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Journal of Vibration Engineering

ISSN:1004-4523

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M.tuberculosis: Manipulationof Host's immune response and potent targets for anti-tubercular drugs

Neelam Thakur*
Assistant Professor, Applied Sciences
E mail: *neelamthakur.uis@cumail.in*

Abstract
Tuberculosis, an ancient disease which is caused by Mycobacterium tuberculosis remains a global burdenof morbidity and mortality in human beings. It is an intracellular pathogen which subverts bactericidal defences of the host in order to survive and flourish in macrophages.Mtb inhibits the phagosomal maturation in order to avoid it's trafficking by inhibiting all those events which leads to phago-lysosomal fusion and is achieved with help of certain effectors of this pathogen which work either solely or in a cumulative manner. Nowadays, treatment of TB engrosses prolong therapies which set hurdles in compliance and supervision of drug treatment regardless of the advances in development of efficient anti-mycobacterial drugs. Moreover, BCG, the only anti-tubercular vaccine is not successful in providingefficient shield against the adult pulmonary tuberculosis. This review has focussed on current advances in the studying the structures of potent molecules and targets which can be employed for the development of novel and effective anti-mycobacterial drugs.

Key words: Immune subversion, phagosomal maturation, BCG vaccine, drug targets

Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis which is a deadly contagious diseaseis causing 1.5 million deaths yearly and 9.6 million new cases of tuberculosis. In accordance with last issued WHO report (1), active TB developed in10.4 million people in 2016 and 1.3 million HIV-negative individual capitulated to this disease, additional 374,000 deaths among HIV-positives individualsThe dire scenario is exacerbated synergistically by many factors such as the prolonged duration of treatment protocol, the scarcity of novel drugs, the emergence of multi-drug resistant (MDR) and extensive drug resistant (XDR), even total drug resistant (TDR) strains of *M. tuberculosis*, increase in co-infection of HIV, and the devious drug supply (3,4). Following an established route of contagion i.e. inhaling aerosolized bacilli,Mtb is promoted to get settled in alveoli through respiratory tract. There, the first line of host defense recognizesMtb by recruitement of resident phagocytic cells i.e. macrophages and dendritic cells but without complete clearing of Mtb (5). Immune cells are recruited to the infection site and a characteristic infective structure known as granulosomais formed which provide protection to phagocytic cells from more attacks by the immune system (6). This is the state of long term infection i.e. clinically latent state, in which Mtb can survive for decades. Progression of infection occurs which results in active TB when there is disruption of homeostasis between the host's immune response and the attacking pathogen i.e. any immunosuppressive condition which leads to progressive devastation of lungs and other tissues. Therefore, HIV-positive patients have higher risk of developing infective TB by establishing a undeviating connection amid two pathologies (7). Mtb has developed arsenal of strategies for subverting many components of host's immune system which includes maturation of phagosomes, fusion of phago-lysosomes, impaired antigen processing and presentation, inhibition of antigen presentation by MHC class II to CD4+ T cells (8-11), impairment of intra-cytoplasmic killing by autophagy and NLRP3-inflammosome killing (12-14). In this review, authors have offered a panoramic overview of immunological "stand off" by preventing phagosomal maturation and for inventing new anti-tubercular drugs , molecular targets are also discussed.

2. Innate Immunity against*M.tuberculosis* Infection

Pathogens as well as host have developed strategies for their protection and their establishment of effective defences (15).*M.tb*enters andresides within alveolar macrophages (AMs) and dendritic cells

(16)which constitute the first line of defense and help in removal of tubercle bacilli (17-19). Onset of adaptive immune response occurs by internalization and antigen presentation to$CD4^+$ T cells by dendritic cells which are first antigen presenting cells (20-22). In alveolar macrophages, after internalising and exposing Mtb to the acidic environment of the phagosome , a signalling mechanism is triggered which leads to fusion of phagosome to lysosome eventually removal of attacking pathogens (23).Mtb bacilli that escape the phago-lysosomal fusion of alveolar macrophages leads to the destruction of these cells which in turn attract blood monocytes and other inflammatory cells (i.e. neutrophils) to the site of infection. Monocytes differentiate into APCs (macrophages and dendritic cells) which along with neutrophils leads to the formation of granulomas for providing a protective environment to esablish the latent stage of infection (24,25).A granuloma consists of a central necrotic core of the granuloma withepitheloid cells and giant Langerhans cells which are multinucleated. Peripheral layers of activated macrophages and layers of T-cells (both $CD4^+$ and $CD8^+$ cells) which prevents the release and spread of Mtb (26). Shielding function of the granuloma is enhanced by the release of proinflammatory and anti-inflammatory cytokines whose production requires a strict control for establishing MTb infection. IFN-γ, TNF-α and IL-1 are required for enhancing protective function of the granuloma and IL-10 is an anti-inflammatory cytokines which regulates the inflammatory response (27-29). IFN-γ helps in mediating Mtb killing by promoting antigen presentation and recruitement of T-lymphocytes (both CD4+ as well as cytotoxic T-lymphocytes) whereas TNF-α promotes the formation of granuloma . IL-1 is secreted by monocytes and APCs, mediates signals through the IL-1 receptors for generating immune response (30,31). On the contrary, IL-10 is an anti-inflammtory cytokine secreted by macrophages and T-cells suppresses the expression of TNF-α which leads to inactivation of macrophages (32). Mtb has certain specific pathogen-associated molecular patterns (PAMPs) detected by pattern recognition factors (PRRs) and are responsible for generation of innate and adaptive immune responses (33).

3. Subversion of Host's Immune System by Mtb proteins

Zmp1: secretory protein

Phagosomal maturation is important step for removal of Mtb bacilli which leads to complete clearance of invading bacteria (18). This pathogen inhibits progression of infection by preventing phago-lysosomal fusion which is critical for it's establishment in host (34-36). A secretory protein Zmp1 which is a metalloprotease of Mtb, encoded by RV0198c gene is implicated in the prevention of phagosome maturation by suppressing activation of inflammosome (37). Inflammosome is a complex of multiple sensor proteins family, nucleotide binding oligomerization domain which leads to the proteolytic activation of pro-caspase-1 into caspase-1. Caspase-1 activates pro-IL-1 into IL-1 in cascade manner which triggers the phagosomal maturation as well early inflammatory response (38-40). Zmp1 protein helps in inhibition of Pro-IL-1 to IL-1 by suppression of caspase -1. Zmp1 is homologous to human peptidase neprilysisn (NEP) and human endothelin-converting enzyme-1 (ECE-1) so could be employedfor the design of drugs especially non-conserved Arg sequences i.e. Arg615 and Arg616 (41). Zmp1 mutant strains are also used for the development of novel anti-TB vaccine in light of newly discovered functions of this protein. Many preclinical studies have shown that, BCG zmp1mutant strains are safe and generates a strong immunological response and increases the efficacy of BCG vaccine . Hence, can be considered a potent candidate for the development of novel anti-TB vaccine and been well thought-out for clinical testing.

SecA2

SecA2, specific ATPase which is required for transporting small proteins (42-44). It is required for replication of Mtb in macrophages as well as mouse (45). In previous studies , it has been shown that secA2 mutant fails to prevent phagosomal maturation and is responsible for establishing infection inside (46).SapM is a secreted phosphatase is exported by *Mtb* SecA2 pathway which leads to suppression of phago-lysosomal fusion as well as its growth in macrophages (47,48).PknG, *Mtb* serine/threonine protein kinase is another effector of Mtb which is also SecA2 dependent and is essential for establishment of infection by arresting phagosomal maturation (49). Importance of SecA2 export of PknG and SapM was established by overexpressing these proteins in secA2 mutant and follow SecA1-dependent pathway (50).

5. Identification of molecular targetsfor effective drug discovery against Mtb

The targets of most of the drugs are vital enzymes implicated in many cellular processes such as protein synthesis, energy metabolism, cell wall synthesis andthe metabolism of several molecules and cofactors (51-52).The active compounds against Mtb and phenotypes-associated genes are screened by a persuasive technique known as phenotype screening (53). This technique is helpful in screening undeviating phenotypic response of Mtb bacilli against many compounds and evaluation of their efficiency in bacterial killing. Many problems related to metabolism and permeability of drugs are circumvented by this technique.New anti-tubercular compounds and targets are discovered due to advancement in this technique and that leads to the development of novel drugs for combating Mtb infection (54). In this review, author has focussed on some of the enzymes which helps in the formation of key metabolites required for Mtb survival and are the potent targets.

1. **Enzymes of purines and pyrimidines Nucleotide synthesis Pathways as potent drug targets**

   DNA and RNA are formed by polymerization of purinesandpyrimidines nucleotides which are synthesized by the de novo and salvage pathways and have been reported as promising targets for the development of anti-mycobacterial drugs both in vitro and vivo (55).All pyrimidine nucleotides are synthesized from a common ancestor i.e. Uridinemonophospahte as pyrimidine synthesis pathways congregate on this molecule.PyrEwhich is encoded by Rv0382c a key molecule is phosphoribosyltransferase enzyme which helps in the phosphorylation of orotic acid into orotate mono phosphate. A metallorganic molecule is present in the active site of this enzyme which interacts with protein part and represents a new target for the development of new antitubercular agents with least anti mycobacterial resistance (56). So new organometallic molecular scaffolds could be a new insight for the development of anti-bacterial drugs and could be a new research area for designing novel molecules(57). This can provide extraordinary opportunities for inventing novel organometallic inhibitory molecules which can help in developing new anti-bacterial drugs against MTB even for MDR and XDR strains.Mtb either scavenge purines from host or synthesize by de novo and salvage pathways(58, 59). Like pyrimidines synthesis, both de novo and salvage pathways of purines nucleotides have a common intermediate, inosine mono phosphate. Inosine monophosphate dehydrogenase (IMPDH) which is required for the dehydrogenation of inosine monophosphate to xanthine monophosphate and only one homologs (guaB2, Rv3411c) is required among three(guaB1, guaB2, and guaB3) (60, 61). A spontaneous mutation in guaB2 gene confers resistance to Mtb but failed to determine the crystal structure of this enzyme. But structure of IMPDH is determined by an alternate strategy which crystallized the homolog of IMPDH which revealed the reasons behind futile recognition of the active molecules in IMPDH mutant. Thus, IMPDH is aproficient target for Mtb drug development and suggested that crystallization of homologous target proteins is an alternate strategy for dissecting the molecular determinants of bacterial resistance to anti-tubercular drugs (62).The PrsA enzyme (Rv1017c), the only enzyme which is needed for conversion of ribose-5- phosphate to phosphoribosylpyrophosphate (PRPP) using ATP(63-68), which is main metabolite involved in a number of biosynthetic pathways i.e. amino acids synthesis and is also plays important role in the synthesis of the mycobacterial cell wall constituents(69-70).An alternate strategy is also preferred for crystallization of MtbPrsA as above and due to high homology, could be a potent molecule for development of novel anti-tubercular drugs (71).

2. **Enzymes of citric acid cycle as Drug Targets**

   Citric acid cycle orTCA (tricarboxylic acid cycle) is highly synchronized and multifarious cycle in which many pathways converge, so is the source of many metabolites which are necessary for survival and homeostasis of any aerobic bacteria in host environment (72). The enzymes of this cycle also constitute important drug target but pose challenge due to sequence similarities with their homologs in human beings. Despite of this, an inhibitor of Mtbfumaratehydratase was discovered (73), which differentially binds with non-conserved residues among the human and Mtb homologs. So there is possibility to discover inhibitors which can specifically target the conserved enzymes of Mtb preferably with noteworthy connotations for antI-mycobacterial drug development. Glyoxylate shunt is one of the most

attractive targets for the Mtb TCA cycle which is an alternate route that bypasses the steps involved in loss of co_2 which are catalyzed by two enzymes, named as isocitrateliase (ICL, Rv0467) andthe malate synthase (MS, Rv1837c). A previous work has reported the importance of ICL enzyme because it's inhibition proved fatal toMtb(71). The efforts have been deputed in elucidating the structures of effective molecules that can be exploited for designing specific inhibitors (72).

9. Conclusions

Tuberculosis is the disease which can be traced back to 70,000 yrs ago, still remains one of the horrific disease and is further aggravated by the coming out of MDR, XDR and TDR and co-infection with HIV.Mtb remains viable within infected host macropahges for a prolonged time and also leads to subversion of innate and adaptive immune responses, these are the reasons which makes it as an efficient pathogen. In macrophages, phagosomal maturation is arrested by many effector molecules of Mtb such as, Zmp-1, Sap M,SecA2, Pkng etc. SecA2 pathway has a broad role in preventing phago-lysosomal fusion which involves SapM, PknG and many other factors that act in a cumulative manner. BCG, the only vaccine against Mtb is not a reliable vaccine, so there is a need of discovering new vaccines as well as drugs which can help in combating Mtb infection. New anti-TB effector molecules are identified by using phenotypic screening and target based approach in a cumulative manner which is very much beneficial in eradicating infection as it's therapy have need of many drugs with different modes of action.Structural biology and in silico methods of unravelling concealed targets could also be predicted as vulnerable targets for curbing Mtb infection. The cellular and molecular mechanisms of Mtb which are reviewed here provides information about new targets which can be exploited for developing novel and effective anti-mycobactercular drugs as well as vaccines.

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