OnLine Journal of Biological Sciences

Review

Strategies of Tuberculosis–HIV Vaccines Design using Immunoinformatic Approach

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Article history
Received: 04-02-2019
Revised: 16-04-2019
Accepted: 10-05-2019

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Abstract: Tuberculosis is amongst the highest cause death worldwide. According to WHO, an estimated of more than a million people fell ill with Tuberculosis (TB) in 2016. The resistances of the *M. tuberculosis* (MTb) bacteria make drugs, not that useful in battle with TB. Vaccination is a better choice to avoid the worst cases of TB. The resistance possessed by MTb bacteria with some of the drugs reported creates its challenge to reduce the high rate of TB related death. One of the concerns is the high mortality rate due to TB associated with HIV. However, there's still no effective vaccine for Tuberculosis-Human Immunodeficiency Virus (TB-HIV). Vaccines for Tuberculosis-HIV even on the way of progress to get the demanding the mutation of the bacteria and challenging scientists to get the right vaccines for this disease. Immunoinformatic is one of the approaches that can speed up the making of the vaccine.

Keywords: Tuberculosis, Human Immunodeficiency Virus, Vaccine, *M. tuberculosis*, Tuberculosis-Human Immunodeficiency Virus

Introduction

Tuberculosis (TB) is recorded as one of the highest causes of death worldwide by 2016 with an estimation of over a million people die from TB (WHO, 2017). This disease is generally induced by the *M. tuberculosis* (*MTb*) which transmitted through the air. Therefore lungs are the first organ infected with the disease (Chiodi and Kaufmann, 2014). People with a good immune system can be affected with TB although there is no very severe or arguable TB sympthon. Hence, Purified Protein Derivative (PPD) and Interferon Release Assay (IGRA) is needed to aid in diagnosis of TB. Vaccination is often considered as an effective strategy for TB control (Doerr and Berger, 2014). The vaccination of TB that increase the immune system from infancy into adulthood would have a significant impact on global TB rates (Styblo and Meijer, 1976). Hence, various ways are being made to develop a vaccine for TB to have high efficacy and no contraindications to HIV. Thus, patients with HIV can receive the vaccine for TB. *Bacillus Chalmette–Guérin* (BCG) is the first licensed vaccines against TB that used globally since 1923. It is estimated that over billions of people have been vaccinated with BCG (Eccles and Mehta, 2011). Although, BCG has reported having a high range of efficacy that is 0-80% against adult pulmonary (Lefford and Mitchison, 1966; Schaible *et al*., 2017). As a vaccine, BCG serves only to make pediatric patients resist severe TB. BCG is seen correlated with its use in people that easy to be infected by bacterial and there is a possibility that the bacterium revert back to it's lethal form cause it possesses a low safety profile that associated with it (Detmer and Glenting, 2006).

Current vaccine development focused on early secreted antigens that related to active bacterial replication such as the *ESAT-6, CFP-10* and *Ag85 family*. These antigens are varying from the whole-cell mycobacterial vaccine. As they are highly immunogenic, TB vaccine has used them extensively in the development and animal models (Voss *et al*., 2018). TB Vaccine planning had a varied type. This planning is varying from vectored vaccines and adjuvanted proteins to whole mycobacterial cell. The adjuvanted proteins and vectored protein generate a response from glycoproteins such as CD4+ and CD8+. Therefore, it is important to cultivate an improved immune mechanism diversity within the current planning. Currently, there is a development in vaccine with a particular focus on broadening vaccine immunogenicity with antibody

responses and improvement to activate significant T lymphocyte feedback (*e.g., T-cells, HLA-E, CD1 and MR1*) (Fletcher and Schrager, 2016). By utilizing, the science of Bioinformatic especially the Immunoinformatic method. This method expected to accelerate the process of getting the latest vaccine for TB-HIV.

Immune System Response to Tuberculosis (TB)

Tuberculosis occurs when *Mycobacterium tuberculosis* (MTb) transmitted via inhalation and infects the lungs (Lerner *et al*., 2015). First, the air that we breathe drives bacteria into mucus. Then from the interaction between bacteria and mucus, it induces our immune response. The macrophages that act as a response from immune have complex of four different protein called the inflammasome (Middleton *et al*., 2002). At this response, this organelle prepares the specific immunity proteins called interleukins. The interleukins are the first line of our defense system against the MTb infection in our lung. Nonetheless, MTb can make its way to the deep airways and alveoli by avoiding the mucus trap. Although, macrophages can also eliminate foreign cells, by digesting and destroying them. MTb can produce protein that inhibits the fusion between phagosome and lysosome in macrophages. Thus, ultimately hindering the macrophages survivability (**Fig. 1**).

Tuberculosis (TB) can multiply and cause local infections. After three weeks of infection, it will infect cell-mediated immunity and immune cells will surround the site of TB infections by creating granuloma. After that, the middle part containing MTb will die and it is known as caseous necrosis. The location where the infection of MTb developed from caseous necrosis is called ghon focus. The ghon focus and infected lymph node are known as the ghon complex. The ghon complex is located in the lower part of the lungs. Symptoms of asymptomatic or flu-like illness usually detect this infection. In some other cases despite being surrounded by immune cells and forming bacterial granulomas, TB can remain alive but are harmless or dormant. This dormant TB can be activated anytime. One of the reasons of the reactivation of inactive TB is when the person loses their stability in their immune system or infected by the virus causes decreased immune system such as Human Immuno Deficiency Virus (HIV).

Tuberculosis (TB) and Human Immuno Deficiency Virus (HIV)

The record of high people infected with TB are because of the spreading occurs through the air. This spreading is inhaled by people and more vulnerable people with a weak immune system will be infected. Because of that, the World Health Organisation (WHO) global tuberculosis program in 1993 began promoting a strategy called as Directly Observed Therapy Short course (DOTS) (Sandhu, 2011). Under the DOTS strategy, anti-tuberculosis medication is swallowed by patients under the supervision of a health worker thereby ensuring that proper medications are given at proper intervals and at the right dose (Otu, 2013). Even though like that as DOTS required a proper information regarding the health record of the patients, this strategy is inefficient in country or city with poor health management. Because of that, people started to shift into vaccination rather than medication.

People with a good immune system can be infected with TB. However, during the infection, *M. tuberculosis* bacteria is still dormant. Then, what about people infected with the Human Immuno Deficiency Virus (HIV)? Patients who are infected with HIV positive and also carrying TB are many times more likely to cultivate active TB than people not infected with HIV living in the same country (Sandhu, 2011).

People infected with HIV positive then get infected with TB usually do not know that they have TB bacteria. HIV positive is undetectable because when TB is scanned with X-ray, it is undefined. The problem of TB resistance to phase 1 drugs from TB treatment also poses challenges in dealing with the disease. Various ways are being made to develop a vaccine for TB to have high efficacy and no contraindications to HIV. Thus, patients with HIV can receive the vaccine for TB.

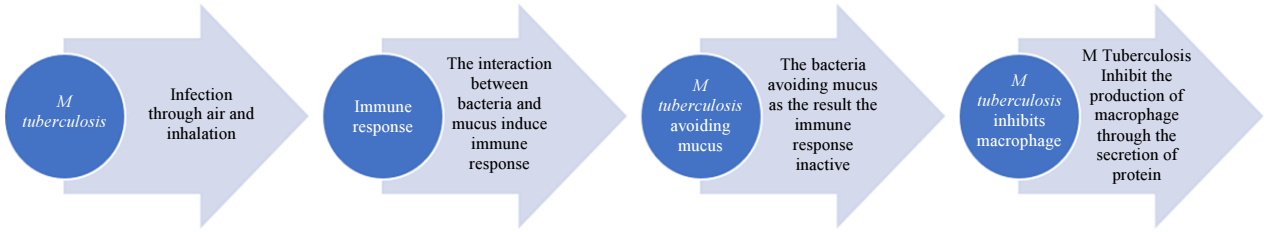

Fig. 1: Review flowchart that represent the Immune system response to Tuberculosis (TB)

Drug Resistance of *Mycobacterium tuberculosis*

TB's resistance to the drug for *M. Tuberculosis* (MTb) is the aftereffect from sudden changes in genes that encode either the enzymes or target of drug. It have been shown in previous studies that deletions, insertion, or resistance-associated point mutations of the first line drugs (streptomycin, rifampin, pyrazinamide, ethambutol and isoniazid) and for several auxiliaries and newer drugs (fluoroquinolones, nitroimidazopyrans, ethionamide and macrolides) are involved in activation of drugs (Somoskovi *et al*., 2001; Stover *et al*., 2000). Detection of the reduction of drug effectiveness in patients with TB could be done through the observation method. When a person with TB takes treatment, he or she will be given a set of medications that he should take at stage 1 for at least three months.

After three months it will be seen changes or effects that occur in patients. If the patient does not undergo any changes to a drug, MTb type that infects the patients are resistant to the drug. This condition means that there are two possibilities. The MTb could be resistant to multi-drug or extremely resistant to drug. The Multi-Drug Resistant TB (MDR-TB) means that the MTb infected are resistant against several drugs for treating TB. The Extremely Drug-Resistant TB (XDR-DR), implies that the MTb infected are resistant almost all phase of medicine uses to treat TB. Both are harder to manage because it means the regular antibiotics cannot kill the MTb.

One of the most infamous drugs for treating Tuberculosis is Rifampin or Rifampicin. Rifampin works as an inducer for the vital enzyme cytochrome p450 (CYP450) in the liver which responsible for the many metabolized drugs including the antiretroviral drug that used for treating people with HIV. The metabolized also proves that the rifampin makes a drug for treating HIV metabolized much faster than normal and lost their effect quicker as well. This dilemma is why the treatment for people with TB-HIV related is harder and dilemmatic.

The Vaccine for Tuberculosis Related to HIV

Relatively it is recorded over ten million people are affected with *Mycobacterium tuberculosis* and HIV or about a third from patients with HIV (WHO, 2004). People infected with both HIV and TB have the highest susceptibility of developing active tuberculosis. It is because HIV weakens the human immune system and making it easier for tuberculosis to active. Currently, BCG which used in human since 1921, is the only licensed TB vaccine and has been used globally especially in newborns infants (Evans *et al*., 2016; Husain *et al*., 2015). BCG contains a varied reduced *M. bovis* concentration at each dose. This concentration has varied effects. The effects are varied according to the quantity of feasible to dead strains used in for one quantity (Bali *et al*., 2015). BCG vaccine shows excellent efficacy in Tuberculosis. However, in people diagnosed with HIV-positive, it can cause severe or even fatal disease. The fatal disease means that the medication is growing uncontrollably and infected the body. Although the mechanism is still uncertain, people with HIV-positive could be infected with TB.

Despite the fact explained in the previous paragraph, TB and HIV still need to be treated. To treat TB, we have to use anti-TB drugs while for HIV we can use anti-retroviral drugs like Zidovudine (ZDV), also known as azidothymidine (AZT) (Ghodke *et al*., 2013). However, the problem is if we start to manage one of them with anti-retroviral drug or anti-TB drug there will be an adverse effect like viral resistance or an exagratted inflamantory resistance commonly called Immune Reconstitution Inflammatory Syndrome (IRIS) (Wong *et al*., 2016). Because of that, an inventive method was invented to restraint the replication of new vaccine in the body. This approach allows the immune system to fight future infection with the tuberculosis bacillus without any secondary effect (Hoft *et al*., 2008).

Development of Vaccine in Trials for Tuberculosis

With over a million deaths worldwide, the World Health Organization (WHO) listed Tuberculosis (TB) among the top 10 causes of death in 2016 (World Health Organization, 2017). This fact positioned tuberculosis to be among the deadliest infectious disease in the world. Not only that, TB alarmingly high numbers of cases with Multidrug Resistant (MDR) and Extensively Drug-Resistant (XDR) variants, prompted the leaders of G20 to emphasize how urgently effective vaccines are needed (Schaible *et al*., 2017).

Currently, there are 16 TB vaccines developed in stages 1, 2, 3 and 13. These vaccines are currently in clinical trials. The vaccines can be divided into virally vectored subunit, adjuvant protein subunit vaccine and whole cell-derived vaccines. The whole cell-derived vaccines is a strategy to make this disease gained growing attraction. It is because of the ongoing difficulties in identifying individual antigens. The attention from the whole-cell derived vaccine strategy could make many scientists start researching a way to generate a protective immune response for MTb. The only known vaccine in this approach is recombinant BCG vaccine known as VPM1002. To ensure the effectiveness of the vaccine, the VPM1002 has been made a stable coding for two different MTb virulence factors, phoP and fadD26.

Another strategy is to induce a stronger immune response and to make the vaccine more potent. To do

that, scientists engineered the vaccine called *Mycolyl transferase*. This vaccine will allow the producing large amounts key protein of *Mycobacterium tuberculosis*. The usual Viral-vectored vaccines strategies are to induce CD4+ and CD8+ T cells in those pre-existing immunity with *Mylcoly transferase* primed by BCG vaccination or exposure to mycobacteria in the environment. The crucial secretion system used in *Mylcoly transferase* from MTb was identified as five different types (ESX1-5) (Abdallah *et al*., 2007). Among those secretion systems, the best characterized of these is ESX1. ESX1 is necessary for the full activation of MTb, which uses ESX1 to translocate MTb inside phagosome into the cytosole inside of infected macrophages where it may persist in a protected environment (Romagnoli *et al*., 2012; van der Wel *et al*., 2007). ESX1 secretes among many antigens such as ESAT-6 and CFP-10. ESAT-6 and CFP-10 form the basis for immunological diagnosis of MTb infection in the Interferon-Gamma Release Assays (IGRAs) (Thoppil and Bishayee, 2011). But, BCG does not express ESAT-6 and CFP-10 which make it lacks ESX1. IGRAs may be used to detect the infection even in patients previously immunized with BCG, as it may not be otherwise distinguished with the classical Mantoux intradermal reaction.

Immunoinformatic Approach in Designing New Vaccine

One of the tools that can speed up vaccine discovery is by immunoinformatic methods. The immunoinformatic is utilised to look an effective antigen for MTb, to develop a new vaccine. In designing a vaccine, it is necessary to know the validated epitope. Because the peptide binds into the human MHC (HLA-DR). The stronger it binds, the stronger response from immune system (Mosaad, 2015). Hence, it is essential to know the right peptide that can binds to human MHC, to get a stronger response. The potential vaccine contained CD8+ epitopes, showed the projected population coverage (PPC) 97.4% global PPC and 92.7% East African PPC (Shah *et al*., 2018). The vaccine candidate must generate an unusual responses from T cell such as cd T cells in order to expand the vaccine immunogenicity. Presently, the immunogenicity of MTb vaccine is appraised through measuring cytokines such as IFN-c measured by ELISPOT and polyfunctional T cells (Méndez-Samperio, 2016).

The most advanced multi-step approaches are through immunoinformatic software which focuses on T-cell epitope identification. These steps include MHC binding, proteasome cleavage, TAP transport. These approaches are benefitted from diminished rate of false positive predictions and better accuracy, significantly lowering the quantities of peptides in clinical trials and research (Rai *et al*., 2012).

The innovative in designing a vaccine for TB-HIV is needed by widening the selection of antigen. The choosing of favorable vaccine antigens has been hindered by the predicament delivering this antigen by the complexity and size of the MTb proteome. The most likely solution to solve that problem is to generate immunotherapeutic vaccines based on T-cell epitope. This epitope represents multiple antigens for TB. Identifying the antigen to generate the immune response to MTb is one of the steps needed in designing a vaccine. The gene complex, Human Leukocyte Antigen (HLA) itself is closely related to the epitope. Each person has their own specific HLA class (Gustiananda, 2011). As a result, the candidate for epitope-based vaccine design is antigen with high binding affinity to several HLA alleles tends to be varied. The other strategy for selecting antigen is to choose a conserved antigen although it redirects feedback from immune into antigenic determinant inside the antigen. This method is not usually recognized during natural immunity the referred epitope known as subdominant epitope. Mouse models have demonstrated this strategy (Orr *et al*., 2014; Weinreich Olsen *et al*., 2000).

The first step in planning research for predicting epitope started from obtaining the sequences of protein from the National Center for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov/). After obtaining the sequence, we will replace or eliminated the sequences of protein that we use to predicting epitopes. *M. tuberculosis is* known to have more than millions of base pairs and approximately around thousands genes (Méndez-Samperio, 2016). To make sure the protein we choose is right we will run the protein against the retrieved antigen using automated BLASTP (Altschul *et al*., 1997; Tambunan *et al*., 2017). To improve the predictions of protein from FASTA secreted by Type II secretion pathway by SignalPor PAPROC-1, we will use VaxiJen, NetMHCIIpan, MHCpred or Propred (Dyrløv Bendtsen *et al*., 2004; Tambunan *et al*., 2016). VaxiJen is used to predict the protein from the SignalP server. Based on the score of each protein shown in VaxiJen, we will choose the top 5 best antigenic protein (Guan *et al*., 2003; Rai *et al*., 2012). Then, IEDB is used to predict the possibility of binding affinity to a wide range of human MHC alleles (Kim *et al*., 2012). We use IEDB because it covers a large variety of human MHC alleles. These alleles exist at high frequency in the global human population and are based on an extensive collection of literature binding data.

TAPPred server can be used to predict the binding of Transport Antigen presentation (TAP) (http://www.imtech.res.in/raghava/tappred/) (Bhasin *et al*., 2007). To predict the T-cell epitopes, we will use EpiJen software that predicts epitopes using the combination of TAP transport, proteasome cleavage and MHC binding (Tambunan *et al*., 2012). Usually, the

effective epitope antigen should have a sequence that does not split the proteome and can be transported by TAP. Every computational algorithm for epitope prediction will produce different information sets. Vaccine design then accomplished by using homology modeling method. Which, this method needs three-dimensional protein molecule structure in PDB format.

Conclusion

The high mortality rate of HIV related to TB is the challenge of finding the best strategies to reduce the mortality rate. One of the causes high TB death rates is because TB is transmitted through the respiratory system and most people are late knowing that they have been infected with TB. This is true, especially to people who have been infected with HIV. This is because they are generally difficult to detect TB, so they are treated too late. Also, people with latent TB who later become infected with HIV have a higher risk of experiencing activated TB. These things make it difficult to deal with patients with TB-HIV. If we handled one, then the other will result in the more severe disease. The immunoinformatic approach can be a tool to speed up the knowledge of Tuberculosis-HIV interactions against the immune system. This approach is the way to find a new vaccine design, that effective for TB and without making any complication HIV related patients. One of the innovations currently developed in vaccine planning for TB-HIV related patients is to develop the BCG vaccine. This planning start with using the immunoinformatic method to find the effective antigens for TB. After that, we will conduct the simulation with software such as MOE 2014. Then, to ensure that this planning works.

Acknowledgment

The authors would like to thank Directorate of Research and Community Engagement (DRPM), Universitas Indonesia for providing support in writing the manuscript. Thanks also goes to Center Library of Universitas Indonesia (LPP LIB) for providing support in English editing.

Funding Information

This project was funded and supported by PIT 9 research project of Universitas Indonesia.

Author's Contributions

Ani Fatonah: Contribute writing the draft manuscript.

Ig Satrio Wicaksono: Contribute proofreading the draft manuscript.

Usman Sumo Friend Tambunan: Contribute supervising the manuscript.

Ethics

This review contains materials from several published articles. The corresponding author confirms that all of the authors have read and approved the manuscript and there are no ethical issues involved.

Conflict of Interest

The authors declare that there is no conflict of interest.

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