based on a genetically modified vaccine virus. Many other strategies are also being used to develop novel vaccines, plus both subunit vaccines such as Hybrid-1, HyVac4 or M72. A few of these vaccines can be well administered without needles making them preferable for areas where HIV is very frequent and few of these vaccines have been fruitfully experienced in humans and are now in extended testing in TB-endemic regions. To support further discovery, researchers across the globe are promoting latest economic models of vaccine advance, including prices, tax incentives and go forward market commitments. This review gives the vital suggestion of various vaccine growth approaches and its effective application in tuberculosis control.

Keywords: New vaccine development strategy for TB

1. Introduction
Tuberculosis in the past also known as phthisis pulmonalis is a widespread disease caused by different strains of Mycobacterium tuberculosis [1]. It was first discovered by Robert Koch in 1882. Tuberculosis belongs to a pathogenic bacteria species of the family Mycobacteriaceae [2]. It has an unusual, waxy coating on its cell surface (due to the presence of my colic acid), as a result it makes a cell incapable for Gram staining. It spreads through the air or through casual events when people who have an active TB infection, cough, sneeze or transmit respiratory fluids through the air [3]. The genome of MTB consists of circular chromosomes having 4,200,000 nucleotides. The G+C content is about 65% and genome contains about 4000 genes. It Plasmids plays an important role in transferring virulence because genes on the plasmids are more easily transferred than genes located on the chromosome. Two main stages are observed in TB Infection: Active TB is a rapidly multiplying stage in which bacteria invade different organs of the body. A person who has an active pulmonary TB disease may spread TB to others by airborne transmission of infectious particles (i-e through cough). Latent TB is a condition in which infected person with TB doesn't develop disease. They have no symptoms and their chest x-ray may be normal. The only manifestation which shows the encounterment of disease may be through the reaction of tuberculin skin test (TST) or interferon-gamma release assay (IGRA).
TB is one of the world's most devastating human diseases as it affects one-third of the world's population. New infections occur at the rate of 1% of the world population each year [4]. An estimated 13.7 million chronic cases were reported globally, in 2007 [5] while in 2013, an estimated 9 million new cases occurred [6] mostly in developing countries [7]. In 1993 World Health Organization (WHO) declared global TB emergency, after that DOTS and WHO's End

TB strategy was adopted for the successful elimination of TB globally.

2. General Manifestation

M. *Tuberculosis* is a slim, Gram-positive bacillus, size ranging from 0.2 – 0.4, 2 – 10. It is an obligate aerobe, non-motile and non-spore former. It shows maximum growth at the pH of 6. 5-6.8 at 37 ºC. For the primary growth of TB enriched or complex media is required. When 10% carbon dioxide is added to enriched media; its growth is much more enhanced. The dry, rough, transparent colonies usually appear within 4 to 6 weeks after incubation. MTB is an acid fast bacterium that forms an acid-stable complexes when aryl methane dyes are added. This acid fast property of MTB is due to the rope like structural arrangement of peptidoglycan [8]. The bacteria can be isolated and stored in labs at –80 degrees. H37Rv is the most commonly studied and used strains of colonies. MTB complexes are mostly found in the well-aerated upper lobes of the lungs, as it is an obligate aerobe. It is a facultative intracellular parasite of macrophages. It has a slow generation time of 15-20 hours and colonies appear after 4-6 weeks on either type of media. [9].

3. Recent researches

The current research is much more geared up towards the understanding of the mechanism of virulence. For example, one such research showed that prokaryotic-and eukaryotic-like iso forms of the glyoxylate cycle enzyme isocitrate lyase (ICL) are together required for fatty acid catabolism and virulence in Mycobacterium tuberculosis. This is an important discovery which provides an insight such as drugs that are glyoxylate cycle inhibitor, which could be used to treat tuberculosis [10]. Another group of scientists found that a newly identified protein having carboxylesterase activity is required for the virulence of Mycobacterium tuberculosis. They also found that the gene MT2282 encodes a protein that is associated with carboxylesterase. When a mutant gene was used to infect mice, the mice's life was prolonged as compared with those that were infected with the wild type strain [11]. There is very little knowledge regarding the host-microbe interaction that happens before M. tuberculosis gets into the macrophages and how M. Tuberculosis adhere to the host is still being researched. One research suggested M. Tuberculosis produces a tiny Pilli that enables them to colonize the host by adhering to the host and invading the macrophages and epithelial cells of the host. The Pilli produced are called MTP. The study is important because MTP could be used as vaccine in the nearby future [12].The currently available BCG vaccine has limitations, and research to develop new TB vaccines is ongoing. A number of researchers are currently in phase I and II clinical trials. Two main approaches are being used to attempt to improve the efficacy of available vaccines. One approach involves adding a subunit vaccine to BCG, while the other strategy is attempting to create new and better live vaccines [13]. Subunit vaccines, for TB disease are currently in trials in South Africa [14].Vaccines are hoping to play a significant role in the treatment of both latent and active disease.

4. Epidemiology

Tuberculosis first reached epidemic proportions in the western world, during 18th and 19th century. The disease flourishes due to lack of knowledge about the disease, and other factors like ignorance, poverty, overcrowding, poor hygienic conditions and particularly during the social disruptions of war and economic depression the disease flourishes more. After HIV/AIDS, Tuberculosis are the second-most common cause of death from infectious disease [15]. Roughly one-third of the world's population has been infected with M. Tuberculosis and new infections occurring in about 1% of the population each year [16]. However 90–95% of tuberculosis infections remain asymptomatic [17]. In 2010, 8.8 million new cases of TB were diagnosed, and 1.20–1.45 million deaths occurred, most of these occurring in developing countries [18]. 0.35 Million deaths out of these 1.45 Million, occur in those also infected with HIV [19]. Estimated 8.6 million chronic cases of active TB cases reported in 2012 [20]. The country with the estimated highest incidence rate of TB in 2007 was Swaziland, with 1,200 cases per 100,000 people. TB is less common in developed countries and is found mainly in urban areas. In different areas of the world rate of TB per 100,000 people were: Africa 332, America 36, Europe 63, Southeast Asia 278, and Western Pacific 139 in 2010 [21].

5. Global TB Control Program

The global TB emergency was declared in 1993 by WHO saying that: "Tuberculosis is out of control in many parts of the world and today is the greatest humanity killer. The disease, preventable and treatable, but it has been grossly neglected and no country is immune to it." [22]. Later on, DOTS strategy for TB control was launched by the WHO in the mid-1990s. A decade later, tuberculosis was still the significant contributor to infectious disease morbidity and mortality. DOTS rank as one of the most cost-effective health interventions. Since 1995, DOTS expansion has increased, ensuring high cure rate. Approximately 60% of the population has been benefited from this care. In 2009, 5.8 million TB cases were notified through DOTS program. The DOTS-Plus program is for multi-drug-resistant tuberculosis (MDR-TB). According to WHO from 2000-2013 through proper diagnosis and treatment 37million lives were saved [23]. To gear up the TB program globally, World TB day is celebrated each year. The purpose to celebrate this day is to raise the awareness about the burden of tuberculosis (TB) worldwide and the status of TB prevention and control efforts. WHOs End TB Strategy envisions a world free of TB with 0% death due to this chronic disease [24]. Through this program different goal, targets and outlines actions are set for the governments and partners to provide patient-centered care, and drive research and innovations needed to end the epidemic and eliminate TB.

6. Pathogenesis of TB infection

6.1 Primary Infection

After the inhalation of infectious droplets from an infected individual, M. Tuberculosis normally enters the host through the mucosal surfaces – usually via the lung, where they are deposited in the peripheral respiratory alveoli, mostly in the middle and lower lobes of the lungs. In the lungs, they are first engulfed by non-specific alveolar macrophages. If the ingested Mycobacterium is not destroyed by the alveolar macrophages then they will start there multiplication in the macrophage until the macrophage bursts and releases the microbes out where they are ingested by inactivated blood macrophages. The blood macrophages, together with T cells are attracted to the lungs by common tactic factors [25]. Without affecting the host cells, the ingested bacteria continuously multiply intracellularly. Some of the bacterial filled macrophages are transported to the hilar lymph nodes

through lymphatic channels from the infected site where, they may disseminate through blood and lymphatic systems to the number of tissues, including the brain, spleen, liver, bone, meninges, and other parts of the body through blood and lymphatic system [26]. The signs and symptoms in the affected tissues are usually absent while a minor inflammatory reaction is observed. However, through chest x-rays or radiological detection, the primary site of infection and some enlarged hilar lymph nodes can often be detected. In immune compromised adults and in infants, dissemination of organisms through blood may occasionally produce life-threatening meningitis [26].

6.2 Reactivation of Infection

The latent TB reactivated usually in the body parts having comparatively high oxygen content and low lymphatic fluid or drainage, most often in the upper part of the lung. The large number of bacilli usually spreads through the lesions damaging the large areas with a serious necrosis. As a result the necrotic material is discharged or spread towards the pulmonary cavity and bronchi, also damaging the small blood vessels. The chronic fever, weight loss and persistent cough are the main symptoms observed [27].

6.3 Virulence Mechanism

The basic mechanism of virulence in M. Tuberculosis are being still unknown as it does not produce any exotoxin. However cell wall component LAM bond with alveolar macrophages and utilize complement receptors (CR1, CR3), surface fibronectin and mannose [28]. Many of the factors and genes contribute to virulence and increased multiplication of the bacterium inside the cell. These genes either enhance the survival of bacteria in the macrophage or by providing the physical and chemical conditions (low pH, high CO2 and high lactic acid) in developing lesion. LAM, my colic acids, phenolic glycolipids (PGL), sulfolipids and proteins have an important role in disrupting the interaction of phagosome–lysosom [30]. It has an envelope which also contributes to its virulence. LAM has an important function that it modulates cytokine production and also affects the T-cell function including antigen presentation [29].

6.4 Signs and symptoms

The significant signs and symptoms of TB infection include:

A bad cough lasts 3 weeks or longer, Pain in the chest, Coughing up blood or sputum (phlegm from deep inside the lungs), Weakness or fatigue, Weight loss, No appetite, Chills, Fever, Sweating at night and Swollen lymph glands. Active tuberculosis infections mostly involve the lungs (in approximately 90% of cases).Symptoms may include prolonged cough producing sputum, chest pain and cough up blood in small amounts. In very rare cases, the infection move towards the pulmonary artery, resulting in massive bleeding [30]. Tuberculosis may become a chronic illness and cause extensive scarring or lesions in the upper lobes of the lungs. In 15–20% of active cases, the infection spreads outside the lungs, causing other kinds of TB, which are collectively denoted as "extra pulmonary tuberculosis" [31]. Extra pulmonary TB, mostly affects immune suppressed persons and young children. In HIV patients, this occurs in more than 50% of cases. Extra pulmonary infection sites include the genitourinary system (urogenital tuberculosis), the lymphatic system the central nervous system (in tuberculosis meningitis), bones and joints (in Potts disease of the spine) [32-34]. When it spreads to the bones, it is also known as "osseous tuberculosis."

6.5 Risk factors

A number of factors make people susceptible to TB infections. The most important risk factor globally is HIV; about 13% of the people with TB are infected by the virus [35]. This problem is particularly more in areas where the HIV prevalence rate is high i-e sub-Saharan Africa. About 5–10% of the people without HIV who are infected with TB develop active disease during their lifetimes; in contrast, 30% of those cows infected with HIV develop the active disease [36]. Other risk factors related to the TB infection include:

Two closely linked factors of TB infection are overcrowding and malnutrition.

6.6 Drug adductors

People living in an area where vulnerable people like prisoners gather, Poor resource communities and medically underprivileged people, Children in close contact with those people who are at high-risk. Healthcare providers serving the TB patients., Chronic lung disease is another significant risk factor, Cigarette smokers have increased risk of TB disease compared to nonsmokers, Other diseases like diabetes mellitus, kidney diseases and alcoholism also increase the risk of developing tuberculosis [37]. Certain medications, such as infliximab (an anti-αTNF monoclonal antibody) and corticosteroids, are also the important risk factors, especially in the developed world [38].

6.7 Vaccination

Today the only available vaccine against TB is almost one-century-old Bacillus Chalmette Guerin-e BCG vaccine. It was developed by Albert Guerin and Camille Chalmette and at the Pasteur Institute in Paris between 1906 and 1919 in Paris. BCG plays an important role in TB control, principally in areas with high levels of the disease. It has been used for prophylaxis of tuberculosis in various countries. It is a live attenuated vaccine derived originally from a strain of *M. bovis that* was attenuated by repeated subcultures. Successful vaccination leads to a minor local lesion, self-limiting multiplication of the organism locally. It is routinely administered to infants in many countries and provides significant protection against severe forms of TB, mostly disseminating and meningeal forms. However, vaccination campaigns have had little impact on the pulmonary TB, which represents the transmissible form of this disease and also the protective ability of BCG against pulmonary TB in adults is incomplete and inconsistent. It is still unclear why the protective effect of early-age BCG vaccination often begins to vanish with adolescence, especially in TB-endemic areas. Nevertheless, in a North American study, BCG's protective efficacy was found to last for over 50 years [39], thus pointing to the importance of possible environmental modulators (co-infections, comorbidity, nutrition, genetics, TB exposure intensity, etc.). Another issue of concern that compromises BCG's utility is that infants with HIV have an increased risk of developing disseminated BCG-oasis [40], bearing similarities to individuals with genetic deficiencies in the interleukin (IL)-12/IL-23/interferon-gamma (IFNγ)/signal transducer and activator of transcription (STAT)1 pathway [41-42]. This implies that TB vaccines need to be developed that not only have an increased ability to induce protective immunity against TB, but also have a better safety profile compared to BCG [43].

6.8 Consequences for the development of new TB vaccine

In the past, it was assumed that only living vaccines (like BCG) could generate the long-lived response necessary

against M. Tuberculosis infection. This has really influenced the search for immunologically relevant TB antigen. However, Andersen and his colleagues in 1994, examined the protective effect of vaccination in mice and guinea pigs with culture-filtrate proteins (CFPs) obtained from log-phase TB cultures, demonstrated that the CD41 T cells transfer the protection [44]. The hypothesis was purposed after studying the antigens isolated from the culture filtrate of actively growing bacteria, states that proteins secreted in the phagosome by living bacilli might be the first antigens which are to be presented to the immune system during the early phase of the infection, so the immune response might be more effective towards these proteins stimulating a protective immune response [45]. When used as vaccines against an acute M. Tuberculosis infections culture filtrate antigens such as Ag85A/B, ESAT-5 and TB10.4 have shown protective results and efficiency. These antigens are present in experimental stages where the main purpose is to boost BCG-induced immunity [46].The vaccines in clinical development so far were all actively replicating bacteria derived, and have all been assessed as prophylactic vaccines [47]. The primary focus of study is their ability to restrict early bacterial growth. It is also proposed that their activity is limited against dormant bacilli. As M. Tuberculosis can survive intracellularly in latent stage for many years by making major gene expression changes. The conclusion from these studies is that such vaccines should contain antigens specifically expressed by the dormant bacteria.

6.9 New targets for vaccine development

A new phase of research on subunit vaccines for TB starts due to an improved knowledge of antigen expression patterns. Subunit vaccines have many advantages compared to BCG‟s: first and the most significant one is that they have an ability to produce defined antigens and other products expressed by the bacteria during different phases of the infection, the other one is that they have an ability to choose a delivery system that stimulates specifically the kind of immune response – a Th1 dominated response – needed and finally, as they need not to be restricted in their growth (or are designed not to require growth in the host) by prior immunity to Mycobacterium, their activity in individuals is not affected by environmental Mycobacterium. In respect to this a study was done in which a mouse was inoculated with six different typical Mycobacterium strains from soil and sputum samples isolated from Caroga district in Northern Malawi (a region in which BCG vaccination has no effect against pulmonary TB). The results show that the two of these strains from the Mycobacterium avium complex found to block the BCG activity completely. Importantly, the subunit vaccine was completely unaffected by prior sensitization [48]. This makes subunit vaccines highly attractive for the boosting strategy. Also, most of the subunit vaccines under development use either non-living or vectors deficient of replication, means that they pose no threat even in HIV-positive individuals. This shows that they are suitable for vaccines in areas where TB and HIV are closely intertwined especially in TB endemic areas. The TB vaccines being developed fall into two categories. The first category is the development of vaccines having an ability to replace the BCG, showing longer and more effective protection. At present, it is unlikely to replace subunit vaccine with BCG's in the near future, as the BCG's are cost effective, safe and extensively used throughout the world. So, the strategy for BCG vaccine replacement mostly focused on recombinant BCG or attenuated TB vaccines. The other strategy involves the designing of vaccines to further boost up the BCG induced immunity by administering into the already BCG vaccinated individuals. Subunit vaccines are not affected by existing anti-mycobaterial immunity, compared with recombinant Mycobacterium vaccines where it is not clear whether the attenuated vaccines are virulent enough to overcome the existing anti-mycobacterium immunity due to earlier environmental exposure to Mycobacterium or a prior BCG vaccination. Therefore, the best choice is to use the Mycobacterium vaccines primarily, and subunit vaccines as a booster. However, because a vaccine administered as a booster so, a booster vaccine should also have an ability to prime an effective immune response in those people or older children who did not receive the BCG vaccine, or receive an ineffective BCG vaccination (incorrectly administered, or with a vaccine that was too old or incorrectly stored) [47]. It is assumed that about two billion people are latently infected with M. Tuberculosis means that any booster vaccine is also necessary to be administered to large numbers of latently infected individuals. Few questions are raised which are needed to be answered, like: Safety related question i-e either the vaccine, which is to be developed is properly screened for safety in M. Tuberculosis-infected individuals? Can this vaccine help the already infected people, who did not prevent a latent infection or did not receive a primary vaccination? Mathematical modeling suggests that a post-exposure vaccine are effective in preventing disease in latent infection individuals which cause a significant decrease in the number of new cases in the short term, but, a combination of pre- and post-exposure vaccine would have a larger effect [48]. The best approach would therefore be a single vaccine that is effective in different stages of infection and can counteract acute and latent infection. However, no such „multistage‟ vaccine currently exists.

6.10 New tools for TB prevention and control

New tools are defined as: Availability of new diagnostics, drugs and vaccines, their optimal adoption in TB control programs and their implementation in areas where they have proven efficacy and impact. Following a few steps are taken to control the spread and to reduce the intensity of tuberculosis disease. Technological advancement in the field of diagnosis, therapies, and vaccines are urgently needed. Increased sensitivity and faster field diagnosis methods are needed to detect M. tuberculosis and to identify drug resistance. Ongoing researches in exploring diagnostic technologies such as simple dipsticks that evaluate blood samples and manipulation of mycobacterium-specific bacteriophages (viruses that infect bacteria) to create culture-based tests [49]. There is also a need for new anti-tuberculosis drugs and vaccines. Drugs that reduce the duration and frequency of treatment will tremendously affect the tuberculosis control for patients to ensure complete treatment [50]. Development of an effective vaccine is critical in controlling and eliminating tuberculosis. For countries having dual epidemics of tuberculosis and HIV, programs are needed in order to provide additional control services (such as preventive therapy for high-risk persons) to accelerate the decline of tuberculosis. The development of simple, inexpensive diagnostics, alternative treatment regimens (especially less expensive and shorter treatment regimens) and improved vaccines should be explored as ways to improve tuberculosis control efforts.

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