REVIEW

Latent tuberculosis infection: What we know about its genetic control?

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SUMMARY

About 90% of all cases of tuberculosis (TB) infection are comprised of latent mycobacterial persistence in the absence of clinical manifestations. In a proportion of latently infected individuals infection eventually reactivates and becomes contagious, seriously influencing epidemiological situation. Mechanisms of Mycobacterium tuberculosis transition to dormancy and TB reactivation are poorly understood, and biological markers of latency remain largely unknown. Data are accumulating that the dynamical equilibrium between the parasite and the host (expressed as a long term asymptomatic infection) and its abrogation (expressed as a reactivation disease) are genetically controlled by both parties. In this short review, the authors summarize the results of experimental studies on genetic regulation of the latent TB infection.

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1. Introduction

Manifestations of Mycobacterium tuberculosis infection in humans varies from an asymptomatic latent possession to a rapid progressive disease. It is generally considered that in the absence of overt dysfunctions in the immune system not more than 3–10 per cent of individuals infected with M. tuberculosis eventually develop clinical disease [1]. Apart from the rare cases of apparently complete eradication of mycobacteria due to yet unknown factors of natural resistance [2], ~90 per cent of infected individuals without clinical manifestations comprise an enormous reservoir of latent tuberculosis infection (LTBI). In some of these latently infected individuals infection transits to the active state, becomes contagious and seriously affects epidemiological situation [3]. Thus, currently the problem of LTBI identification, treatment and prevention is one of the most important in infectious medicine.

Evolution strategy of mycobacterial parasitism, presumably, combines slowly developing infection (ensures long survival of a given bacterial population) with the guaranteed reactivation of a proportion of latent bacterial populations (ensures horizontal transmission). At the present level of biomedical knowledge this combination looks unbeatable since too little is known about the mechanisms of protective immunity to and pathogenesis of TB in general and LTBI in particular. Only success in identification of essential immune mechanisms and biological markers of protection will allow us to adequately modulate biochemical pathways of pathogenesis and assess the performance of novel vaccines and drugs using reliable biological correlates [4]. Nevertheless, despite serious attention to the problem of TB latency and reactivation during last decades, we still do not understand the biology of LTBI and its transition to overt infection [5].

After infecting the host and reaching its organs, predominantly the lung, M. tuberculosis is engulfed by neutrophils and macrophages and falls under pressure of natural and adaptive immune responses. We do not know how often these protective factors totally eradicate the population of the parasite, but in many cases mycobacteria transit to the so called dormant state and acquire elevated level of resistance to external bactericidal factors. In microbiological terms, the dormant state of M. tuberculosis is traditionally defined as inability to replicate in culture combined with extremely low metabolic activity [6]. At the systemic level, infection transits to latency [7] accompanied by formation of highly structured granulomata consisting predominantly of leukocytes and well isolated from the surrounding tissue [9]. Due to
isolated location and depressed metabolism of mycobacteria, this form of infection is difficult to detect using standard biochemical and microbiological methods and to eliminate with common antibiotics. Apparently, LTBI may last asymptptomatically for a very long time and represents the most common variant of tuberculosis infection [10].

In this review the emphasis will be made on genetic aspects of LTBI and its reactivation “from the point of view” of the parasite and the host. Numerous studies on biochemical aspects of dormancy and reactivation were recently reviewed [11,12] and will not be discussed here.

2. Mycobacterial transition to dormancy and reactivation: changes in gene expression

Information about physiology of dormant mycobacteria is scarce [10]. Even precise localization of dormant bacilli is not known [13], and the definition of “dormancy” is still operational: the question whether dormant mycobacteria do not replicate at all, or cell divisions occur at extremely low rates is the subject of debate [14]. Recently a very high level of genome stability was demonstrated in clinical isolates of M. tuberculosis circulating in human populations for more than 30 years [15]. The data provide a strong evidence that dormant mycobacteria do not (or almost do not) replicate.

Not much is known about the mechanisms of mycobacterial transition to dormancy. Apparently, the transition is largely determined by the dosR regulon consisting of ~50 genes [16]. The expression of dosR genes is induced when mycobacteria are cultured under hypoxic conditions (Wayne’s model) [17-19], in cultured macrophages [20], in mice [21] and in guinea pigs [22], i.e., when mycobacterial growth is inhibited by external factors. Earlier it was demonstrated that transition to dormancy is accompanied with up-regulation of the hspX (Rv2031c) gene which also belongs to the dosR regulon and encodes alpha-crystallin [23,24]. Very recently it was found that the dosR directly interacts with the important sigma factor SigA which, in turn, regulates a variety of cellular processes [25]. In addition, it was shown that DATIN, a protein encoded by the Rv0079 gene in the dosR regulon, can stimulate production of inflammatory cytokines involved in granuloma formation and support. The authors suggest that this modulation of the host immune response may serve for keeping infection in the latent state, since granulomas isolate mycobacteria from the surrounding tissues [26].

It should be mentioned that the expression of dosR is required for resuscitation of mycobacterial growth after dormancy. On the other hand, different mutations in dosR do not result in M. tuberculosis death under hypoxic conditions suggesting that transition to dormancy and survival under pressure are regulated not exclusively by the dosR [27]. Moreover, it was found that the initial dosR-related response which starts immediately after the onset of hypoxia is followed by the expression of a massive dosR-independent gene cohort, EHR, including a significant number of transcriptional regulators [19]. The authors suggested that this enduring response rather than the dos-R activity may represent the mechanism responsible for the maintenance of bacterial survival during dormancy/latency. In addition, the expression profiles of dosR differ between mycobacterial strains with different virulence, e.g., H37Rv, H37Ra [28] and W-Beijing [29]. These observations clearly indicate that the role of dosR in virulence, dormancy and resuscitation is not completely understood [30].

Two other genes presumably involved in mycobacterial transition to dormancy and backwards are relA (Rv2583c), whose product seem to prevent the transition to dormancy and/or stimulate resuscitation of growth after dormancy [31], and a transcription regulator from the LuxR family which supports the dormant state of M. tuberculosis [32]. In addition, the hypothesis that the latent state of mycobacteria may depend upon toxin-antitoxin systems [33] starts receiving experimental support. Involvement of the vapBC toxin-antitoxin system in the development of culture-negative state was demonstrated for Mycobacterium smegmatis [34]. Another example of linkage between mycobacterial growth and toxin activity is the MazF toxin in M. tuberculosis, which abrogates protein synthesis by disrupting 23S rRNA molecules at the consensus sequence in the ribosome active center [35].

Dormant mycobacteria are resistant to antibiotics which suggests that a long persistence within the host leads to a marked inhibition or even arrest of their metabolism [36]. Until recently good models of dormancy were lacking, thus very little is known about metabolic and gene expression shifts underlying transition of mycobacteria along the “multiplication → dormancy → reactivation” axis. The majority of experiments aimed on characterization of mycobacterial metabolism in the dormant state were performed using Wayne’s anaerobic model which rather reflects an adaptive response to low oxygen conditions than the state of true deep dormancy [10]. Thus, Rodriguez et al. [37] determined the transcription profile of genes involved in the biosynthesis of mycobacterial cell-wall trehalose-based glycolipids in non-replicating persistently hypoxic mycobacteria (Wayne’s model), and in murine models of chronic and progressive tuberculosis in attempt to understand the role of these molecules in latent infection. A decrease in the transcription of mmtpL8 and mmtpL10 transporter genes and the increased transcription of the pks (polyketidesynthase) genes involved in sulfitolipid and diacyl-trehalose biosynthesis were detected in hypoxic bacilli and in the murine model of chronic infection, whereas all these genes were found to be up-regulated during the progressive disease. In vitro models of dormancy developed in Kaprelyants’ lab allows collecting large amounts of non-culturable M. tuberculosis cells which retain the capacity to resuscitate their growth under certain conditions [38,39]. One of the models is based upon culturing M. tuberculosis in Sauton’s medium without potassium. Under these conditions, more than 99% of bacterial cells transit to dormant, non-culturable state during a prolonged, 60-d stationary phase. The second model was based upon culturing M. tuberculosis under gradual acidification of the medium, resulting in a massive accumulation of ovoid cells with the properties closely resembling those predicted for dormant bacteria [40]. Both type of dormant cells resuscitated growth when cultured in the fresh medium in the presence of supernatants obtained from actively growing mycobacterial cultures. In order to characterize biochemical processes underlying transition to the non-culturable state in this model, the DNA microarray transcriptome analysis was performed [41]. Several hundreds of genes involved in basic metabolic processes — respiration, regulation of transcription and translation, cell wall biosynthesis — appeared to be down-regulated during transition indicating switching off the majority of anabolic reactions and energy producing machinery in the dormant state. Importantly, significant proportion of up-regulated genes encoded catabolic enzymes (beta-glycosidases, proteases, proline-iminopeptidases, alanine dehydrogenases). Thus, up-regulation of the sthA encoding a soluble pyridine–pyridine transhydrogenase that catalyses the conversion of catabolic NADPH to NADH is a marker of the prevalence of the catabolic reactions in non-culturable state. Up regulation of isocitrate lyase gene — the key enzyme in glyoxilate shunt — was also observed in our model, similarly to what has been reported for the Wayne’s model. Remarkably, only two genes from the dosR were up-regulated, whereas 17% of up-regulated genes overlapped with those from the EHR (see above), further suggesting that the latter may play a general role in mycobacterial dormancy whatever was the mechanism of its induction [41]. However, this
transcriptional profiling could well reflect predominantly the processes occurring during transition to dormancy rather than the state of deep dormancy itself. The latter is very likely characterized by a global down-regulation of gene expression. Indeed, another transcriptome analysis performed in “persistent” mycobacteria resistant to main antibiotics demonstrated wide inhibition of gene expression, with only 15 genes up-regulated, including accr encoding α-crystalline and genes for a number of sigma factors [42].

Another important stage of LTBI is mycobacterial transition from dormant state to active growth leading to reactivation TB. An important role of proteins from the Rpf family in this process was demonstrated in numerous studies [12,43–45]; thus up-regulation of the rpf genes’ expression upon reactivation of mycobacteria found in rabbits [46] was expected. The conservative domain of all Rpf proteins is structurally close to lytic transglycosilases, and it was demonstrated that these proteins participate in the cell wall hydrolysis (remodeling), evidently, an important early part of the resuscitation process [47]. More recently, we obtained an evidence that the Rpf proteins might be active at a later stage of resuscitation, whilst the early events are linked with activation of the systems responsible for cAMP biosynthesis [48]. We have found that exogenous free fatty acids stimulated resuscitation of non-culturable M. smegmatis via activation of adenylate cyclase, increasing the cAMP intracellular levels and activating cellular metabolism at the initial stages of resuscitation (lag-phase metabolic reactivation). However, according to the real-time PCR measurements, increase in the Rpf biosynthesis occurred later and correlated not with the lag phase but with the active M. smegmatis growth. We suggest that the whole resuscitation pathway may be divided into three phases: (i) true lag phase, (ii) cAMP-depended metabolic reactivation, and (iii) Rpf-depended secondary growth [48].

3. LTBI and hypothetical role of non-coding short RNAs

Gene expression analysis is a powerful tool for dissecting pathogens’ physiology, but until recently full transcriptional profiles were obtained by hybridization on DNA microarrays – the method which has substantial intrinsic limitations [49]. Situation profoundly improved after introduction of the new generation sequence platforms, such as Illumina, SOLiD and others, applicable to massive sequencing of the whole cellular RNA (RNA-seq) [50–52]. During last 5 years an impressive number of RNA-seq-based studies describing the whole transcriptome of M. tuberculosis were published [53–58]. Analyses of gene expression profiles were based upon comparisons between mycobacterial cultures at lag and log phases of growth or under several stress factors. The most important novel information reported was identification in the M. tuberculosis transcriptome a great number of short non-coding RNAs – genetic elements which regulate gene expression. However, for the studying transcriptome under conditions more closely imitating real infection, e.g., infection of cultured macrophages, DNA microarray approach is still in use [59]. We were the first who applied new generation sequence approaches for the studying the transcriptome of intracellular pathogens in infected tissues in vivo [60]. Similar approaches may be applied to any pathogenic bacteria and help to identify virulence factors, drug targets and epidemiological monitoring. Presently, we intensively use this method to study dynamic switches in transcriptomes of M. tuberculosis and Mycobacterium avium following infection of murine hosts genetically susceptible and resistant to these bacteria, and the first characteristics of gene groups whose expression profiles depend upon the stage of infectious process are accumulating [60–62].

The short non-coding RNAs represent, perhaps, the hottest spot in bacterial genetics during the last decade. Being involved in regulation of transcription, translation and mRNA stability, these molecules are used by bacteria for rapid switches in global gene expression profiles for physiological responses to environmental changes [63]. This is particularly important for the pathogenic bacteria who have to regulate gene expression in response to rapidly changing conditions (temperature, pH, factors of immunity, etc.) within the host [64]. The principle role of short RNAs in regulation of expression of genes determining virulence was demonstrated in Chlamydia trachomatis, Clostridium perfringens, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pyogenes, Vibrio cholerae and Yersinia pestis (see [65] for the review).

In the genus Mycobacterium short RNAs were found in M. tuberculosis, Mycobacterium bovis, M. avium, M. smegmatis and Mycobacterium marinum [55,61,66–68]. Taking into consideration that the dosR regulon which apparently is important in LTBI also regulates the expression of non-coding short RNAs [54], the role of short RNAs in the transition from activity to dormancy and backwards is entirely possible.

4. Mycobacterial transition to dormancy and reactivation: genetics of the host

Immune response against M. tuberculosis was characterized in considerable detail [69–71], however, it is not clear what combination of immune reactions not only restricts mycobacterial growth but also protects from the lung tissue damage. Even less is known about local immune responses in the lungs and lymphoid organs during latent infection [72,73]. The simplest model of M. tuberculosis persistence is based upon infection of relatively resistant mice of B6 inbred strain [74] with a low dose (~100 CFU) of mycobacteria via aerogenic route. After initial acute phase of mycobacterial growth, infection is taken under control by the immune system and bacterial loads in organs remain stable for a few months. However, eventually all infected mice dye of lung pathology [75]. This model of chronic TB is convenient due to its simplicity but by no means universal because does not provide information about the processes that take place in genetically more susceptible or more resistant hosts.

The classical Cornell model [76,77] and its modifications are based upon infection of mice of different strains with different doses of virulent mycobacteria, followed by antibiotic chemotherapy for 1–2 month till no culturable bacilli can be recovered from lungs and spleens [78,79]. From 12 to 28 weeks after treatment withdrawal (depending upon details of challenge and treatment) an active infectious process with recovery of culturable bacilli from organs re-develops in a proportion of mice. Importantly, all research teams reported reactivation of infection only in some mice although genetically identical inbred animals were used in most experiments [80]. This phenotypic variability within experimental groups substantially interferes with the reliable modeling of latency/reactivation and deciphering molecular mechanisms which underlay switches in immune system at different stages of the disease. With this regard, we need refined and improved animal models of LTBI. The fact that clinical forms of TB develop in a small proportion of primarily infected individuals, as well as variations in the disease phenotypes observed in patients and experimental animals [12,69], clearly indicate that genetics of the host plays an important role in dormancy and reactivation control [81,82]. Unfortunately, after an early experimental work [83] in which detailed analyses of immune responses was not performed due to the lack of appropriate methodology in 1980-ies, genetic aspects of TB relapse in the Cornell-like model were studied only in Apt’s lab [84,85]. We demonstrated that in genetically TB-susceptible mice reactivation occurs in 100 per cent of animals and characterized some shifts in immune response, lung pathology and gene expression profiles along the course of infection. It is
highly desirable that similar experiments are repeated by independent researches and in other mouse strain combinations. In particular, mouse strains carrying the susceptible allele of the Ipr1 gene on different genetic backgrounds deserve special attention since allow modeling various types of TB pathology (necrotic lesions, miliary TB, chronic infection) [82].

In the extensive literature on chronic and latent TB (see [86] for the review) it is emphasized that transition to latency, its stability and reactivation equally depend upon genetics of the host and the pathogen. Nevertheless, very few attempts were undertaken to study genetic variation in the two interacting species in a unified experimental system, mostly due to a very complex nature, duration and cost of corresponding experiments. Our three labs recently accomplished the first 12-mo experiment in which mice of genetically TB-susceptible and relatively resistant strains, which substantially differed in parameters of immune response and pathology under the Cornell-like conditions [84], were infected with the wild type M. tuberculosis H37Rv and its derivative bearing quadruple KO mutations disrupting four out of five genes encoding proteins of the Rpf family (ΔACDE). Previously it was shown that such multiple Rpf-KO mutants express strongly attenuated phenotypes in the mouse models of infection [44,87]. Before injection, both mycobacterial strains underwent procedures leading to the non-culturable, dormant state exactly as described previously [40]. The results displayed in Figure 1 clearly demonstrate that the major parameters of reactivation disease critically depend upon genetics of both the parasite and the host. This model provides a tool for the studying the key shifts in immune responses and gene expression profiles accompanying transitions along the “dormancy → reactivation → effective/ineffective control” axis, and experiments with genetically and physiologically manipulated bacteria injected in mice with different genetic TB susceptibility are presently in progress.

5. Treatment against LTBI reactivation: chemotherapy in mice

Experimental assessment of the efficacy of anti-TB chemotherapy includes two substantially different criteria: (i) achievement of the organ culture-negative state of the host; (ii) complete eradication of infection. The first state is much easier to reach but very often a few weeks or months after treatment withdrawal infection reactivates. The proportion of animals demonstrating reactivation after drug-driven dormancy and the speed of

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Different disease progression after infection of genetically susceptible I/St and resistant B6 mice with established *in vitro* dormant wild type and quadruple Rpf-KO (ΔACDE) strains of *M. tuberculosis* H37Rv. Mycobacteria of the two strains were cultured and stored as described in [40] until fully losing the capacity to form agar colonies. Mice were infected via trachea with ~10^6 directly counted microbial cells in 50 μl of PBS containing Tween 80. CFU counts in lungs (A, C) and spleens (B) were estimated at month 8 and 12 (w. t. — black bars, ΔACDE — white bars). All B6 mice cleared ΔACDE infection in the lungs by month 12; in I/St mice infection level declined but bacteria were not eradicated (C). All B6 mice survived more than a year and showed ~0.7 log decline in the lung CFU counts between months 8 and 12 of infection with the w. t. dormant bacteria (A, C); all I/St mice died by day 300 of infection (D).
reactivation depend upon many factors, including the dose of infection, virulence of mycobacteria, combination of drugs and time of treatment [88,89]. Unfortunately, the influence of host genetics on the efficacy of treatment has never been systematically addressed [90], although sporadic publications clearly indicate the importance of genetic diversity for the outcome of treatment [84,91].

Any given drug regimen capable to achieve the culture-negative state of the host does not guarantee eradication of infection and reactivation prevention. Small populations of mycobacteria that were not totally killed in the course of treatment become dormant and lose susceptibility to the very drug regimen which provided culture conversion to negative. Experimental studies of the last decade clearly indicate that culture-negative conversion and even reactivation prevention are most effectively achieved by application of drug combinations that include the classical first- or second-line antibiotics and the newly developed drug candidates. Thus, it was demonstrated in BALB/c mice infected with M. tuberculosis H37Rv via aerosol route that the addition of Nitromidazopyran (PA-824) to a standard 6- mo course of therapy (INH + Rif + PZA for 2 months plus INH + Rif for 4 months) allows to reach culture negative conversion after 4-mo instead of 6-mo treatment [89]. PA-824 also increased bactericidal activity of Rif + PZA combination [92]. Nevertheless, new regimens did not surpass the old ones regarding the prevention of infection relapse. One possible option to increase the efficacy of relapse prevention is, perhaps, combination of chemo- and immune therapies, as suggested in a proof-of-principle study [93].

Application of a new and very effective drug, an ATP synthase inhibitor TMC-207, to B6 mice infected intravenously led to a rapid culture conversion to negative, even when the drug was administered alone, but reactivation of infection occurred in almost all animals. Importantly, combination of TMC-207 with PZA substantially decreased the proportion of relapse, and combination of TMC-207 + PZA with another new drug candidate, SQ109, totally abolished reactivation [94]. More complex combinations of TMC-207 with less conventional drugs demonstrated even higher efficacy in the BALB/c aerosol infection model: 8 weeks of treatment with TMC-207 + PZA + CFZ (Clofazimin) or TMC-207 + PZA + RPT (Rifampentin) was sufficient for preventing reactivation of infection for at least 3 months post the treatment withdrawal, and TMC-207 + PZA + RPT + CFZ combination achieved this effect after 6 weeks [95]. In a close perspective, TMC-207, recently approved for the clinic, as suggested in a proof-of-principle study [93].

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References


Shleeva MO, Kudykina YK, Vostroknutova GN, Suzina NE, Mulyukin AL. Multiple small RNAs identified in mycobacterial hiv bcc are also expressed in Mycobacterium tuberculosis and Mycobacterium smegmatis. Nucleic Acids Res 2010;38:4067–78.


