Tuberculosis xxx (2014) 1–7

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

REVIEW

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

Tatiana Kondratieva [a], Tatyana Azhikina [b], Boris Nikonenko [a], Arseny Kaprelyants [c], Alexander Apt [a, *]

[a] Central Institute for Tuberculosis, Moscow, Russia
[b] Institute of Bioorganic Chemistry, Moscow, Russia
[c] Institute of Biochemistry, Moscow, Russia

ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form
10 June 2014
Accepted 20 June 2014

Keywords:
Latent tuberculosis
Gene expression
Short RNA
Host genetics
Treatment
Animal models

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

* Corresponding author. Laboratory for Immunogenetics, Central Institute for Tuberculosis, Yauza alley, 2, Moscow 107564, Russia.
E-mail address: asapt@aha.ru (A. Apt).

http://dx.doi.org/10.1016/j.tube.2014.06.009
1472-9792/

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

Please cite this article in press as: Kondratieva T, et al., Latent tuberculosis infection: What we know about its genetic control?, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.06.009

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 only15 genes up-regulated, including *acr2* 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

Please cite this article in press as: Kondratieva T, et al., Latent tuberculosis infection: What we know about its genetic control?, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.06.009

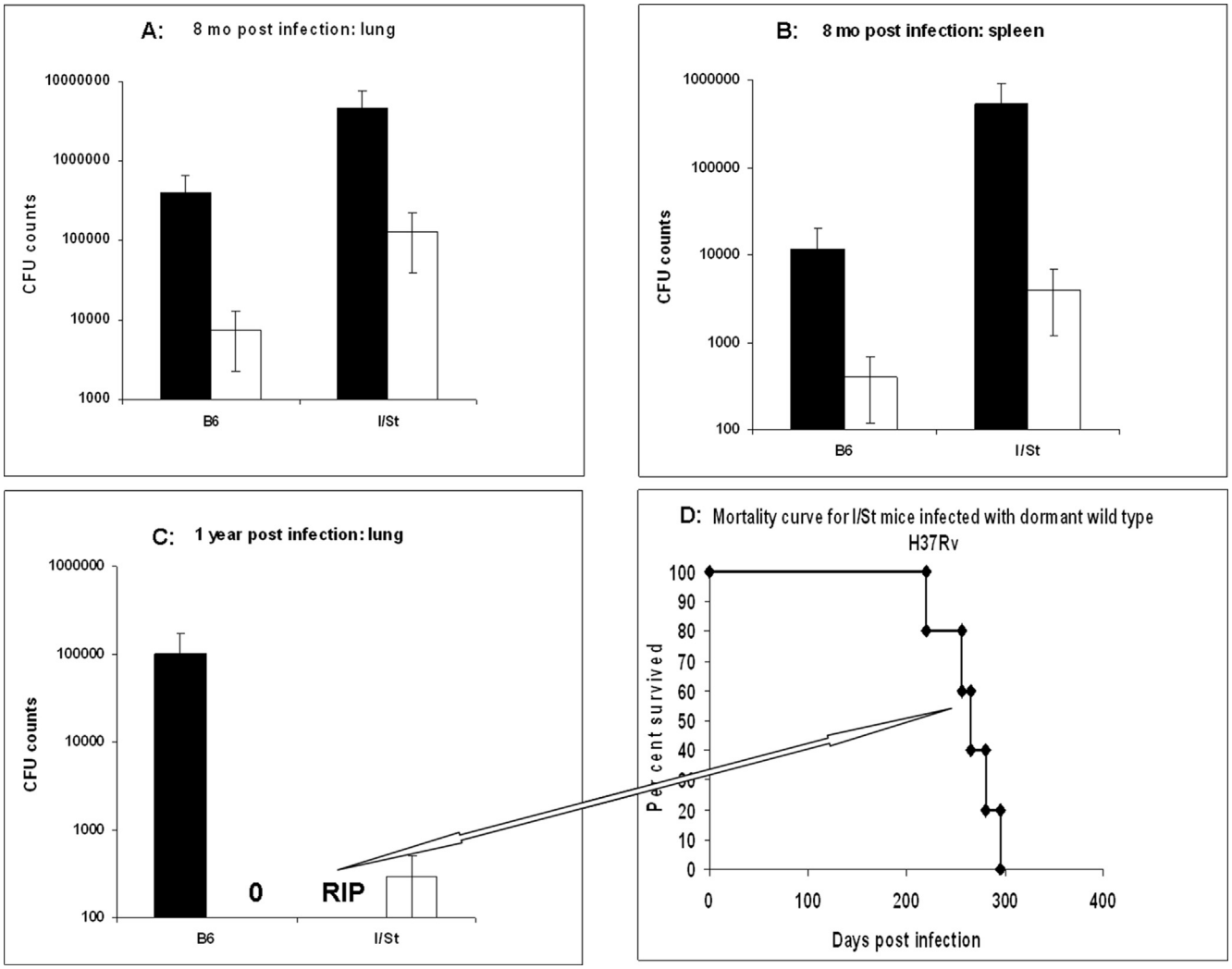

Figure 1. Different disease progression after infection of genetically susceptible I/St and resistant B6 mice with established *in vitro* dormant wild type and quadruple *Rpf*-KO (ΔACDE) strains of *M. tuberculosis* H37Rv. Mycobacteria of the two strains were cultured and stored as described in [40] until fully losing the capacity to form agar colonies. Mice were infected via trachea with ~10^4 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 cleaned 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).

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

Please cite this article in press as: Kondratieva T, et al., Latent tuberculosis infection: What we know about its genetic control?, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.06.009

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 systemically 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 Nitroimidazopyran (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 (Rifapentin) 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 clinical use, may markedly reduce the duration of TB patients' treatment [88] and, hopefully, help to deal with reactivation problem.

Acknowledgments

The work of the authors is financially supported by the grants 13-04-40070-H, 13-04-40071-H and 13-04-40072-H (parts of the Complex Project 13-04-40070-K), grant 14-04-01688 (all – from the Russian Foundation for Basic Research), and MIT-Skoltech contract 182 MRA.

Competing interest: None declared.

Ethical approval: Not required.

References

[1] Barry CE 3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, Schnappinger D, Wilkinson RJ, Young D. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol 2009;7:845–55.

[2] Cobat A, Gallant CJ, Simkin L, Black GF, Stanley K, Hughes J, Doherty TM, Hanekom WA, Eley B, Jaïs JP, Boland-Auge A, van Helden P, Casanova JL, Abel L, Hoal EG, Schurr E, Alcaïs A. Two loci control tuberculin skin test reactivity in an area hyperendemic for tuberculosis. J Exp Med 2009;206:2583–91.

[3] Lillebaek T, Andersen AB, Dirksen A, Smith E, Skovgaard LT, Kok-Jensen A. Persistent high incidence of tuberculosis in immigrants in a low-incidence country. Emerg Infect Dis 2002;8:679–84.

[4] Kaufmann SH. Future vaccination strategies against tuberculosis: thinking outside the box. Immunity 2010;33:567–77.

[5] Ottenhoff TH, Kaufmann SH. Vaccines against tuberculosis: where are we and where do we need to go? PLoS Pathog 2012;8:e1002607.

[6] Gangadharam PR. Mycobacterial dormancy. Tuber Lung Dis 1995;76:477–9.

[7] Ahmad S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. Clin Dev Immunol 2011;2011:814943.

[8] Cardona PJ. A dynamic reinfection hypothesis of latent tuberculosis infection. Infection 2009;37:80–6.

[9] Ulrichs T, Kaufmann SH. New insights into the function of granulomas in human tuberculosis. J Pathol 2006;208:261–9.

[10] Chao MC, Rubin EJ. Letting sleeping dos lie: does dormancy play a role in tuberculosis? Annu Rev Microbiol 2010;64:293–311.

[11] Dworkin J, Shah IM. Exit from dormancy in microbial organisms. Nat Rev Microbiol 2010;8:890–6.

[12] Gengenbacher M, Kaufmann SH. Mycobacterium tuberculosis: success through dormancy. FEMS Microbiol Rev 2012;36:514–32.

[13] Ehlers S. Lazy, dynamic or minimally recrudescent? on the elusive nature and location of the mycobacterium responsible for latent tuberculosis. Infection 2009;37:87–95.

[14] Munoz-Elias EJ, Timm J, Botha T, Chan WT, Gomez JE, McKinney JD. Replication dynamics of Mycobacterium tuberculosis in chronically infected mice. Infect Immun 2005;73:546–51.

[15] Yang Z, Rosenthal M, Rosenberg NA, Talarico S, Zhang L, Marrs C, Thomsen VØ, Lillebaek T, Andersen AB. How dormant is Mycobacterium tuberculosis during latency? A study integrating genomics and molecular epidemiology. Infect Genet Evol 2011;11:1164–7.

[16] Park HD, Guinn KM, Harrell MI, Liao R, Voskuil MI, Tompa M, Schoolnik GK, Sherman DR. Rv3133c/dosR is a transcription factor that mediates the hypoxic response of Mycobacterium tuberculosis. Mol Microbiol 2003;48:833–43.

[17] Sherman DR, Voskuil M, Schnappinger D, Liao R, Harrell MI, Schoolnik GK. Regulation of the Mycobacterium tuberculosis hypoxic response gene encoding alpha -crystallin. Proc Natl Acad Sci USA 2001;98:7534–9.

[18] Voskuil MI, Schnappinger D, Visconti KC, Harrell MI, Dolganov GM, Sherman DR, Schoolnik GK. Inhibition of respiration by nitric oxide induces a Mycobacterium tuberculosis dormancy program. J Exp Med 2003;198:705–13.

[19] Rustad TR, Harrell MI, Liao R, Sherman DR. The enduring hypoxic response of Mycobacterium tuberculosis. PLoS One 2008;3:e1502.

[20] Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK. Transcriptional adaptation of Mycobacterium tuberculosis within macrophages: insights into the phagosomal environment. J Exp Med 2003;198:693–704.

[21] Karakousis PC, Yoshimatsu T, Lamichhane G, Woolwine SC, Nuermberger EL, Grosset J, Bishai WR. Dormancy phenotype displayed by extracellular Mycobacterium tuberculosis within artificial granulomas in mice. J Exp Med 2004;200:647–57.

[22] Sharma D, Bose A, Shakila H, Das TK, Tyagi JS, Ramanathan VD. Expression of mycobacterial cell division protein, FtsZ, and dormancy proteins, DevR and Acr, within lung granulomas throughout guinea pig infection. FEMS Immunol Med Microbiol 2006;48:329–36.

[23] Yuan Y, Crane DD, Simpson RM, Zhu YQ, Hickey MJ, Sherman DR, Barry CE 3rd. The 16-kDa alpha-crystallin (Acr) protein of Mycobacterium tuberculosis is required for growth in macrophages. Proc Natl Acad Sci USA 1998;95:9578–83.

[24] Zhang Y. Persistent and dormant tubercle bacilli and latent tuberculosis. Front Biosci 2004;9:1136–56.

[25] Gautam US, Sikri K, Vashist A, Singh V, Tyagi JS. Essentiality of DevR/DosR interaction with SigA for the dormancy survival program in Mycobacterium tuberculosis. J Bacteriol 2014;196:790–9.

[26] Kumar A, Lewin A, Rani PS, Qureshi IA, Devi S, Majid M, Kamal E, Marek S, Hasnain SE, Ahmed N. Dormancy associated translation inhibitor (DATIN/Rv0079) of Mycobacterium tuberculosis interacts with TLR2 and induces proinflammatory cytokine expression. Cytokine 2013;64:258–64.

[27] Leistikow RL, Morton RA, Bartek IL, Frimpong I, Wagner K, Voskuil MI. The Mycobacterium tuberculosis DosR regulon assists in metabolic homeostasis and enables rapid recovery from nonrespiring dormancy. J Bacteriol 2010;192:1662–70.

[28] Malhotra V, Tyagi JS, Clark-Curtiss JE. DevR-mediated adaptive response in Mycobacterium tuberculosis H37Ra: links to asparagine metabolism. Tuberculosis (Edinb) 2009;89:169–74.

[29] Minch K, Rustad T, Sherman DR. Mycobacterium tuberculosis growth following aerobic expression of the DosR regulon. PLoS One 2012;7:e35935.

[30] Boon C, Dick T. How Mycobacterium tuberculosis goes to sleep: the dormancy survival regulator DosR a decade later. Future Microbiol 2012;7:513–8.

[31] Murphy DJ, Brown JR. Identification of gene targets against dormant phase Mycobacterium tuberculosis infections. BMC Infect Dis 2007;7:84.

[32] Hong Y, Zhou X, Fang H, Yu D, Li C, Sun B. Cyclic di-GMP mediates Mycobacterium tuberculosis dormancy and pathogenecity. Tuberculosis (Edinb) 2013;93:625–34.

Please cite this article in press as: Kondratieva T, et al., Latent tuberculosis infection: What we know about its genetic control?, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.06.009

[33] Arcus VL, Rainey PB, Turner SJ. The PIN-domain toxin-antitoxin array in mycobacteria. Trends Microbiol 2005;13:360–5.
[34] Demidenok OI, Kaprelyants AS, Goncharenko AV. Toxin-antitoxin vapBC locus participates in formation of the dormant state in Mycobacterium smegmatis. FEMS Microbiol Lett 2014;352:69–77.
[35] Schifano JM, Woychik NA. 23S rRNA as an a-Maz-ing new bacterial toxin target. RNA Biol 2014;11:101–5.
[36] Flynn JL, Chan J. Tuberculosis: latency and reactivation. Infect Immun 2001;69:4195–201.
[37] Rodríguez JE, Ramírez AS, Salas LP, Helguera-Repetto C, Gonzalez-y-Merchand J, Soto CY, Hernández-Pando R. Transcription of genes involved in sulfolipid and polyacyltrehalose biosynthesis of Mycobacterium tuberculosis in experimental latent tuberculosis infection. PLoS One 2013;8:e58378.
[38] Mukamolova G, Salina E, Kaprelyants A. Mechanisms of latent tuberculosis: dormancy and Resuscitation of Mycobacterium tuberculosis, vol. 1. National Institute of Allergy and Infectious Diseases, NIH; 2008. pp. 83–90. Frontiers in Research.
[39] Shleeva MO, Salina EG, Kaprel'iants AS. Dormant form of Mycobacterium tuberculosis. Mikrobiologiia 2010;79:3–15.
[40] Shleeva MO, Kudykina YK, Vostroknutova GN, Suzina NE, Mulyukin AL, Kaprelyants AS. Dormant ovoid cells of Mycobacterium tuberculosis are formed in response to gradual external acidification. Tuberculosis (Edinb) 2011;91:146–54.
[41] Salina EG, Mollenkopf HJ, Kaufmann SH, Kaprelyants AS. M. tuberculosis gene expression during transition to the “Non-Culturable” state. Acta Naturae 2009;1:73–7.
[42] Keren I, Minami S, Rubin E, Lewis K. Characterization and transcriptome analysis of Mycobacterium tuberculosis persisters. MBio 2011;2. e00100–11.
[43] Biketov S, Potapov V, Ganina E, Downing K, Kana BD, Kaprelyants A. The role of resuscitation promoting factors in pathogenesis and reactivation of Mycobacterium tuberculosis during intra-peritoneal infection in mice. BMC Infect Dis 2007;7:146.
[44] Downing KJ, Mischenko VV, Shleeva MO, Young DI, Young M, Kaprelyants AS, Apt AS, Mizrahi V. Mutants of Mycobacterium tuberculosis lacking three of the five rpf-like genes are defective for growth in vivo and for resuscitation in vitro. Infect Immun 2005;73:3038–43.
[45] Kana BD, Mizrahi V. Resuscitation promoting factors in bacterial population dynamics during TB infection. Drug Discov Today Dis Mech 2010;7. e13–e8.
[46] Kesavan AK, Brooks M, Tufariello J, Chan J, Manabe YC. Tuberculosis genes expressed during persistence and reactivation in the resistant rabbit model. Tuberculosis (Edinb) 2009;89:17–21.
[47] Cohen-Gonsaud M, Barthe P, Bagnéris C, Henderson B, Ward J, Roumestand C, Keep NH. The structure of a resuscitation-promoting factor domain from Mycobacterium tuberculosis shows homology to lysozymes. Nat Struct Mol Biol 2005;12:270–3.
[48] Shleeva M, Goncharenko A, Kudykina Y, Young D, Young M, Kaprelyants A. Cyclic amp-dependent resuscitation of dormant mycobacteria by exogenous free fatty acids. PLoS One 2013;8:e82914.
[49] Shendure J. The beginning of the end for microarrays? Nat Methods 2008;5:585–7.
[50] McGinn S, Gut IG. DNA sequencing - spanning the generations. N Biotechnol 2013;30:366–72.
[51] Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet 2010;11:31–46.
[52] Ozsolak F. Third-generation sequencing techniques and applications to drug discovery. Expert Opin Drug Discov 2012;7:231–43.
[53] Arnvig K, Young D. Non-coding RNA and its potential role in Mycobacterium tuberculosis pathogenesis. RNA Biol 2012;9:427–36.
[54] Arnvig KB, Comas I, Thomson NR, Houghton J, Boshoff HI, Croucher NJ, Rose G, Perkins TT, Parkhill J, Dougan G, Young DB. Sequence-based analysis uncovers an abundance of non-coding RNA in the total transcriptome of Mycobacterium tuberculosis. PLoS Pathog 2011;7:e1002342.
[55] Arnvig KB, Young DB. Identification of small RNAs in Mycobacterium tuberculosis. Mol Microbiol 2009;73:397–408.
[56] Miotto P, Forti F, Ambrosi A, Pellin D, Veiga DF, Balazsi G, Gennaro ML, Di Serio C, Ghisotti D, Cirillo DM. Genome-wide discovery of small RNAs in Mycobacterium tuberculosis. PLoS One 2012;7:e51950.
[57] Pellin D, Miotto P, Ambrosi A, Cirillo DM, Di Serio C. A genome-wide identification analysis of small regulatory RNAs in Mycobacterium tuberculosis by RNA-Seq and conservation analysis. PLoS One 2012;7:e32723.
[58] Uplekar S, Rougemont J, Cole ST, Sala C. High-resolution transcriptome and genome-wide dynamics of RNA polymerase and NusA in Mycobacterium tuberculosis. Nucleic Acids Res 2013;41:961–77.
[59] Mukhopadhyay S, Nair S, Ghosh S. Pathogenesis in tuberculosis: transcriptomic approaches to unraveling virulence mechanisms and finding new drug targets. FEMS Microbiol Rev 2012;36:463–85.
[60] Azhikina T, Skvortsov T, Radaeva T, Mardanov A, Ravin N, Apt A, Sverdlov E. A new technique for obtaining whole pathogen transcriptomes from infected host tissues. Biotechniques 2010;48:139–44.
[61] Ignatov D, Malakho S, Majorov K, Skvortsov T, Apt A, Azhikina T. RNA-seq analysis of Mycobacterium avium non-coding transcriptome. PLoS One 2013;8:e74209.
[62] Skvortsov TA, Ignatov DV, Majorov KB, Apt AS, Azhikina TL. Mycobacterium tuberculosis transcriptome profiling in mice with genetically different susceptibility to tuberculosis. Acta Naturae 2013;5:62–9.
[63] Waters LS, Storz G. Regulatory RNAs in bacteria. Cell 2009;136:615–28.
[64] Lease RA, Smith D, McDonough K, Belfort M. The small noncoding DsrA RNA is an acid resistance regulator in Escherichia coli. J Bacteriol 2004;186:6179–85.
[65] Toledo-Arana A, Repoila F, Cossart P. Small noncoding RNAs controlling pathogenesis. Curr Opin Microbiol 2007;10:182–8.
[66] DiChiara JM, Contreras-Martinez LM, Livny J, Smith D, McDonough KA, Belfort M. Multiple small RNAs identified in Mycobacterium bovis BCG are also expressed in Mycobacterium tuberculosis and Mycobacterium smegmatis. Nucleic Acids Res 2010;38:4067–78.
[67] Wang S, Dong X, Zhu Y, Wang C, Sun G, Luo T, Tian W, Zheng H, Gao Q. Revealing of Mycobacterium marinum Transcriptome by RNA-seq. PLoS One 2013;8:e75828.
[68] Ignatov DV, Mefodieva LG, Maiorov KB, Skvortsov TA, Azhikina TL. Novel small RNAs from Mycobacterium avium. Russ J Bioorg Chem 2012;38:447–9.
[69] Apt AS. Are mouse models of human mycobacterial diseases relevant? Genetics says: 'yes!'. Immunology 2011;134:109–15.
[70] Apt AS, Kondrat'eva TK. Tuberculosis: pathogenesis, immune responses and genetics of the host. Mol Biol Mosk 2008;42:880–90.
[71] Russell DG. Who puts the tubercle in tuberculosis? Nat Rev Microbiol 2007;5:39–47.
[72] North RJ, Jung YJ. Immunity to tuberculosis. Annu Rev Immunol 2004;22:599–623.
[73] Wayne LG. Dormancy of Mycobacterium tuberculosis and latency of disease. Eur J Clin Microbiol Infect Dis 1994;13:908–14.
[74] Medina E, North RJ. Resistance ranking of some common inbred mouse strains to Mycobacterium tuberculosis and relationship to major histocompatibility complex haplotype and Nramp1 genotype. Immunology 1998;93:270–4.
[75] Rhoades ER, Frank AA, Orme IM. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent Mycobacterium tuberculosis. Tuber Lung Dis 1997;78:57–66.
[76] McCune RM, Feldmann FM, Lambert HP, McDermott W. Microbial persistence. I. The capacity of tubercle bacilli to survive sterilization in mouse tissues. J Exp Med 1966;123:445–68.
[77] McCune RM, Feldmann FM, McDermott W. Microbial persistence. II. Characteristics of the sterile state of tubercle bacilli. J Exp Med 1966;123:469–86.
[78] de Wit D, Wootton M, Dhillon J, Mitchison DA. The bacterial DNA content of mouse organs in the Cornell model of dormant tuberculosis. Tuber Lung Dis 1995;76:555–62.
[79] Dhillon J, Mitchison DA. Effect of vaccines in a murine model of dormant tuberculosis. Tuber Lung Dis 1994;75:61–4.
[80] Scanga CA, Mohan VP, Joseph H, Yu K, Chan J, Flynn JL. Reactivation of latent tuberculosis: variations on the Cornell murine model. Infect Immun 1999;67:4531–8.
[81] Kondratieva E, Logunova N, Majorov K, Averbakh M, Apt A. Host genetics in granuloma formation: human-like lung pathology in mice with reciprocal genetic susceptibility to M. tuberculosis and M. avium. PLoS One 2010;5:e10515.
[82] Schurr E, Kramnik I. Genetic control of host susceptibility to tuberculosis. In: Kaufmann S, Britten W, editors. Handbook of tuberculosis. Weinheim, Germany: Wiley-VCH; 2008.
[83] Lecoeur HF, Lagrange PH, Truffot-Pernot C, Gheorghiu M, Grosset J. Relapses after stopping chemotherapy for experimental tuberculosis in genetically resistant and susceptible strains of mice. Clin Exp Immunol 1989;76:458–62.
[84] Radaeva TV, Nikonenko BV, Mischenko VV, Averbakh MM Jr, Apt AS. Direct comparison of low-dose and Cornell-like models of chronic and reactivation tuberculosis in genetically susceptible I/St and resistant B6 mice. Tuberculosis (Edinb) 2005;85:65–72.
[85] Radaeva TV, Kondratieva EV, Sosunov VV, Majorov KB, Apt A. A human-like TB in genetically susceptible mice followed by the true dormancy in a Cornell-like model. Tuberculosis (Edinb) 2008;88:576–85.
[86] Russell DG. Mycobacterium tuberculosis and the intimate discourse of a chronic infection. Immunol Rev 2011;240:252–68.
[87] Kondratieva T, Rubakova E, Kana BD, Biketov S, Potapov V, Kaprelyants A, Apt A. Mycobacterium tuberculosis attenuated by multiple deletions of rpf genes effectively protects mice against TB infection. Tuberculosis (Edinb) 2011;91:219–23.
[88] Ibrahim M, Truffot-Pernot C, Andries K, Jarlier V, Veziris N. Sterilizing activity of R207910 (TMC207)-containing regimens in the murine model of tuberculosis. Am J Respir Crit Care Med 2009;180:553–7.
[89] Nuermberger E, Rosenthal I, Tyagi S, Williams KN, Almeida D, Peloquin CA, Bishai WR, Grosset JH. Combination chemotherapy with the nitroimidazopyran PA-824 and first-line drugs in a murine model of tuberculosis. Antimicrob Agents Chemother 2006;50:2621–5.
[90] Nikonenko BV, Apt AS. Drug testing in mouse models of tuberculosis and nontuberculous mycobacterial infections. Tuberculosis (Edinb) 2013;93:285–90.
[91] Driver ER, Ryan GJ, Hoff DR, Irwin SM, Basaraba RJ, Kramnik I, Lenaerts AJ. Evaluation of a mouse model of necrotic granuloma formation using C3HeB/FeJ mice for testing of drugs against Mycobacterium tuberculosis. Antimicrob Agents Chemother 2012;56:3181–95.
[92] Tasneen R, Tyagi S, Williams K, Grosset J, Nuermberger E. Enhanced bactericidal activity of rifampin and/or pyrazinamide when combined with PA-824 in a murine model of tuberculosis. Antimicrob Agents Chemother 2008;52:3664–8.

Please cite this article in press as: Kondratieva T, et al., Latent tuberculosis infection: What we know about its genetic control?, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.06.009

[93] Buccheri S, Reljic R, Caccamo N, Meraviglia S, Ivanyi J, Salerno A, Dieli F. Prevention of the post-chemotherapy relapse of tuberculous infection by combined immunotherapy. Tuberculosis (Edinb) 2009;89:91–4.
[94] Sacksteder KA, Protopopova M, Barry CE 3rd, Andries K, Nacy CA. Discovery and development of SQ109: a new antitubercular drug with a novel mechanism of action. Future Microbiol 2012;7:823–37.
[95] Williams K, Minkowski A, Amoabeng O, Peloquin CA, Taylor D, Andries K, Wallis RS, Mdluli KE, Nuermberger EL. Sterilizing activities of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis. Antimicrob Agents Chemother 2012;56:3114–20.

Please cite this article in press as: Kondratieva T, et al., Latent tuberculosis infection: What we know about its genetic control?, Tuberculosis (2014), http://dx.doi.org/10.1016/j.tube.2014.06.009