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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.

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 [7,8] accompanied by formation of highly structured granulomata consisting predominantly of leukocytes and well isolated from the surrounding tissue [9]. Due to

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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 asymptomatically 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]. These 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 α-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 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 persistent 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 *mmpL8* and *mmpL10* transporter genes and the increased transcription of the *pks* (polyketidesynthase) genes involved in sulfolipid and diacyltrehalose 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