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REVIEW

Tuberculosis: A disease without boundaries

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SUMMARY

Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis* (MTB) that usually affects the lungs leading to severe coughing, fever, and chest pains. Although current research in the past four years has provided valuable insight into TB transmission, diagnosis, and treatment, much remains to be discovered to effectively decrease the incidence of and eventually eradicate TB. The disease still puts a strain on public health, being only second to HIV/AIDS in causing high mortality rates. This review will highlight the history of TB as well as provide an overview of the current literature on epidemiology, pathogenesis and the immune response, treatment, and control of TB. In this race to combat a disease that knows no boundaries, it is necessary to have a conceptual and clear understanding of TB overall with the hope of providing better treatment through novel and collaborative research and public health efforts.

1. Introduction: the history of tuberculosis from the 1800s to the present

"Just sleep and eat nutritious foods" was the advice given to patients in the 1800s infected with tuberculosis (TB), or formerly known as consumption [1], an airborne disease that usually affects the lungs leading to severe coughing, fever, and chest pains [2,3]. This mysterious disease, whose Latin-originated name describes the rod shape of the bacillus, became better understood when the German microbiologist Robert Koch announced that *Mycobacterium tuberculosis* caused TB in 1882 [1]. This revolutionary finding, along with the later discoveries of tuberculin in 1890 and the Bacillus-Calmette Guérin (BCG) vaccine in 1908 and antituberculosis drugs starting in 1943, offered hope for the eradication of a disease deadlier than the plaque.

Mortality rates significantly declined from the early to mid-20th century; however, funding for research dwindled and between 1970 and 1990, drug and vaccine developments slowed [1,3]. With the onset of the AIDS pandemic and resistant strains, TB rates rose once again, and with that, interest in TB research and prevention [1]. Though by this time, the diagnostic and treatment tools necessary to combat the disease were largely obsolete and strategies to control and prevent the disease were developed, including the Directly Observed Treatment Short-Course (DOTS) program in 1993, with the addition of a DOTS-plus program to address multidrug resistant (MDR) TB in 1998 [1,3].

Although current research in the past four years has provided valuable insight into TB transmission, diagnosis, and treatment, much remains to be discovered to effectively decrease the incidence of and eventually eradicate TB [1,4]. The disease still puts a strain on public health, being only second to HIV/AIDS in causing high mortality rates [2]. It has been reported that in 2011 alone, there were about 8.7 million new cases and 1.4 million deaths due to TB [1,2,5], with about two billion people latently infected [3]. The

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purpose of this review is to highlight the current literature on epidemiology, pathogenesis, treatment, and control of TB, in order to better understand the disease in hopes of providing better treatment through novel research and public health efforts.

2. Epidemiology, transmission, diagnostic tools: prevalence, airborne transmission, TST/IGRAs

There are certain risk groups that are more susceptible to getting infected including: young adults (more commonly males), those in developing countries, health care workers who are around the disease frequently, and those whose immune systems are weak, as in those who have HIV or smoke [2,4]. In fact, TB is the leading cause of death in those infected with HIV and HIV-TB comorbidity has been widely studied [4,6]. Additionally, foreign-born individuals and those who reside in impoverished areas or where malnutrition is prevalent are more likely to get infected [2]. The host's own deficiency in interleukin (IL)-12 promoting the T helper (Th) 1 response may be another factor in the increased susceptibility to infection [7]. There are other conditions that may pose a high-risk for susceptibility to MTB infection such as diabetes, ageing, long-term use of corticosteroids, TNF-α blockers, polymorphism in vitamin D receptors, polymorphism in IL-12 and IFN-γ genes. However, these other conditions will not be discussed in detail here.

MTB infection is acquired by inhalation of infectious aerosol particles released from close contacts [6,8]. A majority of individuals who inhale MTB mount an effective response in the lungs leading to successful inhibition in the growth of MTB, resulting in the bacteria becoming dormant; this condition is often referred to as latent tuberculosis or LTBI [9]; immunocompetent latent individuals are infected with MTB but do not present symptoms and do not transmit the disease to others [2,6]. It is well-known that 1/3rd of the entire world's population is latently infected with MTB [6]. From latent infection, the infection can progress to an active state [5]. About 5–10% of LTBI cases are at risk to progressing from infection to active (primary) TB [6]. Those with HIV and other immunocompromised individuals, such as those with cancer or currently taking immunosuppressing medication have a higher risk of developing active TB.

The World Health Organization (WHO) reported that “one-third of the world's population has been infected with TB” [2]. Holding true to Robert Koch's statement that the disease is deadlier than the plaque or cholera [1,6], about 9 million people were infected with TB and about 1.5 million succumbed to the disease in 2013 [2]. In 2004, TB was responsible for 2.5% of all deaths in the world [8]. The household is often the site of exposure in high- and low-burden countries, though infection rates are higher in areas such as hospitals or prisons [4]. Prevalence of the disease in such settings depends on virulence, innate immunity, and susceptibility.

While TB can be present in any society in any country, a majority of those deaths reported, about 95%, occurred in low- and middle-income countries where resources are more limited, with a majority of cases appearing in India and China [2,4]. Those with HIV are most at-risk for getting infected with TB, and about 80% of HIV-infected people with TB live in sub-Saharan Africa [4,8]. In contrast, in low-burden countries like the United States, only 10% of people with TB are infected with HIV; in 2008, only 12, 904 TB cases were reported with the incidence being 4.2 per 100,000. While diagnostic advancements have been made in the past four years, 80% of TB cases worldwide are concentrated in twenty-two countries [1] twenty-seven countries including India, China, and Russia, are responsible for about 85% of MDR TB cases [4]. Unfortunately, more recent data is not available due to the limits of global surveillance and reporting systems [8]. Additionally, there still remains an incomplete understanding how one person can acquire the disease while another doesn't, though they are exposed to the same risk factors, or how to better determine latent to active TB progression [5,7].

Since a majority of people with TB have latent infection [6], the development of new diagnostic and screening tools and standards has become necessary in order to control the disease [9,10]. Interferon-gamma release assays (IGRAs) are used to diagnose LTBI, although the tuberculin skin test (TST) still remains the most cost-effective test [9]. The TST and IGRA work by measuring the response of T cells to TB antigens [6].

2.1. TST

In the traditional TST, tuberculin purified protein derivative (PPD) mix of proteins from TB are intradermally injected into a person, causing a type IV delayed hypersensitivity skin reaction, if the individual was either previously exposed to mycobacterial proteins present in the vaccine or previously exposed to the mycobacteria infection [9]. To determine if the person is infected with TB, the size of the skin reaction is measured; the usual standard is between 48 and 72 h and a cut-off from 0.74 at 5 mm to 0.40 at 15 mm. However, the TST is known to lead to false-positive responses in those who are BCG vaccinated and to false-negative responses in immunosuppressed individuals.

2.2. IGRAs

The IGRAs are more sensitive and specific (81–88% compared to 70% sensitivity for the TST) [10]; however, they are expensive and technical [4]. They detect the release of cytokine IFN-γ from T cells that react to antigens not found in the BCG vaccine [4,10]. A blood sample is taken from an individual and the release of IFN-γ is measured. Guidelines constantly change for IGRA use. In Canada and in some European countries, it has even been suggested that IGRAs and the TST be used together to diagnose LTBI, but these tests are not definitive [9,10].

Overall, having a better understanding of how the disease develops in individuals from a latent to active TB by identifying risk factors associated with high- and low-burden countries will help lead to the development of better diagnostic tools and will improve our understanding of the immune response in TB.

3. Pathogenesis and immune response: the interactions between MTB and the host cell

de Martino et al. [7] describe the initial combat of TB once it invades the host using the quote: “All is decided the first day, which gets the longest day”. Once the bacterium *M. tuberculosis* (MTB) is inhaled via droplets spread through person-to-person contact, macrophages can phagocytose and kill the bacilli. However, if the bacilli are not killed, during that initial interaction, they can proliferate within dendritic cells and alveolar macrophages at a rapid rate, signaling the production of IL-1-α, IL-1β, and other host proinflammatory cytokines.

This response is mediated by pattern recognition receptors (PRRs) [7,11] expressed by macrophages and dendritic cells that recognize pathogen-associated molecular patterns (PAMPS) expressed on MTB [11]. Toll-like receptors (TLRs) help uptake MTB, which induces an intracellular signaling cascade to produce the cytokines. However, anti-inflammatory cytokines help the infection by opposing host cell proinflammatory responses. During the initial innate immune response, MTB proliferates within the host cell, inducing cell death via the virulence factor ESX1 type VII secretion

system, on its own terms and when it is ready, delaying the adaptive immune response [7,12].

In doing so, TB is a unique disease in that a detectable cellular response occurs after a much longer time-about 2–12 weeks after infection [4,6]. Dendritic cells transport *mycobacteria* to the mediastinal lymph node (MLN), where antigen-presenting cells (APCs) activate T cells [13]. Bacilli are loaded onto major histocompatibility complex (MHC) class II, priming Th 1 IFN-γ-secreting antigen-specific T cells that move to the lung. With the activation of the adaptive immune response, both effector $CD4^+$ and $CD8^+$ T-cells are induced [7,13]; MTB can accumulate mutations during this time [7] and cytokines are continually produced, such as IFN-γ [13]. *Mycobacteria* can change the expression of their genes and therefore their surface antigens to evade T-cell recognition [7].

After the continuous production of cytokines, T cells limit the mobility of macrophages and activate them for enhanced function [13]. A type 1 "immune granuloma", composed of macrophages, neutrophils, monocytes, dendritic cells, and T cells, is then formed in the lung that might control MTB growth, as some studies have shown, though it is now thought that MTB can hijack the granuloma for its own benefit. MTB alter the immune response of the granuloma through IL-10, which works to suppress the activation of T cells and macrophages. Interestingly, knockout (KO) studies in IL-10 mice demonstrate a small reduction in bacterial load, suggesting that, through evolution, MTB might utilize multiple pathways for its own benefit. A "chronic granuloma", that additionally is composed of foamy and epitheloid macrophages and a fibrotic encapsulation, can result, leading to the morphological and functional alterations of the granuloma; these alterations are highly dynamic and can vary between individuals and even within an individual, though it is unclear whether these alterations are mediated by the host or by the bacteria.

There is a delay between MTB infection and T cell response [6,7,13]. Up until this stage, TB is suppressed to an inactive, or latent form, where infected individuals do not present with symptoms and cannot transmit the disease [6,7]. In the case of LTBI, as mentioned, mycobacterial growth is controlled (though bacterial replication does occur) by adaptive immunity before the disease can develop [4], by containing MTB within granulomas [6,11,13] or by nitric oxide (NO) in mice or antimicrobial peptides in humans [14]. Latently infected people display healed granulomas, "marked by central calcification in conjunction with fibrotic encapsulation containing no detectible bacilli" [4,13]. Encapsulation may prevent bacterial escape or limit immune intrusion, but this is unclear [13].

However, at some point, even after many years of latency, exogenous factors [14], like the secreted protein resembling resuscitation-promoting factor (Rpf), can be activated, where bacteria are reverted from a suppressed state to an active state and can resume cell division [7,12]. Rpf gene deletions might be a factor in the progression from latency to reactivation [12], where it has been shown that RpfB and RpfE are important for resuscitation [14]. Additionally, toxin-antitoxin (TA) gene pairs, or plasmid maintenance factors [14], encoded by MTB play a role in determining the switch from latency to reactivation by producing a toxin that gets neutralized by antitoxin that is then degraded; the toxin can then kill the cell [12,14]. Although, the regulation between latent and active TB and the actual state of the bacterium during latency remains to be further studied [14].

It is at the adaptive immune stage that TB can clinically manifest in multiple ways, once it switches from a latent to active state; clinical manifestation depends on where *mycobacteria* proliferate [4,6,14]. Proliferation can occur in the spine, hips, and the gastrointestinal tract, but in most cases, proliferation occurs in the lung; about 85% of TB patients present with pulmonary symptoms [6]. Clinical symptoms of pleural TB include chronic cough, night sweat, blood-tinged sputum, weight loss, shortness of breath, fever, chest pain, and pleurisy (or inflammation of the pleura membrane surrounding the lungs) [4,6]. Cloudy patches and pleural effusion can appear in chest radiographs [6]. However, elderly people might not show these signs and symptoms [4].

In order to study the interaction between the host immune response and the MTB, several animal models are used, including the murine (or mouse), guinea pig, rabbit, and monkey models [14]. The preferred animal models to date are the murine and monkey (or macaque) models [13–15]. While the murine model is more cost-effective and more easily available than the macaque model, it lacks a paucibacillary state present in human latent infection that the macaque model does possess [14,15]. Although the two are different and cannot imitate human TB perfectly due to a variety of factors and complexities, findings using each combined have provided valuable insight into TB characteristics, pathogenesis, and treatment [14,15]. Here, the focus will be on the contribution of the murine and macaque models to our understanding of human TB latency, granulomas, treatment [13,14], and HIV co-infection [14,15].

In the murine model, expression studies conducted in 2004 that analyzed the change in gene expression profiles during MTB chronic infections *in vivo* have shown that genes change their transcription levels in the lungs, similar to *in vitro* dormancy models [14]. Earlier studies conducted using the Cornell model of dormancy developed around the 1960s have shown that mice treated with certain drugs can develop reactivation; without this treatment, an actively replicating dormant population is present in mice. Unlike the murine model, clinically latent infection in the cynomolgus model can be directly detected and reactivation does not require the drug regimen needed by the Cornell model. Latent infection granulomas from the macaque model more closely resemble those in humans [14,15], which could add to our understanding of how humans with latent disease respond to antibiotic treatments [14] and could translate from murine studies to humans about the role of granulomas in preventing and controlling the spread of MTB in the future [13].

Translational studies should be interpreted with caution [14], as modeling HIV, a high risk factor associated with TB, has proved challenging as mice are not hosts of HIV [15]. Although humanized and BLT (bone marrow, liver, thymic) mice can be used, the macaque model can be infected with a retrovirus similar to HIV, simian immunodeficiency virus (SIV). Recently, MTB antibiotic treatment was shown to reduce HIV transgene expression in HIV transgenic mice, while mice infected with MTB demonstrated an increase in HIV expression at bacterial replication sites and sites of inflammation. The MTB/SIV co-infection macaque model has played an important role in investigating the cellular and molecular mechanisms of TB reactivation by HIV infection and in the immunologic and microbial factors associated with MTB and HIV co-infection. Using this model, an inverse relationship between risk of MTB infection and CD4 T cell levels has been observed: when the former increases, that latter decreases; this finding has great implications for highly susceptible HIV patients [15]. Taken together, these findings can help in the creation of novel treatment regimens.

4. Treatment and vaccines: antituberculosis drugs, BCG vaccine, and drug resistance

The course of TB treatment depends on whether the individual is in the latent or active stage and on his or her probability of risk. If a person has recently come into contact with an infected individual and a TST is negative, LTBI treatment can be started and continued if the TST result is positive after a 12-week window; HIV patients usually continue treatment though the TST result might be negative

[6]. Treatment of TB usually involves a drug cocktail, or a mixture of multiple drugs, with an intensive initial 2-month phase followed by a slower 4- to 6-month continuation phase [4].

The main antituberculosis drugs used in the chemotherapy of TB are: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM) [4,6]. Depending on the status of the exposed individual, the regimen changes and can be complex to follow. A drug regimen chart created by the Centers for Disease Control and Prevention (CDC) outlines the intervals and doses for drug treatment during specific phases [8]. For example, if the MTB isolate is fully susceptible, either EMB or SM are discontinued, and PZA can be discontinued after two months of treatment. INH and RIF are continued for four months. Treatment can last from six to nine months [6,8] or even up to twenty months [6].

In addition to the five main antituberculosis drugs, the *Mycobacterium bovis* BCG vaccine is the current vaccine used to mimic the natural immune response to infection [16]. Although the BCG vaccine has been widely administered for more than eighty years and strongly induces Th 1 cells, its efficacy is highly variable, according to a recent review by Andersen [16]. It has only limited protection in adults with pulmonary TB in high-TB-endemic regions. The need for a better vaccine is evident; however, limitations in our knowledge of which aspects of BCG immunity are important for long-lasting protection against MTB prevent successful efforts. Over the past ten years, there has been a race to develop a better vaccine, and the Modified-Vaccinia-Ankara (MVA) 85A vaccine was introduced [16]. Unfortunately, excitement over MVA85A clinical trials was undermined as the vaccine could not improve protection against TB.

Why is there such a complex regimen involving multiple drugs to treat TB? The answer lies in the fast spontaneous mutation rate of MTB [4,6]. Although drug susceptibility tests are always performed to monitor resistance [8], previous treatment, not completing treatment, not complying with treatment, and improper or inadequate regimens can confer drug resistance [2,6,8]. According to the fourth Global Drug Resistance Surveillance Project conducted from 2002 to 2007 by the WHO [2], about 17% of new resistance cases had resistance to at least one antituberculosis drug and about 2.9% had multi-drug resistance (MDR), or resistance to at least INH and RIF. INH resistance is the most common type [6]. A WHO report in 2009 estimated about 3.3% of TB cases were MDR.

Drug resistance continues to pose a major health concern. In some areas, such as Azerbaijan, it was reported in 2007 that 56.3% of new cases were resistant to any drug and 22.3% were MDR [8]. Annual mortality was estimated in 2009 to be 150,000 worldwide [6], with about 480,000 developing MDR-TB in 2013 with the most cases found in India, China, and Russia [2]. In recent years, extensive-drug resistance (XDR), or MDR plus resistance to second-line drugs, has become a worldwide concern, with about 24% of XDR cases among MDR cases in Russia in 2006 [8], and accounting for about 9% of MDR cases in the world in 2013 [2].

5. Control of TB: achievements, DOTS, Stop TB Strategy

Fortunately, the WHO reports that some countries have continued to have major declines in TB cases [2]; in Cambodia alone the TB prevalence fell by about 50%. Due to diagnostic and treatment efforts, about 37 million lives were saved between 2000 and 2013. [2]. However, maintaining this success has mostly been complicated by HIV, MDR, XDR, the inefficacy of the BCG vaccine, false positive screening tests, non-compliance to treatment regimens, the complexity of MTB, and the stall in developing novel therapeutics.

In some countries, like India, China, and Russia, TB rates are only dropping slowly and still remain high [2]. To address this concern, an additional treatment regimen is undergone by patients called directly observed therapy short-term (DOTS), which closely monitors treatment adherence and completion [6], with the goal of efficiency and cost-effectiveness [1]. With the emergence of MDR-TB, DOTS-plus was initiated as a more rigorous treatment strategy [1,4].

The WHO drafted a Stop TB Strategy [2], which outlines six aims to be implemented by all countries to reduce TB on both local and national scales, with the overall goal of enhancing TB treatment, health care experiences, and research. It is the world's hope that at least some of these aims will be achieved in the near future.

6. Conclusion: where are we and where do we need to go?

Understanding TB has progressed from the advice in the 1800s to “just sleep and eat nutritious foods” [1]. IGRAs can better diagnose LTBI than TST [4,10] and the macaque model can provide insight into HIV co-infection as it closely resembles human TB [13–15]. However, a more complete understanding of latent development [3,4,6,7,11–14] and of the protective and pathological immune responses and interactions in TB [4,6,7,11–13,16] are greatly needed in order to develop efficacious drugs and vaccines [6,16]. Possibly measuring an individual's transcriptional signature could allow for a more personalized monitoring of the disease. This could help reduce the number of MDR cases [2,4,6,8] and might be a better alternative to DOTS therapy [1,4,6]. It is remarkable that this disease has posed a threat to public health since the 1800s, yet therapeutic advancements have been futile and slow. In this race to combat a disease that knows no boundaries, it is necessary to have a conceptual and clear understanding of TB overall. This can only be enforced if collaborative efforts among research, public health officials, primary care professionals, and the general public continue to push the boundaries of our current knowledge, mitigating the global burden of TB.

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