Review article

Challenging *Mycobacterium tuberculosis* dormancy mechanisms and their immunodiagnostic potential

Alexandre Silva Chaves, Michele Fernandes Rodrigues, Ana Márcia Menezes Mattos, Henrique Couto Teixeira*

Department of Parasitology, Microbiology and Immunology, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil

**Article Info**

Article history:
Received 15 June 2015
Accepted 17 August 2015
Available online 8 September 2015

Keywords:
Tuberculosis
Latency
DosR antigens
Diagnosis

**Abstract**

*Mycobacterium tuberculosis* is the etiologic agent of tuberculosis, one of the world's greatest causes of morbidity and mortality due to infectious disease. Many evolutionary mechanisms have contributed to its high level of adaptation as a host pathogen. Prior to become dormant, a group of about 50 genes related to metabolic changes are transcribed by the DosR regulon, one of the most complex and important systems of host-pathogen interaction. This genetic mechanism allows the mycobacteria to persist during long time periods, establishing the so-called latent infection. Even in the presence of a competent immune response, the host cannot eliminate the pathogen, only managing to keep it surrounded by an unfavorable microenvironment for its growth. However, conditions such as immunosuppression may reestablish optimal conditions for bacterial growth, culminating in the onset of active disease. The interactions between the pathogen and its host are still not completely elucidated. Nonetheless, many studies are being carried out in order to clarify this complex relationship, thus creating new possibilities for patient approach and laboratory screening.

© 2015 Elsevier Editora Ltda. All rights reserved.

**Introduction**

*Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB), an infectious disease that remains a major global health issue. Each year millions of people are accounted for infection, ranking TB as the second leading cause of death among infectious diseases, after the human immunodeficiency virus (HIV) infection. In 2013, nine million new cases of TB were notified leading to 1.5 million deaths despite the availability of treatment.

One of the hallmarks of *M. tuberculosis* is the ability to establish a latent infection, capable of long persistence in the host, even in the presence of a functional immune system. This persistent subclinical infection is driven by a low number of bacilli, which are kept in check by the host's immune response.
system, a situation thought to exist in approximately one-third of the world’s population. Of those individuals, 5–10% may develop clinical manifestations of active disease at some point in their lifetime. Most TB cases occur within 2–5 years of the initial infection, especially in young children. Malnutrition, tobacco smoke, indoor air pollution, alcoholism, silicosis, insulin dependent diabetes, renal failure, malignancy, HIV infection, and immune suppressive treatment, are considered risk factors for reactivation.

An important component of a public health response against TB in high-income countries is the investigation of contacts of known TB patients. In contrast, in low- and middle-income countries this policy is still being implemented. In terms of prevalence, 29.1% of the contacts in high-income countries are latently infected, while 1.4% have active disease. In low- and middle-income realities, 51.5% of the contacts have latent TB infection (LTBI), with 3.1% of contacts developing overt disease. Interestingly, in these settings, reactivation rates of up to 40% are observed in children under 2 years old, predominantly within the first year after infection. Healthcare workers are another significant risk group especially in low- and middle-income countries. The prevalence of LTBI among healthcare workers is, on average, 54%, emphasizing the importance of TB control in hospitals. However, there is no agreement on the most common place for LTBI transmission, in addition, there is no gold standard for its diagnosis, which generates inaccuracy and misinterpretations depending on the methodology chosen.

Currently in use, but far from gold standard

There are two main immunological approaches for screening M. tuberculosis latently infected individuals: the tuberculin skin test (TST), which is an in vivo assay, and the interferon-γ release assays (IGRAs), which comprises two ex vivo methods. For the execution of the TST, a determined amount of purified protein derivative (PPD) is injected via the intradermal route into the forearm of the patient. The PPD is a protein extract from the culture supernatants of M. tuberculosis. If the reaction is positive, there is an indication of infection. Regarding the IGRAs, there are two distinct tools, an enzyme-linked immunosorbent assay (ELISA)-based technique, and an enzyme-linked immunospot (ELISpot)-based method. Their most popular commercial versions are QuantIFERON®-TB Gold In-Tube (QFN-GIT; Cellestis Ltd, Carnegie, Victoria, Australia); and T-SPOT®TB (Oxford Immunotec Ltd, Memphis, Tennessee, USA), respectively. In a general sense, both enzyme-linked assays verify the immunological memory of the host. While the ELISA-based test determines the concentration of IFN-γ in supernatants of M. tuberculosis antigen-stimulated cell cultures, the ELISpot-based assay allows a quantitative assessment of IFN-γ-secreting cells in response to M. tuberculosis-specific antigens.

The sensitivity of the TST, which is the most widespread test, is extremely dependent on the patient’s immunological status, being lower in immunocompromised patients. There is growing evidence that the sensitivity of the IGRAs are higher than that of the TST; however, the lack of studies carried out in immunocompromised subjects and the impossibility of a clear differentiation between active disease and LTBI are major setbacks. As another important consideration, the sensitivity of TST is also dependent on the choice of the cut-off value, thus, entangling with the specificity, which is affected by the cross-reactivity between M. tuberculosis antigens and environmental mycobacteria. Various groups have performed reviews and meta-analysis of sensitivity and specificity values to help clinicians and agencies to develop guidelines toward the diagnosis of LTBI. According to Pai et al., the sensitivity of IGRAs scored a value of 70% for QFN-GIT and 90% for T-SPOT.TB, while TST reached a mark of 77%. Diesl et al. verified that IGRAs were more reliable for the identification of non-infected individuals, as compared to TST. The specificity for QFN-GIT, T-SPOT.TB, and TST were 99.4%, 98%, and 88.7%, respectively. However, in high-incidence settings, a positive IGRA may not necessarily indicate TB, as much as a negative IGRA or negative TST cannot rule out the possibility of active disease.

In high-income countries, in which low TB rates are found, LTBI screening is recommended in the subjects at increased risk of developing the disease as a matter of public health. However the lack of clear cut-off values for serial testing and unclear interpretation make useful tools like the IGRAs still hard to be implemented in all realities. Besides, the exact proportion of positive tests for LTBI and the number of patients that still have not received preventive chemotherapy is still unknown. Public health policies and clinical management for the identification and treatment of latently infected subjects would be improved with a better understanding of the nature of LTBI.

The key mechanism for latent infection

For survival and persistence in extreme conditions, M. tuberculosis must be able to sense environmental signals and utilize them to trigger its adaptation machinery, then making its endurance possible in a new ambient. Low oxygen tension, oxidative stress, and NO are factors that are frequently associated with the establishment and maintenance of LTBI. In this context, the transcription factor Rv3133c, named dormancy survival regulator (DosR), directly coordinates the expression of approximately 50 genes, which altogether make up the regulon DosR, preparing the metabolic changes that will allow the mycobacteria to enter dormancy.

Under aerobic conditions, the transcription factor PhoP (Rv0757) is responsible for the maintenance of basal levels of Rv3133c. When hypoxia is set, Rv3133c may have more five-fold increase compared to its original values, enabling the entire regulon to be induced. Remarkably, the first cellular changes can occur under low concentrations of Rv3133c. So, the first genes to be transcribed are those related to protein stability and homeostatic regulation, such as hspx (ru2031) and ru1738, respectively, preparing the cell for further metabolic changes.

Though strictly aerobic, M. tuberculosis may face low oxygen levels during the course of infection, typically in late granulomas, which are characteristically avascular, inflammatory, and necrotic. Those conditions have been demonstrated as hypoxic. In vivo models that mimic hypoxic conditions
have shown that the more oxygen is consumed, the more mycobacterial growth decreases, until it eventually ceases.\(^{27}\) A decrease in both in vitro and in vivo oxygen tension leads to a state of non-replication, which causes the latent infection as a mechanism of adaptation.\(^{28}\) Given the necessary conditions for the dormancy state induction, the DosR regulon is activated through the action of two histidine kinase sensors, DosT (Rv2027c) and DosS (Rv3132c). Both of them are capable of associations with divalent gases such as NO, CO, and O\(_2\), the regulators of their activity.\(^{29}\)

The absence of aerobic respiration renders few metabolic options to the bacilli. Thus, the induction of the DosR regulon must be triggered prior to complete hypoxia, allowing adaptation and survival mechanisms to be transcribed and translated right before ordinary energy sources are depleted.\(^{30}\) Despite its importance, the role DosR regulon plays reaches its apex during the preparation for the dormancy state, changing energy sources and reducing energy expenditure.\(^{31}\) When dormancy is fully set, another group of genes called *enduring hypoxic response* (EHR) takes over until the conditions for optimal growth are once more resumed.\(^{32}\)

To better comprehend the host-pathogen relationship during dormancy, various research groups have been carrying out experiments to unravel the mechanisms by which *M. tuberculosis* survives the environmental changes elicited by the host’s immune system. Bold et al.\(^ {33}\) demonstrated that the first changes in gene expression during the chronic phase would be sufficient to decrease the frequencies of protective CD\(^4\) IFN-\(\gamma\) T cells in the lungs of C57Bl/6 mice. As a result of the down regulation of genes expressed during the active growth phase followed by the up regulation of those expressed during dormancy, the antigen presenting cells are not able to perform a proper activation of CD\(^4\) T cells. Thus, there is not sufficient production of IFN-\(\gamma\) to control the infection.\(^ {34}\) Besides, as stated by Mehra et al.,\(^ {35}\) the activation of the DosR prevents the immune system to establish a proper T helper 1-type of response against the pathogen.

However, one cannot associate the establishment of dormancy with a state in which *M. tuberculosis* would be fully protected from the immune system in all situations. Actually, as demonstrated by Mariotti et al.,\(^ {36}\) dormant mycobacteria lack the capacity to subvert the host immune system due to its inability to block the phagosome maturation. Such findings could be associated with persistent antigen presentation to CD\(^4\) T cells by dendritic cells. However, it may be considered unexpected that dormant mycobacteria is recognized by T lymphocytes with higher efficiency than active growing mycobacteria. It is unlikely that dormant mycobacteria could infect tissue macrophages during latency, as they do not replicate and cannot invade host cells unless they reactivate to sense the environment.\(^ {30}\) Another mechanism for host protection against *M. tuberculosis* infection was recently described by Venkatasubramanian et al.\(^ {37}\) A particular subset of regulatory T cells, named CD\(^4\) CD25\(^+\) FoxP\(^3\) D4GD1\(^+\) T cells, was found to contribute in the contention of *M. tuberculosis* in both human and murine models. These cells do not produce immunomodulatory cytokines, such as TGF-\(\beta\) or IL-10, secreting Rho GDP dissociation inhibitor (D4GD1) instead. This factor acts on macrophages, enhancing the production of IL-1\(\beta\), TNF-\(\alpha\), and reactive oxygen species, indirectly contributing, to DosR regulon’s activation, as a mechanism of mycobacterial growth arrest.\(^ {37}\)

Recent works like the one carried out by Ryndak et al.,\(^ {38}\) have shed some light toward the niches where the mycobacteria actually actively grows. Remarkably, it was verified that alveolar epithelial cells are interesting sites for mycobacterial active growth, prior to macrophage infection.\(^ {38}\) However, the physical location during LTBI is poorly understood. It is conceivable that dormant bacteria reside in cells which have a scarce ability to act as antigen presentation cells, and subverted macrophages in mature granulomas.\(^ {39}\) These structures physically separate T lymphocytes from infected cells by a fibrous wall of caseum,\(^ {40}\) limiting their interactions, creating a reservoir for *M. tuberculosis*.\(^ {41}\)

### DosR antigens and diagnosis

Diagnosing TB continues to be a challenge, especially in low-income countries with high prevalence of the disease.\(^ {1}\) Classic methods such as sputum smears and cultures are relatively cheap and widespread; however, their major drawbacks are low sensitivity for direct sputum analysis and the long time required for the culture.\(^ {42}\) Molecular biology is also an alternative. Despite the rapid diagnosis afforded by this technique, its relatively high cost and moderate sensitivity are factors that make it unsuitable for all realities.\(^ {43}\) Thus, the search for alternative approaches implementing new markers and antigens capable of a better differentiation between LTBI and overt disease have become the subjects of many research groups.\(^ {1,5,42}\)

Among the most important and well-studied DosR regulon-encoded genes, the gene that codifies for the protein Rv2031c, also known as the 16 kDa antigen, whose levels elevate as dormancy sets in, and is kept at low amounts during mycobacterial exponential growth.\(^ {39,44}\) This antigen, as demonstrated by Demissie et al.,\(^ {45}\) elicited a higher IFN-\(\gamma\) response by T cells from asymptomatic subjects with history of previous infection by *M. tuberculosis* in both Ethiopian and Gambian populations. The opposite happened toward the recognition of the active phase antigen ESAT-6, which was better suited for people with active disease or people who had been in recent contact with tuberculous patients.\(^ {45}\) In agreement, Goletti et al.\(^ {46}\) observed a correlation between LTBI and higher IFN-\(\gamma\) responses elicited by DosR-encoded antigens. The following antigens were tested via ELISA: Rv2626c, Rv2627c, Rv2628, Rv2031c and Rv2032. However, only Rv2628 elicited a high and significant response for both latently infected and recently cured TB groups. The ability of Rv2628 for predicting LTBI had a specificity of 87.5%, and sensitivity of 76%. As internal control, QFN-GIT did not detect a response capable of discriminating different stages of the disease. In another study, IFN-\(\gamma\) response against mycobacterial dormancy antigens was higher in those subjects who tested positive for TST, while the response against the active phase antigen CFP-10 was significantly greater in people with active disease. The dormancy antigens Rv1733c, Rv2029c, Rv2627c, and Rv2628 were the ones that elicited the highest IFN-\(\gamma\) responses in latently infected subjects.\(^ {47}\)

A remarkable fact concerning the dormancy related antigens is that they do not show any boost effect related to
M. bovis BCG vaccination. It was well demonstrated by Lin et al. in both animal and human models for the following antigens: Rv1733c, Rv1738, Rv2029c, Rv2031c, Rv2032c, Rv2627c, and Rv2628. The main responsive cellular type to the dormancy antigens in terms of IFN-γ production, is the CD4+ CD45RO+ T cells, which belongs to the T cell memory subtype. The same is also valid for the TST, characterizing a phenomenon found in individuals infected by mycobacteria, but not in healthy subjects. Among the best DosR encoded antigens recognized by T cells, the Rv1733c, Rv2029c, and Rv2031c are the most expressive concerning CD4+, and CD8+ polyfunctional T cells in subjects exposed to M. tuberculosis. Both cellular types are phenotypically different from those generated by M. bovis BCG vaccination, which are CD27+, while those found in latently infected individuals do not express this marker.

Caccamo et al. verified that the frequencies of polyfunctional CD4+ IFN-γ− IL-2− TNF-α+ T cells were significantly higher in patients with active disease, decreasing as chemotherapy progresses. The post-chemotherapy phenotypic pattern consisted mainly of CD4+ IFN-γ− IL-2− and in minor proportion CD4+ IFN-γ− T cells, which was the pattern found in latently infected patients. According to Seder et al., the initial antigenic load dictates the phenotypic pattern of the lymphocytes, so it is expected that the new pattern will be closely related to the formation of memory cells. This is also in accordance with the pattern of cytokines found after chemotherapy.

An interesting approach toward dormancy antigens is their association with humoral immunity. Thus, the serological detection of antibodies against Rv2031, for example, would provide potential new markers. Raja et al. observed that IgA, IgG, and IgM antibodies against Rv2031c were detected in serum of culture-positive and both smear-positive and smear-negative TB patients, indicating that it could be used as a screening strategy. Interestingly, the combination of IgA, IgG and IgM, generated sensitivities and specificities of 83% and 93%, respectively, allowing the differentiation between active pulmonary disease and healthy subjects. In another study, Brazilian healthcare workers recently exposed to M. tuberculosis, showed higher anti-Rv2031c IgM titers than those with previous infection, or uninfected subjects, suggesting that Rv2031c would be a suitable marker for recent infection. Moreover, studies from our laboratory have shown a relation between successful treatment of pulmonary TB and serum IgG1 levels against Rv2031c, ESAT-6 and CFP-10 antigens. During the first three months of treatment, a significant increase in IgG1 antibody titers against Rv2031c was noticed. However, by the sixth month of treatment, IgG1 levels dropped to titers comparable to the ones elicited by the healthy controls, as indicative of successful chemotherapy. Concerning the levels of IgG1 against ESAT-6 and CFP-10, they were already high prior to chemotherapy, but as it progressed, they also dropped to control levels. Apparently, this increase in antibody levels is a consequence of the release of antigens from cytosol during the lysis of the mycobacteria, thus increasing the titers of specific antibodies after the first term of treatment. Such findings are also in agreement with a recent study published by Belay et al., in which the levels of IFN-γ, TNF-α, and IL-10 in response to Rv2031c stimulation were significantly higher in patients with LTBI after the beginning of the prophylactic chemotherapy.

What should be taken into account?

One of the most important features of M. tuberculosis for its success as a pathogen is its high level of adaptation to the host. The consensus is that the bacteria, when in a state of latent infection, stops growing and enters a stationary phase due to the granuloma walling off. Eventually, when favorable conditions are re-established in the surrounding environment, the mycobacteria resumes its normal growth. Despite the important role played by the granulomas during this process, the exact localization of the dormant mycobacteria is still not well known. Garton et al. showed that high numbers of mycobacteria sharing typical dormancy patterns can be found in the sputum of patients with active disease. Thus, what conventionally is known as latent infection LTBI or dormant state is in fact, part of a greater spectrum in which different levels of replications are found.

As verified by Arlehamn et al. in a study carried out with latently infected subjects, the same T cell memory phenotype described by Schuck et al. was also accounted for the majority of responsive T cells for both CFP-10 and Rv2031c antigens. The phenotype in question is composed by CD4+ CD45RA− CCR7− effector memory T cells. Moreover, it is unknown whether or not there is a certain degree of replication in latently infected patients in a way that the memory effector lymphocyte subtype is kept. However, this is the major subtype partaking in the immune response against M. tuberculosis. Still, further studies are necessary in order to determine if this pattern of response is due to persistence of certain antigens, an undetected bacterial replication, or to longevity of the immune response even in the absence of an antigenic stimulation.

The different responses of peripheral blood cells and the ones at the site of infection is a matter of great importance. As stated by Chiaccio et al., there is a higher frequency of monofunctional IFN-γ-secreting effector memory T cells specific to RD1 (active phase genomic locus) antigens and to the DosR-encoded antigen Rv2628 in the bronchoalveolar lavage of patients with active TB than in their peripheral blood. Taking this into consideration, the approach toward latently infected patients possesses an interesting feature. While there was a predominant Rv2628 response in the peripheral blood, there were not any differences between both RD1 and DosR antigens in the bronchoalveolar lavage. So, one can assume that the response to a DosR antigen in patients with active TB, and antigens related to active disease in latently infected patients could be an indicator of the continuum spanned by the life cycle of the mycobacteria.

Another important point to be explored is the possibility of cross-reaction between dormancy antigens and those originated from environmental non-tuberculous mycobacteria, which are facultative intracellular bacteria that live in specific niches in the environment. Apparently, 41 of the 50 antigens codified by the DosR regulon have homologues in different species of environmental mycobacteria, and in some common environmental bacteria. Lin et al. carried out an
experiment testing subjects infected with *Mycobacterium marinum* or *Mycobacterium kansasi* on the responsiveness of their peripheral blood cells against stimulus with DosR antigens. The results indicated that the antigens Rv1733c and Rv2627 were highly cross-reactive antigens, but despite the great level of homology of the antigens Rv2029c, Rv2626c, and Rv2031 in environmental mycobacteria, virtually no cross-reactions were observed.

Apart from the points previously discussed, two key aspects should be taken into account toward the use of these antigens as diagnostic tools: the genotype of the *M. tuberculosis* strain and the ethnicity of the population to be tested. To exemplify this topic, Riaño et al. observed that latently infected subjects from Colombia produced higher IFN-γ levels in response to the following DosR antigens: Rv1737c, Rv2029c, and Rv2628. However, the antigens Rv1733c, Rv1735c, and Rv2006 have not elicited a significant IFN-γ response. In contrast, Rv1733c, Rv1735c, and Rv2006 were ranked among the best recognized antigens in terms of IFN-γ response by latently infected populations from South Africa, Ghana, and Uganda.

### Concluding remarks

In this review article we briefly discussed some mechanisms by which *M. tuberculosis* is able to keep itself at a low metabolic profile, allowing it to survive in an aggressive environment as is frequently found in its host. Such mechanisms work under fine genetic control, and its products may affect bacterial structure and metabolism leading to escape the host's immune system. As previously observed, DosR regulon-encoded antigens may be used as a powerful factor to improve the current understanding about the relationship between the host immune response and the establishment of LTBI. However, the main point to be taken into consideration is that latent infection is actually part of a greater spectrum in which the life cycle of *M. tuberculosis* is inserted. Thus, this must be taken into consideration when analyzing the overall immune response against the pathogen as well as in research for new diagnostic tools for tuberculosis control.

### Conflicts of interest

The authors declare no conflicts of interest.

### Acknowledgements

The authors gratefully acknowledge the Brazilian funding agencies CNPq, FAPEMIG and CAPES. A.S.C. was given a master fellowship from CAPES, Brazil.

### References

1. World Health Organization, WHO. Global Tuberculosis Report 2014. Geneva, Switzerland: WHO; 2014. http://www.who.int/tb/publications/global_report/gtbr14_main_text.pdf [accessed 20.03.15].

2. Baena A, Porcelli SA. Evasion and subversion of antigen presentation by *Mycobacterium tuberculosis*. Tissue Antigens. 2009;74:189–204.

3. Tufariello JM, Chan J, Flynn JL. Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. Lancet Infect Dis. 2003;3:578–90.

4. Koul A, Arnould E, Lounis N, Guillemon J, Andries K. The challenge of new drug discovery for tuberculosis. Nature. 2011;469:483–90.

5. Lin PL, Flynn JL. Understanding latent tuberculosis: a moving target. J Immunol. 2010;185:15–22.

6. Kizza FN, List J, Nkwata AK, et al. Prevalence of latent tuberculosis infection and associated risk factors in an urban African setting. BMC Infect Dis. 2015;15:165.

7. Fox G, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2013;41:140–56.

8. Dheda K, van Zyl Smit R, Badri M, Pai M. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. Curr Opin Pulm Med. 2009;15:188–200.

9. Joshi R, Reingold AL, Menzies D, Pai M. Tuberculosis among health-care workers in low- and middle-income countries: a systematic review. PLoS Med. 2006;3:e494.

10. Legesse M, Ameni G, Mamo G, Medhin G, Bjune G, Abebe F. Community-based cross-sectional survey of latent tuberculosis infection in Afar pastoralists, Ethiopia, using QuantiFERON-TB Gold In-Tube and tuberculin skin test. BMC Infect Dis. 2011;11:89.

11. Lalvani A, Millington KA. T cell-based diagnosis of childhood tuberculosis infection. Curr Opin Infect Dis. 2007;20:264–71.

12. Brock I, Munk ME, Kok-Jensen A, Andersen P. Performance of whole blood IFN-gamma test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. Int J Tuberc Lung Dis. 2001;5:462–7.

13. Lalvani A, Pareek M. Interferon gamma release assays: principles and practice. Enferm Infecc Microbiol Clin. 2010;28:245–52.

14. Redelman-Sidi G, Sepkowitz KA. IFN-gamma release assays in the diagnosis of latent tuberculosis infection among immunocompromised adults. Am J Respir Crit Care Med. 2013;188:422–31.

15. Sester M, Sester U, Clauer P, et al. Tuberculin skin testing underestimates a high prevalence of latent tuberculosis infection in hemodialysis patients. Kidney Int. 2004;65:1826–34.

16. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med. 2008;149:177–84.

17. Diel R, Goletti D, Ferrara G, et al. Interferon-gamma release assays for the diagnosis of latent *Mycobacterium tuberculosis* infection: a systematic review and meta-analysis. Eur Respir J. 2011;37:88–99.

18. Pai M, Banaei N. Occupational screening of health care workers for tuberculosis infection: tuberculin skin testing or interferon-gamma release assays? Occup Med (Lond). 2013;63:458–60.

19. Chao JD, Papaviniasusundaram KG, Zheng X, et al. Convergence of Ser/Thr and two-component signaling to coordinate expression of the dormancy regulon in *Mycobacterium tuberculosis*. J Biol Chem. 2010;285:29239–46.

20. Wayne LG, Sohaskey CD. Nonreplicating persistence of *Mycobacterium tuberculosis*. Annu Rev Microbiol. 2001;55:139–63.

21. Kumar A, Deshane JS, Crossman DK, et al. Heme oxygenase-1-derived carbon monoxide induces the
22. Taneyea NK, Dhingra S, Mittal A, Naresh M, Tyagi JS. Mycobacterium tuberculosis transcriptional adaptation, growth arrest and dormancy phenotype development is triggered by vitamin C. PLoS ONE. 2010;5:e10860.

23. Bartek IL, Rutherford R, Grppo V, et al. The DosR regulon of M. tuberculosis and antibacterial tolerance. Tuberculosis (Edinb). 2009;89:310–6.

24. Majumdar SD, Vashist A, Dhingra S, et al. Appropriate DevR (DosR)-mediated signaling determines transcriptional response, hypoxic viability and virulence of Mycobacterium tuberculosis. PLoS ONE. 2012;7:e35847.

25. Chauhan S, Sharma D, Singh A, Surlalia A, Tyagi JS. Comprehensive insights into Mycobacterium tuberculosis DevR (DosR) regulon activation switch. Nucleic Acids Res. 2011;39:7400–14.

26. Via LE, Lin PL, Ray SM, et al. Tuberculous granulomas are hypoxic in guinea pigs, rabbits, and nonhuman primates. Infect Immun. 2008;76:2333–40.

27. Wayne LG, Hayes LG. An in vitro model for sequential study of shiftdown of Mycobacterium tuberculosis through two stages of nonreplicating persistence. Infect Immun. 1996;64:2062–9.

28. Sohaskey CD. Nitrate enhances the survival of Mycobacterium tuberculosis during inhibition of respiration. J Bacteriol. 2008;190:2981–6.

29. Cho HY, Cho HJ, Kim YM, Oh JI, Kang BS. Structural Insight into the Heme-based Redox Sensing by DosS from Mycobacterium tuberculosis. J Biol Chem. 2009;284:13057–67.

30. 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.

31. Park H-D, Guinn KM, Harrell MJ, et al. Rv3135c/DosS is a transcriptional inhibitor of the hypoxic response of Mycobacterium tuberculosis. Mol Microbiol. 2003;48:833–43.

32. Rustad TR, Harrell MJ, Liao R, Sherman DR. The enduring hypoxic response of Mycobacterium tuberculosis. PLoS ONE. 2008;3:e1502.

33. Bold TD, Banaei N, Wolf AJ, Ernst JD. Suboptimal activation of antigen-specific CD4+ effector cells enables persistence of M. tuberculosis in vivo. PLoS Pathog. 2011;7:e1002063.

34. Jung YJ, LaCourse R, Ryan L, North RJ. Virulent but not avirulent Mycobacterium tuberculosis can evade the growth inhibitory action of a T helper 1-dependent, nitric oxide Synthase 2-independent defense in mice. J Exp Med. 2002;196:991–8.

35. Mehra S, Foreman TW, Didier PJ, et al. The DosR regulon modulates adaptive immunity and is essential for Mycobacterium tuberculosis persistence. Am J Respir Crit Care Med. 2015;191:1185–96.

36. Mariotti S, Pardini M, Gagliardi MC, et al. Dormant Mycobacterium tuberculosis fails to block phagosome maturation and shows unexpected capacity to stimulate specific human T lymphocytes. J Immunol. 2013;191:274–82.

37. Venkatasubramanian S, Dhiman R, Paidipally P, et al. A rho GDP dissociation inhibitor produced by apoptotic T-cells inhibits growth of Mycobacterium tuberculosis. PLoS Pathog. 2015;11:e1004617.

38. Ryndak MB, Singh KK, Peng Z, Laal S. Transcriptional profile of Mycobacterium tuberculosis replicating in type II alveolar epithelial cells. PLoS ONE. 2015;10:e0123745.

39. 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.

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

41. Gideon HP, Flynn JL. Latent tuberculosis: what is the host sees? Immunol Res. 2011;50:202–12.

42. Chegou NN, Hoek KG, Kriel M, Warren RM, Victor TC, Walzl G. Tuberculosis assays: past, present and future. Expert Rev Anti Infect Ther. 2011;9:457–69.

43. Trebuca A, Enarson DA, Chiang CY, et al. Xpert(R) MTB/RIF for national tuberculosis programmes in low-income countries: when, where and how? Int J Tuberc Lung Dis. 2011;15:1567–72.

44. Hu Y, Coates AR. Transcription of the stationary-phase-associated hspX gene of Mycobacterium tuberculosis is inversely related to synthesis of the 16-kidalton protein. J Bacteriol. 1999;181:1380–7.

45. Demissie A, Leyten EM, Abebe M, et al. Recognition of stage-specific mycobacterial antigens differentiates between acute and latent infections with Mycobacterium tuberculosis. Clin Vaccine Immunol. 2006;13:179–86.

46. Goletti D, Butera O, Vanini V, et al. Response to Rv2628 latency antigen associates with cured tuberculosis and remote infection. Eur Respir J. 2010;36:135–42.

47. Leyten EM, Lin MY, Franken KL, et al. Human T-cell responses to 25 novel antigens encoded by genes of the dormancy regulon of Mycobacterium tuberculosis. Microbes Infect. 2006;8:2052–60.

48. Lin MY, Geluk A, Smith SG, et al. Lack of immune responses to Mycobacterium tuberculosis DosR regulon proteins following Mycobacterium bovis BCG vaccination. Infect Immun. 2007;75:3523–30.

49. Schuck SD, Mueller H, Kunitz F, et al. Identification of T-cell antigens specific for latent Mycobacterium tuberculosis infection. PLoS ONE. 2009;4:e5590.

50. Commandeur S, Lin MY, van Meijgaarden KE, et al. Double- and monofunctional CD4(+) and CD8(-) T cells respond to Mycobacterium tuberculosis DosR antigens and peptides in long-term latently infected individuals. Eur J Immunol. 2011;41:2925–36.

51. Adekambi T, Ibegbu CC, Kalokhe AS, Yu T, Ray SM, Renganarajan A. Distinct effector memory CD4+ T cell signatures in latent Mycobacterium tuberculosis infection, BCG vaccination and clinically resolved tuberculosis. PLoS ONE. 2012;7:e36046.

52. Caccamo N, Guggino G, Joosten SA, et al. Multifunctional CD4(+) T cells correlate with active Mycobacterium tuberculosis infection. Eur J Immunol. 2010;40:2211–20.

53. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol. 2008;8:247–58.

54. Chowdhury IH, Ahmed AM, Choudhuri S, et al. Alteration of serum inflammatory cytokines in active pulmonary tuberculosis following anti-tuberculosis drug therapy. Mol Immunol. 2014;62:159–68.

55. Raja A, Uma Devi KR, Ramalingam B, Brennan PJ, Immunoglobulin G, A, and M responses in serum and circulating immune complexes elicited by the 16-kidalton antigen of Mycobacterium tuberculosis. Clin Diag Lab Immunol. 2002;9:308–12.

56. Rabahi MF, Junqueira-Kipnis AP, Dos Reis MC, Oelemann W, Conde MB. Humoral response to HspX and HspC8 to previous and recent infection by Mycobacterium tuberculosis. BMC Infect Dis. 2011;11:50:202–12.

57. Mattos AM, Almeida CdeS, Franken KL, et al. Increased IgG1, IFN-gamma, TNF-alpha and IL-6 responses to Mycobacterium tuberculosis antigens in patients with tuberculosis are lower after chemotherapy. Int Immunol. 2010;22:775–82.

58. de Souza FM, do Prado TN, Pinheiro JdosS, et al. Comparison of interferon-gamma release assay to two cut-off points of tuberculin skin test to detect latent Mycobacterium tuberculosis infection in primary health care workers. PLoS ONE. 2014;9:e102773.
59. Belay M, Legesse M, Mihret A, et al. Pro- and anti-inflammatory cytokines against Rv2031 are elevated during latent tuberculosis: a study in cohorts of tuberculosis patients, household contacts and community controls in an endemic setting. PLoS ONE. 2015;10:e0124134.

60. Caws M, Thwaites G, Dunstan S, et al. The influence of host and bacterial genotype on the development of disseminated disease with Mycobacterium tuberculosis. PLoS Pathog. 2008;4:e1000034.

61. Garton NJ, Waddell SJ, Sherratt AL, et al. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. PLoS Med. 2008;5:e75.

62. Lin PL, Rodgers M, Smith L, et al. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. Infect Immun. 2009;77:4631–42.

63. Arlehamn CS, Sidney J, Henderson R, et al. Dissecting mechanisms of immunodominance to the common tuberculosis antigens ESAT-6, CFP10, Rv2031c (hspX), Rv2654c (TB7.7), and Rv1038c (EsxJ). J Immunol. 2012;188:5020–31.

64. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol. 2004;22:745–63.

65. Chiacchio T, Petruccioli F, Vanini V, et al. Higher frequency of T-cell response to M. tuberculosis latency antigen Rv2628 at the site of active tuberculosis disease than in peripheral blood. PLoS ONE. 2011;6:e27539.

66. Arend SM, van Soolingen D, Ottenhoff TH. Diagnosis and treatment of lung infection with nontuberculous mycobacteria. Curr Opin Pulm Med. 2009;15:201–8.

67. Lin MY, Reddy TBK, Arend SM, et al. Cross-reactive immunity to Mycobacterium tuberculosis DosR regulon-encoded antigens in individuals infecte with environmental, nontuberculous mycobacteria. Infect Immun. 2009;77:9.

68. Riano F, Arroyo L, Paris S, et al. T cell responses to DosR and Rpf proteins in actively and latently infected individuals from Colombia. Tuberculosis (Edinb). 2012;92:148–59.

69. Black GF, Thiel BA, Ota MO, et al. Immunogenicity of novel DosR regulon-encoded candidate antigens of Mycobacterium tuberculosis in three high-burden populations in Africa. Clin Vaccine Immunol. 2009;16:1203–12.