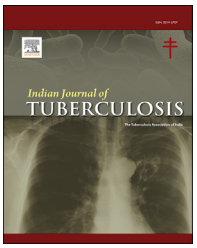
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review article

The roles of latency-associated antigens in tuberculosis vaccines

Arshid Yousefi Avarvand [a], *Farzad Khademi* [b], *Mohsen Tafaghodi* [c], *Zahra Ahmadipour* [d], *Bagher Moradi* [e], *Zahra Meshkat* [d,*]

[a] *Department of Medical Laboratory Sciences, School of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[b] *Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran*
[c] *Nanotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[d] *Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[e] *Esfarayen Faculty of Medical Sciences, Esfarayen, Iran*

ARTICLE INFO

Article history:
Received 23 December 2016
Accepted 26 April 2019
Available online xxx

Keywords:
Latency-associated antigens
Mycobacterium tuberculosis
Vaccine

ABSTRACT

Tuberculosis (TB) is a re-emerging disease and is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). TB is currently one of the leading causes of morbidity and mortality, worldwide. The only available vaccine against TB infection, Bacillus Calmette–Guérin (BCG), fails to adequately protect against reactivation of latent infections in adults. Furthermore, recently developed subunit vaccines, which are in various stages of clinical trials, are all prophylactic vaccines based on proteins expressed in replicating stage of *M. tuberculosis* and they are not preventive of reactivation of latent TB infection. Thus, an appropriate subunit post-exposure vaccine needs to be developed to control all forms of TB infection. To produce a multi-stage subunit vaccine, scientists should combine the early secreted *M. tuberculosis* antigens with latency antigens. For this purpose, some latency proteins are known which could be important antigens in the production of specific humoral and cellular immune responses in latent *M. tuberculosis* infected individuals. Several studies have evaluated the immunogenicity of these proteins in improving the TB vaccines. The present study is a comprehensive review of several studies on the role of the latency antigens in the development of TB vaccines. Overall, the studies indicate that the latency-associated antigens including the resuscitation-promoting factors, the Dormancy of survival regulon (DosR) proteins and the starvation stimulant proteins are potential candidates for the development of subunit vaccines against TB infection.

* *Corresponding author*. Tel: +98 5138012453; Fax: +98 5138409612.
E-mail address: meshkatz@mums.ac.ir (Z. Meshkat).

https://doi.org/10.1016/j.ijtb.2019.04.012
0019-5707/

Please cite this article as: Yousefi Avarvand A et al., The roles of latency-associated antigens in tuberculosis vaccines, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2019.04.012

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), is a re-emerging infectious disease and is one of the leading causes of morbidity and mortality in many countries, especially in developing countries.[1] According to the latest report by the World Health Organization (WHO) in 2018, about 10 million people had TB, which led to1.3 million deaths. This state is further exacerbated due toTB/HIV co-infection, multidrug-resistant (MDR) *M. tuberculosis* strains and the appearance of extremely drug-resistant (XDR) *M. tuberculosis* strains.[2] Currently, vaccination is one of the most effective approaches in prevention and control TB infection. The TB vaccine, Bacillus Calmette–Guérin (BCG), is the only available approved vaccine against TB and has been widely used in many countries for decades.[3] BCG provides highly variable efficacies against adult pulmonary TB infection with a range of 0–80% protection. BCG also fails to adequately protect against reactivation of latent infections in adults. Furthermore, the current vaccine suffers from inability to eliminate latent TB infection, and loss of efficacy over time and in immune compromised patients.[4,5] One of the important differences between *M. tuberculosis* and other pathogens is the survival of bacteria in an intracellular habitat in the human cells for decades and its establishment as a latent infection. About one-third of the global population has latent TB infection and about 5–10% of them will eventually progress active TB infection whenever their immune status suppresses. Patients with latent TB infection are the major reservoir of adult TB infection.[6,7] Most TB vaccines that recently entered different phases of clinical trials were based on proteins expressed in replicating stages. Such TB vaccines can prevent active TB infection (prophylactic vaccine) but not latent TB infection.[6] Thus, a more effective subunit post-exposure vaccine is needed to control all forms of TB infection in adults, especially latent infection. To produce a multi-stage subunit vaccine, researchers should combine early secreted antigens such as Ag85B with *M. tuberculosis* latency antigens such as HspX.[6] Recently, some multi-stage subunit vaccines *i.e.* Hybrid 56 + IC31 and ID93+GLA-SE, containing dormancy antigens, have entered clinical trials.[8,9] Latency-associated antigens have shown to elicit appropriate and strong immune and protective responses.[10] These antigens include: 1) the Dormancy of survival regulon (DosR) proteins which are responsible for adaptation to hypoxia. These constitute the latency expressing proteins; 2) nutrient starvation proteins; and 3) enduring hypoxic response (EHR) genes which comprise many genes of the DosR regulon.[11,12] Recently, scientists have used several *M. tuberculosis* proteins homologous to the resuscitation-promoting factor (rpfa-A-E) of *Micrococcus luteus*. These proteins are involved in resuscitation and reactivation of dormant TB infection and to control reactivated *M. tuberculosis*. These proteins lead to specific humoral and cellular immune responses in latently *M. tuberculosis* infected individuals. Thus, *M. tuberculosis* resuscitation-promoting factors proteins (Rpf), particularly RpfB which memory T-cells able to respond to them can be used as antigens for novel TB subunit vaccines.[13,14] DosR-regulated protein HspX is an important antigen that leads to specific humoral and cellular immune responses in latently *M. tuberculosis* infected individuals.[15] This protein is known to be targeted by $CD4^+$ and $CD8^+$ T cells. On the other hand, several of other latency antigens such as RV2628c, RV1813, RV2660, RV0072, RV1737 and RV2031c have been shown to induce strong Th1-mediated immune responses.[16,17] Some previous studies have suggested that latency-associated antigens in all kinds of TB vaccines can induce strong humoral and/or cellular Th1-mediated immune responses.[16–18] Therefore, it could be hypothesized that a heterologous prime boost vaccine, including a combination of dormant antigens to induce a specific immune response, is more protective than the homologous prime boost vaccine. The present review article focuses on dormancy-related antigens of *M. tuberculosis* as potentially ideal candidates in the development of new vaccine against TB.

2. Resuscitation-promoting factors

Resuscitation-promoting factors (Rpf) are proteins with peptidoglycan-hydrolyzing activity and are vital for mycobacterial virulence, especially for resuscitation from dormancy. These proteins were first reported in *M. luteus* as a secretory protein. Mutant *M. tuberculosis* strains with a deficiency in Rpf show inability in replication, reactivation and in resistance to stress probably due to changes in their cell wall structure.[19] There are five *rpf* genes in the *M. tuberculosis* genome (*rpf*A-E) which are very similar to those of Rpf from *M. luteus*.[14] Rpf proteins are potential targets for the host immune response. Rpf are key virulence factors for *M. tuberculosis* and responses to Rpf could be protective. These have been evaluated in cellular experiments and mouse models.[14] Previous studies have demonstrated that RpfB and RpfE produce protective immune responses *in-vitro* and are potential candidates for the development of vaccine.[20] RpfB and RpfE induce the maturation of dendritic cells (DCs) and these activated DCs polarize T-cell proliferation toward Th1 phenotype. RpfE also induces Th17 development which in addition to Th1 is needed for appropriate protection against *M. tuberculosis*.[20,21] The immune responses induced by Rpf antigens and their protective efficacy against challenge with virulent *M. tuberculosis* have been evaluated in animal models.[22] Lee *et al* demonstrated that when C57BL/6 mice were vaccinated intramuscularly with RpfB using a plasmid DNA vector, it can elicit a significant poly functional $CD8^+$T cell responses in mice, suggesting that RpfB DNA immunization may be protective against TB infection.[21] In another study RpfB and RpfD were reported to be the most antigenic in the tested models. It was also indicated that RpfB protein is the most immunogenic antigen among the five Rpf proteins of *M. tuberculosis*, implying that Rpf proteins can be used as an antigen in new TB vaccines.[14] In conclusion, RpfB and RpfE induce immune responses associated with resistance to *M. tuberculosis* and provide some levels of protection in animal models. Further human-based studies are needed to evaluate if the observation could be replicated in humans and whether Rpf should be considered as

Please cite this article as: Yousefi Avarvand A et al., The roles of latency-associated antigens in tuberculosis vaccines, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2019.04.012

antigens for development of a subunit vaccine against *M. tuberculosis*.[22]

3. Dormancy of survival regulon (DosR)

A region in the genome of *M. tuberculosis* called DosR regulon contains 50 latency-associated genes and is active during latency. Most latency-associated antigens related to DosR regulon including Rv2031c, Rv2029c, Rv2628c, Rv1737c, Rv1733c and Rv0081 were evaluated for their immunogenic characteristics in different studies.[16] This review summarizes the possible role of DosR regulon proteins as effective candidates for development of subunit vaccines against TB infection.

3.1. *Rv2031c (gene name:* hspX*; gene length: 435 bp; 144 amino acids)*

An important antigen from the DosR regulon is a stress protein induced by hypoxia and nutrient scarcity is called heat-shock protein X orα-crystallin ((14 kDa antigen) (HSP16.3)).[23] The expression of HspX protein is regulated by DosR regulon in different environmental conditions.[24] HspX is associated with bacterial growth, escape from the host immune system and prolonged bacterial survival in macrophages during latent infections.[25,26] One of the most crucial immune responses against *M. tuberculosis* infection is activation of T helper1 (Th1) and cytotoxic T (Tc) cells. HspX strongly induces Th1 cytokines such as IFN-γ (interferon gamma) and TNF-α (tumor necrosis alpha) followed by induction of cellular and humoral immune responses in the latent phase of TB infection.[27,28] Several studies have shown the efficacy of HspX protein for the induction of strong Th1-medited immune responses and its potential to be a suitable antigen candidate for vaccination against TB infection. As an example, in a study conducted by Yuan *et al*, a DNA vaccine was constructed expressing a fusion protein of *M. tuberculosis* antigens including Ag85B, Esat6 and HspX. After vaccination of mice with DNA vaccine, a significant increase in antigen-specific IFN-γ against HspX antigen and higher levels of HspX specific T cell proliferation was observed, compared to vaccination with BCG.[29] Geon *et al* evaluated immune responses against Ag85A and HspX antigens in the mice model. Ag85A and HspX increased the level of IFN-γ responses. Also, after mice challenge with *M. tuberculosis*, HspX subunit vaccine induced significant protective immunity.[30] In another study, Yuan *et al* developed a new recombinant BCG expressing high levels of Ag85B and HspX. After vaccination of mice with this vaccine, the immunization of new vaccine compared to BCG. It was observed that the new vaccine was able to protect mice against intranasal infection of *M. tuberculosis* better than that of BCG.[31] Similar results were shown by Xin *et al*, Marongiu *et al*, de Sousa *et al*, Geluk et *al* and Costa *et al* confirmed the immunogenicity of HspX antigen.[9,25,28,32,33] Several studies indicated that HspX protein either in combination with adjuvants or as encapsulated with nanoparticles can strongly induce the immune system. In a study conducted by Khademi *et al*, HspX antigen was shown to efficiently induce mucosal and systemic immune responses in BALB/c mice, when combined with a replicating bacilli antigen, as multi-stage subunit vaccine (HspX/EsxS-fused protein), alone or as encapsulated in PLGA and PLGA:DDA hybrid nanoparticles.[34] Additionally, they showed that adding DOTAP and MPLA adjuvants to HspX/EsxS-fused protein could enhance immune responses after subcutaneous and nasal immunization of BALB/c mice.[34,35] In another study by Mansury *et al*, HspX protein in combination with PPE44 and EsxV proteins were encapsulated into DOTAP liposome. They showed that this formulation was able to induce a strong Th1-mediated response.[36] Amini *et al* reported a multi-component vaccine containing HspX in combination with PPE44 and EsxV antigens adsorbed on calcium phosphate nanoparticles could induce strong cellular immunity in an animal model.[37] Niu *et al* developed a new multi-sage subunit vaccine against TB infection containing ESAT6-Ag85B-MPT64-Mtb8.4-HspX antigens along with two adjuvants, DDA and Poly I:C. They showed that this new vaccine could induce a stronger immunity compared to traditional BCG vaccine.[38]

3.2. *Rv2029c (gene name:* pfkB*; gene length: 1020 bp; 339 amino acids)*

M. tuberculosis pfkB gene encodes a 35 kDa-latency protein with kinase and phosphotransferase activity. This *M. tuberculosis* latency antigen is DosR-regulon-encoded antigen and as a probable 6-phosphofructokinase (*pfkB*) is involved in glycolysis: converts sugar-1-P to sugar-1, 6-P.[39] The immunogenicity of this latency protein was evaluated in different studies. Arroyo *et al* reported that *M. tuberculosis* latency antigen Rv2029c was able to induce higher immune responses of T cells ($CD4^+$ or $CD8^+$) producing IFN-γ and TNF-α in latently *M. tuberculosis* infected individuals.[39] The immunogenicity profile of Rv2029c antigen has also been investigated by Mensah and colleagues.[40] They reported that latency antigen Rv2029c was able to induce higher levels of IFN-γ, Granzyme B, TNF-α and IL-17 and also low levels of IL-10 and sIL-2R-α in peripheral blood mononuclear cell.

3.3. *RV2628c (gene name:* Rv2628*; gene length: 363 bp; 120 amino acids)*

M. tuberculosis Rv2628 gene encodes a 13 kDa-latency antigen RV2628c protein with unknown function. It was reported that this *M. tuberculosis* DosR-regulon-encoded antigen can induce strong long-term IFN-γ responses. As an example, Goletti *et al* study showed that this latency antigen may induce immune-mediated protection against TB infection.[17] Similar results DosR-regulon-encoded protein Rv2029c, Mensah and colleagues were reported for DosR-regulon-encoded protein Rv2628.[40]

3.4. *Rv1737c(gene name:* narK2*; gene length: 1188 bp; 395 amino acids)*

The protein expressed by this gene *i.e.* nitrate/nitrite transporter NarK2 is involved in the excretion of nitrite across the membrane. Arroyo *et al* assessed the DosR antigens Rv1737c

Please cite this article as: Yousefi Avarvand A et al., The roles of latency-associated antigens in tuberculosis vaccines, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2019.04.012

immunogenicity and reported that it is capable of promoting T cells responses.[39]

3.5. *Rv1733c (gene name:* Rv1733c*; gene length: 633 bp; 210 amino acids)*

Rv1733c is a probable conserved trans membrane protein with unknown function. It was indicated as a potent inducer of T cell antigen in bioinformatics analysis.[16] However, this DosR-regulon-encoded antigen is also known to be immunogenic *in vitro* experiments conducted by Mensah *et al*, Kassa *et al* and Commander *et al*[40–42]

3.6. *Rv0081 (gene name:* Rv0081*; gene length: 345 bp; 114 amino acid)*

Twelve kDaRv0081 antigens belong to the latency-associated antigens and is encoded by DosR regulon.[16] This *M. tuberculosis* sDosR-regulon-encoded antigen is a probable transcriptional regulatory protein and involved in transcriptional mechanism. Kassa *et al* reports on the immune response against Rv0081 antigen confirmed Rv0081 antigen as a potent immunogenic antigen.[41]

4. The starvation regulon

4.1. *Rv2660c (gene name:* Rv2660c*; gene length: 228 bp; 75 amino acid)*

A collection of genes including Rv2660 and Rv2659 called the starvation regulon and expressed in response to nutrient deprivation by *M. tuberculosis*.[16] Govender *et al* suggested that the starvation stimulon gene product Rv2660 is a suitable antigenic candidate in a post-infection vaccine against TB infection. They showed that Rv2660 induced IFN-γ production in latently *M. tuberculosis* infected individuals.[43]

5. Conclusion

In all literature reviewed in the current study, it has been shown that the latency-associated antigens including resuscitation-promoting factors, the DosR regulon encoded antigens and the starvation regulon encoded antigens can stimulate cellular immune responses against latent TB infection. Therefore, they could be potential candidate antigens for immunization as a prophylactic vaccine against the latent stage of TB infection and could prevent the reactivation of latent infection.

REFERENCES

1. Steingart KR, Ng V, Henry M, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6(10):664–674.
2. Organization World Health (WHO). *Global Tuberculosis Report 2018*. 2018.
3. Lucia S, Mihaescu T. History of BCG vaccine. *Medicina*. 2013;8(1):53–58.
4. Mosavat A, Soleimanpour S, Farsiani H, et al. Fused Mycobacterium tuberculosis multi-stage immunogens with an Fc-delivery system as a promising approach for the development of a tuberculosis vaccine. *Infect Genet Evol*. 2016;39:163–172.
5. Farsiani H, Mosavat A, Soleimanpour S, et al. Fc-based delivery system enhances immunogenicity of a tuberculosis subunit vaccine candidate consisting of the ESAT-6: CFP-10 complex. *Mol Bio Syst*. 2016;12(7):2189–2201.
6. Soleimanpour S, Farsiani H, Mosavat A, et al. APC targeting enhances immunogenicity of a novel multistage Fc-fusion tuberculosis vaccine in mice. *Appl Microbiol Biotechnol*. 2015;99(24):10467–10480.
7. Ohara N. Current status of tuberculosis and recombinant bacillus Calmette-Guérin vaccines. *J Oral Biosci*. 2012;54(2):92–95.
8. Khademi F, Derakhshan M, Yousefi-Avarvand A, Tafaghodi M, Soleimanpour S. Multi-stage subunit vaccines against *Mycobacterium tuberculosis*: an alternative to the BCG vaccine or a BCG-prime boost? *Expert Rev Vaccines*. 2018;17(1):31–44.
9. Xin Q, Niu H, Li Z, et al. Subunit vaccine consisting of multi-stage antigens has high protective efficacy against *Mycobacterium tuberculosis* infection in mice. *PLoS One*. 2013;8(8), e72745.
10. Moradi J, Tabrizi M, Izad M, Mosavari N, Feizabadi MM. Designing a novel multi-epitope DNA-based vaccine against tuberculosis: in silico approach. *Jundishapur J Microbiol*. 2017;10(3), e43950.
11. Sun X, Zhang L, Jiang J, et al. Transcription factors Rv0081 and Rv3334 connect the early and the enduring hypoxic response of *Mycobacterium tuberculosis*. *Virulence*. 2018;9(1):1468–1482.
12. Bose T, Das C, Dutta A, Mahamkali V, Sadhu S, Mande SS. Understanding the role of interactions between host and Mycobacterium tuberculosis under hypoxic condition: an in silico approach. *BMC Genomics*. 2018;19(1):555.
13. Zhu W, Plikaytis BB, Shinnick TM. Resuscitation factors from mycobacteria: homologs of *Micrococcus luteus* proteins. *Tuberculosis*. 2003;83(4):261–269.
14. Romano M, Aryan E, Korf H, et al. Potential of *Mycobacterium tuberculosis* resuscitation-promoting factors as antigens in novel tuberculosis sub-unit vaccines. *Microb Infect*. 2012;14(1):86–95.
15. Silva BD, Tannus-Silva DG, Rabahi MF, Kipnis A, Junqueira-Kipnis AP. The use of *Mycobacterium tuberculosis* HspX and GlcB proteins to identify latent tuberculosis in rheumatoid arthritis patients. *Memórias do Instituto Oswaldo Cruz*. 2014;109(1):29–37.
16. Meier NR, Jacobsen M, Ottenhoff TH, Ritz N. A systematic review on novel *Mycobacterium tuberculosis* antigens and their discriminatory potential for the diagnosis of latent and active tuberculosis. *Front Immunol*. 2018;9:2476.
17. 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(1):135–142.
18. Su H, Zhu S, Zhu L, et al. *Mycobacterium tuberculosis* latent antigen Rv2029c from the multistage DNA vaccine A39 drives TH1 responses via TLR-mediated macrophage activation. *Front Microbiol*. 2017;8:2266.
19. Kana BD, Gordhan BG, Downing KJ, et al. The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth in vitro. *Mol Microbiol*. 2008;67(3):672–684.
20. Choi HG, Kim WS, Back YW, et al. *Mycobacterium tuberculosis* RpfE promotes simultaneous Th1-and Th17-type T-cell

Please cite this article as: Yousefi Avarvand A et al., The roles of latency-associated antigens in tuberculosis vaccines, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2019.04.012

immunity via TLR4-dependent maturation of dendritic cells. *Eur J Immunol*. 2015;45(7):1957–1971.
21. Lee J, Kim J, Lee J, Shin SJ, Shin EC. DNA immunization of *Mycobacterium tuberculosis* resuscitation-promoting factor B elicits polyfunctional CD8+ T cell responses. *Clin Exp Vaccine Res*. 2014;3(2):235–243.
22. Rosser A, Stover C, Pareek M, Mukamolova GV. Resuscitation-promoting factors are important determinants of the pathophysiology in *Mycobacterium tuberculosis* infection. *Crit Rev Microbiol*. 2017;43(5):621–630.
23. Khademi F, Yousefi-Avarvand A, Derakhshan M, et al. *Mycobacterium tuberculosis* HspX/EsxS fusion protein: gene cloning, protein expression, and purification in *Escherichia coli*. *Rep Biochem Mol Biol*. 2017;6(1):15–21.
24. Aagaard C, Dietrich J, Doherty M, Andersen P. TB vaccines: current status and future perspectives. *Immunol Cell Biol*. 2009;87:279–286.
25. Marongiu L, Donini M, Toffali L, Zenaro E, Dusi S. ESAT-6 and HspX improve the effectiveness of BCG to induce human dendritic cells-dependent Th1 and NK cells activation. *PLoS One*. 2013;8, e75684.
26. Wang X, Zhang J, Liang J, et al. Protection against *Mycobacterium tuberculosis* infection offered by a new multistage subunit vaccine correlates with increased number of IFN-γ+ IL-2+ CD4+ and IFN-γ+ CD8+ T cells. *PLoS One*. 2015;10, e0122560.
27. Li Q, Yu H, Zhang Y, et al. Immunogenicity and protective efficacy of a fusion protein vaccine consisting of antigen Ag85B and HspX against *Mycobacterium tuberculosis* infection in mice. *Scand J Immunol*. 2011;73:568–576.
28. de Sousa EM, da Costa AC, Trentini MM, de AraújoFilho JA, Kipnis A, Junqueira-Kipnis AP. Immunogenicity of a fusion protein containing immunodominant epitopes of Ag85C, MPT51, and HspX from *Mycobacterium tuberculosis* in mice and active TB infection. *PLoS One*. 2012;7, e47781.
29. Yuan W, Dong N, Zhang L, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine expressing a fusion protein of Ag85B-Esat6-HspX in mice. *Vaccine*. 2012;30:2490–2497.
30. Jeon B-Y, Kim S-C, Eum S-Y, Cho S-N. The immunity and protective effects of antigen 85A and heat-shock protein X against progressive tuberculosis. *Microb Infect*. 2011;13:284–290.
31. Yuan X, Teng X, Jing Y, et al. A live attenuated BCG vaccine overexpressing multistage antigens Ag85B and HspX provides superior protection against *Mycobacterium tuberculosis* infection. *Appl Microbiol Biotechnol*. 2015;99:10587–10595.
32. Geluk A, Lin MY, van Meijgaarden KE, et al. T-cell recognition of the HspX protein of *Mycobacterium tuberculosis* correlates with latent *M. tuberculosis* infection but not with *M. bovis* BCG vaccination. *Infect Immun*. 2007;75:2914–2921.
33. da Costa AC, de Oliveira Costa-Junior A, de Oliveira FM, et al. A new recombinant BCG vaccine induces specific Th17 and Th1 effector cells with higher protective efficacy against tuberculosis. *PLoS One*. 2014;9, e112848.
34. Khademi F, Derakhshan M, Yousefi-Avarvand A, Najafi A, Tafaghodi M. A novel antigen of *Mycobacterium tuberculosis* and MPLA adjuvant co-entrapped into PLGA:DDA hybrid nanoparticles stimulates mucosal and systemic immunity. *Microb Pathog*. 2018;125:507–513.
35. Khademi F, Sahebkar A, Fasihi-Ramandi M, Taheri RA. Induction of strong immune response against a multicomponent antigen of *Mycobacterium tuberculosis* in BALB/c mice using PLGA and DOTAP adjuvant. *Apmis*. 2018;126(6):509–514.
36. Mansury D, Ghazvini K, Jamehdar SA, et al. Increasing cellular immune response in liposomal formulations of DOTAP encapsulated by fusion protein Hspx, PPE44, and Esxv, as a potential tuberculosis vaccine candidate. *Rep Biochem Mol Biol*. 2019;7(2):156–166.
37. Amini Y, Moradi B, Tafaghodi M, Meshkat Z, Ghazvini K, Fasihi-Ramandi M. TB trifusion antigen adsorbed on calcium phosphate nanoparticles stimulates strong cellular immunity in mice. *Biotechnol Bioprocess Eng*. 2016;21(5):653–658.
38. Niu H, Peng J, Bai C, et al. Multi-stage tuberculosis subunit vaccine candidate LT69 provides high protection against *Mycobacterium tuberculosis* infection in mice. *PLoS One*. 2015;10, e0130641.
39. Arroyo L, Rojas M, Franken KL, Ottenhoff TH, Barrera LF. Multifunctional T cell response to DosR and Rpf antigens is associated with protection in long-term *Mycobacterium tuberculosis*-infected individuals in Colombia. *Clin Vaccine Immunol*. 2016;23(10):813–824.
40. Mensah GI, Addo KK, Tetteh JA, et al. Cytokine response to selected MTB antigens in Ghanaian TB patients, before and at 2 weeks of anti-TB therapy is characterized by high expression of IFN-γ and Granzyme B and inter-individual variation. *BMC Infect Dis*. 2014;14(1):495.
41. Kassa D, Ran L, Geberemeskel W, et al. Analysis of immune responses against a wide range of *Mycobacterium tuberculosis* antigens in patients with active pulmonary tuberculosis. *Clin Vaccine Immunol*. 2012;19(12):1907–1915.
42. Commandeur S, Lin MY, van Meijgaarden KE, et al. Double- and monofunctional CD4+ and CD8+ T-cell responses to *Mycobacterium tuberculosis* DosR antigens and peptides in long-term latently infected individuals. *Eur J Immunol*. 2011;41(10):2925–2936.
43. Govender L, Abel B, Hughes EJ, et al. Higher human CD4 T cell response to novel *Mycobacterium tuberculosis* latency associated antigens Rv2660 and Rv2659 in latent infection compared with tuberculosis disease. *Vaccine*. 2010;29(1):51–57.

Please cite this article as: Yousefi Avarvand A et al., The roles of latency-associated antigens in tuberculosis vaccines, Indian Journal of Tuberculosis, https://doi.org/10.1016/j.ijtb.2019.04.012