Tropical Medicine and International Health doi:10.1111/j.1365-3156.2010.02665.x

VOLUME OO NO OO

Viewpoint

Scientific dogmas, paradoxes and mysteries of latent *Mycobacterium tuberculosis* infection

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Summary Worldwide, there are nearly 10 million new cases of active TB and 1.8 million associated deaths every year. WHO estimates that one-third of the world's population is infected with *Mycobacterium tuberculosis (Mtb)*, forming a huge latent *Mtb* global reservoir. This renders the prospect of ever eliminating *Mtb* from the human race almost impossible. Several controversial issues regarding host-pathogen interactions and existing prevention and eradication strategies for latent *Mtb* infections need to be critically re-examined. In this viewpoint, widely held assumptions on *Mtb* latency and isoniazid monotherapy and chemoprophylaxis are challenged. We highlight the need for future research to resolve these issues and to develop evidence-based strategies for better understanding of equilibrium and escape of *Mtb* in the human body, eventually leading to global recommendations for elimination of the latent *Mtb* state through informed policy and practice. Until such strategies and policies are realized, WHO and TB experts will have to settle for global TB control rather than eradication.

keywords *Mycobacterium tuberculosis,* tuberculosis, latency, isoniazid, immunology, preventive therapy, re-activation, MDR/XDR-TB

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is a global health catastrophe. There are nearly 10 million new cases of active TB and 1.8 million people die from it every year (WHO 2010) making it one of the most important causes of death worldwide from an infectious disease. Global tuberculosis control ultimately depends on identification of the two billion people who, according to WHO estimates, are infected with *Mycobacterium tuberculosis* (*Mtb*) (Sudre *et al.* 1992; Corbett *et al.* 2003). Current strategies for identification and treatment for latent TB are inadequate (Sterling 2008; Parris *et al.* 1998; Lobue & Menzies 2010). The latest Europe TBNET consensus statement on latent *Mtb* infection re-emphasises the lack of accurate scientific knowledge on mycobacterial mechanisms of immune evasion, replication, equilibrium and escape (Mack *et al.* 2009). Furthermore, several controversial issues regarding host-pathogen interactions and existing prevention and eradication strategies for latent *Mtb* infections need to be critically re-examined, particularly the current scientific basis, dogmas, paradoxes and mysteries of latent *Mtb* infection.

Definitions: infection, active disease and latency

The probability of development of active clinical tuberculous disease after being infected with *Mtb* inhaled from infected aerosols from an infectious patient with active TB is very small (Behar *et al.* 2010). No more than 10% of

those infected with *Mtb* develop symptoms and signs of active disease over a lifetime; the actual figure depends on geographical location, numbers of organisms and strain type, genetic background, immunosuppression including co-infection with HIV, social, environmental and other risk factors (Sterling 2008; Lin & Ottenhoff 2008). The risk of developing *active tuberculous disease* after initial infection is thought to be highest within the first year (estimated at 1%) and second year (estimated at 0.3%); thereafter the risk diminishes considerably. Thus the majority of immunocompetent individuals (>90% of those infected) either eliminate *Mtb* or contain it in a 'latent state' in which an equilibrium is established between host and pathogen.

'Latent TB' is a clinical condition that occurs after an individual is infected with *Mtb*, the infection is established, and the elicited host immune response holds the *Mtb* bacilli in a quiescent state, thereby preventing active replication and tissue damage. Thus *Mtb* bacilli are present in host tissue and yet there are no clinical symptoms or signs of active tuberculous disease. Latency has been proven to exist since *Mtb* bacilli have been cultured from tissues obtained from healthy individuals who died from traffic accidents and had no pathological evidence of active disease (Opie & Aronson 1927). This state of *Mtb* latency is assumed to be lifelong (Ehlers 2009). While this dogma persists, long term latency may be related to *Mtb* strain differences and high human density-adjusted strains. Importantly, re-activation of latent *Mtb* bacilli can occur at any time in the infected individual's lifetime. The actual number of people developing active tuberculous disease later in life varies considerably and depends on the waning of effective host immune responses, occurrence of chronic diseases such as diabetes, alcoholic liver disease, malnutrition, immunosuppression due to HIV co-infection and steroids or other immunosuppressive drugs. When active disease occurs in later life, it becomes difficult to ascertain whether it is due to reactivation of latent *Mtb* bacilli or a new infection with a different *Mtb* strain.

Mtb latency and size of the global problem

According to WHO estimates, 2 billion people globally are latently infected with *Mtb* (Sudre et al. 1992). How this figure was calculated and why it has become a dogma in the literature over the past two decades requires explanation since this huge latent *Mtb* human reservoir would render the prospect of eliminating *Mtb* from the human race seemingly impossible. Latent TB is diagnosed by the absence of clinical disease (symptoms and signs) and positive intradermal skin test reactivity with purified protein derivative (PPD) of *Mtb*. The tuberculin skin test (TST) and/or the more recent positive interferon gamma release assays (IGRAs) lack accuracy for the purpose. An accurate study to survey worldwide prevalence of latent TB would be impossible due to logistical reasons. Thus epidemiological models based on sets of complex formulae, using epidemiological indicators of case notification rates, predicted incidence of disease, mortality from TB, annual risk of infection (from tuberculin skin test surveys), rate of change of the annual risk of infection in the past, and the age-specific prevalences of infection, are used by WHO to estimate latent TB prevalence rates and of dual *Mtb*/HIV co-infection rates for each country, region and continent. In 1990 WHO estimated that one third (1.7 billion people) of the world's population was infected with *Mtb* and that 3 million people were co-infected with *Mtb* and HIV (2.375 million in Africa) (Sudre *et al.* 1992). Since then the TB and HIV epidemics have progressed, the figures have not reduced significantly (WHO 2010) and the emergence of drug resistant *Mtb* strains throughout the world adds an another complex dimension to TB control (Migliori *et al.* 2010).

Mycobacterial escape and equilibrium

Several clinical, immunological and pharmacological issues relating to *Mtb* latency need to be unravelled before effective progress can be made towards eradicating the *Mtb* reservoir. *Mtb* has over thousands of years developed an elaborate survival mechanism in humans, allowing it to remain in a dormant state in the human body after initial infection (Behar *et al.* 2010). In most immunocompetent individuals, the immune system engages a lifelong battle against latent *Mtb* infection, either eradicating it or limiting its replication and containing its spread (Parris *et al.* 1998; Lobue and Menzies 2010; Behar *et al.* 2010; Lin & Ottenhoff 2008; Opie & Aronson 1927; Ehlers 2009; Henderson *et al.* 2010). Among the unresolved mysteries of latent *Mtb* are the nature and anatomical situation of persisting tubercle bacilli (Grange 1992). Various morphological variants of the bacilli such as minute Much's granules and cell wall-free forms have been postulated but none has been clearly proven (Stanford 1987). Also, while it is often stated that persisting bacilli are sequestered in dense fibrous scars of healed primary lesions, studies based on *in situ* PCR suggest that they are more widely distributed throughout apparently normal lung tissue (Hernández-Pando *et al.* 2000). In addition, such DNA is widely distributed in adipose tissue of individuals with latent TB (Neyrolles *et al.* 2006). Although most cases of post-primary TB occur in the upper regions of the lung, there is no reason to assume that latent bacilli are restricted to this anatomical situation (Balasubramanian *et al.* 1994).

One generally accepted postulate of latent TB assumes that *Mtb* bacilli are in a true dormant state, undergoing no replication. Such a state could be a response to tissue factors such as anoxia, but although the DosR regulon enables *Mtb* to adapt to anaerobic conditions *in vitro*, and to survive for long periods under such conditions, its relevance in human TB remains uncertain (Honaker *et al.* 2009). Recent data cast doubt on the assumption of an 'inactive' latent state, as there is constant metabolic activity within the *Mtb* bacilli. More recently, there is increasing evidence to support the hypothesis that molecular chaperones are secreted as intercellular signalling molecules, which in turn can control metabolic activity at, and composition of, the cell wall (Henderson *et al.* 2010). Resuscitation-promoting factors have also been identified and the role of these in *Mtb* latency and *Mtb* re-activation is being investigated (Biketov *et al.* 2007).

A further bacterial regulatory mechanism dependent on tissue factors favouring latency involves Wag31, a protein homologous to DivIVA proteins present in all Gram positive bacteria and which, by an effect on peptidoglycan synthesis, determines cell morphology and division. In mycobacteria, depletion of Wag31 under conditions conducive to dormancy causes a weakening of the cell wall, initially at one pole but with the eventual formation of circular forms (Kang *et al.* 2008). This could well be the initiating phase in formation of the postulated cell wall-free, non acid-fast persisting bacilli.

These proposed forms of latency are certainly not mutually exclusive and may coexist, though there may be considerable differences between the nature of bacilli persisting after initial infection with limited tissue damage, and those persisting after therapy for post-primary TB, when there is extensive tissue damage and dense fibrous scarring.

Mycobacterial eradication

TB eradication will require the identification and preventive treatment of high risk individuals who are latently infected with *Mtb*. However, accurate and direct identification of latent *Mtb* infection is not possible due to technical deficiencies. Available diagnostic tests used to identify individuals latently infected with *Mtb*, the *in vivo* tuberculin skin test (TST) and the *ex vivo* interferon-gamma release assays (IGRAs), are designed to identify an adaptive immune response against, but not necessarily latent infection with, *Mtb*. The proportion of individuals who truly remain infected with *Mtb* after tuberculin skin test or IGRA conversion is unknown. Emerging data show that IGRAs are not useful for distinguishing active disease from latent *Mtb* infection in high TB and HIV endemic areas. It is also uncertain how long adaptive immune responses towards *Mtb* antigens persist in the absence of live mycobacteria. Clinical management and public health care policies for preventive chemotherapy against *Mtb* need improving and will require more sensitive and specific tests which can distinguish between active disease and latent TB (Mack *et al.* 2009; Marais *et al.* 2009).

From the viewpoint of epidemiology and disease control, the most important form of *Mtb* latency is that occurring between primary infection and the emergence of post-primary, usually infectious, pulmonary TB. The classical rationale of preventing emergence of active disease from a latent state involves administering a limited duration course of a single or combination of anti-*Mtb* drugs. WHO currently recommends isoniazid monotherapy for treatment of latent TB and for secondary prophylaxis after treatment for active TB in those at risk (Sudre et al. 1992; Lobue & Menzies 2010). Isoniazid preventive therapy is recommended for risk groups who are HIV-infected, receiving immunosuppressive therapy, or in close contact with sputum smear positive active TB patients, such as health care workers, prison staff, household contacts and immigrants to TB non-endemic areas who are latently infected.

In high endemic HIV/AIDS and TB areas, particularly sub-Saharan Africa, a large proportion of HIV-infected patients are co-infected with *Mtb*. WHO recommends isoniazid preventive therapy (IPT) for preventing re-activation of latent *Mtb* infections progressing to active TB (WHO 1998). However, despite clear guidelines, this policy has not been implemented widely in most TB and HIV/AIDS endemic areas. There is also widespread concern that such preventive therapy may lead to the emergence of initial resistance to isoniazid, the most potent and important first line bactericidal drug crucial to the rapid reduction of the organism load in the early phase of chemotherapy. Furthermore, the issue of the role of isoniazid prophylaxis in patients infected with MDR or XDR strains of *Mtb* remains unclear.

The paradox of isoniazid monotherapy dogma

Isoniazid is a potent first-line bactericidal drug which can rapidly reduce the *Mtb* organism load at start of chemotherapy (Mitchison 1985). Despite several decades of research, numerous theories and expert discussion groups, the pharmacological and physiological basis of the use of INH monotherapy for IPT has eluded scientists and TB experts (Vilchèze & Jacobs 2007). For INH to be effective, it requires active division and replication of *Mtb* bacilli. There is strong evidence from clinical trials that isoniazid monotherapy is highly effective in preventing latent *Mtb*

infection progressing to active TB (WHO 1998). This paradox, of a highly effective impact of INH on dormant, non-replicative metabolic state *Mtb* (that should render it physiologically resistant to killing by INH), has not yet been explained and continues to baffle scientists.

Revisiting alternative modes of action of isoniazid

Isoniazid is a pro-drug that requires oxidative activation by the mycobacterial catalase-peroxidase enzyme KatG and the most frequent resistance-determining mutations occur in the gene encoding this enzyme (Timmins *et al.* 2004). The usual target for INH is the enzyme enoyl-acyl carrier protein reductase which is involved in the synthesis of mycolic acid, a major constituent of the mycobacterial cell wall (Argyrou *et al.* 2006). Some INH resistance-determining mutations occur in the *inhA* gene that encodes this enzyme, or in its promoter region. If, however, the latent *Mtb* bacillus is in a cell wall-free form, and therefore lacking mycolic acid as a result of inhibition of Wag31 or of other mechanisms, INH would not be expected to have a bactericidal effect (Kang *et al.* 2008). This indicates that INH has a quite different mode of action on such latent *Mtb* bacilli. INH, to be effective in TB latency, requires effective T cell responses. This is based on the observation that INH only offers protection to TST-positive individuals in TB and HIV endemic areas, despite the fact that even TST-negative people are likely to have latent TB. It appears possible that an interaction between isoniazid and the human T cell response is required before isoniazid can be effective in latency. The isoniazid-T cell interaction may be mediated through endogenous vaccination by released mycobacterial antigens.

Multi-drug resistant TB (MDR-TB), defined as resistance of *Mtb* to INH and rifampicin, is an important problem globally (Migliori *et al.* 2010). Currently there are no methods by which patients with latent TB due to INH resistant strains of *Mtb* can be identified. This poses another dilemma for national TB programs which are following WHO guidelines using IPT. A fundamental and crucial question arises as to whether INH is effective against latency caused by infection with MDR strains of *Mtb*, which by definition are resistant to INH. This may be answered through current longitudinal cohort studies of patients in highly MDR-TB endemic areas who are being given INH by national TB programmes (Fraser *et al.* 2006).

Other drugs for treatment of latent TB

While unravelling the mysteries of latency and mechanisms of action of INH continues, other clinical approaches in eradicating the huge *Mtb* human reservoir include further studies for better selection of combination of drug candidates to eradicate latent *Mtb*. Two large-scale trials are ongoing (Lobue & Menzies 2010); one compares efficacy and effectiveness of 9 months of INH with 4 months rifampicin; the other compares 9 months of daily self-administered INH with 3 months of once-weekly INH combined with rifapentine. The results of these two trials may shape future recommendations for preventive therapy. However as with INH, these will not shed light on the fundamental scientific basis underlying the mechanism of action of these bactericidal drugs on latent *Mtb* bacilli.

Conclusion

The challenges posed by *Mtb* infection, its natural history, its interactions with the immune system and its mechanisms for evasion require more serious investments for basic science research (Sudre *et al.* 1992; Hernández-Pando *et al.* 2000). The task of unravelling the mysteries of survival mechanisms evolved by *Mtb* to overcome the human immune response, and its successful use of the human population to survive and thrive over centuries, is a daunting one. The current scientific basis underlying *Mtb* dormancy, its dogmas and paradoxes, and the persisting mysteries of *Mtb* and its interaction with the immune system leave a huge knowledge gap. From the current perspective, it appears that the human race will continue to be outwitted by *Mtb,* and WHO and TB experts will have to settle for trying to achieve global TB control rather than global eradication of *Mtb*.

Acknowledgements

Alimuddin Zumla, Justin O'Grady, Matthew Bates, Peter Mwaba, Keertan Dheda, Markus Maeurer, Michael Hoelscher receive grant support from EuropeAID (ADAT), EU-FW7, UK-MRC, SIDA, and EDCTP. Alimuddin Zumla also acknowledges support from NIHR-UCLH CBRC.

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