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

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Treatment of latent tuberculosis (LTBI) is a vital component of tuberculosis (TB) elimination but is not efficiently implemented with currently available diagnostics and therapeutics. The tuberculin skin test and interferon-γ 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 LTBI, 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 might enable future improved diagnostic and treatment strategies.

The challenge of TB 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 alone [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 the 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]. 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 TB, and the practice of effective infection control are important and will lead to further reductions in TB incidence. However, this alone will not achieve TB elimination by the mid-21st century [3]. Models suggest that a combination of interventions will be required to meet this target and that treating those with latent TB 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 the risk of development of active disease and shorter treatment regimens for LTBI are clearly required, but significant advances in understanding of the biology of LTBI are needed to achieve this.

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What is LTBI and how is it diagnosed?

LTBI conceptually denotes a state in which *Mycobacterium tuberculosis* persists within its host without causing symptoms or signs while 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 TB, was developed, unsuccessfully, as a therapy for TB 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

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individuals with occult infection. This diagnostic test, refined over 40 years, is still used by measuring the induration formed 48–72 h after intradermal injection of 2–10 units of purified protein derivative (PPD) of tuberculin. This tuberculin skin test (TST) has guided understanding of the epidemiology of LTBI. Following exposure to an infectious individual with pulmonary TB, up to 45% of close contacts become TST positive [6]. In total, 5–10% of these individuals (if immunocompetent) will develop disease, most 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 one-third of the population of the world 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 Bacillus Calmette-Guérin (BCG)] have been partially addressed by a new generation of antigen-specific interferon-γ (IFN-γ) release assays (IGRA). In these assays, IFN-γ release is measured following overnight stimulation of peripheral blood with two TB antigens, early secretory antigenic target 6 (ESAT-6) and culture filtrate protein-10 (CFP-10), which are not present in BCG and many NTMs. The same depth of epidemiological studies does 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; neither 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, the significance of reversion from positive to negative in either test, or whether positivity for TST or IGRA is associated with protection from re-infection.

The immunological basis of TST and IGRA

The components of the TB-specific immune response measured by TST and IGRA can now be examined in more detail by multiparameter 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 h 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 full 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 re-infection is unlikely and antigen is likely to have been cleared; it has also recently 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. It also brings into question whether all individuals classified as having LTBI with these diagnostic tests harbor viable organisms with the potential to reactivate.

The spectrum of TB

Conversion to TST and IGRA positivity might coincide with the ability of the host 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 crosstalk facilitates containment of the infection by providing an inhospitable environment for bacillary replication. Developments in intravital imaging have demonstrated that, at least during 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 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), which increases with prolonged hypoxia [22]. These changes occur at physiologically relevant oxygen tensions below 1 mm Hg, which have been measured within live, infected animals [23]. This *in vitro* 'dormancy' is often equated with clinical latency and has influenced attempts to develop novel and more specific diagnostics tests and vaccines for LTBI. The immune responses to DosR and EHR antigens have been investigated and although several 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 TB. 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 coexist 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 probable. 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 useful, especially for targeting dormant mycobacteria (see below).

These considerations have led to a rethinking of the active disease *versus* latent infection paradigm. Several recent publications [27–29] have argued that TB is best represented as a more dynamic spectrum of infection states, reflecting the shifting balance in the host–pathogen interaction (Fig. 1).

Developing diagnostic tests that identify individuals at key points along this spectrum would allow for more rational treatment; some considered to have LTBI might not require treatment, whereas others might require treatment similar to those with active disease. Transcriptomics might prove useful in this regard. Recently, a distinct 393-transcript signature that distinguished active TB was identified. This signature correlated with the radiographic extent of disease and normalized with treatment of active TB. In total, 10–25% of the latent cases also clustered with active disease. The significance of this is unclear, but might represent individuals with a high burden of 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 would provide a fresh understanding of the biology of TB. In addition to 'omics'-based approaches, further advances in immunological assays beyond IGRAs using ESAT-6, CFP-10 and TB7.7 (Rv2654) (used in currently available commercial assays) might enable the 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-γ or identifying differences in the polyfunctionality or phenotype of antigen-specific T cells [31,32]. For example, heparin-binding hemagglutinin (HBHA), a surface-expressed adhesin, has been shown to induce stronger IFN-γ responses in peripheral blood of latently infected individuals than in those with active disease who have greater tumor necrosis factor (TNF)-α responses to this antigen and Rv2628 (DosR-encoded), appears 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 enable one to consider the implication of dynamic changes in responses with serial testing. Conversions and reversion of IGRA status, as well as sudden changes in the magnitude of positive response, are frequently encountered and, although it is tempting to think this could be informative about infection state, longitudinal studies will be required to determine the 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 TB 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 of treatment with isoniazid appears to provide optimal protection in HIV−ve populations, resulting in up to a 90% reduction in TB in treatment completers [38]. The situation is different in HIV-infected individuals, where 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; this suggests ineffectual treatment of latent infection rather than that re-infection is responsible. Additionally, incidence of infection was lower in the 6-month arm if antiretroviral treatment (ARV) had been started [39]. This suggests a role for a functional immune system to assist in the chemotherapeutic eradication of infection. Finally, some evidence suggests that treatment of LTBI boosts the IFN-γ response to several TB antigens within the first month of treatment, which might contribute to clearance of infection [40].

Unlike active disease, monotherapy is often used in treating LTBI, 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-analyses 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. The authors demonstrated that mutation rates in latent and active infection were similar, suggesting that development of resistant mutants in latent infection is at least theoretically possible [42]. How this compares to latent infection in humans has yet to be established, but again highlights a potential risk of monotherapy.

Novel chemotherapeutic strategies for latent tuberculosis

Although well tolerated, isoniazid might not be the most rational choice of drug for LTBI. Isoniazid inhibits synthesis of mycolic acids, which are 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]. Therefore, early bactericidal activity [*i.e.* the evaluation of reduction in colony forming units (cfu) over the first few days or weeks of therapy] does not predict sterilizing activity (*i.e.* the 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 LTBI are desirable. Several newer anti-tuberculous agents, such 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

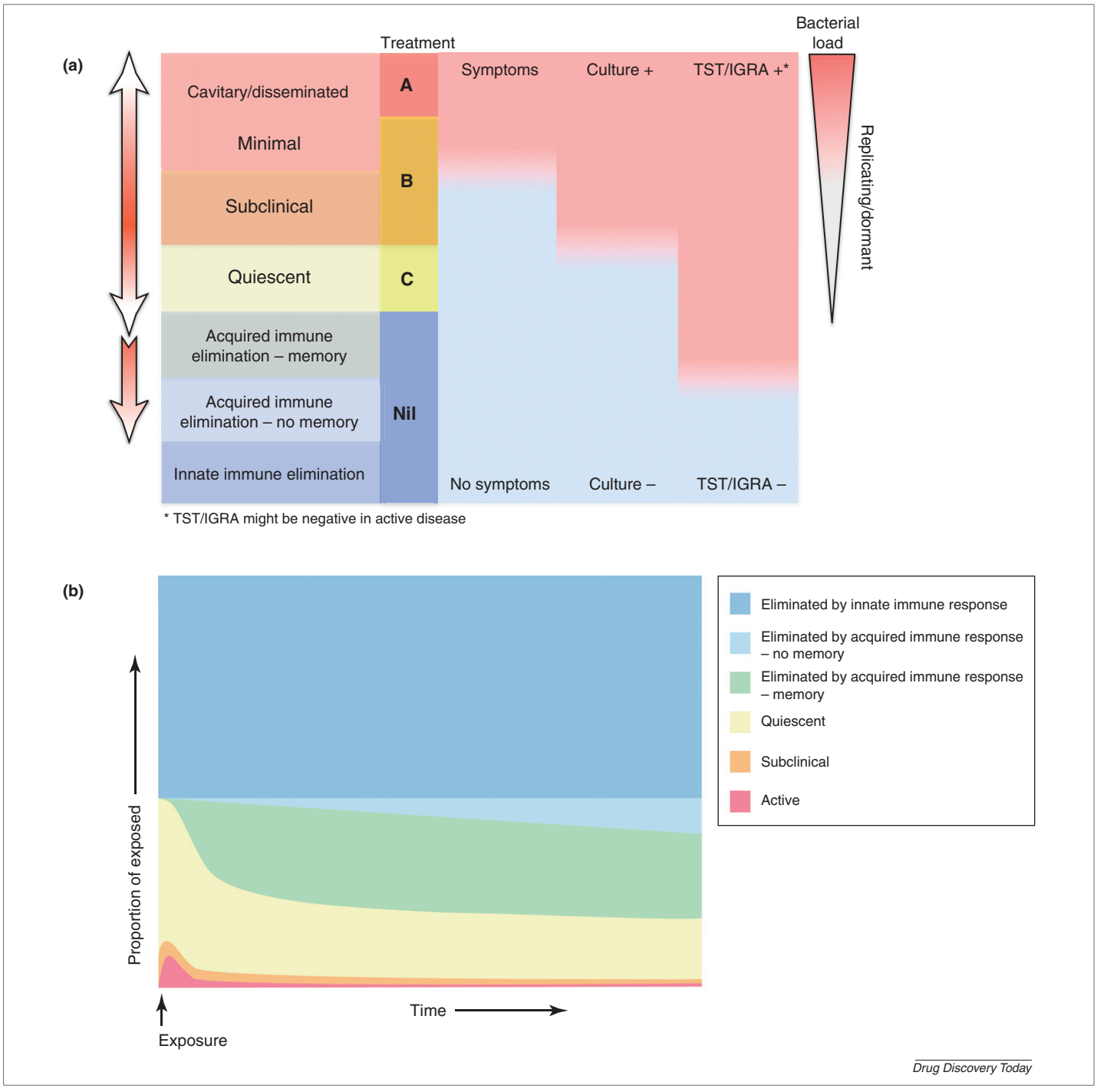

FIGURE 1

The spectrum of tuberculosis. **(a)** In this model, after initial exposure, the bacillus can be eliminated by innate immune mechanisms (*i.e.* mucocillary, neutrophil, macrophage, among others). Once infection is established and an acquired immune response has been generated, interferon-γ (IFN-γ) release assays (IGRA) or tuberculin skin test (TST) might become positive. Infection can be eliminated by the acquired immune response but if antigen-specific effector memory persists, TST or IGRA might remain positive and it is possible a degree of protection from re-infection is present. Over time, memory responses might wane, resulting in reversion of TST or IGRA. In these scenarios in which the bacillus was eliminated, no further treatment would be required, although prophylactic vaccination might further reduce the risk of re-infection. If the bacillus is controlled but not eliminated by the acquired immune response, the individual might 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-tumor necrosis factor (TNF) therapy] during this state might lead to rapid progression to active disease. The dynamic nature of this state might 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 subclinical 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 the bacillary load and the proportion of bacilli in dormant state. **(b)** 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 (the graph depicts all exposed individuals over time).

TABLE 1

Current treatment regimens for LTBI

Drug[a]	Dose (mg)	Duration	Frequency	Comment
INH	300	6–9 months	Daily	Standard first-line in HIV− and HIV+
INH	300	36 months	Daily	Recent evidence for greater efficacy in HIV+
INH	900	6–9 months	Twice weekly	Alternative regimen; enables 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

[a] INH, isoniazid; PZA, pyrazinamide; RIF, rifampicin; RPE, rifapentine.

TABLE 2

Novel TB drugs in phase II trials that might be of use in the treatment of LTBI.

Drug	Class	Mechanism of action
PA-824	Nitroimidazole	Inhibits cell wall synthesis Toxic reactive nitrogen species released following bioreduction Bactericidal/sterilizing Active against replicating and non-replicating bacilli
OPC-67683	Nitroimidazole	Toxic reactive nitrogen species release following bioreduction Bactericidal/sterilizing Active against replicating and non-replicating bacilli
TMC207	Diarylquinoline	Inhibits mycobacterial ATP synthase Bactericidal/sterilizing Active against replicating and non-replicating bacilli

to that of rifampicin and isoniazid, and also the superior sterilizing activity of rifapentine in combination with isoniazid [46]. Encouragingly, a recent clinical study has demonstrated that 12 doses of rifapentine and isoniazid given weekly for 3 months is effective preventive treatment for LTBI [47]. Although animal models provide an important way to select candidate regimens, accurately representing human LTBI is a challenge and all animal models have their drawbacks. Mice, in particular, although 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 those seen in humans following low dose infection and, although their high cost and large size are a disadvantage, they might 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 might 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 yet been identified directly *in vivo* and, as a result, various models have been developed for their study. Within activated macrophages, largely owing to exposure to nitric oxide, mycobacteria induce most of the DosR regulon genes. Transcriptional changes promote fatty acid metabolism as well as 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 that, through dysregulation of low-density lipoprotein (LDL) uptake, develop numerous intracellular lipid bodies that provide a carbon and energy source for the mycobacteria. In addition, foamy macrophages have impaired phagocytic and bactericidal function and, hence, could 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 efforts to target this enzyme have proven difficult thus far [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 show a dramatic complete reversal of the tricarboxylic acid cycle (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 might materialize to be subjected to high-throughput drug discovery methods.

Toxin/anti-toxin systems (TA) might also facilitate persistence of *M. tuberculosis*. TA systems comprise a set of two 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 that 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 upregulated 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 crucial. *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* might also lead to development of novel chemotherapeutic agents.

Knowledge of how *M. tuberculosis* protects itself from the hostile intracellular environment could provide other targets. Recently, it was shown *in vitro* that, in hypoxic environments, *M. tuberculosis* can use nitrate as an effective terminal electron acceptor and that nitrate respiration enables 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 (*i.e.* the transition from dormant to metabolically active state) might 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 five resuscitation-promoting factor (Rpf)-like genes (RpfA–E), which although not individually crucial, 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]. Rpfs also enhance recovery of organisms from culture. The exact mechanisms by which Rpfs mediate resuscitation are 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 the immune response to Rpfs, thus preventing resuscitation, would be novel strategies to control LTBI.

Enhancing the susceptibility of persistent organisms to antimicrobials might 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 *Escherichia coli* and *Staphylococcus 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 TB in humans remains to be seen, but the principle of co-administration of an agent that 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 is suboptimal. One approach to post-exposure vaccination is to direct the immune response towards persisting organisms by presenting it with antigens upregulated 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 two mouse models of LTBI resulted in significant reductions in cfu at necropsy [64]. An alternative approach is to combine vaccination with drug treatment of LTBI with a view to boosting the immune response further to allow shorter treatment duration. A phase II study has recently been completed using RUTI® [65], a vaccine based on a detoxified fragment of *M. tuberculosis*, used as an adjunct to 1 month of isoniazid therapy.

Concluding remarks

Diagnostic and therapeutic approaches for LTBI over the past century have often arisen as a result of the implementation and refinement of techniques and medication not rationally designed for their purpose. As a result, current diagnostics identify many individuals as having LTBI who will never develop active disease

BOX 1

Fundamental questions regarding the biology of LTBI.

- Do all persons with positive IGRA or TST have persistent mycobacterial infection?
- What immune mechanisms govern the progression, control or elimination of infection?
- What is the protective effect of LTBI against re-infection and to what degree does treatment of LTBI enhance this?
- Are bacilli to be found exclusively within granulomas in LTBI?
- What are the differences in host–pathogen interaction and outcome of infection between strains of *Mycobacterium tuberculosis*?
- What are the genetic factors that govern host susceptibility to infection and progression to disease?

and, although efficacious treatments strategies exist, their length, inconvenience and poor targeting mean that 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 enable development of practical diagnostics to identify only those at risk of developing active disease and implementable efficacious treatments of short duration (Box 1). Ultimately, this will contribute to making the aspiration of TB eradication a reality.

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