Review paper

Immune evasion strategies of *Mycobacterium tuberculosis*

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Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), remains a leading cause of global mortality. This review explores *M. tuberculosis*'s immune evasion mechanisms, including its inhibition of phagosome-lysosome fusion, resistance to reactive oxygen and nitrogen species, and interference with host signaling pathways. *M. tuberculosis* secretes proteins such as PknF, which inhibits NLRP3 inflammasome activation, and employs lipid-mediated defenses against host immune responses. The bacterium's persistence in granulomas complicates eradication and enables it to develop antibiotic resistance. Existing treatments, such as first-line antibiotics, face limitations due to this rising drug resistance. Research into the role of proteins such as ESAT-6, DRAM1, and BNIP3 in *M. tuberculosis* pathogenesis may lead to new drug targets. Additionally, host-directed therapies, including Vps18-mediated lysosomal repair, have shown potential in improving TB treatment outcomes. Novel therapies, including small-molecule inhibitors targeting bacterial kinases, host-directed therapies, and phage therapy, offer promising alternatives. Traditional healing methods, such as the spread of 'good nigudah' often reveals scientific phenomena at work, such as the presence of immunogenic extracellular vesicles (EVs) abundant in *M tuberculosis* antigen in the breath aerosols of tuberculosis smear-negative, hymn-chanting congregation of the faithful. Dismissing such traditional practices that provide herd immunity, as hearsay, may hence prove detrimental. Understanding *M. tuberculosis*'s pathogenesis and immune evasion is crucial for developing effective treatments and vaccines against this persistent pathogen.

Keywords

Mycobacterium tuberculosis, Tuberculosis, Immune evasion, Immune response, Drug resistance, Host-pathogen interactions, Latent tuberculosis, Host directed therapy

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1. Introduction

Infectious diseases are one of the leading causes of death; in 2019, approximately 13.7 million people died due to infectious diseases (1). Infectious diseases have the capacity to rapidly spread across countries and impact populations worldwide. They have a substantial effect on economies, social well-being and human health. They have a significantly and disproportionately larger impact on poorly developed countries. Due to slow economic, political and social development, these countries struggle more to reduce the effect of these diseases on their population as compared to developed countries like the United States of America or Germany. For example, in the recent pandemic of COVID-19, countries like Bangladesh and Afghanistan faced health crises and experienced a significant drop in the price of goods. Food insecurity was also a major issue in these countries; according to a survey conducted by the Bangladesh National Nutrition Council (BNNC), 75% of respondents indicated that they did not have sufficient access to food at home, while 91% stated that they did not have sufficient money to buy food (2). This was due to the high expenditure on healthcare and medicines for the treatment of COVID-19. Although the recent COVID-19 pandemic is under control, infectious diseases remain a constant threat.

1.1 *Symptoms*

M. Tuberculosis is an infection characterized by a range of symptoms that can significantly affect the respiratory system and overall health. The bacteria is more commonly known to infect the lungs but it can also affect vital organs like the brain, spine and kidneys (3).

M. Tuberculosis is spread through the air when someone coughs, sneezes or propagates droplets of any form. Breathing in bacteria laden droplets may not make the recipient sick (4). If someone is infected with HIV, has been infected with *M. Tuberculosis* in the last two years, has an alcohol and drug addiction or has other health problems like diabetes, they are at a much higher risk of getting infected with *M. Tuberculosis* (5).

There are three stages of symptom progression. The primary infection is the first stage where immune cells can capture and destroy the germs. However, some bacteria may survive and multiply in the host. This latent infection is the stage following the primary infection. Immune system cells surround lung tissue harboring *M. Tuberculosis* germs. This is termed as a granuloma. If the immune system manages to keep the bacteria in check, they cannot cause any more damage. Active disease occurs when the immune system fails to suppress the infection. Infectious agents spread sickness throughout the body, including in the lungs (6).

Pulmonary infection presents with symptoms of chest pain, wheezing and difficulty in breathing, coughing with mucus and occasionally with blood, fever, night sweats and fatigue (7). Without any treatment, these symptoms worsen and can often be fatal. In addition to the presence of pulmonary *M. Tuberculosis* in the lungs, systemic Tuberculosis can spread throughout the body (7). Invasive systemic infection has the potential to cause Meningitis, high levels of white blood cells in the urine, Addison's disease, Hepatitis, and/or Lymphadenitis (8). The stigma surrounding *M. Tuberculosis* can

lead to feelings of isolation, depression, anxiousness and helplessness.

1.2 *The immune system*

The immune system is the intricate defense mechanism of the body that fights infections and other diseases. The immune system is categorized by the rapidity and the type of response, the innate or non-specific response and the adaptive or specific response. The innate immune system is the first line of defense, and it is found in all multicellular organisms; its purpose is to prevent infections and attack the invading pathogens before they cause too much damage. It consists of physical barriers such as the skin, nose hairs and mucus as well as specialized cells like the Dendritic cells and Neutrophils (9). The response is rapid, but the limitation is that the response is very non-specific and may not be effective enough to purge the body of the infectious agent(s). The adaptive immune response; on the other hand; is extremely specific and only targets a particular pathogen. In order to target the pathogen, it must first be able to identify it. This results in the adaptive immune system having a longer response time of a few days or weeks (9). The adaptive system also has the ability to remember the pathogen for a period of time; this helps 'build immunity'; which in turn, reduces the time until an effective immune response is launched during a re-infection (9).

1.3 *Mycobacterium tuberculosis*

Among the large variety of pathogens that can cause infectious diseases, *Mycobacterium Tuberculosis* is one of the most prominent ones and is the 13th leading cause of death in the world (10). The bacterium was identified by Dr. Robert Koch in 1882 (10). One in seven residents of the United States and Europe died due to this fatal (at that time) infectious disease during this time period (10). Today, almost half of the African continent has an incidence rate of more than 300 cases per 100,000 while all of India has an incidence rate of 100-299 per 100,000. While a quarter of the global population is infected with latent Tuberculosis, ~ 5-10% will go on to develop active disease and become symptomatic and infectious with weakness, fever, night sweats and bouts of cough, occasionally with blood.

Mycobacterium Tuberculosis is a member of the genus Mycobacterium that also includes the causative agent of leprosy, *Mycobacterium leprae*. The distinguishing characteristic of mycobacteria is that the cell wall is thicker than that of other bacteria (11). *M. tuberculosis* is a high G+C Gram-positive bacteria; the term 'G+C' refers to the percentage of Guanine and Cytosine in the DNA (12). The bacterium is rod shaped, which helps it concentrate proteins in one part of the cell despite its small size (0.2 mm long); the cell poles also participate in various cellular functions such as chromosome replication and segregation (11,12). This specifically optimizes growth *via* cell polarization mechanisms that allow for the growth machinery, including cell-wall enzymes, membranes, and the cytoskeleton to be concentrated near the site of cell growth.

2. Discussion

Mycobacterium Tuberculosis is known to evade the human immune system and has evolutionary adapted to escape the innate and adaptive immune systems, living in a latent state in a quarter of the world's population. This paper will discuss the evasion mechanisms of *Mycobacterium Tuberculosis*,

specifically its inhibition of phagosome-lysosome fusion, resistance to reactive oxygen and nitrogen in the body and interference with host cell signaling. The effect of granulomas and existing treatments will be briefly discussed as well.

2.1 Evasion strategies

2.1.1 *Inhibition of Phagosome and Lysosome fusion*

After a bacterium or a disease-causing organism enters the body, it is detected as foreign and is taken up by specialized cells – phagocytes. An example of a phagocyte is a macrophage, a specialized cell which is responsible for recognition and phagocytosis of harmful organisms that enter the body (13). A key process in the immune response after pathogens are internalized *via* phagocytosis is the fusion of the phagosome and the lysosome; organelles found inside the phagocytic cell. The phagosome is a vesicle that forms by invagination of the plasma membrane with its associated lipids and proteins during phagocytosis of microbes or microbial proteins (14). The lysosome in contrast is a membrane bound organelle that contains digestive enzymes; which break down waste materials and also have the capacity to digest the entire cell (15).

Once the phagosome has matured, it migrates to the lysosome-rich perinuclear region, where the lysosome and phagosome membranes fuse with the help of fusion proteins (16). Membrane fusion is defined as the process by which two separate lipid membranes combine to form one continuous bilayer (17). Under normal circumstances, this process uses acidic conditions to fuse and develop into phagolysosomes which are vital in eliminating a pathogen from the host (18).

M. tuberculosis can inhibit this fusion process. This is executed by the manipulation of host signaling pathways. The bacterium prevents phagosome acidification – and hence lysosome fusion - by preventing the recruitment and assembly of VTPase, a proton pump which acidifies the phagosome (19).

The mycobacterium is also able to use the production of fatty acids to its advantage by hindering lysosomal transport. Lysosomal transportation is essentially the movement of lysosomes within a cell; this is a vital process for endocytosis, autophagy and most importantly, phagocytosis. *M Tuberculosis* is able to impair the normal function of lysosomes by producing complex glycolipids within its cell wall, some of which include, phosphatidylinositol mannosides (PIM) and mannose-capped lipoarabinomannan (ManLAM). These glycolipids can interfere with the acquisition of phosphatidylinositol 3-phosphate (PI3P), a phospholipid found in the host's cell membrane and involved in protein trafficking, and thus alter the dynamics of RAB proteins, which are generally involved in lysosomal transport (20). As a result of this cell membrane damage, there is a disruption in the normal fusion process of the phagosome and lysosome, and the bacteria successfully avoids the exposure to the harsh acidic environment of the lysosome, which allows it to survive in the phagosome for a longer period of time and replicate.

In some rare cases where acidification is not prevented, the mycobacterium is still able to

survive. Even though acidic pH tends to arrest the growth of *M. tuberculosis*, it was observed that the pathogen was able to survive and replicate within macrophages and phagolysosomes. Metabolomic analysis found that *M. tuberculosis* responded to acidic pH by altering its metabolism to preferentially assimilate lipids such as oleic acid over carbohydrates such as glycerol. This diversion of metabolic flux was achieved due to the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) being impaired in acid-exposed *M. tuberculosis,* in turn likely contributing to a reduction in glycolytic flux (21).

2.1.1.1 Small molecule drugs and targets

Pyrazinamide

Pyrazinamide is thought to combat the bacterium's ability to prevent acidification. It is a highly specific prodrug that works specifically against *M. tuberculosis*. *In vitro* and *in vivo,* the medication is only active at slightly acidic pH which is a range between 5.5 to 5.9. Pyrazinamide diffuses into active *M. tuberculosis* cells that express the pyrazinamidase enzyme, which transforms the prodrug pyrazinamide to its active form, pyrazinoic acid. Pyrazinoic acid inhibits the mycobacterial fatty acid synthase FAS I. This in turn interferes with the bacterium's capacity to manufacture new fatty acids, which are necessary for its growth and replication (22, 23). To begin, in the near neutral pH of the mycobacterium, the deprotonated Pyrazinoate anion (pK_a of Pyrazinoic acid is reported to be between 2.9 and 3.6) is effluxed out of the mycobacterium by efflux pumps (24). A fraction of the anion is protonated extracellularly, which; being uncharged then diffuses back into the bacterium; where it is de-protonated again, thus releasing protons and acidifying the intracellular bacterial milieu. This cycle of protonation and de-protonation continues and more pyrazinoic acid accumulates inside the bacterium as the bacterial intracellular pH decreases. The drug hence acts as an uncoupler of bacterial oxidative phosphorylation, although several lines of evidence contradict this singular mechanism (25,26). Resistance to the drug can develop by mutations in the pyrazinamidase enzyme (27) and overexpression of efflux pumps (28).

It has also been suggested that intracellular pyrazinoic acid buildup alters membrane potential and interferes with energy production (29) both of which are required for *M. tuberculosis* survival at acidic pH. Pyrazinoic acid binds to the ribosomal protein S1 (RpsA), preventing trans-translation. This may explain the drug's ability to kill latent mycobacteria, (30) although evidence to the contrary exists (31).

Bedaquiline

Bedaquiline, formulated as the fumarate salt, inhibits mycobacterial ATP synthase and is active against multidrug resistant (MDR) *M. tuberculosis* (32). It has a four-orders-of-magnitude more binding affinity to the mycobacterium ATP synthase than it does to the human mitochondrial ATP-synthase (33), thus accounting for its minimal side effects. Bedaquiline, by itself, possesses bacteriostatic/cidal activity due to its mycobacterial ATP synthase inhibition. However, it also reprograms the host macrophages by the activation of transcription factor EB associated overexpression of lysosome associated genes (34). Multiple antimicrobial defense

mechanisms are hence activated in the host macrophage cells, including the enhancement of phagosome-lysosome fusion, and increased autophagy. This host immunity enhancing effect could be one of the reasons that this drug is active against MDR-TB.

Repurposed lysosome acidification inducing drugs

Imatinib induced inhibition of Abl tyrosine kinase; an enzyme that controls lysosome acidification in macrophages; inhibited the growth of intracellular *M. tuberculosis* in a murine model. The mechanism of lysosome acidification was correlated with an increase of the transcription and expression of the proton pumping enzyme vacuolar-type H(+)-adenosine triphosphatase (V-ATPase) (35). The V-ATPase mediated lysosomal acidification also provides a mechanism for modulating senescence *via* the lysosome-mitochondrial axis (36), as does lysosome acidification triggered by PGC1α agonists (37) and (38) which are master regulators of mitochondrial biosynthesis. Hence, a wide variety of FDA approved compounds such as tyrosine kinase inhibitors, V-ATPase assembly facilitators and PGC1α agonists acidify lysosomes and can thus be repurposed for use as host-directed therapies for inhibiting *M. tuberculosis*.

2.1.1.2 Protein targets

Receptor-interacting protein kinase-3 (RIP3) can be used to treat infections by increasing autophagy, limiting bacterial survival, encouraging autophagosome production, and facilitating phagosome-lysosome fusion. Strategies may include increasing RIP3 activity, activating autophagy, creating RIP3-targeted medicines, studying the RHIM domain, and conducting clinical trials for translational research. Targeting RIP3 can improve autophagy, clearing tuberculosis germs and enhancing human defenses against mycobacterial infections (39).

Another prominent protein that can be manipulated as a potential treatment is the BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3). M. Tuberculosis -infected macrophages can activate BNIP3, which induces mitophagy. Mitophagy is the selective degradation of mitochondria by autophagy. In contrast, BNIP3-deficient macrophages do not stimulate mitophagy, resulting in lower mitochondrial membrane potential in response to *M. Tuberculosis* infection. BNIP3 deficiency can thus cause an accumulation of damaged mitochondria, which results in higher amounts of mitochondrial reactive oxygen species (mROS), decreasing *M. tuberculosis* intracellular survival. As a result, (inhibiting) BNIP3 may provide a unique therapeutic target for tuberculosis treatment (40).

Additionally, there exist various cellular responses to repair the damaged cell membranes. The endosomal sorting complex required for transport (ESCRT) is one of them. ESCRT helps repair phagosomal cell membranes and more recently, has been shown to repair mycobacterium induced phagosome damage (41). It has also been suggested that galectins and the mammalian target of rapamycin (mTOR) work together to help remove or repair phagosomal membrane damage caused by *M. Tuberculosis*. In order to narrow the several host factors which, help repair *M. Tuberculosis* induced cell damage, a genome wide CRIPSR screen was performed. It was found that Vacuolar protein sorting-

associated protein 18 (Vps18), a member of the HOPS and CORVET trafficking complexes, was a host factor required to repair *M. Tuberculosis* damaged phagosomal membranes.

It was discovered that Vps18 knockouts, were more prevalent in the population of cells with greater phagosomal membrane damage. *M. Tuberculosis* infection of monoclonal Vps18-knockout cells elevated transcriptional markers of phagosome membrane damage, as well as bacterial co-localization with endosomal membrane damage markers. Vps18 colocalized with *M. Tuberculosis* early after infection, regardless of phagosomal membrane disruption. Furthermore, *M. Tuberculosis* developed more robustly in Vps18-knockout cells; however, this increased mycobacterial growth did not lead to increased macrophage cell mortality. The loss of Vps18 lowered the efficiency of the first-line anti-TB drug pyrazinamide (41). After a series of tests, with Vps18, the most effective Vps-C complex, it was found that cells with Vps18 knockout infected with *M. tuberculosis* presented with significantly greater membrane damage than those with Vps-18 (41).

Damage-Regulated Autophagy Modulator 1 (DRAM1)

Damage-Regulated Autophagy Modulator 1 (DRAM1) is an infection-inducible membrane protein whose role in the immune response is not fully known. DRAM1 localized to mycobacteria in a punctate pattern early after phagocytosis, progressing to full DRAM1 envelopment of the bacterium over time. During the same time period, DRAM1-positive mycobacteria colocalized with the light chain 3 (LC3) marker for autophagosomes and the LysoTracker and LAMP1 markers for (endo)lysosomes. Knockdown studies demonstrated that DRAM1 was needed for LC3 recruitment and acidification of mycobacterium-containing vesicles. A decrease in the level of LAMP1 resulted in less fusion of lysosomes with mycobacteria-containing vesicles. In mouse macrophages, DRAM1 knockdown reduced autophagy markers and increased infection rates. These studies highlight DRAM1's importance in promoting the trafficking of mycobacteria through autophagic pathways, making it is a prospective target for therapeutically modulating macrophage microbicidal activity (42).

ESAT - 6

Virulence factors are molecules that assist the bacterium in colonizing the host (52). ESAT-6 is a protein secreted by *M tuberculosis* which plays a crucial role in the bacterium's virulence. It was first discovered in 1995 and was initially considered as a potential target for a tuberculosis vaccine and diagnostic tool due to the strong immune response it elicited in infected individuals. The secretion of ESAT-6 is dependent on a specialized secretory system called ESX-1, which is essential for *M tuberculosis* survival within host cells (14).

The genes responsible for the ESX-1 system, found on the esx-1 operon, are recognized by T cells, suggesting that the host's immune system exerts selective pressure on these proteins. Mutants that lack ESX-1 or the RD1 region in general; which contains the genes for ESAT-6 and other proteins; are less virulent, indicating the importance of ESAT-6 secretion for *M tuberculosis* virulence. Interestingly, the non-pathogenic species *M*

smegmatis also possesses an ESX-1 system, but it does not confer virulence (14), suggesting that the ESX-1 system may be necessary, but not sufficient for virulence.

The regulation of ESAT-6 secretion is complex and involves multiple genes and mechanisms. For instance, the gene WhiB6 plays a role in promoting ESAT-6 production. Moreover, the amount of ESAT-6 secreted by *M tuberculosis* is associated with its virulence; clinical *M tuberculosis* isolates produce more ESAT-6 than laboratory strains. This suggests that the ability to produce and secrete ESAT-6 is closely tied to *M tuberculosis* virulence (14).

A 15-year contact tuberculosis study investigated the metaphysical idea of "Nigudah-immunity," which originated in ancient tantric healing traditions. In ancient societies such as India and China, indigenous knowledge systems contained methods such as bodily fluid and aerosol inoculation, which may have influenced public health concepts such as vaccine development. In 1994, a healer in northern India utilized aerosol-based inoculation during *Kirtan* chanting, believing it distributed "good-nigudah," which were envisaged as microscopic creatures that prevented disease. Although originally concerned with tuberculosis transmission, researchers studied this traditional technique using focus group discussions, examining its metaphysical basis in Ayurveda and Hindu philosophy. The notion was that good-nigudah, derived from hazardous infections, could provide community protection (43).

The study examined TB infection rates among *Kirtan* chanting members and non-participants exposed to a smear-positive TB case. The results showed that *Kirtan* participants had no TB infections, whereas non-participants had a 9% infection rate. This led to additional metaphysical modeling and the discovery of extracellular vesicles (EVs) abundant in *M tuberculosis* antigen in the breath aerosols of smear-negative tuberculosis patients. In preliminary experiments, the extracted EVs, particularly those containing the ESAT-6 antigen, demonstrated immunogenicity by stimulating immunological responses in mice. These findings form the foundation for future study on the impact of traditional practices in immunity and public health and the possibility of herd immunity (43).

In conclusion, ESAT-6 is a crucial factor in *M tuberculosis* virulence and its survival within host cells. Understanding the mechanisms behind ESAT-6 secretion and its regulation could lead to new approaches for tackling Tuberculosis infections (14). Scientists could possibly conduct an experiment using a knock-out model of *M tuberculosis* and develop an effective vaccine.

2.1.2 *Resistance to Oxygen and Nitrogen*

The innate immune system is known for its rapid, yet unspecific response to pathogens. One step in the initial response of the immune system against pathogens is the secretion of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within the biological mileau of a phagocyte. While these compounds are unstable, they are highly reactive. They can be synthesized by various enzymatic reactions in aerobic organisms and prolonged exposure to them can result in cellular damage which essentially helps with eradicating pathogens (44).

ROS and RNS are produced in the phagolysosme by recruiting diverse proteins. For example, NADPH oxidase is a protein complex that is known to produce superoxide (O^{2-}). This oxide can dismutate (reaction between two usually identical molecules in which one is reduced and other one is oxidized) to hydrogen peroxide (H_2O_2), which is an extremely reactive chemical with known anti-microbial properties (45,46). Following this, H_2O_2 reacts with oxygen ions (O^{2-}) and creates other radicals such as hydroxyl radicals (OH^-) and single oxygen. Hypochlorous acid (HOCl) is formed when the myeloperoxidase enzyme reacts H_2O_2 with Cl^- ions. Microbes' RNA, proteins, and lipids can be structurally and functionally disrupted by the various types of ROS.

Microbial RNA molecules can be oxidized by HO• produced from the Fenton reaction by detaching the hydrogen from the C-H bonds, resulting in numerous oxidation adducts, including 8-oxo-7,8-dihydroguanosine (8-oxo-G), 8-oxo-7,8-dihydroadenosine (8-oxo-A), 5-hydroxyuridine (5-HO-U), and 5-hydrocytosine (5-HO-C). Although ROS affects all four nucleobases, the most common oxidation lesion detected in cells is 8-oxo-G, since guanosine has the lowest reduction potential. Under healthy settings, the frequency of 8-oxo-G is ~ 1 per 100,000 unaltered guanosines. This alteration has a high propensity to generate mutations in the open reading frame, resulting in changes in mirobial gene expression and the production of abnormal proteins. Furthermore, 8-oxo-G can significantly limit the rate of peptide bond formation, severely affecting microbial protein synthesis.

The nitric oxide synthase 2 generates nitric oxide radicals. Citrulline and nitric oxide (NO) are produced by this enzyme from arginine and oxygen. NO can interact with O_2 inside the phagosome to form the extremely toxic peroxynitrite (ONOO), which can alter the proteins and DNA of ingested microbes. Additionally, NO can directly harm bacterial enzymes and influence the growth of microbes (47). For example, *M tuberculosis* can develop biofilms to defend itself from immunological responses and medicines. NO disrupts these biofilms, making the bacteria more vulnerable. This interruption could prevent *M tuberculosis* from causing chronic infections (48).

Most pathogens are severely damaged by the actions of RNS and ROS. This damage limits their growth and eventually contributes to their clearance from the host. However, in the case of *M tuberculosis*, the bacterium has developed three main strategies to protect itself from the negative effects of ROS/RNS. First, it has a very complex cell wall composition which includes Mycolic acid that provides a physical barrier against the diffusion of ROS/RNS molecules into the bacterial cytoplasm. Second, the bacterium expresses a range of enzymes that can neutralize ROS/RNS molecules thus minimizing their damaging effects. Last, it possesses a highly evolved DNA repair mechanism, so even if the oxygen and nitrogen species do damage its DNA, it is able to efficiently recover (49).

The complex cell wall of *M tuberculosis* is composed of substances that can scavenge ROS and prevent their penetration into the cell. Some of these substances are lipoarabinomannan (LAM), cyclopropanated

mycolic acids, and phenolic glycolipid I (PGL-1) (50). These molecules not only prevent the penetration of ROS but also that of RNS.

To neutralize reactive oxygen and nitrogen species *M Tuberculosis* has a repertoire of several enzymes including catalase, peroxidase, superoxide dismutase, thioredoxin, and glutathione reductase (51). These enzymes can neutralize, remove, or repair ROS and RNS-induced harm to bacterial proteins and DNA (50 Voskuil et al.). The imbalance of production of reactive oxygen species and antioxidant defenses is defined as oxidative stress and can cause the negative effects on microbial DNA and proteins as described above (52). Interestingly, *M Tuberculosis*, can inhibit oxidative stress, by expressing the thioredoxin reductase TrxB2 enzyme. For the optimum performance of its proteins, this enzyme aids in maintaining a reductive intracellular environment (53). Drugs that target TrxB2 can kill *M Tuberculosis* during an infection because TrxB2 is crucial for the growth and survival of the pathogen (51).

The mycobacterium's thioredoxin system is composed of thioredoxin reductase (encoded by trxB2), thioredoxin (Trx) and NADPH. TrxB2 catalyzes the disulfide-thiol exchange of thioredoxins and thioredoxin-like proteins using electrons from NADPH (51). It thus helps the mycobacterium survive oxidative stress. Researchers found that when they decreased the activity of TrxB2, *M tuberculosis* became more sensitive to oxidative stress. However, TrxB2 does more than just protect *M tuberculosis* from oxidative stress. It also engages in cross-talk with other important processes in the mycobacterium, such as DNA repair and cell division (54). When TrxB2 activity was reduced, *M tuberculosis* became more sensitive to certain antibiotics and chemicals that targeted the cell wall. This suggests that TrxB2 is involved in maintaining the integrity of *M tuberculosis* cell wall as well.

Additionally, a bifunctional RelA/SpoT homolog (RSH) enzyme, designated RelMtb constitutes the so-called stringent response of *M tuberculosis* in the induction of antibiotic tolerance. It is mediated by tetra- or penta-phosphorylated guanosine [(p)ppGpp] and inorganic polyphosphate [poly(P)] and induces metabolic quiescence, thereby suppressing the activity of numerous antibiotic targets. RelMtb possesses dual (p)ppGpp synthesis and hydrolysis activities. Its constitutive activity maintains basal levels of (p)ppGpp, which are required for bacterial growth and biofilm formation both *in vitro* and *in vivo* (55). Pyrazinoic acid inhibits Rv2783c; a constituent of the RNA degradosome, a multi-enzyme complex which also modulates p(pp)Gpp hydrolysis, thereby adding this target to the drug's repertoire (56).

Overall, these findings suggest that TrxB2 promotes *M tuberculosis* immune escape by several mechanisms and could be a promising target for new Tuberculosis drugs. Researchers have developed a way to partially reduce the activity of TrxB2, which could be used to screen for potential drugs that target this protein. This method of partial activity reduction could also be used to study other essential proteins in *M tuberculosis* and other pathogens.

TrxB2 depletion caused rapid lytic death in replicating *M tuberculosis*, with significant cell elongation preceding the lysis. This suggested a connection between redox-homeostasis and cell-envelope integrity in *M tuberculosis*, since inactivation of TrxB2 may impair transglycosylation and contribute to bacterial lysis. Transglycosylation is an extremely efficient process for incorporating highly modified bases such as queuine into RNA. This observation also suggests that TrxB2 depletion may increase the microbial cell wall permeability to certain compounds, although it did not make the mycobacterium susceptible to all high molecular weight antibiotics.

A mycobacterial gene, pcaA, is required for cording and mycolic acid cyclopropane ring synthesis in the cell wall of *M tuberculosis* (57). A murine study found that pcaA was crucial in the formation of the structure of glycolipids like trehalose dimycolate (TDM), making them proinflammatory and enhancing their ability to interact with host immune cells. Many present anti-tuberculosis medications work by targeting processes involved in mycobacterial cell wall production, among others (48).

Specifically, studies have shown that *M Tuberculosis* strains lacking functional pcaA exhibit reduced growth and pathogenesis during initial infection stages in mice. The absence of cyclopropane modification leads to decreased inflammatory responses from macrophages and less severe granulomatous inflammation in infected mice. This highlights how genetic determinants like pcaA play a critical role in shaping the pathogenic strategies employed by *M tuberculosis* for long-term persistence within its host (54). Hence, the pcaA gene and its products could also serve as potential target for new developments in treatments and drugs. There are currently no medications that directly target the pcaA gene or its product(s) in *M tuberculosis*. However, research into novel pharmacological targets for tuberculosis is continuing, and enzymes encoded by pcaA may be investigated as targets for future drug development efforts (54, 58).

2.1.3 *Interference with host signaling*

Once *M tuberculosis* establishes itself inside the macrophage, the bacterium is able to interfere with the host's signaling pathways. The bacterium is able to manipulate cytokine signaling. Cytokines are signaling proteins that help the immune system to mount a defense against pathogens and control inflammation. This section will evaluate how *M tuberculosis* is able to interfere with this key process.

M Tuberculosis uses its ability to interfere with host signaling pathways by inhibiting pro-inflammatory signaling and interfering with host kinases. Host kinases are essentially biological catalysts or enzymes that add phosphate groups to other biomolecules such as proteins, lipids and nucleotides, which lead to changes in their function (59).

Host signaling is one of the key processes of communication between cells. An essential part of the immune system that transmits information from the receptors to the nucleus, where transcription of vital immunity genes is initiated, is the cell signaling machinery. Signal transduction is the process in which binding of an extracellular messenger to the cell surface receptor is translated into changes

in biochemistry, cell biology, and gene transcription that make it possible for the cell to respond to the information that was received (60). Protein phosphorylation, in which protein kinases play a significant role, plays a crucial role in closely controlling the majority of cellular functions and cell signaling pathways. Pathogens have developed a variety of strategies to disrupt crucial signal transduction pathways like the nuclear factor kB (NF-kB) and mitogen activated protein kinase (MAPK) pathways. Additionally, pathogens release effectors that hijack cell cycle machinery, modify vesicular trafficking, and manipulate the actin cytoskeleton and its regulators (61).

One of the most important signaling molecules during infection defense is Interferon- gamma (IFN-y). It stimulates the macrophages to 'attack' and kill foreign bodies and pathogens, however, it does not do the same for *M tuberculosis*. The binding of IFN-y to its receptor on a cell leads to the phosphorylation of binding sites on the intracellular part of the receptor by JAK. This phosphorylation activates these binding sites and allows two STAT proteins to bind to them. The STAT proteins then are also phosphorylated by JAK and this phosphorylation leads to their dimerization. The STAT dimer then dissociates from the receptor and ingresses into the nucleus where it can activate the transcription of genes that help fight the infection. This series of tightly orchestrated events constitutes the Janus kinase-STAT pathway (62).

This pathway is not affected by *M tuberculosis*, which is focused on the transcription of IFN-gamma responsive genes. STAT1alpha tyrosine and serine phosphorylation, dimerization, nuclear translocation, and DNA binding are all ostensibly unaffected in *M tuberculosis* infected cells (62).

Certain macrophage responses to IFN-gamma are inhibited by *M tuberculosis* and its components. Although some reports suggest that mycobacteria limit IFN receptor expression or Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling, a majority of investigations have shown that *M Tuberculosis* does not affect these early stages of IFN signaling. Most IFN-induced genes are not inhibited by *M. tuberculosis*, which indicates that inhibitory mechanisms must instead affect the subsequent stages in signaling and transcriptional regulation. This is consistent with the preservation of proximal IFN signaling (63).

M tuberculosis can also affect the outcome of infected cells by regulating the toll like receptor signaling pathway. Toll-like receptors (TLRs) are the important mediators of inflammatory pathways in the gut which play a major role in mediating the immune responses towards a wide variety of pathogen-derived ligands and link adaptive immunity with the innate immunity. The NLRP3 (nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3) inflammasome is a complex that is responsible for the activation of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18. *M tuberculosis* inhibits the activation of the NLRP3 inflammasome by its phosphokinase, PknF (63). PknF, in turn, regulates Rv1747, a molecule that controls *M tuberculosis* synthesis of certain lipids (63). These lipids often suppress

NLRP3 inflammasome activation. Without PknF, fewer of these lipids are generated, allowing the inflammasome to function more efficiently. Importantly, this suppression by PknF occurs independently of the mycobacterium's other strategies for interacting with host cells (63). A study evaluated the vulnerability of mice lacking IL-1β or its receptor to wild-type mice infected with *M tuberculosis*. Interleukin-1 beta (IL-1β) is a cytokine protein that is encoded by the IL1B gene. Knockout mice, including $Il1r1^{-/-}$ and $Il1b^{-/-}$ are unable to manufacture or respond to IL-1β. Researchers tested the role of IL-1β signaling in protecting against *M tuberculosis* infection by infecting mice and analyzing their responses (63). Mice lacking the IL-1 receptor or IL-1β were more susceptible to *M tuberculosis*, highlighting the significance of IL-1β in host defenses. Interestingly, *M. Tuberculosis* has developed techniques to evade or decrease inflammasome activation, thereby affecting IL-1β levels. Excessive IL-1β synthesis during *M tuberculosis* infection can cause inflammation and neutrophil recruitment (63).

M. tuberculosis inhibits NLRP3 inflammasome activation using a method that is independent of the ESX-1 secretion system, in contrast to the mycobacterium's ability to inhibit the AIM2 inflammasome via an ESX-1 dependent mechanism (63,64). *M tuberculosis* infection reduces the LPS/ATP-induced K^+ efflux and increases xanthine oxidase (XO) activity, resulting in lower cytosolic ROS levels. PknF, a *M tuberculosis* serine threonine kinase, inhibits NLRP3-dependent IL-1β production and pyroptosis in mouse and human primary macrophages (63). K^+ efflux, Cl^- efflux, and ROS production are all involved in PknF-mediated NLRP3 inflammasome suppression. Furthermore, the *M tuberculosis* pknF mutant increases XO activity compared to *M tuberculosis* infected cells, hence enhancing XO-mediated ROS generation (64). Overall, it appears that PknF inhibits the specific NLRP3 inflammasome activation pathway, which is only marginally activated during a *M. Tuberculosis* infection. Targeting PknF could restore proper inflammasome activation, thereby enhancing the host's ability to control and eliminate *M. tuberculosis* infection. Potential approaches might include developing small-molecule inhibitors that specifically block PknF activity or modulate the affected ion channels and ROS pathways to reinstate effective immune responses.

In conclusion, *M Tuberculosis* infected macrophages exhibit a significant reduction in the IFN-gamma-induced association of STAT1 with the transcriptional coactivators CREB binding protein and p300. This suggests that the mycobacterium either directly or indirectly disrupts this protein-protein interaction, which is necessary for transcriptional responses to IFN-gamma. The effects of live bacteria can be replicated by gamma-irradiated *M tuberculosis* and isolated cell walls, showing that the bacterial components that initiate the suppression of IFN-gamma responses are constitutively produced (62).

2.2 Impact of granulomas

A granuloma is a clump or cluster of white blood cells and other tissue formed as a reaction of the body to infections or inflammation. It is normally found in the lungs, skin and other parts of the body (65). It forms when droplets containing *M*

tuberculosis bacteria accumulate in newly infected lungs. Because it is newly infected, resident alveolar macrophages stimulate the immune response, in the form of phagocytosis. Alveolar macrophages are the best targets for *M tuberculosis* because they are specifically adapted to eradicate tiny airborne particles through phagocytosis and are not the ideal white blood cell for inducing strong inflammatory responses and producing anti-microbial chemicals such as nitric oxide (NO) and reactive oxygen intermediates (ROI) (66).

Following this initial response, the macrophages help transport the bacterium out of the airway and into the interstitium of the lungs (the space between the air sacs and the small blood vessels that surround the air sacs), where it is consequently able to evade immune-mediated killing using several virulence factors (67). Simultaneously, as the infection continues to advance, there is persistent recruitment and aggregation of macrophages at the site of infection. This leads to the formation of a granuloma (67).

2.3 Existing tests and treatments

Vaccines train the immune system to effectively control infections, thus reducing the need for antibiotics, and consequently, prolonging the time to antibiotic resistance. They have the ability to decrease the host time to response to bacterial infections. Preclinical prediction of human vaccine efficacy is very difficult due to the lack of animal models which can reproduce the human response to *M tuberculosis*. Despite this challenge, the Bacille Calmette-Guérin (BCG) Vaccine was developed and has been shown to be effective. The vaccine originated from the observation that cows were also susceptible to infection with *M tuberculosis*. This specific form of *M Tuberculosis* was termed *Mycobacterium bovis*. In the early 20th century, scientists were able to develop the BCG vaccine, which uses a significantly less infectious and aggressive version of the mycobacterium (Müller). This vaccine is still commonly used even though it is ineffective in adults, yet it was shown to be effective in children.

Since so many people carry latent Tuberculosis and feel no symptoms, diagnosing latent infection carriers is challenging. Latently infected people are carriers which allow the bacterium to multiply and evolve, and eventually develop antibiotic resistance. Additionally when they become immunocompromised due to old age or other infections, Tuberculosis can break out again and they infect other people in their community.

Only two tests—the skin test and the blood test for tuberculosis—can identify both latent and active patients. If a person has a positive *M. Tuberculosis* test result and a medical examination does not reveal active *M. Tuberculosis* disease, a diagnosis of latent *M. Tuberculosis* infection is made (68).

Latent tuberculosis is usually tested by the Tuberculin skin test (TST); however, it has several limitations concerning its sensitivity and specificity (68 Al-Orainey). The test essentially observes the patient's reaction to an injection of tuberculin, a combination of purified proteins (69). During the test, 0.1 milliliters of tuberculin is injected just under the skin of the patient's forearm. It is not the most reliable test as it shows a positive result

in people who have had Bacille Calmette-Guérin (BCG) vaccination or have been exposed to other species of mycobacteria.

As an alternative to antibiotics, immunotherapy could be a potential treatment for *M. Tuberculosis*. Immunotherapy uses substances that enhance the immune system. The substances used include cytokines which are proteins that help carry out communication between cells to activate the immune system ("How Immunotherapy Is Used to Treat Cancer"). For example, cytokines like IFNα and IFNβ would prove beneficial as they help in the recruitment of phagocytes in the lungs; immunotherapy with such cytokines among others may be a possible preventive measure (70).

The typical treatment for *M. Tuberculosis* uses the four first line drugs: Isoniazid, Rifampicin, Ethambutol and Pyrazinamide in different combinations and for different time periods. Each of these drugs has a specific function. Isoniazid and Ethambutol can prevent the formation of bacterial cell walls after being activated by the bacterial catalase-peroxidase enzyme KatG in Mycobacterium tuberculosis (71). Rifampicin inhibits bacterial DNA-dependent RNA polymerase (72). Pyrazinamide prevents the creation of fatty acids by changing a particular enzyme into an active form. This damages the cell wall and prevents the bacteria from producing the energy they require to survive. Although drugs, which mainly target the multifaceted cell wall, have successfully been developed to combat the bacteria, it rapidly mutates and becomes resistant to the drug; presently, it is resistant to numerous antibiotics like bedaquiline and fluoroquinolone (73). Such strains bring us back to the pre-antibiotic era and stress the urgent need to develop alternative strategies to combat the disease. An alternative treatment could be phage therapy, where mycobacteriophages are used. Mycobacteriophages are viruses that infect mycobacteria. They are natural antagonists of mycobacteria and are equipped with mechanisms to lyse mycobacteria (74), which refers to the disintegration of cell by rupture of the cell wall. More specifically, mycobacteriophages encode proteins that act as endolysins which are enzymes that specifically hydrolyze the bacterial cell wall.

The bacterial cell wall of *M. Tuberculosis* is exponentially more complex and dynamic than other gram-positive and gram-negative pathogens. It is composed of a thick mycolyl-arabinogalactan-peptidoglycan complex core (mAGP) involved in virulence and persistence.

In order to get past this obstacle, many mycobacteriophages encode another enzyme located downstream of lysA, named Lysin (LysB). This enzyme cleaves the ester bonds between mycolic acid and the mAGP, thus obtaining cellular access. Although conflicting evidence exists, it is generally believed that bacterial resistance to endolysins is low, making this a promising alternative to solving the ever-growing resistance problem (75). Yet, even though the concept of phage therapy has been around for almost a century, it is still considered to be in its experimental stages and it has not been approved for human use yet.

To summarize, new antibiotic drugs alone may be ineffective against the bacteria as it is constantly evolving and mutating.

Granulomas can impact the effectiveness of *M. Tuberculosis* treatment by creating physical and metabolic barriers that affect the diffusion of antibiotics and by harboring dormant bacteria. However, with appropriate drug choices, extended treatment durations, and strong immune responses, *M. Tuberculosis* can be effectively treated, even when granulomas are present.

3. Perspectives

Tuberculosis remains one of the deadliest infectious diseases, disproportionately affecting developing countries. *M. tuberculosis* is primarily transmitted through airborne droplets and can persist in a latent state within macrophages. The bacterium has evolved multiple strategies to evade immune responses, including inhibiting phagosome-lysosome fusion, resisting oxidative stress, and manipulating host cell signaling.

M. tuberculosis prevents phagosome-lysosome fusion by blocking V-ATPase recruitment, thus avoiding acidification and degradation within macrophages. It also produces glycolipids that impair lysosomal transport, further preventing its elimination. Even when exposed to acidic conditions, *M. tuberculosis* adapts its metabolism to survive, preferring lipid assimilation over carbohydrate metabolism.

Resistance to reactive oxygen and nitrogen species (ROS/RNS) is another crucial survival strategy. The bacterium's thick, lipid-rich cell wall prevents ROS penetration, and it produces detoxifying enzymes such as catalase, superoxide dismutase, and thioredoxin reductase (TrxB2) to neutralize oxidative damage. Additionally, *M. tuberculosis* employs a stringent response mediated by RelMtb to induce metabolic quiescence, contributing to antibiotic tolerance.

M. tuberculosis also interferes with host cell signaling pathways. It inhibits pro-inflammatory cytokines like IL-1β through PknF, a serine/threonine kinase. PknF regulates bacterial lipid synthesis, suppressing inflammasome activation and weakening host defenses. Knockout models demonstrate that this pathway is critical for bacterial persistence in macrophages. Granuloma formation plays a dual role in tuberculosis pathogenesis. While granulomas encapsulate *M. tuberculosis*, preventing its spread, they also create an environment where the bacteria can persist in a dormant state. This latency complicates treatment, as the bacteria can reactivate when the host's immunity declines.

Existing TB treatments rely on antibiotics, including isoniazid, rifampicin, ethambutol, and pyrazinamide. However, the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains threatens their efficacy. Immunotherapy, such as IFN-γ treatment, enhances macrophage function and may improve treatment outcomes. Host-directed therapies, targeting bacterial enzymes like TrxB2 and PknF, offer promising new strategies.

Phage therapy is another emerging approach that employs mycobacteriophages to selectively target and lyse *M. tuberculosis*. These viruses encode enzymes that degrade bacterial cell walls, potentially overcoming antibiotic resistance. Although still in experimental stages, phage therapy holds

promise as an alternative to traditional treatments.

Research into the role of proteins such as ESAT-6, DRAM1, and BNIP3 in *M. tuberculosis* pathogenesis may lead to new drug targets. ESAT-6 is crucial for virulence and immune system evasion, while DRAM1 and BNIP3 are involved in autophagy and mitophagy pathways that regulate bacterial survival within host cells. Additionally, host-directed therapies, including Vps18-mediated lysosomal repair, have shown potential in improving TB treatment outcomes.

Overall, tuberculosis remains a formidable public health challenge due to its complex interactions with the host immune system and its ability to develop resistance to existing treatments. Advancing our understanding of *M. tuberculosis* molecular mechanisms is essential for developing novel therapeutics and vaccines to effectively combat the disease.

4. Conclusion

Tuberculosis continues to be a significant global health threat, particularly in low-income regions. *M. tuberculosis* has evolved multiple immune evasion mechanisms, including inhibition of phagosome-lysosome fusion, resistance to oxidative stress, and manipulation of host signaling pathways. These strategies allow it to persist within the host and contribute to latent infections. The rise of drug-resistant strains underscores the need for alternative therapeutic strategies. While current treatments such as first-line antibiotics remain essential, emerging approaches—including small-molecule inhibitors, immunotherapy, and phage therapy—offer hope for more effective control of tuberculosis. Research into host-directed therapies and novel drug targets such as TrxB2, PknF, and ESAT-6 will be critical in overcoming treatment challenges. Additionally, investigation into empirical traditional or folk medicine that demonstrates curative properties should be actively explored for undercurrents of scientific mechanisms at work. A comprehensive understanding of *M. tuberculosis* pathogenesis will be essential for developing new therapeutics and ultimately achieving global tuberculosis eradication.

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Abbreviations

BNNC: Bangladesh National Nutrition Council, PIM: Phosphatidylinositol mannosides, ManLAM: Mannose-capped lipoarabinomannan, PI3P: Phosphatidylinositol 3-phosphate, FAS: Fatty acid synthase, RpsA: Ribosomal protein S1, 1-a PGC1a: Peroxisome proliferator-activated receptor gamma coactivator, RIP3: Receptor-interacting protein kinase-3, BNIP3:

BCL2/adenovirus E1B 19 kDa protein-interacting protein 3, mROS: Mitochondrial reactive oxygen species, ESCRT: Endosomal sorting complex required for transport, DRAM1: Damage-Regulated Autophagy Modulator 1, LC3: Light Chain 3, Evs: Extracellular Vesicles, mTOR: Mammalian Target Of Rapamycin, ROS: Reactive Oxygen Species, HOCl: Hypochlorous acid, 8-oxo-G: 8-oxo-7,8-dihydroguanosine, 8-oxo-A: 8-oxo-7,8-dihydroadenosine, 5-HO-U: 5-HydrOxyUridine, 5-HO-C: 5-HydrOCytosine, NO: Nitric Oxide, ONOO: Peroxynitrite, LAM: lipoarabinomannan, PGL-1: Phenolic GlycoLipid 1, TDM: Trehalose dimycolate, NF-kB: Nuclear Factor kB, MAPK: Mitogen Activated Protein Kinase, IFN-γ: Interferon-gamma, JAK-STAT: Janus Kinase-Signal Transducer and Activator of Transcription, TLRs: Toll-Like Receptors, NLRP3: Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3, IL-1β: Interleukin -1β, PknF: Phosphokinase, XO: Xanthine Oxidase, ROI: Reactive Oxygen Intermediates, BCG: Bacille Calmette-Guérin, mAGP: Mycolyl-arabinogalactan-peptidoglycan, LysB: Lysin B, AR: Actively Replicating, NR: Non Replicating, MSC: Mesenchymal Stem Cells, E3: Ubiquitin Ligase, UPS: Ubiquitin-Proteasome System, mTORC1: Mechanistic Target of Rapamycin Complex 1, HDT: Host-Directed Therapy, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, V-ATPase: vacuolar-type H(+)-adenosine triphosphatase.

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