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Latent tuberculosis: what the host "sees"?

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Abstract

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), is the most successful pathogen of mankind and remains a major threat to global health as the leading cause of death due to a bacterial pathogen. Yet 90–95% of those who are infected with MTB remain otherwise healthy. These people are classified as "latently infected," but remain a reservoir from which active TB cases will continue to develop ("reactivation tuberculosis"). Latent infection is defined by the absence of clinical symptoms of TB in addition to a delayed hypersensitivity reaction to the purified protein derivative of MTB used in tuberculin skin test or a T-cell response to MTB-specific antigens. In the absence of reliable control measures for tuberculosis, understanding latent MTB infection and subsequent reactivation is a research priority. This review aims to summarize the recent findings in human and non-human primate models of tuberculosis that have led to new concepts of latent tuberculosis.

Keywords

Tuberculosis; *Mycobacterium tuberculosis* infection; Latent tuberculosis; Non-human Primate models

Introduction

Mycobacterium tuberculosis (MTB) remains a major threat to global health. The latest World Health Organization (WHO) global burden of tuberculosis (TB) estimates are that almost one-third of the world's population is infected with MTB, with 8.9–9.9 million incident cases, 9.6–13.0 million prevalent cases, 1.1–1.7 million deaths among HIV-1 uninfected, and an additional 0.45–0.62 million deaths in HIV-1 infected persons [1]. Furthermore, one-third of the world's population that is considered latently infected remains a reservoir from which active TB disease will continue to develop for the foreseeable future, thus presenting a major obstacle to achieving global control of TB.

A major breakthrough in the history of tuberculosis was the successful attenuation of *M. bovis* for use as a vaccine: Bacille Calmette-Guérin (BCG), developed in 1921. It remains the only available vaccine for tuberculosis worldwide, with over 120 million doses administered each year [2]. BCG immunization is considered effective in children, providing 80% protection against severe and disseminated tuberculosis, such as tuberculous meningitis and miliary disease [3, 4]. BCG reduces the risk of TB in adults, by an average of 50% as shown in a meta-analysis, however various reports suggests wide range of efficacy from 0 to 80% in different populations [5, 6]. Comparative genomics studies reveled genetic

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divergence with various BCG vaccine strains [7–11]. Potential influence of these genetic differences, through antigenic variation, on the protective immunity and efficacy of BCG immunization with various vaccine strains has generated considerable concerns internationally [12]. The genetic diversity of different BCG vaccine strains has also been shown to have variable effect on immunogenicity, reviewed in [13].

Current control strategies against TB in most developing countries are still largely dependent on the partially effective BCG vaccine, and the early diagnosis and treatment of active TB. In latently infected person at high risk, drug treatment with isoniazid as preventive therapy is recommended, as well as antiretroviral therapy for HIV-1 infected persons, since TB is the major killer of HIV+ persons worldwide [14].

MTB infection is spread by airborne droplet nuclei, which contain the pathogen expelled from the lungs and airways of those with active TB. The infectious droplet nuclei are inhaled and lodge in the alveoli and in the alveolar sac where MTB is engulfed by alveolar macrophages. These macrophages invade the subtending epithelial layer, which leads to a local inflammatory response that results in recruitment of mononuclear cells from neighboring blood vessels, providing fresh host cells for the expanding bacterial population. These cells initiate the formation of the granuloma, the hallmark of tuberculosis disease pathology. With the development of an acquired immune response and the arrival of lymphocytes, the granuloma acquires a more organized, stratified structure [15]. The development of immune response about 4–6 weeks after the primary infection is indicated by a positive DTH (delayed type hypersensitivity) reaction to Tuberculin. The balance between host immunity (protective and pathologic) and bacillary multiplication determines the outcome of infection.

An encounter with MTB is classically regarded to give rise to three outcomes: (1) a minority of the population rapidly develop primary active TB disease—with clinical symptoms; (2) the majority of infected persons show no disease symptoms but develop an effective acquired immune response and are referred to as having latent infection; and (iii) a proportion of latently infected persons will reactivate and develop post-primary active TB.

After infection with MTB, 5–10% of individuals (mainly infants or children) will develop progressive clinical disease referred to as primary active TB. Primary TB usually occurs within 1–2 years after the initial infection. This results from local bacillary multiplication and spread in the lung and/or blood. Spread through the blood can seed bacilli in various tissues and organs. Post-primary, or secondary, TB can occur many years after infection owing to loss of immune control and the reactivation of bacilli. The immune response of the patient results in a pathological lesion that is characterized by localized, often extensive tissue damage, and cavitations. The characteristic features of active post-primary TB can include extensive lung destruction with cavitation, positive sputum smear (most often), and upper lobe involvement, however these are not exclusive. Patients with cavitary lesions (i.e., granulomas that break through to an airway) are the main transmitters of infection. In latent TB, the host immune response is capable of controlling the infection but falls short of eradicating the pathogen. Latent TB is defined on solely on the evidence of sensitization by mycobacterial proteins that is a positive result in either the Tuberculin skin test (TST) reaction to purified protein derivative of MTB or an in vitro interferon-gamma (IFN-γ) release assay to MTB-specific antigens (described below) [16], in the absence of clinical symptoms or isolated bacteria from the patient.

Vaccination with BCG can cause difficulties in interpretation of the TST, due to the genetic similarities of BCG and MTB. Although the available epidemiologic evidence supports that tuberculin reactivity due to BCG vaccination fades 5–10 years after vaccination, it is still not

possible to completely differentiate individuals that are PPD + as infected or vaccinated. Advances in genomic research resulted in the landmark discovery of a genomic region of difference (RD1) that is absent in BCG but present in MTB, which led to the development of in vitro T-cell-based immunodiagnostics measuring Interferon-gamma (IFN-γ) release in response to the RD1-encoded immunodominant antigens ESAT-6 and CFP-10. The assays show promising results for diagnosis of MTB infection with the operational advantage of requiring only one patient visit, higher sensitivity compared with the TST and unaffected by prior BCG vaccination [17]. This was considered a major advance in the last decade [16, 18, 19]. These assays are also less affected by the immune status of the person tested [20]. However, as with the TST, these assays do not differentiate between MTB infection and active TB [21]. Thus, diagnostics for determining disease status (i.e., active, latent, or reactivation) are still a major impediment to control of TB. In contrast to the risk of MTB infection, which is largely determined by exogenous factors, i.e., exposure, the risk of developing active TB following MTB infection is largely endogenous, determined by the individual's immune status or by the virulence of the MTB strain.

"Latent tuberculosis": the changing paradigm

The term latent tuberculosis was coined by Clemens von Pirquet, who developed the tuberculin skin test (TST) in 1907 using Koch's tuberculin (a crude mixture of mycobacterial products). He introduced the term "latent tuberculosis" to describe children who did not manifest any symptoms of tuberculosis but had a positive response to tuberculin. In 1908, Charles Mantoux introduced the use of cannulated needle and syringe to inject tuberculin intracutaneously in the current format and Florence Seibert further developed purified protein derivative (PPD), which is currently in use [2, 22].

The term "latent" TB appears to be a very useful clinical concept to prioritize management of TB. However, considering "latent" TB as a homogenous entity poses potential limits to rational development of new drug compounds, vaccine candidates' biomarkers, as well as to determining the risk each individual has for reactivation. A significant improvement in the understanding of the MTB "latency" is necessary to be able to put forth the research priorities for control of TB.

The interplay between MTB and the human host is multi-faceted and complex, and poorly understood. New technologies have contributed to the development of new concepts about this interaction. With particular relevance to this review, recent studies on the nature of immune response, histology of lung granuloma, imaging studies (especially in non-human primate models), and treatment response strongly support that there is marked heterogeneity within the classifications of active and latent TB [23, 24].

Although latent TB infection traditionally implied that the MTB was in some inactive form within the body, a more current and evolving concept is that the definition of latent TB encompasses a diverse range of individual states, from those who have completely cleared the infection to those who are incubating actively replicating bacteria in the absence of clinical symptoms [23, 25]. Similarly, active TB in humans and non-human primates is characterized by diverse pathological presentations, ranging from small granulomas, caseous hypoxic lesions containing variable numbers of bacteria, to liquefied cavities with a massive load of replicating organisms to extrapulmonary or miliary disease [23, 24]. In addition, there is also increasing observation of sub-clinical active infection in TB prevalence surveys [26].

Therefore, it was proposed that the clinical diversity in tuberculosis reflects the relative numbers, type, and anatomical distribution of lesions and it is better that the MTB infection may be viewed as a continuous spectrum extending from sterilizing immunity, to subclinical

active disease, to fulminant active disease, with conventional designations of latent infection and active disease corresponding to partially overlapping regions of biological heterogeneity [23], and as described in Fig. 1 (can be found in color in online version).

The major drawback currently with the changing view of latent TB is that none of the diagnostic assays are sufficiently sensitive or specific to assign/define a particular person to a "place on the spectrum." This hinders development of newer drugs and vaccine candidates and the management of those at high risk before they develop active disease. A research priority therefore is to understand the spectrum of tuberculosis, with the available technological advances, so that combinatorial markers can be defined that address the scale of the spectrum. The numerous questions that need to be addressed include: Where is the bacterium in latent TB? What pathologic or immunologic features define the spectrum? Can we measure MTB products and correlate these with the spectrum of the tuberculosis, what are the correlates of protection? What is the role of T or B cells in the spectrum of latent tuberculosis? What is the breaking point of reactivation and dissemination? These could be addressed by a comprehensive study of immunological and cellular markers combined with evaluation of transcriptional profiles, use of advanced real-time imaging technologies, and at the tissue and organ level to understand the complexity of all stages of MTB infection.

Granulomas and latent tuberculosis

The granuloma is the pathologic hallmark of TB. A granuloma is an organized structural collection of immune cells that forms in response to pulmonary inflammation due to the interaction of host immune cells with the antigenic stimuli of the bacillus, resulting in the recruitment of multiple cell types. The primary cellular component of the granuloma is the macrophage [27]. They initiate and form the major cell type in granulomas. In addition, $CD4^+$, $CD8^+$ T cells, B cells, neutrophils, and fibroblasts are present. The macrophages primarily harbor the bacilli and also capable of killing these microbes. Their functions include antimycobacterial effectors, pro-and anti-inflammatory cytokine production, secretion of chemokines, and proteins associated with tissue modeling.

The granuloma functions both as the niche in which the bacillus can grow or persist and the immunological environment in which host cells interact to control and prevent dissemination. In human TB, a spectrum of granuloma types is observed in both active and latent TB. Thus, the mere formation of granuloma is insufficient to control of infection, but the proper functioning of granuloma determines the ultimate outcome of infection [27]. The types of granuloma include caseous granulomas, comprising both T and B lymphocytes, macrophages, and neutrophils. Caseous granuloma in latent infection can be calcified with few inflammatory cells. Non-necrotic granulomas are primarily macrophages, with fewer lymphocytes and are found primarily in active disease. Necrotic neutrophilic granuloma and completely fibrotic granulomas can also be observed in infected patients [27, 28]. The role of macrophages in granulomas has been recently reviewed [27].

To study and understand the formation and function of granulomas requires obtaining lung tissue with granuloma, which is very difficult in humans. Therefore, animal model systems are necessary for detailed study of tuberculosis. There are many different animal models of tuberculosis, with varying similarities to humans. Non-human primates, primarily macaques, are remarkably similar to humans in terms of infection outcome and presentation as well as pathology and have the additional advantage of a large number of immunological tools being available for manipulation of the system. The granulomas seen in active and latent monkeys are extremely similar to those seen in human TB [28–31]. Lin et al. [28] showed that a spectrum of lesions that could be found within and between monkeys of the same clinical classification (active or latent) and that these types of lesions are relatively specific

to the disease state, as has been described for humans [32] classified as having latent infection typically have at least one, and often several, granulomas in one lung lobe and in an associated draining thoracic lymph node(s). This is the classic Ghon complex, described decades ago in human latent tuberculosis. These granulomas are generally caseous with partial or total mineralization, often with extensive peripheral fibrous connective tissue deposition. Completely fibrotic (sclerotic) lesions are occasionally observed. On the other hand, monkeys with active TB often have a range of lesion types, including caseous with or without peripheral fibrosis, non-necrotizing (primarily epithelioid macrophages with a lymphocytic component), and suppurative (with significant neutrophilic infiltrate) [28]. Mineralized or completely fibrotic lesions indicative of more chronic immunologic responses to subclinical (latent) infection are occasionally observed in monkeys with active TB. The identification of subclinical "percolating" monkeys indicated that some bacilli occasionally escape from the confines of a granuloma to appear in the airways, even in latency [28].

Where is the bacterium in latent TB?

Latent TB is not defined by the isolation of bacilli from the infected host, since the very definition of latent TB precludes the presence of bacilli in easily accessible sites (e.g., sputum). The actual "state" of bacilli during latent tuberculosis continues to haunt researchers for more than 100 years. Reports from autopsy studies performed in the early 20th century suggest viable bacteria could be isolated from tissue samples in those from TB endemic regions who died of causes unrelated to TB [33–36].

In humans, it is not feasible to evaluate the existence of MTB in healthy latent TB cases. Non-human primate models present potential alternative and advantage for further understanding of the nature of MTB during latent infection. In the NHP model, low-dose infection with MTB leads to development of latent TB or active TB. MTB can be sometimes cultured from airways or by gastric aspirate (GA) in the first 2 months post-infection in those monkeys that develop latent TB. Further, at necropsy, in the monkeys that did not present with any clinical symptoms of TB (latent TB), there were fewer granulomas in the lung and draining lymph nodes when compared with those monkeys that developed active disease. Lower numbers of MTB were cultured from the involved lung and lymph nodes in latent TB monkeys. In addition to latent and active TB, small numbers of monkeys showed no clinical symptoms of TB, but occasionally had positive MTB culture from bronchoalveolar lavage (BAL) or GA several months after or even years after infection. These monkeys were thought to represent subclinical disease and were termed as percolators [28]. Thus, a spectrum of infection outcomes, including a spectrum of latency is supported by this realistic model system.

The common belief is that in latent TB, due to the "potent" immune response generated by the host, the bacillus stops growing and enters a state of stationary phase, eventually becoming non-replicating, while retaining the ability to resume growth under favorable circumstances within the granuloma [37]. These MTB are often referred to as dormant, persistent, non-replicating [38–43] However, there is very little data to support that this is actually the case, and in fact the physiologic and metabolic status of the bacilli in latent TB remains a mystery. The physical location of bacilli during latent infection also remains poorly understood. The knowledge of the physical nature of MTB during latency is important for development of new treatment, prevention (pre- and post-exposure), and diagnostic options [43]. Although it is commonly believed that viable or non-replicative bacteria is contained within the granuloma during latent TB, old autopsy studies suggest that viable bacteria may be present in apparently normal lung tissue from individuals who had tuberculous lesions [34, 44] but no clinical disease.

The persistent bacilli are thought to encounter depletion of nutrients, shifts in pH, production of growth limiting products, and reduced oxygen or increased nitric oxide within the granuloma; this has led to the development of various in vitro models of latency [41]. Bacterial culture models are subject to various stress conditions like hypoxia [38], nutritional starvation [45], acidic pH [46], and nitric oxide, which could mimic some of the microenvironments encountered by the bacillus in vivo. MTB subject to these culture conditions (stress) induce distinct set of gene expression profiles [45–48], different from normal optimum growth conditions. From a variety of human, animal and in vitro studies, it is apparent that the oxygen tension is intimately associated with the outcome of MTB infection [31, 40, 42, 49–51]. A detailed analysis of MTB genes that are switched on predominantly during conditions thought to mimic latency (hypoxia) is considered a research priority to lead to the identification of new antigenic targets for anti-TB strategies [52]. Under these circumstances of hypoxia, it is recognized that an early response is the coordinated upregulation of genes under the control of two sensor kinases (dosS and dosT) and a response regulator (dosR). This is supported based on the observation that some of these candidate genes were found to encode a number of MTB antigens with the potential to induce a strong T-cell IFN-γ responses [53–57]. Acr-1 (Rv2031c, hpsX) is one of the well-described dosR regulated antigens shown to induce a dominant B- and T-cell response [57, 58]. However, although there are reports suggesting that some of these antigens are preferentially recognized in those with latent infection[53–55, 57, 58], other studies also show that there is a great overlap of responses to these antigen in both persons with active and latent TB [59, 60]. More recently, it has been shown that a second wave of genes are induced by more prolonged hypoxia with only a small overlap with the dosR regulon: this has been called the enduring hypoxic response (EHR) and includes a large number of transcriptional regulators [47, 49].

Another line of evidence against the concept of “dormant” bacilli as the only bacterial population in latent TB is that the established effective drug treatment regimens for latent TB. The use of 6–12 months of isoniazid preventive therapy (IPT) is associated with a reactivation risk reduction of 60% (95% CI 48–69) in immunocompetent individuals [61] and possible eradication of the infection [62]. Isonaizid acts by inhibiting cell wall synthesis and therefore is only active against actively replicating organisms. These observations suggests that a portion of bacilli in persons with latent TB are replicating at least part of the time and the possible explanation therefore is that the latent infection cycles through a range of metabolic states over time, rendering a number of bacilli susceptible to the drug during the course of 6–12 months of preventive therapy [63–65]. A complementary explanation is that there are various bacterial populations at any one time within different or the same lesions in a latently infected person. Additional support for these ideas comes from Ford et al. [66], where they observed the distribution of SNPs in MTB and isolated from various disease states in Cynomolgus macaques, and from this calculated the mutation rates. They found a similar mutation rate during latency, active disease, and reactivation as in a logarithmically growing culture. The authors suggest that the MTB might be actively replicating during the entire course of clinical latency balanced by robust killing. In any event, this study supports more active replication and mutation in latency than previously thought and suggests that this might be the reason why the isoniazid monotherapy for latent TB is a risk factor for the emergence of isoniazid resistance.

Advances in real-time imaging technology have provided a window into lungs of infected humans, often during imaging for diagnostic purposes (e.g., lung diseases). Computed tomography (CT) can identify lesions (granulomas) in lungs of latently infected persons. Positron emission tomography (PET) scanning using ^{18}F-fluorode-oxyglucose (FDG) as a probe, which identifies areas of metabolically active cells, indicates that these lesions span a range of FDG avidity, from “cold” to “hot” [67–69]. These suggest that at least in terms of

inflammation, latent infection is not always a “dormant” process, but instead is dynamic. Studies in macaques with MTB infection support the findings in humans, and this could help in understanding the course of MTB infection.

T-cell response in latent TB

T lymphocytes are considered critical to overcome acute MTB infection and also found in the granuloma, mediating the inflammatory balance in histological and flow cytometry studies [70, 71]. The majority of T cells at the disease site in humans are cytotoxic T cells, Th1 cells, Th17 cells, and regulatory T cells (T regs) that modulate the cytokine production by other cell types [72].

The primary role of $CD4^+$ T cells is to produce cytokines that assist and orchestrate other immune cells in the environment. Human studies on individuals co-infected with HIV-1 and MTB provide evidence that a reduction in CD4 T cells increases the risk of developing TB, and an increase in $CD4^+$ T cell numbers with antiretroviral therapy correlates with the decrease in susceptibility to develop active TB, suggesting that these T cells play an important role in the protection against TB [14].

MTB-specific $CD4^+$ T cells produce primarily type 1 or Th1 cytokines, which include interferon-gamma (IFN-γ), IL-2, and tumor necrosis factor (TNF) [73]. The protective role of IFN-γ was best demonstrated by the fact that people with defective type 1 cytokine pathway poorly control non-pathogenic mycobacteria [74]. Administration of anti-TNF monoclonal antibody (infliximab) was shown to reactivate TB in patients [75]. Although IFN-γ is essential to human defense against mycobacteria, it is increasingly recognized that PBMC secretion of IFN-γ is a poor correlate of protection in field studies of tuberculosis [73]. This is in part due to the fact that IFN-γ secretion increases when the antigenic (bacterial) load increases. Greater attention to markers such as IL-2, which might better reflect immunological memory, is now being paid [76].

The emerging picture is that distinct IFN-γ/IL-2 functional profiles correlate with different models of infection. In a study assessing the capacity for IFN-γ and IL-2 secretion by MTB-specific T cells in HIV-uninfected persons with active TB indicated a co-dominance of IFN-γ^+ single positive and IFN-γ^+/IL-2^+ double positive T cell, followed by a shift to a dominance of IFN-γ^+/IL-2^+ double positive and IL-2 single positive T cells after treatment [76]. Similarly, Caccmo et al. [77] described that poly functional (IFN-γ^+TNF$^+$IL-2^+) CD4 T cells correlate with higher bacterial load and active tuberculosis with predominant effector phenotype, while those with latent TB had IFN-γ^+IL-2^+ or IFN-γ^+ single cytokine producing $CD4^+$ T cells with effector and central memory phenotype. In addition, they also found that the proportion of poly functional $CD4^+$ T cell decreased after 6 months of treatment and presented with a similar functional phenotype that of latent TB. The authors conclude that the poly-functional T cells correlate to the disease progression and bacterial load. Similar results were also reported in a TB prevalence study from Gambia [78].

In HIV-1 infected persons, disease progression and increase in viral load was found to correlate with the loss of IL-2 secretory function by $CD4^+$ T cells [79]. In another study, during immune reconstitution of HIV-1 infected MTB sensitized patients, central memory $CD4^+$ T cells ($CD27^+$ $CD45RA^-$ and $CD27^+$ $CCR5^-$) expanded during combined antiretroviral therapy while effector and terminally differentiated T cells decreased and these changes were thought to correlate to a decrease in susceptibility to tuberculosis [80]. These novel findings suggest that central memory $CD4^+$ T cell responses might be a better correlate of protection than polyfunctional terminally differentiated $CD4^+$ T cells. In our NHP study, reactivation of latent TB monkeys with SIV coinfection occurred (<17 weeks) or late (>26 weeks) after SIV infection. In the early reactivating monkeys, there were

significantly more polyfunctional $CD4^+$ T cells 3–5 weeks post-infection than the late reactivating monkeys. These MTB-specific polyfunctional T cells were better correlates of antigen load (i.e., disease status) than of protection [72]. These findings are in line with the idea proposed by Seder et al. [81] that the amount of initial antigen exposure will govern the extent of differentiation, and functionality of the cells.

IL-17 producing $CD4^+$ T cells were shown to mediate the recruitment of protective Th1 cells to lung up on MTB challenge in mice [82]. However, the role of IL-17 in the containment of MTB in humans is not known (reviewed in [83]). IL-17 also known to contribute to the recruitment of neutrophils, which have been suggested to be an important factor in active TB [84]. Regulatory T (T_{reg}) cells participate in the regulation of the inflammation. In NHP, T_{reg} are recruited from the blood to the airways soon after MTB infection, and those monkeys which developed latent TB had higher levels of T_{reg} in blood before infection as opposed to those that developed active TB disease [85]. In a human TB contact study from Gambia, similar decrease in $Foxp^{3+}$ expression levels in peripheral blood was reported in recently infected persons, suggesting migration of T_{reg} from periphery to the site of infection [86]. Although many T-cell types have been described that may participate in control of TB, the balance of these types at the level of the granuloma may be the most important factor, so that antimicrobial (inflammatory) responses and those that modulate pathology (anti-inflammatory) can work to kill organisms without damaging the host tissues.

Humoral response and latent TB

The critical role of cellular immune response to TB has been well studied. However, the contribution of the humoral (B-cell-mediated) response to the initial infection, containment, and the maintenance of the latent TB infection remain poorly defined. In fact, many believe that the humoral response against MTB may not be relevant for protection [87]. However, recent studies indicate that B cells and possibly antibodies may have a potential role in control of TB. B cells are a major cellular component of granuloma in humans, non-human primates, and in mice infected with MTB [72, 88]. B cells are capable of producing antibodies and cytokines and present antigens to T cells. In addition, antibodies, through interactions with the Fcγ receptors on other cells, including macrophages, may modulate the immune response at the granuloma level. Recent studies in mice show promising evidence that B cells in fact might play an important role in providing optimal immune responses to tuberculosis, modulating susceptibility, cytokine production, neutrophilic infiltration, macrophage function, and also T-cell responses [89]. The potential role of B cells in tuberculosis and how they regulate the immune control of TB are beginning to be appreciated (reviewed in [89]).

Studies evaluating human gene expression profile for suggestive signatures of susceptibility or resistance to tuberculosis describe FcGR1B, FcGR1B (CD64) as the most differently regulated genes in person with active TB in both high endemic African populations and in Caucasian populations [90, 91]. A major function of the family of Fc receptors for IgG (FcγRs) is binding of antibodies by their constant domain, and also can stimulate simultaneous trigger activating and inhibitory signaling pathways to set threshold for cell activation, and thus generate a well-balanced immune response. Understanding the role of B cells during the course of latent tuberculosis is also very important.

Biomarkers in Latent TB

The need for biomarker arises from the lack of suitable tests to detect the presence of viable MTB in the spectrum of tuberculosis. Host biomarkers might be ideal in assisting early diagnosis of tuberculosis, monitoring of treatment response, provide correlates of risk or protection, and defining the spectrum of tuberculosis. Biomarker discovery has been a

research priority in the recent years, reviewed by Walzl et al. [92]. Candidate biomarkers aimed to differentiate disease outcome: active or latent or extent of disease, treatment response, and to correlate risk or protection after vaccination. However, detecting latent tuberculosis remains difficult owing to the lack of gold standard or considerable overlap of immune response due to the bimodal (active or latent) sampling process. The concept of a spectrum of latency underlines the challenge of developing a single biomarker that would differentiate active and latent TB. Instead, biomarkers that provide a "position on the spectrum" will likely need to be developed so that the relative risk of reactivation for an individual can be assessed.

Reactivation

Reactivation of latent tuberculosis can occur years or decades after the primary infection in humans, which suggests that the latent infection is a dynamic process between the host immune system and the bacterial replication. The risk factors for reactivation include immunosuppression, e.g., HIV, steroids, or anti-TNF therapy; malnutrition, smoking, alcohol, diabetics, renal failure, and malignancy. Reactivation studies can tell us what the breaking points are for control of infection, and identification of the changes that lead to reactivation may suggest important correlates of protection.

In humans, an increased incidence of reactivation of latent tuberculosis was observed in patients receiving TNF neutralizing agents for inflammatory conditions [75, 93, 94]. TNF plays a critical role in immune response to TB including macrophage activation, apoptosis, chemokine, and adhesion molecule expression. In NHP model, it is shown that Cynomolgus macaques receiving TNF neutralizing agents had uncontrolled and disseminated disease by 8 weeks after MTB infection, and ~70% of latently infected monkeys reactivated the disease with 6 weeks of anti-TNF treatment [95].

HIV is the most potent risk factor for the development or reactivation of TB. Following the acquisition of HIV, the risk of active TB in individuals with latent TB increases to over 10% lifetime risk to over 10% each year [96]. It is hypothesized that HIV co-infection has a fundamental impact on the spectrum of the host–pathogen relationship with a general shift toward poor immune control, high bacillary numbers, and subsequent development of active infection and symptomatic disease [97]. The risk of developing TB in those living with HIV depends on the degree of immunosuppression, socioeconomic status, and the TB incidence pressure.

In human studies, lower $CD4^+$ T-cell levels shown to correlate with the increase in susceptibility to the reactivation or reinfection of TB. In a study comparing the $CD4^+$ T cells in HIV-1 infected and HIV-1 uninfected, TB sensitized person, HIV-1 infected persons had a total CD4 T-cell deficit in addition to the impaired MTB antigen-specific CD4 T-cell function, and this was suggested to be potential risk factor [98]. Similarly, in NHP TB-SIV co-infection studies, risk of reactivation of TB was associated with the $CD4^+$ T-cell depletion during the acute phase of SIV infection [99].

The problem with the existing concept of bimodal classification of TB is that there is no prospect to identify those who are at the higher end of the latency spectrum, and therefore, impossible to underscore the position (s) within the spectrum that is most likely to develop active disease. On the other hand, the concept of "TB spectrum" provides a better model to identify and define the position (s) within latent TB, as the position in the spectrum is most likely to depict those at higher risk of reactivation.

Prevention strategies

Vaccine: BCG was one of the first live-attenuated vaccines to be used in humans. It remains the only available and commonly used TB vaccine. In developing countries, the majority of infants are BCG-vaccinated, yet these are the countries that maintain the highest rates of tuberculosis, underlining the fundamental failure of BCG as a vaccine against tuberculosis. However, BCG vaccination is considered to be effective in children preventing sever forms of TB and also reduces the risk of TB in adults. [3–6]. Kaniga et al., investigated whether delayed BCG administration (at 10 weeks instead of at birth) had more BCG-specific "polyfunctional" CD4 T cells at 1 year of age than infants who received BCG on day 1 of life, and the results suggest that modifications in BCG vaccination practices may affect efficacy of the vaccine and should be studied further [100]. In HIV-1 infected infants, BCG disease is common, both in the presence and in the absence of ART [101, 102]. WHO has therefore recommended that BCG not be given to HIV-exposed infants at birth, but only once they are shown to be HIV-uninfected [103]. This unsatisfactory scenario underscores the need for better, safer vaccines against TB.

The future of TB vaccination is likely to involve a heterologous prime-boost strategy. The "prime" vaccine is likely to be a BCG modified to become safer and more immunogenic, or even attenuated and modified MTB. The current vaccines in the development and strategies that could be used are summarized in [104–106]. Appropriate vaccine strategies to contain tuberculosis should include post-exposure prophylaxis that can prevent reactivation of latent infection. One such candidate is a "multistage" vaccination strategy in which the early antigens Ag85B and 6-kDa early secretory antigenic target (ESAT-6) are combined with the latency-associated protein Rv2660c (H56 vaccine). This has shown evidence of pre- and post-exposure protection in a murine model of tuberculosis [107].

Current treatment strategy for latent TB: WHO promotes a three "I's" strategy to combat the epidemic, in addition to its directly observed therapy, short course (DOTS) strategy for treatment of incident disease. These I's are intensified case finding, infection control, and 6–9 months' isoniazid preventive therapy (WHO: http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf). Isoniazid preventive therapy, administered to those with evidence from TST of sensitization by TB, is a cornerstone of control in many areas of the world with low incidence. Such preventive therapy, administered to patients who are positive on TST and infected with HIV, in areas of high incidence also provides around 60% protection, although the duration of protection is limited [108]. This feature, combined with the logistics surrounding administration and reading of the skin test, poor adherence outside clinical trials, and fear of increased isoniazid resistance, has discouraged widespread adoption of the measure. These problems have spurred interest in finding shorter, safer, and cheaper alternative regimens, with similar efficacy. The current shorter regimens on clinical trials are reviewed in [109, 110].

In summary, recent advances in technology, human studies, and animal model development have increased our understanding of the various infection outcomes with MTB. However, there is still much to learn and understand about both active and latent TB, and the spectrum of latency in particular. Translational strategies must arise from the fundamental breakthroughs in the study of TB to have a major impact on TB control worldwide. Only new strategies will curb the epidemic and eventually lead to the end of TB.

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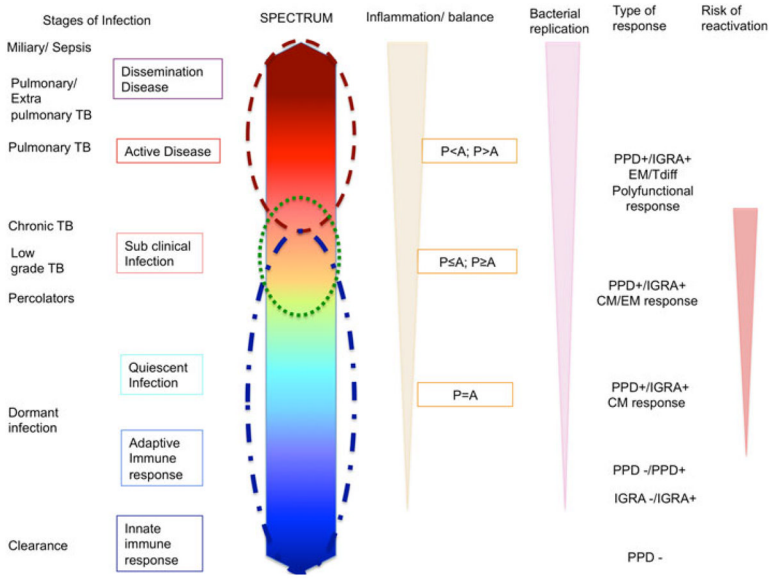

Fig. 1.

The outcome of MTB infection can be viewed as spectrum "*heat map*" with corresponding stages of infection from bacterial clearance (cold-*lower end* [*blue*]) to disseminated disease (hot-*upper end* [*dark red*]). The classical, bimodal classification based on the presence or absence of clinical symptoms: active (marked in *dashes* [*red*]) and latent (marked in *dashes-dots* [*blue*]) TB are marked, to indicate the variability within those categories. In addition, recent studies support the existence of subclinical infection (marked in *dots* [*green*]), which overlaps with both active and latent TB. Bacterial replication is expected to increase up the spectrum of infection. The inflammatory factors [Pro- (P) and Anti- (A) inflammatory] are at balance at the lower end of the spectrum controlling bacterial replication, while as the infection advances up the spectrum, this balance is lost resulting in increase in bacterial burden and/or increased pathology. Similarly, the position in the spectrum depicts the risk of reactivation, higher on the spectrum are at higher risk. Treatment with either combined antiretroviral therapy (cART), isoniazide preventive therapy (IPT) or anti tubercular therapy (ATT), shifts one down the spectrum, and therefore, less susceptible to reactivation. Detectable response to PPD or in IGRA may vary from negative to positive within the latent spectrum, although this is speculative. The type of T cells response in in vitro assays range from less central memory (CM) response at the lower end of the spectrum to effector memory (EM) and terminally differentiated (Tdiff) phenotype with increase in functionality of the T cells as they go higher the spectrum; again this has not been proven. Adapted from [23–25] (Color figure online)