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Understanding latent tuberculosis: the key to improved diagnostic and novel treatment strategies

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Abstract

Treatment of latent tuberculosis (LTBI) is a vital component of tuberculosis elimination but is not efficiently implemented with available diagnostics and therapeutics. The tuberculin skin test and interferon gamma release assays can inform that infection has occurred but do not prove that it persists. Treatment of LTBI with isoniazid targets actively replicating bacilli but not non-replicating populations, prolonging treatment duration.

Developing more predictive diagnostic tests and treatments of shorter duration requires a greater understanding of the biology of latent tuberculosis, from both host and bacillary perspectives. In this article we discuss the basis of current diagnosis and treatment of LTBI and review recent developments in understanding the biology of latency that may enable future improved diagnostic and treatment strategies.

Keywords

latent; tuberculosis; LTBI; diagnosis; treatment

The challenge of Tuberculosis control and elimination

Tuberculosis (TB) is curable but globally it remains a leading cause of death; 1.45 million people died from this disease in 2010[1]. The challenges to TB control are numerous, compounded by the complex biology of the disease. However, strategies focusing on prompt identification of infectious cases, close monitoring of treatment as well as broader political engagement and infrastructure development have resulted in progress, with global incidence rate now gradually declining from a recent peak of 1400 cases per million per year[1]. The international health community has set itself the ambitious target of eliminating TB as a global public health problem, defined as less than 1 case per million per year, by 2050[2].

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Teaser: Widespread treatment of latent tuberculosis will only be possible with more predictive diagnostic tests and shorter treatments. Developing these will require a greater understanding of the biology of latent tuberculosis.

This target will only be achieved by a sustained decline in TB incidence, far in excess of what is currently being achieved. Optimal implementation of existing TB control measures, especially intensifying efforts to identify and treat cases of active tuberculosis and the practice of effective infection control is important and will lead to further reductions in TB incidence. However, this alone will not achieve TB elimination by mid century[3]. Models suggest that a combination of interventions will be required to meet this target and that treating those with latent tuberculosis infection (LTBI) could make a significant contribution[4]. However, current diagnostics, which classify 2 billion individuals as having LTBI (although only 10% will subsequently develop active disease), and current therapeutics (which advise up to 9 months of isoniazid), make mass treatment of LTBI impractical. Diagnostics that predict risk of development of active disease and shorter treatment regimens for LTBI are clearly required but significant advances in our understanding of the biology of LTBI are needed to achieve this.

What is Latent tuberculosis and how is it diagnosed?

Latent tuberculosis conceptually denotes a state in which *Mycobacterium tuberculosis* persists within its host without causing symptoms or signs whilst maintaining viability with the potential to replicate and cause symptomatic disease. Identifying bacilli in latently infected individuals is not currently feasible and therefore LTBI is inferred solely through evidence that immune sensitization has occurred.

Tuberculin, a heat-killed culture filtrate of tuberculosis, was developed, unsuccessfully, as a therapy for tuberculosis at the end of the 19th century[5]. Its diagnostic potential for LTBI, however, was recognized as it caused an easily visualized delayed hypersensitivity reaction in individuals with occult infection. This diagnostic test, refined over 40 years, is still used today by measuring the induration formed 48–72 hours after intradermal injection of 2–10 units of purified protein derivative (PPD) of tuberculin. This tuberculin skin test (TST) has guided our understanding of the epidemiology of latent tuberculosis. Following exposure to an infectious individual with pulmonary tuberculosis up to 45% of close contacts become TST positive[6]. Five-10% of these individuals (if immunocompetent) will develop disease, the majority within the first 5 years with the risk falling off rapidly after the first year. Despite this declining risk over time “latency” periods of greater than 30 years have been documented[7]. Extrapolation from TST surveys indicate that approximately 1/3 of the world’s population will have a positive TST and the inference has been this represents the proportion infected with TB[8]. TST sensitivity (which is poor in the immunocompromised) and specificity (the test shows cross reactivity with non-tuberculous mycobacteria (NTM) and BCG) have been partially addressed by a new generation of antigen-specific interferon-gamma release assays (IGRA). In these assays, interferon gamma release is measured following overnight stimulation of peripheral blood with two TB antigens, ESAT-6 and CFP-10, that are not present in BCG and many NTMs. The same depth of epidemiological studies do not exist for IGRAs and although they appear to correlate more closely with exposure[9] the improvement in predictive value of IGRA over TST has not been dramatic[10]. Importantly, neither TST nor IGRA distinguish active from latent disease nor can they be used to stratify those at greatest and lowest risk of active disease. Discordance between IGRA and TST has been documented but the significance of this is still not clear. In addition little is known about the long- term dynamics of TST responses or IGRA reactivity or the significance of reversion from positive to negative in either test or if positivity for TST or IGRA may be associated with protection from reinfection.

The immunologic basis of TST and IGRA

The components of the tuberculosis specific immune response measured by TST and IGRA can now be examined in more detail by multi-parameter flow-cytometry, intravital imaging and immunohistochemistry. The lymphocytic infiltration that causes induration associated with a positive TST has recently been shown to relate to CD4 cells with a memory phenotype (CD45RO)[11]. Intravital imaging of delayed hypersensitivity reaction in a rat model has confirmed that skin homing antigen specific effector memory T cells are engaged with resident antigen presenting cells within 3 hours of challenge[12]. In addition antigen-specific effector memory cells are recruited to the airways of TST+ve but not TST-ve individuals following bronchoscopic administration of PPD years after skin test conversion[13]. The mechanisms governing homeostasis of the effector memory compartment and lineage relationship between central and effector memory cells are not yet fully established[14]. However, evidence that effector memory responses can persist in the absence of ongoing antigenic stimulation for decades is clear from studies of smallpox vaccine recipients[15]. In addition, following successful treatment for active TB, IGRAs can remain positive for decades even in regions of extremely low TB prevalence where reinfection is very unlikely and antigen is likely to have been cleared, recently it has also been shown that effector memory T cells are the major contributory cell type to IFN- γ production in positive IGRAs in this setting[16]. This raises the possibility that IGRA tests for LTBI could remain positive in the absence of persisting antigen as a result of IFN- γ release from antigen specific effector memory T cells and brings into question whether all individuals classified as having latent TB with these diagnostic tests harbour viable organisms with the potential to reactivate.

The spectrum of tuberculosis

Conversion to TST and IGRA positivity may coincide with host ability to form granuloma around sites of tuberculous infection[17]. These granulomas are collections of cells of the adaptive and innate immune systems in which cytokine-mediated cross-talk facilitates containment of the infection by providing an inhospitable environment for bacillary replication. Developments in intravital imaging have demonstrated that, at least in the first few months of infection, granulomas are dynamic structures with continuous recruitment of fresh lymphocytes around a more stable macrophage core[18]. This initial immune control can fail either through an over exuberant pro-inflammatory response leading to necrosis and liquefaction of tissue allowing extracellular growth, or by immunosuppression leading to suboptimal granuloma formation with consequent dissemination. In time, however, granulomas resolve with fibrosis and mineralization coinciding with reductions in cellular infiltration. The recovery of viable bacilli from such lesions is much less frequent than from caseous or cellular lesions[19].

Within the granuloma the bacillus must adapt to a variety of environment stresses including reduced oxygen tension, nutrient deprivation, nitric oxide and low pH, conditions explored using a variety of *in vitro* models. These models have been used to demonstrate that *M. tuberculosis* is capable of an extensive repertoire of metabolic realignments to enter a defined non-replicating state[20]. This initial hypoxic response (although it is also induced by other conditions) encoded by the Dormancy survival (Dos) regulon[21] is followed by induction of a larger set of genes termed the Enduring Hypoxic Response (EHR) that increases with prolonged hypoxia[22]. These changes occur at physiologically relevant oxygen tensions below 1 mmHg that have been measured within live, infected animals[23]. This *in vitro* “dormancy” is often equated with clinical latency and this has influenced attempts to develop novel and more specific diagnostics tests and vaccines for latent tuberculosis. The immune responses to DosR and EHR antigens have been investigated and

although a number of these antigens have been found to be immunogenic in latently infected individuals[24–26] few are preferentially recognized by persons with latent rather than active tuberculosis. The linkage between *in vitro* dormancy and clinical latency is highly overly simplified however, as both actively replicating and hypoxic dormant populations of bacilli are likely to co-exist in the same individual in different lesions[20]. There is a growing understanding that tuberculous lesions are highly local, dynamic structures that wax and wane over time and that a simple dichotomous classification of “active” and “latent” is no longer likely. This view would explain the somewhat paradoxical fact that isoniazid, which is active against replicating bacilli, nonetheless represents an effective treatment for latent infection. Understanding how mycobacteria switch metabolic states and developing drugs that might interfere with this would obviously be highly useful (Targeting dormant mycobacteria, *vide infra*).

These considerations have led to a rethinking of the active disease vs latent infection paradigm. Several recent publications[27–29] have argued that tuberculosis is best represented as a more dynamic spectrum of infection states reflecting the shifting balance in the host pathogen interaction.

Developing diagnostic tests that identify individuals at key points along this spectrum would allow for more rational treatment; some considered to have latent tuberculosis may not require treatment while others may require treatment similar to active disease. Transcriptomics may prove useful in this regard. Recently, a distinct 393-transcript signature that distinguished active tuberculosis was identified. This transcript signature correlated with radiographic extent of disease and normalized with treatment of active TB. Ten-25% of the latent cases also clustered with active disease. The significance of this is unclear but may represent individuals with a high burden latent infection or subclinical disease[30]. Apart from providing a diagnostic test, this unbiased approach has the potential to uncover biological differences between subgroups that provide a fresh understanding of the biology of tuberculosis. In addition to “omics” based approaches, further advances in immunological assays beyond IGRAs using ESAT-6, CFP-10 and TB7.7 (used in currently available commercial assays) may allow distinction of subgroups of latent infection, discrimination between active and latent infection and the development of more predictive diagnostic tests. This could be achieved through use of novel antigens, evaluating cytokine release other than IFN α or identifying differences in poly-functionality or phenotype of antigen specific T cells[31, 32]. Heparin-binding hemagglutinin (HBHA), a surface expressed adhesin, for example, has been shown to induce stronger IFN γ responses in peripheral blood of latently infected individuals than those with active disease who have greater TNF α responses to this antigen and Rv2628 (DosR encoded) and appear to induce stronger IFN γ responses in peripheral blood of those with remote rather than recently acquired latent infection[33, 34]. More extensive and adequately controlled studies will be needed to confirm these findings and although the discriminatory value of responses to these and other antigens appears modest, it shows how this approach could be used to develop stage specific tests. In addition, these *ex vivo* assays allow us to consider the implication of dynamic changes in responses with serial testing. Conversions and reversion of IGRA status, as well as sudden changes magnitude of positive response are frequently encountered and while it is tempting to think this could be informative about infection state, longitudinal studies will be required to determine prognostic implications of these observations[35].

Isoniazid preventive therapy and principles of treating latent tuberculosis

By the early 1950s several anti-tubercular agents were available, with isoniazid being best tolerated and most effective. The observation that children with asymptomatic primary tuberculosis treated with isoniazid experienced fewer cases of disseminated disease led to

the idea that isoniazid could be used as a preventive treatment in asymptomatic infected individuals[36]. Over subsequent decades thousands of TST+ve individuals were recruited into randomized control trials establishing the efficacy of isoniazid[37]. From these studies approximately 9 months isoniazid appear to provide optimal protection in HIV-ve populations resulting in up to 90% reduction in tuberculosis in treatment completers[38]. The situation is different in HIV infected individuals, 36 months of isoniazid is superior to 6 months of isoniazid at preventing disease with incidence rates diverging after approximately 200 days because of increased disease in the TST+ve group suggesting that ineffectual treatment of latent infection rather than reinfection is responsible. Additionally, incidence of infection was lower in the 6 month arm if anti-retroviral treatment (ARV) had been commenced[39]. This suggests a role for a functional immune system to assist the chemotherapeutic eradication of infection. Finally, some evidence suggests that treatment of latent TB boosts the IFN- γ response to a number of TB antigens within the first month of treatment, which may contribute to clearance of infection[40].

Unlike active disease, monotherapy is often used in treating latent tuberculosis, the justification being that with lower bacillary load, the stochastic appearance of resistant mutants is unlikely. Studies, however, are rarely designed to address this particular question and meta-analysis of reported isoniazid resistance rates in placebo-controlled trials have not excluded the possibility that prophylactic monotherapy leads to resistance[41]. In the context of HIV-associated TB inadvertent monotherapy for subclinical infection resulting from suboptimal screening is always of concern. A recent study assessed the mutation rate in latent infection by sequencing isolates from macaques with latent or active infection and comparing them to the known infecting strain. They demonstrated that mutation rates in latent and active infection were similar suggesting that development of resistant mutants in latent infection may at least be theoretically possible[42]. How this compares to latent infection humans has yet to be established but again highlights a potential risk of monotherapy.

Novel chemotherapeutic strategies for latent tuberculosis

Although well tolerated, isoniazid may not be the most rational choice of drug for latent tuberculosis. Isoniazid inhibits synthesis of mycolic acids, key cell wall constituents and displays a biphasic killing with rapid early bactericidal activity against actively replicating bacilli but much less efficacy in killing bacilli with low metabolic activity[43]. Early bactericidal activity (evaluation of reduction in colony forming units (cfu) over the first few days or weeks of therapy) therefore does not predict sterilizing activity (efficacy of preventing relapse in human or animal models) of a drug[44]. Drugs such as rifampicin and pyrazinamide, which have more potent sterilizing activity, allow treatment of LTBI to be shortened (table 1). In addition to finding shorter and better-tolerated regimens, novel regimens effective against multi-drug resistant latent tuberculosis are desirable. Several newer anti-tuberculous agents such as rifapentine, TMC-207 and moxifloxacin (table 2) appear to have potent sterilizing activity[45]. A murine model of LTBI has demonstrated that TMC-207 has sterilizing ability equal to rifampicin and isoniazid and also the superior sterilizing activity of rifapentine in combination with isoniazid[46]. Encouragingly a recent clinical study has demonstrated 12 doses of rifapentine and isoniazid given weekly for 3 months is effective preventive treatment for latent tuberculosis[47]. Although animal models provide an important way to select candidate regimens, accurately representing human latent tuberculosis is a challenge and all animal models have their drawbacks. Mice, in particular, though having practical advantages develop chronic rather than latent infection with relatively high bacillary load and without hypoxic lesions, thus the metabolic state of the population of bacilli in this model probably does not reflect that found in human latent infection. Macaques have been shown to develop infection states similar to humans

following low dose infection and although their high cost and large size are a disadvantage they may provide a useful model to evaluate promising leads[48]. Functional imaging using FDG-PET/CT (or indeed novel radiotracers) to quantify metabolically active cells recruited to granuloma may also provide a mechanism to evaluate novel therapies in humans.

Targeting dormant mycobacteria

Treatment duration in all infection states is likely to be shortened further still by therapies specifically targeting non-replicating organisms. Understanding the physiology of dormant populations of *M. tuberculosis* is the key to developing such treatments. Dormant bacilli have not been identified directly *in vivo* and as a result various models have been developed for their study. Within activated macrophages, largely due to exposure to nitric oxide, mycobacteria induce most of the DosR regulon genes. Transcriptional changes promote fatty acid metabolism as well, intensified iron scavenging, anaerobic respiration and cell wall remodeling[49]. There is also some evidence to suggest that *M. tuberculosis* via the oxygenated mycolic acids within its cell wall, can trigger differentiation of macrophages into foamy macrophages which, through dysregulation of LDL uptake develop numerous intracellular lipid bodies which provide a carbon and energy source for the mycobacteria. In addition foamy macrophages have impaired phagocytic and bacteriocidal function and hence may provide a nutrient rich niche for persisting mycobacteria[50]. Targeting vital metabolic pathways of dormant mycobacteria not present in mammalian cells is an obvious strategy. Isocitrate lyase, a key enzyme in the glyoxylate bypass pathway, is an attractive option although thus far efforts to target this enzyme have proven difficult[51]. One of the primary difficulties encountered by the bacillus in adapting to hypoxia is in disposing of excess reducing equivalents generated by both anabolic and catabolic processes [52]. Recent metabolomic analyses of organisms locked in anaerobic stasis shows a dramatic complete reversal of the TCA cycle with active secretion of succinate providing a means for maintaining an energized membrane to allow ATP synthesis[53]. As progress continues to be made in deciphering the metabolism of *M. tuberculosis*[54] further potential targets may materialize to be subjected to high throughput drug discovery methods.

Toxin/anti-toxin systems (TA) may also facilitate persistence of *M. tuberculosis*. TA systems comprise of a set of 2 or more closely linked genes, which encode a protein toxic to cellular function and an antitoxin capable of inhibiting the toxin. Many of these “toxins” cleave mRNA to facilitate rapid realignments of metabolism and prioritize translation either selectively or generally. *M. tuberculosis* has at least 30 encoded functional TA systems, more than any other organism and the majority of these are not present in non-tuberculous mycobacteria. Their role in mediating persistence has yet to be clarified but several TA systems have been shown to be up-regulated as part of the stress response to both hypoxia and macrophage infection but not as part of the Dos regulon[55]. Translation remains an important function even in non-replicating cells and as ribosome abundance becomes limiting the normally unnecessary process of *trans*-translation becomes critical. *Trans*-translation is dependent upon transfer-messenger RNA (tmRNA) which frees ribosomes that have stalled mid-message by displacing the existing mRNA and tagging the nascent protein for degradation. Recent evidence has shown that the mechanism of pyrazinamide involves binding directly to the ribosomal protein S1 and inhibiting *trans*-translation[56]. Further clarification of these complex mechanisms of translational adaptation in non-replicating *M. tuberculosis* may also lead to development of novel chemotherapeutic agents.

Knowledge of how *M. tuberculosis* protects itself from succumbing to the hostile intracellular environment may provide other targets. Recently it has been shown *in vitro*, that in hypoxic environments *M. tuberculosis* can use nitrate as an effective terminal electron acceptor and that nitrate respiration allows it to maintain resistance to acidic

environments. Respiratory nitrate reductase mutants are more susceptible to acidic environments, providing a possible target for novel therapeutics [57]

Interfering with the mechanics of resuscitation (transition from dormant to metabolically active state) may also lead to novel treatment strategies. Resuscitation promoting factors first identified in *Micrococcus luteus* are muralytic (cell wall degrading) enzymes that stimulate growth and facilitate resuscitation of G-C rich Gram-positive bacteria. *M. tuberculosis* has 5 Rpf-like genes (RpfA-RpfE) which although not individually critical, in combination appear to be required for growth and revival following periods of non-replication. Rpf has been detected in *M. tuberculosis* infected human tissue using immunocytochemical staining [58] and immune responses to RpfA and RpfD are present in mycobacteria exposed persons[59]. Rpf also enhances recovery of organisms from culture. The exact mechanisms by which Rpf mediates resuscitation is unclear, whether their muralytic action facilitates diffusion of substrates and nutrients necessary for resuscitation or whether fragments of released muropeptides have immunomodulatory or signaling properties needs to be established[60]. However the possibility of developing chemotherapeutics to stimulate resuscitation thus making the organism more susceptible to antimicrobials or developing vaccines to enhance immune response to Rpf thus preventing resuscitation would be novel strategies to control latent tuberculosis.

Enhancing susceptibility of persistent organisms to antimicrobials may also prove fruitful. In zebrafish embryos, it has recently been demonstrated that efflux pumps induced within intracellular environments can mediate drug tolerance in mycobacteria and that susceptibility can be restored by efflux pump inhibitors such as verapamil[61]. Additionally studies using *in vitro* and mouse models of bacterial persistence have shown that aminoglycoside tolerant, persisting populations of *E. coli* and *S. aureus* become susceptible to aminoglycosides if sugars such as mannitol and fructose are co-administered, promoting carbohydrate metabolism which generates sufficient proton motive force to increase aminoglycoside uptake by the organism[62]. How relevant these discoveries are to tuberculosis in humans remains to be seen but the principle of co-administration of an agent which alters the metabolic state of the organism rendering it more susceptible to antimicrobial killing is a novel strategy.

Vaccination strategies for Latent infection

BCG provides some protection against disseminated disease but limited protection against acquiring infection. Individuals with LTBI often have vigorous immune responses against tuberculous antigens, however it is possible that this is preferentially directed against a particular subset of metabolically active organisms and that immune surveillance of persistent non-replicating populations may be sub-optimal. One approach to post-exposure vaccination is to direct the immune response towards persisting organisms by presenting it with antigens up-regulated by these persisting organisms[63]. Recently a multistage vaccine, comprising Ag85B, ESAT-6 and Rv2660 (a component of the enduring hypoxic response) administered post exposure in 2 mouse models of LTBI resulted in significant reductions in cfu at necropsy[64]. An alternative approach is to combine vaccination with drug treatment of latent tuberculosis with a view to boosting the immune response further to allow shorter treatment duration. RUTI[65], a vaccine based on detoxified fragment of *M. tuberculosis*, used as an adjunct to 1 month of isoniazid therapy has recently completed phase II study.

Conclusions

Diagnostic and therapeutic approaches for latent tuberculosis over the last century have often arisen as a result of implementation and refinement of techniques and medication not

rationally designed for their purpose. As a result current diagnostics identify many individuals as having latent tuberculosis that will never develop active disease and though efficacious treatments strategies exist, their length, inconvenience and poor targeting mean they are often not implemented in practice. Fundamental questions about the adaptation and fate of the tubercle bacillus within its human host still need to be addressed to allow development of practical diagnostics to identify just those at risk of developing active disease and implementable efficacious treatments of short duration (table 3). Ultimately this will contribute to making the aspiration of tuberculosis eradication a reality.

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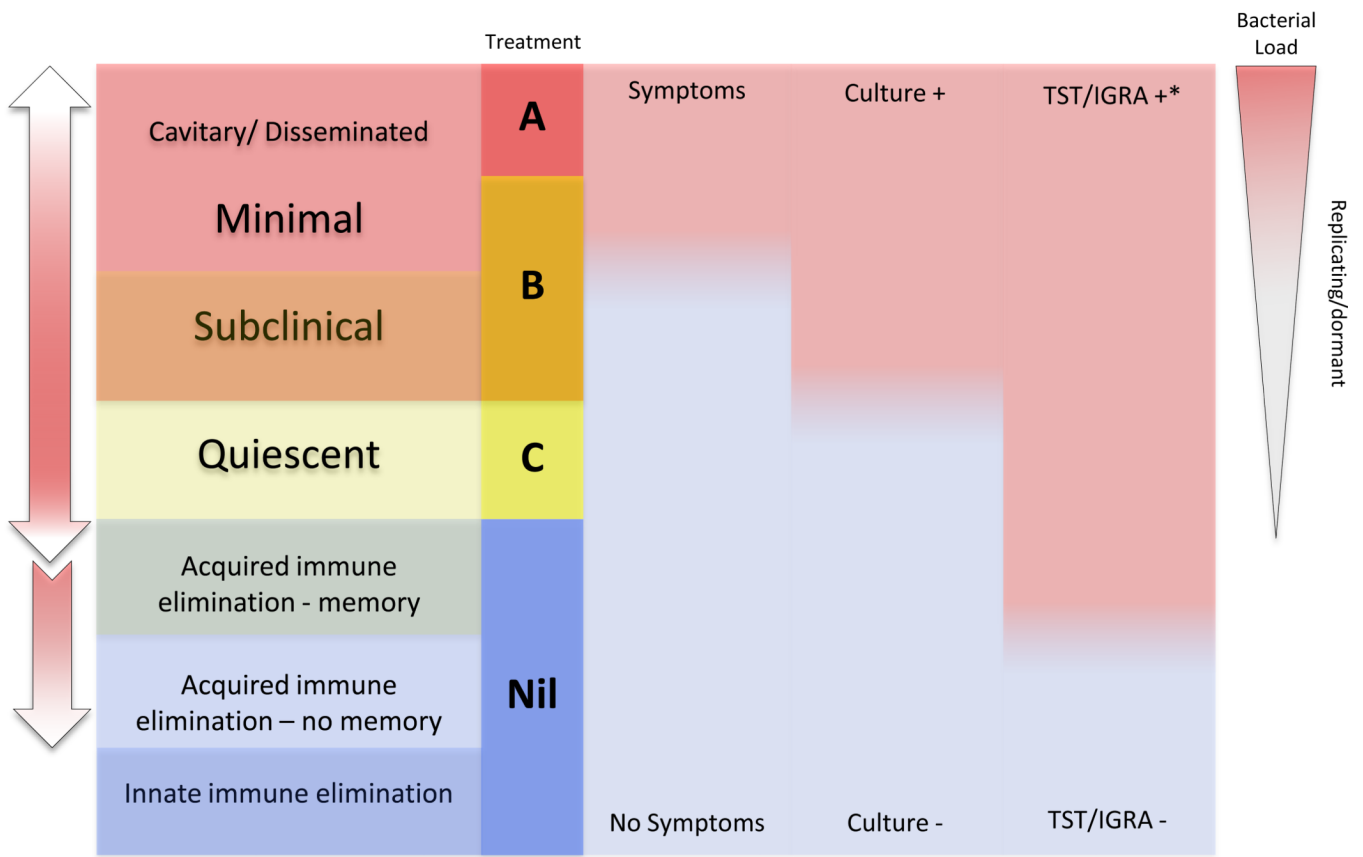
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(a)



* TST/IGRA may be negative in active disease

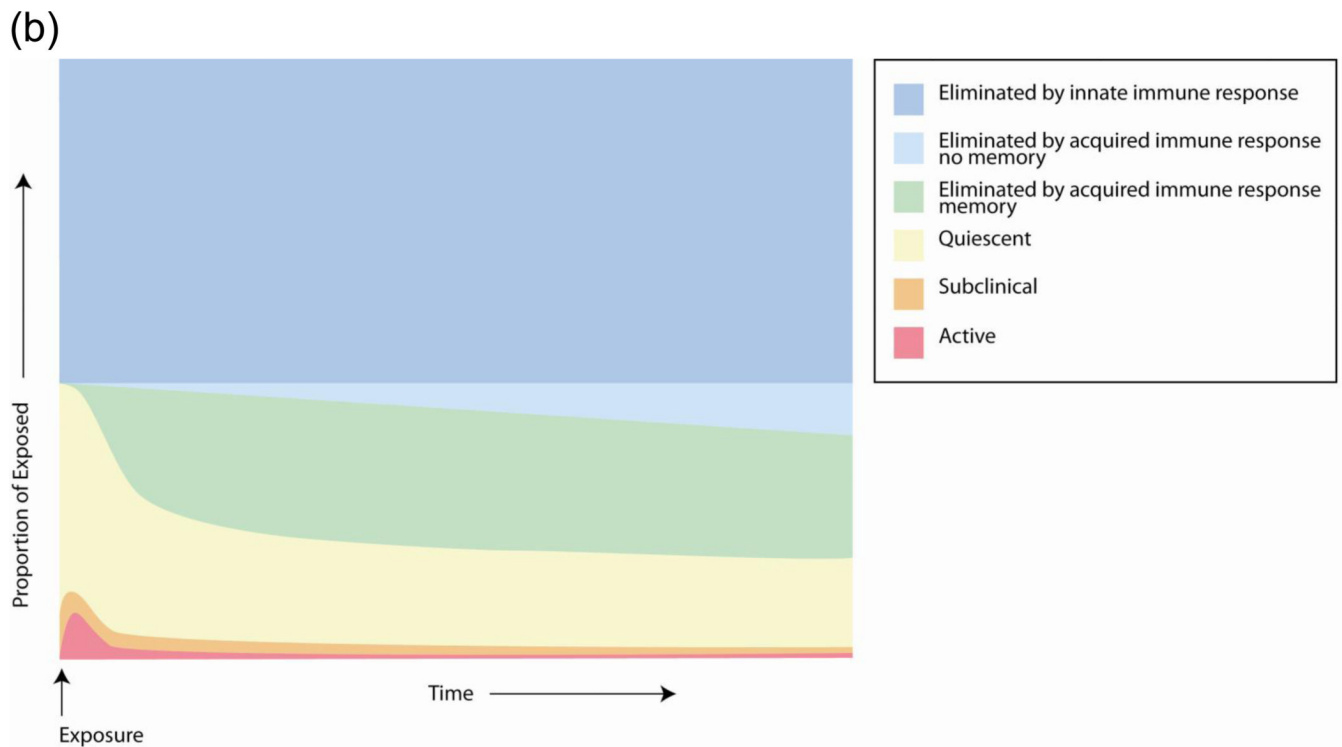


Fig. 1. The Spectrum of Tuberculosis

A. In this model after initial exposure, the bacillus may be eliminated by innate immune mechanisms (mucociliary, neutrophil, macrophage etc.). Once infection is established and an acquired immune response has been generated IGRA or TST may become positive. Infection may be eliminated by the acquired immune response but if antigen specific effector memory persists TST or IGRA may remain positive and it is possible a degree of protection from reinfection may be present. Over time memory responses may wane resulting in reversion of TST or IGRA. In these scenarios in which the bacillus was eliminated no further treatment would be required but prophylactic vaccination may further reduce risk of re infection. If the bacillus is controlled but not eliminated by the acquired immune response the individual may enter a state of quiescent infection in which both symptoms and culturable bacilli are absent and with a greater proportion of bacilli in a dormant rather than replicative state. Immunosuppression (e.g. HIV infection or anti TNF therapy) during this state may lead to rapid progression to active disease. The dynamic nature of this state may result in fluctuation in bacillary load and metabolic state of bacilli with a probability of the immune system regaining control reducing as bacillary load increases, if bacilli are culturable and symptoms and signs absent this would be regarded as a sub-clinical state. Optimal treatment regimens for infection with regard to duration of treatment, number of drugs and mechanism of active of drugs would vary according to bacillary load and proportion of bacilli in dormant state.

B. Shows for a hypothetical group of people exposed to TB, a prediction of how the proportion of individuals in the proposed infection states might vary in the years following single exposure (graph depicts all exposed individuals over time).

Table 1

Current regimens for LTBI

Drugs	Dose (mg)	Duration	Frequency	Comment
INH	300	6–9 months	Daily	Standard 1 st line in HIV–/HIV+
INH	300	36 months	Daily	Recent evidence for greater efficacy in HIV+
INH	900	6–9 months	Twice weekly	Alternative regimen- allows directly observed therapy
RIF	600	4 months	Daily	Alternative regimen
RIF+INH	600+300	3 months	Daily	Alternative regimen
RPE+INH	900+900	3 months	Once weekly	Recently reported as effective in HIV+ and HIV–
RIF+PZA	600+2000	2 months	Daily	Hepatotoxicity issues in HIV–ve - no longer routinely recommended

INH = Isoniazid, RIF = Rifampicin, PZA = Pyrazinamide, RPE = Rifapentine,

Table 2

Novel TB drugs in phase 2 trials that may potentially be of use in treatment of LTBI

Drug	Class	Mechanism of Action
PA-824	Nitroimidazole	<ul style="list-style-type: none">• Inhibits cell wall synthesis• Toxic reactive nitrogen species released following bioreduction.• Bactericidal/Sterilizing.• Active against replicating and non-replicating bacilli
OPC-67683	Nitroimidazole	<ul style="list-style-type: none">• Toxic reactive nitrogen species release following bioreduction.• Bactericidal/Sterilizing.• Active against replicating and non-replicating bacilli
TMC207	Diarylquinoline	<ul style="list-style-type: none">• Inhibits mycobacterial ATP synthase• Bactericidal/Sterilizing.• Active against replicating and non-replicating bacilli

Table 3

Fundamental questions regarding biology of LTBI

Fundamental questions regarding biology of LTBI
1. Do all those with positive IGRA or TST have persistent mycobacterial infection?
2. What immune mechanisms govern progression, control or elimination of infection?
3. What is the protective effect of latent tuberculosis on re-infection and to what degree does treatment of LTBI enhance this?
4. Are latent bacilli exclusively to be found within granulomas?
5. What are the differences in host pathogen interaction and outcome of infection between strains of <i>M. tuberculosis</i> ?
6. What are the genetic factors that govern host susceptibility to infection and progression to disease?