Front. Biol. 2018, 13(4): 293–296
https://doi.org/10.1007/s11515-018-1494-2

REVIEW

HspX protein as a candidate vaccine against *Mycobacterium tuberculosis*: an overview

Arshid Yousefi-Avarvand[1,2], Mohsen Tafaghodi[3], Saman Soleimanpour (✉)[1,2,4], Farzad Khademi[5]

[1] *Antimicrobial Resistance Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[2] *Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[3] *Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*
[4] *Reference Tuberculosis Laboratory of Northeast-Iran, Mashhad University of Medical Sciences, Mashhad, Iran*
[5] *Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran*

BACKGROUND: Tuberculosis (TB) is a contagious infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). This disease with two million deaths per year has the highest mortality rate among bacterial infections. The only available vaccine against TB is BCG vaccine. BCG is an effective vaccine against TB in childhood, however, due to some limitations, has not proper efficiency in adults. Also, BCG cannot produce an adequately protective response against reactivation of latent infections.
OBJECTIVE: In the present study we will review the most recent findings about contribution of HspX protein in the vaccines against tuberculosis.
METHODS: Therefore, many attempts have been made to improve BCG or to find its replacement. Most of the subunit vaccines for TB in various phases of clinical trials were constructed as prophylactic vaccines using Mtb proteins expressed in the replicating stage. These vaccines might prevent active TB but not reactivation of latent tuberculosis infection (LTBI). A literature search was performed on various online databases (PubMed, Scopus, and Google Scholar) regarding the roles of HspX protein in tuberculosis vaccines.
RESULTS: Ideal subunit post-exposure vaccines should target all forms of TB infection, including active symptomatic and dormant (latent) asymptomatic forms. Among these subunit vaccines, HspX is the most important latent phase antigen of *M. tuberculosis* with a strong immunological response. There are many studies that have evaluated the immunogenicity of this protein to improve TB vaccine.
CONCLUSION: According to the studies, HspX protein is a good candidate for development of subunit vaccines against TB infection.

Keywords HspX protein, *Mycobacterium tuberculosis*, vaccine

Introduction

Mycobacterium tuberculosis (*Mtb*) causes persistent infection known as tuberculosis (TB). This disease is one of the main health problems in the world due to 2 million morbidity and mortality annually (Yuan et al., 2012). Nowadays, TB vaccination is one of the best manners to prevent and control this infectious disease (Junqueira-Kipnis et al., 2014; Niu et al., 2015). The only approved vaccine against TB is BCG vaccine, which is a live attenuated strain of *Mycobacterium bovis* (BacilleCalmette–Guerin). BCG vaccine is able to protect newborns and children against TB, but its protection against adult pulmonary TB ranging from 0% to 80% (Xin et al., 2013). The inefficiency of BCG vaccine is attributed to some limitations including: (i) inability to eliminate the latent TB, (ii) decreased protectivity during the time, (iii) inability to protect adults against pulmonary TB, and (iv) problems in patients with immune deficiency (Britton and Palendira, 2003, Andersen and Kaufmann, 2014, Soleimanpour et al., 2015). About one third of the global population has latent TB infection. They are the major reservoir of adult TB infection

Received March 23, 2018; accepted May 7, 2018
Correspondence: Saman Soleimanpour
E-mail: Soleimanpours@mums.ac.ir

(Yuan et al., 2015). Therefore, new vaccines to control the TB disease in adults, especially in latent infection, are needed. In the same time the increasing cases of multidrug resistant (MDR) and the extensively drug resistant (TDR) TB have increased the difficulties in elimination of disease (Xin et al., 2013). These are progresses in order to improve or replace the current vaccine (Yuan et al., 2012). The effective vaccination strategies against TB are recombinant BCG, attenuated *M. tuberculosis*, DNA vaccines and protein subunit vaccines (Mir et al., 2009). The protein based subunit vaccines are effective vaccine candidates have potentials in long-term protection against the disease (Niu et al., 2015). To design a good protein subunit vaccine, various immunodominant antigens of *M. tuberculosis* has been tested. The present review has focused on a dormancy-related antigen of *M. tuberculosis* as an ideal candidate in the development of a new vaccine against TB.

Heat-shock protein X antigen (HspX antigen)

HspX is α-crystalline protein or a heat shock protein of *M. tuberculosis* which also called Hsp16.3 (molecular weight; 16.3 kDa), and Acr (HspX encoding gene, Rv2031c). HspX is produced during the lag phase, and also slightly during the active phase, or under stress conditions such as hypoxia, nutrient scarcity, and the presence of nitric oxide (Shi et al., 2010; Niu et al., 2011; Yuan et al., 2012; O'Garra et al., 2013; Mosavat et al., 2016). Under these conditions its expression could be reached to 25% of the total bacterial protein expression (Geluk et al., 2007). HspX expression co-regulated with DosR regulon (48 genes) in different environmental conditions (Aagaard et al., 2009). This protein is involved in the long term survival of bacteria in macrophages and it escape from the host innate immune system by increasing the stability of the proteins and cellular structures during the latent phase of infection (Shi et al., 2010; Taylor et al., 2012; Jung et al., 2014; Wang et al., 2015). HspX also causes slow-growing of bacteria (Marongiu et al., 2013). The most important immune response against *M. tuberculosis* infection is activation of cell-mediated immunity (Th1 and Tc) (O'Garra et al., 2013). HspX is an important dormancy antigen which could be effectively recognized by both human $CD4^+$ and $CD8^+$ T cells and strongly induces Th1 cytokines such as IFN-γ and TNF-α. It can induce strong cellular and humoral immune responses in latent phase of tuberculosis infection (LTBI) (Lin et al., 2007; Li et al., 2011; de Sousa et al., 2012; Soleimanpour et al., 2015).

Role of Hsp X in tuberculosis vaccines

To improve TB vaccine, many studies have performed in pre-clinical and clinical phases. They showed immunogenicity and efficacy of HspX protein for induction of strong Th1 immune responses and production of high levels of Th1 cytokines, especially in latent TB (Shi et al., 2010; Yuan et al., 2012; Silva et al., 2014). Therefore, HspX is a good antigen candidate for vaccination against TB (Jeon et al., 2011). In this article several studies performed on the immunogenicity and efficacy of HspX protein, have been reviewed.

In a study conducted by Chunwei et al.,, a recombinant BCG strain (rBCG) was constructed which expressed high levels of HspX protein. After vaccination of mice with this vaccine, a significant increase in antigen specific IFN-γ against HspX antigen was observed. Their findings suggest that HspX protein may be promising target for developing rBCG (Shi et al., 2010).

Yuan et al., were constructed a plasmid DNA vaccine expressing a fusion protein of *M. tuberculosis* antigens including Ag85B, Esat6 and HspX. They were assessed the immunogenicity of this vaccine in a mice model. Vaccination with this DNA vaccine elicited high levels of antigen specific IFN-γ. They were also observed higher levels of HspX specific T cell proliferation, as compared with vaccination with BCG (Yuan et al., 2012).

Similar study to Yuan et al., a new recombinant BCG by overexpressing immunodominant multistage antigens of Ag85B and HspX were constructed. After vaccination of C57BL/6 mice with vaccine, immunogenicity of this new vaccine compared with the immunogenicity of BCG. The results showed that new recombinant BCG had better protection against intranasal infection of *M. tuberculosis* than BCG (Yuan et al., 2015).

Hongxia et al., were used a multistage fusion protein of Mtb10.4 and HspX and evaluated its immunogenicity in vivo. This protein was strongly recognized by human T cells and generated strong humoral and cell-mediated immunity. Their study showed that Mtb10.4–HspX fusion protein vaccine induced strong antigen specific immune response in both humans and mice (Niu et al., 2011).

Trentini et al., were demonstrated significant differences between the mice immunized with HspX and non-vaccinated animals. HspX-containing vaccines were immunogenic. Mice immunized with CpG DNA and HspX showed strong humoral and cell-mediated (mainly TNF-α and IFN- γ) responses (Trentini et al., 2014).

In a study performed by Jeon et al.,, the immune responses against Ag85A and HspX antigens were evaluated in mice model. This recombinant protein (Ag85A and HspX of *M. tuberculosis*) was used as a vaccine candidate. After 30 days from the last immunization, mice immunized with this vaccine were challenged with *M. tuberculosis*. Ag85A and HspX antigens induced high levels of IFN-γ responses. At day 30 after the challenge, the protective subunit vaccine against tuberculosis was Ag85A, but HspX subunit vaccine induced significant protective effect after 90 days from the challenge with *M. tuberculosis* (Jeon, Kim et al., 2011).

Liang et al. (2015) produced a new recombinant BCG strain which overexpressed Ag85A, Ag85B, and HspX

antigens of *M. tuberculosis*. They were evaluated immunogenicity of this vaccine in C57BL/6 mice. Their results showed that recombinant BCG was induced strong Th1 immune responses in the infected mice and increased the IFN-γ and IL-12 levels. In addition, this recombinant BCG was able to induce stronger immune protection against primary TB infection in C57BL/6 mice.

In the study of Geluk et al.,, levels of IFN-γ responses to HspX antigen in *M. tuberculosis*-exposed individuals were significantly higher than the *M. tuberculosis*-unexposed BCG vaccines. Their result revealed that vaccination with BCG cannot induce T cell responses against the latency antigens such as HspX (Geluk et al., 2007).

Niu et al., reported a new subunit vaccine against TB. This multicomponent and multistage vaccine contained ESAT6-Ag85B-MPT64-Mtb8.4-HspX antigens along with two adjuvants,N, N'-dimethyl-N, N'-dioctadecylammonium bromide and polyriboinosinicpolyribocytidylic acid. After challenge of immunized mice with *M. tuberculosis* aerosol, this formulation could induce strong immunogenicity and high protective efficacy, even higher than traditional BCG vaccine (Niu, Peng et al., 2015).

As discussed in the study by Li et al. (2011), HspX antigen when combined with the replicating bacilli antigens, as multistage subunit vaccine, can enhance BCG primed immunity and also provides a better protection against both growing and non-growing bacteria.

The immunogenicity profile of a recombinant *M. tuberculosis* Ag85C-MPT51-HspX fusion protein has been investigated in mice and human by de Sousa et al., They showed the immunogenicity of fusion protein both in mice and humans (de Sousa et al., 2012).

In another study by Xin et al.,, Mtb10.4-HspX could improve BCG-primed protective efficacy against *M. tuberculosis* infection in mice (Xin et al., 2013). Marongiu et al., indicated that ESAT-6 and HspX together enable BCG-treated human DCs to elicit T and NK cells immune responses. Therefore, ESAT-6 and HspX may serve as promising candidates for improving the effectiveness of the BCG vaccine as a booster vaccine (Marongiu et al., 2013).

HspX antigen of *M. tuberculosis* could protect the immunized mice against aerosol challenge. Administration of this antigen as a booster for BCG vaccine could increase the protective efficacy (Taylor et al., 2012). The immunogenicity of a recombinant *M. tuberculosis* vaccine consisting of HspX and four other multistage antigens was evaluated in C57BL/6 mice. This novel vaccine could provide significantly better immunogenicity against *M. tuberculosis* infection than the control groups (Wang, Zhang et al., 2015). A new recombinant BCG vaccine expressing Ag85C, MPT51, and HspX of *M. tuberculosis* was constructed by Costa et al., Immunogenicity of this novel vaccine was tested in mice model and demonstrated that specific immune response to vaccine was induced in the lungs and spleen cells. After vaccination with this new vaccine, the bacterial load of the lung was lower than the bacterial load of the lung after vaccination with BCG (da Costa et al., 2014).

Conclusion

According to the studies reviewed in this article, the immunogenicity of HspX antigen against TB infection could be confirmed. In all studies, HspX antigen could stimulate strong immune responses against latent TB. Therefore, it may act as an appropriate candidate antigen for immunization against latent phase of infection. Also, HspX antigen was able to prevent reactivation of latent TB infection. Therefore, it could be recommended as a prophylactic vaccine. Finally, it was observed that the association of HspX antigen with immunogenic antigens of early phase and preparation of multistage subunit vaccine could induce strong immune responses against different stages of *M. tuberculosis* infection. Such these multistage antigens could be used as effective candidates for protection against TB.

Acknowledgements

The authors are grateful to our colleagues in Mashhad University of Medical Sciences for their sincerely cooperation.

Compliance with ethics guidelines

Authors declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Aagaard C, Dietrich J, Doherty M, Andersen P (2009). TB vaccines: current status and future perspectives. Immunol Cell Biol, 87(4): 279–286

Andersen P, Kaufmann S H (2014). Novel vaccination strategies against tuberculosis. Cold Spring HarbPerspect Med, 4(6): a018523

Britton W J, Palendira U (2003). Improving vaccines against tuberculosis. Immunol Cell Biol, 81(1): 34–45

Chen L, Xu M, Wang Z Y, Chen B W, Du W X, Su C, Shen X B, Zhao A H, Dong N, Wang Y J, Wang G Z (2010). The development and preliminary evaluation of a new *Mycobacterium tuberculosis* vaccine comprising Ag85b, HspX and CFP-10:ESAT-6 fusion protein with CpG DNA and aluminum hydroxide adjuvants. FEMS Immunol Med Microbiol, 59(1): 42–52

da Costa A C, Costa-Júnior A O, de Oliveira F M, Nogueira S V, Rosa J D, Resende D P, Kipnis A, Junqueira-Kipnis A P (2014). A new recombinant BCG vaccine induces specific Th17 and Th1 effector cells with higher protective efficacy against tuberculosis. PLoS One, 9(11): e112848

de Sousa E M, da Costa A C, Trentini M M, de AraújoFilho J A, Kipnis A, Junqueira-Kipnis A P (2012). Immunogenicity of a fusion protein

containing immunodominant epitopes of Ag85C, MPT51, and HspX from *Mycobacterium tuberculosis* in mice and active TB infection. PLoS One, 7(10): e47781

Geluk A, Lin M Y, van Meijgaarden K E, Leyten E M, Franken K L, Ottenhoff T H, Klein M R (2007). 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, 75(6): 2914–2921

Jeon B Y, Kim S C, Eum S Y, Cho S N (2011). The immunity and protective effects of antigen 85A and heat-shock protein X against progressive tuberculosis. Microbes Infect, 13(3): 284–290

Jung I D, Shin S J, Lee M G, Kang T H, Han H D, Lee S J, Kim W S, Kim H M, Park W S, Kim H W, Yun C H, Lee E K, Wu T C, Park Y M (2014). Enhancement of tumor-specific T cell-mediated immunity in dendritic cell-based vaccines by *Mycobacterium tuberculosis* heat shock protein X. J Immunol, 193(3): 1233–1245

Junqueira-Kipnis A P, Marques Neto L M, Kipnis A (2014). Role of fused *Mycobacterium tuberculosis* immunogens and adjuvants in modern Tuberculosis vaccines. Front Immunol, 5: 188

Li Q, Yu H, Zhang Y, Wang B, Jiang W, Da Z, Xian Q, Wang Y, Liu X, Zhu B (2011). Immunogenicity and protective efficacy of a fusion protein vaccine consisting of antigen Ag85B and HspX against *Mycobacterium tuberculosis* infection in mice. S cand J Immunol, 73 (6): 568–576

Liang J, Teng X, Yuan X, Zhang Y, Shi C, Yue T, Zhou L, Li J, Fan X (2015). Enhanced and durable protective immune responses induced by a cocktail of recombinant BCG strains expressing antigens of multistage of *Mycobacterium tuberculosis.* MolImmunol, 66(2): 392–401

Lin M Y, Geluk A, Smith S G, Stewart A L, Friggen A H, Franken K L, Verduyn M J, van Meijgaarden K E, Voskuil M I, Dockrell H M, Huygen K, Ottenhoff T H, Klein M R (2007). Lack of immune responses to *Mycobacterium tuberculosis* DosR regulon proteins following *Mycobacterium bovis* BCG vaccination. I nfect Immun, 75 (7): 3523–3530

Marongiu L, Donini M, Toffali L, Zenaro E, Dusi S (2013). ESAT-6 and HspX improve the effectiveness of BCG to induce human dendritic cells-dependent Th1 and NK cells activation. PLoS One, 8 (10): e75684

Mir F A, Kaufmann S H, Eddine A N (2009). A multicistronic DNA vaccine induces significant protection against tuberculosis in mice and offers flexibility in the expressed antigen repertoire. Clin Vaccine Immunol, 16(10): 1467–1475

Mosavat A, Soleimanpour S, Farsiani H, Sadeghian H, Ghazvini K, Sankian M, Jamehdar S A, Rezaee S A (2016). 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, 39: 163–172

Niu H, Hu L, Li Q, Da Z, Wang B, Tang K, Xin Q, Yu H, Zhang Y, Wang Y, Ma X, Zhu B (2011). Construction and evaluation of a multistage *Mycobacterium tuberculosis* subunit vaccine candidate Mtb10.4-HspX. Vaccine, 29(51): 9451–9458

Niu H, Peng J, Bai C, Liu X, Hu L, Luo Y, Wang B, Zhang Y, Chen J, Yu H, Xian Q, Zhu B (2015). Multi-Stage Tuberculosis Subunit Vaccine Candidate LT69 Provides High Protection against *Mycobacterium tuberculosis* Infection in Mice. PLoS One, 10(6): e0130641

O'Garra A, Redford P S, McNab F W, Bloom C I, Wilkinson R J, Berry M P (2013). The immune response in tuberculosis. Annu Rev Immunol, 31(1): 475–527

Shi C, Chen L, Chen Z, Zhang Y, Zhou Z, Lu J, Fu R, Wang C, Fang Z, Fan X (2010). Enhanced protection against tuberculosis by vaccination with recombinant BCG over-expressing HspX protein. Vaccine, 28(32): 5237–5244

Silva B D, Tannus-Silva D G, Rabahi M F, Kipnis A, Junqueira-Kipnis A P (2014). The use of *Mycobacterium tuberculosis*HspX and GlcB proteins to identify latent tuberculosis in rheumatoid arthritis patients. Mem Inst Oswaldo Cruz, 109(1): 29–37

Soleimanpour S, Farsiani H, Mosavat A, Ghazvini K, Eydgahi M R, Sankian M, Sadeghian H, Meshkat Z, Rezaee S A (2015). APC targeting enhances immunogenicity of a novel multistage Fc-fusion tuberculosis vaccine in mice. ApplMicrobiol Biotechnol, 99(24): 10467–10480

Taylor J L, Wieczorek A, Keyser A R, Grover A, Flinkstrom R, Karls R K, Bielefeldt-Ohmann H, Dobos K M, Izzo A A (2012). HspX-mediated protection against tuberculosis depends on its chaperoning of a mycobacterial molecule. Immunol Cell Biol, 90(10): 945–954

Trentini M M, de Oliveira F M, Gaeti M P, Batista A C, Lima E M, Kipnis A, Junqueira-Kipnis A P (2014). Microstructured liposome subunit vaccines reduce lung inflammation and bacterial load after *Mycobacterium tuberculosis* infection. Vaccine, 32(34): 4324–4332

Wang X, Zhang J, Liang J, Zhang Y, Teng X, Yuan X, Fan X (2015). 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, 10 (3): e0122560

Xin Q, Niu H, Li Z, Zhang G, Hu L, Wang B, Li J, Yu H, Liu W, Wang Y, Da Z, Li R, Xian Q, Wang Y, Zhang Y, Jing T, Ma X, Zhu B (2013). Subunit vaccine consisting of multi-stage antigens has high protective efficacy against *Mycobacterium tuberculosis* infection in mice. PLoS One, 8(8): e72745

Yuan W, Dong N, Zhang L, Liu J, Lin S, Xiang Z, Qiao H, Tong W, Qin C (2012). Immunogenicity and protective efficacy of a tuberculosis DNA vaccine expressing a fusion protein of Ag85B-Esat6-HspX in mice. Vaccine, 30(14): 2490–2497

Yuan X, Teng X, Jing Y, M a J, Tian M, Yu Q, Zhou L, Wang R, Wang W, Li L, Fan X (2015). A live attenuated BCG vaccine overexpressing multistage antigens Ag85B and HspX provides superior protection against *Mycobacterium tuberculosis* infection. Appl Microbiol Biotechnol, 99(24): 10587–10595