Evasion of the Immune System by Mycobacterium tuberculosis: A Special Review on Macrophages

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Abstract

Mycobacterium tuberculosis, the bacterium that caused tuberculosis, is estimated to affect 10 million people worldwide in 2019. This bacterium is an intracellular pathogen that is spread through the inhalation of bacterial aerosol particles. The innate immune system in the lungs is prepared to phagocytize these bacteria, particularly macrophages, dendritic cells, monocytes, and neutrophils. M. tuberculosis can evade attacks by the host immune system and has developed strategies to infect successfully, especially macrophages. This intracellular bacterium can inhibit phagolysosome fusion, which is associated with lipoarabinomannan (LAM) in the bacterial cell wall. M. tuberculosis also can persist in phagolysosomes by inhibiting acidification and also inhibiting the action of NOX2 from producing ROS. This ability also allows these bacteria to avoid autophagy within macrophages. Knowledge of the power of these bacteria to manipulate and evade the immune system, especially macrophages, is beneficial in developing medicines and vaccines in the future.

Keywords— macrophages, Mycobacterium tuberculosis, immune system evasion

Abstrak

Mycobacterium tuberculosis, merupakan bakteri yang menyebabkan penyakit tuberkulosis yang diperkirakan menyebabkan kematian lebih dari 10 juta orang di seluruh dunia. Bakteri ini merupakan pathogen intraselular yang menular melalui inhalasi dari aerosol partikel yang mengandung bakteri. Sistem imun didapat pada paru akan bersiaga untuk menfagositosis bakteri ini melalui makrofag, sel dendritic, monosit, dan netrofil. M. tuberculosis dapat menhindari serangan sistem imun host terutama makrofag dan mengembangkan strategi agar dapat berhasil menginfeksi host. Bakteri intraselular ini dapat menghambat fusi fagolisosom, salah satunya akibat adanya lipoarabinomannan (LAM) pada dinding sel bakteri. M. tuberculosis juga dapat tetap bertahan di dalam fagolisosom dengan cara menghambat asidifikasi dan menghambat aktivitas NOX2 dalam memproduksi ROS. Kemampuan ini memungkinkan bakteri ini untuk menghindari autofagi di dalam makrofag. Pengetahuan mengenai kemampuan bakteri ini dalam memanipulasi dan menghindari sistem imun host, terutama makrofag sangat berperan dalam pengembangan obat-obatan dan vaksin terhadap M. tuberculosis di masa mendatang.

Kata kunci- makrofag, Mycobaterium tuberculosis, penghindaran sistem imun

I. INTRODUCTION

The most common cause of death globally and one of the oldest illnesses known to humans, tuberculosis, is brought on by the bacteria Mycobacterium tuberculosis. Globally, in 2021 an estimated 10.6 million people suffered from tuberculosis. Deaths from tuberculosis in 2021 are estimated to be 1.4 million in HIV-negative and as many as 187,000 deaths in HIV-positive patients.[1]

M. tuberculosis is an intracellular pathogen spread by inhaling bacterial aerosol particles. In particular, macrophages, dendritic cells, monocytes, and neutrophils are prepared to phagocytize these microbes as part of the innate immune system in the lungs. In the initial phase of the innate immune reaction, local macrophages are attracted to the infection site, where bacterial phagocytosis will take place. This process causes a local immune response so that more and more immune cells will go to the infection's location, causing granulomas' formation. Granulomas can protect bacteria from further attack by the immune system and can last for decades.[2,3]

Macrophages are one of the defenses of the innate immune system against pathogenic bacteria. However, *M. tuberculosis* can escape from the immune system. [4] *M. tuberculosis* can evade attacks by the host immune system and has developed strategies to infect it successfully. This paper will further discuss the evasion process of the acquired immune system by *M. tuberculosis*, especially against macrophages.

II. LITERATURE REVIEW

CHARACTERISTICS OF MYCOBACTERIUM TUBERCULOSIS

Aerobic, non-spore, and non-motile describe the bacillus known as Mycobacterium tuberculosis. These bacteria have a cell wall made of N-acetyl muramic acid and mycolic acid, ranging in size from 0.2 to 0.6 m. (70-90 carbon). The DNA has a high G+C content (61–71 mol%) and a lengthy cleavage period of roughly 20–36 hours.[5]

A pathogen that lives inside cells, M. tuberculosis can endure in host macrophages. The thick mycolic acid cell wall of this bacterium hinders the slow absorption of nutrients, which slows the growth of these bacteria. However, this thick mycolic acid makes bacteria more resistant to lysosomal enzyme-induced destruction. Mycolic acid is present in the outer layer, while arabinogalactan, phosphatidyl-myo inositol mannosides (PMIs), and peptidoglycan comprise most of the mycobacterium in the inner layer. In addition to mycolic acid, the outer layer contains mannoglycoprotein, lipomannan, and mannose-capped lipoarabinomannan (Man-LAM). Mycobacteria have mannan and arabinomannan on their outside, making up their outer capsule.[6]

With a tripartite structure in its carbohydrate core, MPI anchor, and different mannose capping patterns, Man-LAM is a heterogeneous lipoglycan. Man-LAM is a product of the glycosylation of PIM, which uses mannose (mannan) and arabinose (Arabin) residues. Man-LAM is a critical component of MTB virulence. All pathogenic mycobacteria have this mannose-capping motif.[6]

M. TUBERCULOSIS INFECTION MECHANISM

Only a small number of *M. tuberculosis* droplets from infected individuals reach the alveoli; most are trapped in the upper respiratory passages and swept out by ciliated mucosal cells. Then, using complement receptors, mannose receptors, or type A scavenger receptors, mycobacteria attach to the alveolar cell surface of macrophages. After phagocytosis, mycobacteria can survive in macrophage phagosomes by various mechanisms, such as

inhibiting phagosome maturation, reducing acidity in the phagosome, and avoiding autophagocytotic. After successfully inhibiting phagosome maturation, bacterial multiplication begins, and eventually, the macrophages will rupture and release mycobacteria which will be phagocytosed again by other macrophages. This cycle will repeat until the spread of bacteria is expanded.[4,5,9]

Dendritic cells or monocytes will carry *M. tuberculosis* to the lung lymph nodes to activate T cells. This action will recruit immune cells, including T and B cells, into the lung parenchyma cells to form granulomas. Bacteria will replicate actively within the granuloma; the granuloma will fail to harbor the bacteria. *M. tuberculosis* will spread to other organs, including the brain, through the blood vessels or re-enter the respiratory tract so that it can infect other humans. This phase is highly infectious and causes symptoms; the patient is referred to as sick with TB.[10]

IMMUNE RESPONSE TO BACTERIAL INFECTION

The body's immune system may identify encroaching invaders through cell surface or cytosol receptors, also called pattern-recognition receptors (PRRs). In addition to serving as a particular molecular detector in bacteria, PRR triggers phagocytosis and opens up signaling pathways that produce cytokines and chemokines. Pathogen-associated molecular patterns (PAMPs) from viruses, bacteria, fungi, and parasites are recognized by Toll-like receptors (TLRs). The Myddosome, a multiprotein signaling complex, is formed by the recruitment of particular adapter molecules (such as MyD88) by the TLR during the detection of each PAMP. TLRs' signals will affect the release of pathogen-killing inflammatory cytokines, chemokines, and antimicrobial peptides.[2,4]

Nucleotide oligomerization domain (NOD)-like receptors are cytoplasmic PRRs that recognize PAMPs and TLRs. NOD1 and NOD2 detect bacterial peptidoglycan and activate the NF-kB and MAP kinase (MAPK) pathways to cause inflammation. NOD proteins can assemble into multiprotein inflammasomes. This complex is activated by identifying pathogenic molecules in the intracellular and extracellular compartments, causing caspase-1 and pro-IL-1B to become IL-1B. These cytokines help macrophages fuse their phagosomes and lysosomes to destroy bacteria. They also start the initial inflammatory response.[2,4,5]

IMMUNE RESPONSE TO M. TUBERCULOSIS INFECTION

Recognizing pathogens is the first step in triggering the innate immune system as a defense mechanism in host cells. M. tuberculosis's pathogen-associated molecular pattern (PAMP) is detected by several receptors that control bacterial uptake by opsonization or non-opsonization. C-type lectin (mannose receptor, DC-SIGN, Dectin-1, Dectin-2, Mincle) is one of these receptors, as are complement receptors (complement receptor 3), collectin (surfactant proteins A and D, Mannose-binding lectin), scavenger receptors (MARCO, SR-A1, CD36, SR-B1), Fc receptors (FcgR), glycophosphatidyl-inositol (TLR-2, TLR-4, TLR -9). In M. tuberculosis, surface antigens such as phosphatidyl-inositol mannosides (PIM), phenolic glycolipids (PGL), trehalose dimycolate (TDM), peptidoglycan, and other bacterial components are identified by receptors on the cell surface and phagocytosis that is mediated intracellularly. Additionally, PRR can detect DNA or bacterial second messengers in the cytosol, such as cGAS and STING, which can trigger the production of cytokines farther down the signaling pathway and regulate autophagy. The muramyl dipeptide is one of the PAMPs recognized by the cytosolic PRR known as

the nucleotide domain-like receptor (NLR) oligomerization.[4,11,12]

The TLR signaling pathway significantly influences immunity against M. tuberculosis. Mice deficient in TLR adapter proteins, such as MyD88, are susceptible to M. tuberculosis infection. This is likely caused by decreased NOS2 expression, impaired IL-1 activation or IL-1 receptor pathway activation, impaired IFN- signaling to macrophages, and impaired macrophage responsiveness to IL-12 and TNF-. By ligand-receptor binding to IL-1 receptor-associated kinase (IRAK) and activating several downstream pathways, such as NF-B, mitogen-activated protein kinase (MAPK), and activator protein 1, MyD88 signaling integrates signals from TLRs and IL-1 receptor families (AP1).[4,13]

Macrophages can destroy infections by generating reactive oxygen and nitrogen species, acidifying the phagosome, and fusing the phagosome with the lysosome. M. tuberculosis, however, has several ways to stop this action once it has entered the cell; one is by preventing phagolysosome union. T lymphocytes, which are activated by macrophages, also perform a protective role against M. tuberculosis. Through receptors that bind to the lip-glycan lipoarabinomannan (LAM) on the surface of bacterial cells, dendritic cells that serve as a surveillance system around the airways, arteries, and connective tissue will identify bacteria. Through TLR-2, this lipid adjuvant from LAM activates APC. To eliminate intracellular M. tuberculosis, dendritic cells, and APC will activate CD4 and CD8. Without needing APC activation, natural killer (NK) cells also operate as a bactericidal against M. tuberculosis. Monocytes and other macrophages are activated by the IFN, TNF, and interleukin-2 released by T cells that have been activated. IFN stimulates macrophages to produce more TNF, hazardous oxygen species, and nitric oxide, promoting granulomas' development.[5]

Infection with M. tuberculosis necessitates strict regulation of cytokine synthesis, both pro-inflammatory and anti-inflammatory. Granuloma development is significantly influenced by TNF-α, IFN-γ, and IL-1, and also by IL-10, which is a non-inflammatory response. A pro-inflammatory cytokine called TNF- encourages the development of granulomas, whereas IFN- encourages antigen presentation, the attraction of CD4+ cells, and the activation of cytotoxic T lymphocytes to mediate mycobacterial death. The primary producers of the pro-inflammatory cytokine IL-1 are dendritic cells, macrophages, and monocytes.[3]

When M. tuberculosis is present, macrophages and T cells produce the anti-inflammatory cytokines IL-10 in contrast to pro-inflammatory cytokines. By lowering TNF expression and T-cell IFN production, IL-10 inhibits macrophage activity and aids in the survival of M. tuberculosis.[3]
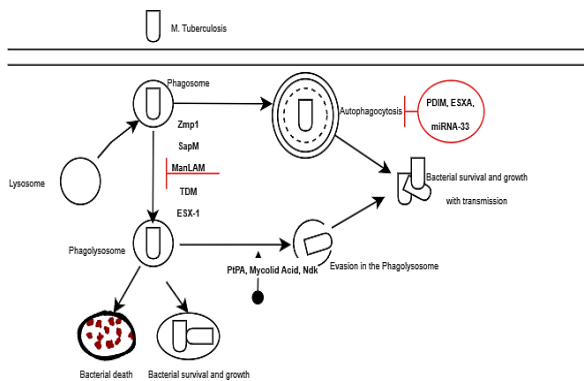
MECHANISM OF M. TUBERCULOSIS IMMUNE EVASION IN MACROPHAGES

INHIBITION OF PHAGOSOME MATURATION IN MACROPHAGES

Macrophages are the first line of defense against *M. tuberculosis* by phagocytosis. Elimination occurs by the fusion of phagosomes with lysosomes which contain acid and hydrolytic enzymes. However, by preventing phagosome maturation and phagolysosome formation, *M. tuberculosis* can live and reproduce in phagosomes through various mechanisms.[4,14,15]

Macrophages can recognize pathogens through PAMPs on the surface of bacterial cells and bind to receptors on the surface of macrophage cells. The bond between the receptor and the ligand will cause the activation of intracellular signaling resulting in a re-arrangement of the actin cytoskeleton, forming a pseudopod. This pseudopod will surround the bacterium and create a

phagocyte cup to internalize bacteria into the phagosome. The proteins needed for maturation will be successively engulfed and fragmented by this phagosome as it interacts with endocytic organelles (forming early and late endosomes and lysosomes). Phagosome maturation is the process that is carried out because the early phagosome cannot eliminate foreign particles.[16]

FIGURE 1. MECHANISM OF M. TUBERCULOSIS IMMUNE EVASION IN MACROPHAGES

The early phagosome gradually becomes acidified as it matures to create the ideal conditions for breaking foreign particles. Ras-associated binding GTPase (Rab GTPase), vacuolar ATPase (V-ATPase), acid hydrolase, acid protease, and major histocompatibility complex (MHC) class II components are just a few of the proteins that are involved in this process. Phagolysosomes are created when lysosomes fuse with the phagosome. They have an acidic atmosphere and are oxidized so that they can use different hydrolytic enzymes to destroy bacteria. The cooperation of Rab proteins, including Rab5 and Rab7, is necessary for phagosome maturation. This protein functions as a regulatory control for various endosome and phagosome phases.[16]

Several proteins in *M. tuberculosis* are essential in inhibiting this phagosome maturation process, including Rv0198c (Zmp1). Through the suppression of caspase-1, this secretory protein functions as a Zn metalloproteinase and prevents the conversion pro-IL-1 into IL-1.[14] This leads to suppressing IL-1β-mediated inflammatory activation and inhibiting phagosome maturation. Research has shown that suppression of zmp1 will restore caspase-1 activation, IL-1β production, and phagosome maturation into phagolysosomes. Research also indicates that the administration of IL-1β can increase the clearance of bacteria in phagolysosomes. Exotoxins or proteosomes like SapM (Rv3310), acid phosphatase/phosphomonoesterase, and serine/threonine kinase can also prevent phagolysosome union (PknG-Rv0410c).[3,4,14]

Lipoarabinomannan (LAM), one of the components of cell walls, was also found to block phagolysosome fusion. This inhibition occurs by releasing the enzyme which works against host PI3P, such as phosphoinositol-3-phosphate phosphatase; therefore, the fusion between the phagosome membrane and lysosome does not happen. ManLAM blocks Ca2+/calmodulin- and Rab5-dependent phosphatidylinositol 3-kinase hVPS34 recruitment to mycobacterial phagosomes. ManLAM activates p38 MAP kinase, which phosphorylates GDI (GDP dissociation inhibitor) to inhibit Rab5 function. Increased Rab5 recall inactivates it.[8]

Trehalose dimycolate (TDM) is a cell wall component that delays phagosome maturation. CLR, TLR2, CD14, and the scavenger receptor MARCO recognize TDM. Through syk/CARD9 signaling in the bone marrow that produces macrophages in mice (mBMDM), TDM modulates the signaling that initiates pro-inflammatory cytokines (TNF- and NO) and anti-inflammatory cytokines (IL-10). Other virulence factors inhibit pathogen recognition receptor (PRR) signaling, specifically TLR2 signaling. Among them are 60kDa chaperonin-2 (GroEL2), sulfated glycolipids, ESAT-6, and lipoprotein LpqT.[8,14-17]

A secretory system of *M. tuberculosis* called ESX-1 is also known to mediate the

disruption of phagosome integrity and prevent its maturation. This system is thought to be mediated by ESAT-6. ESX-1 mediates phagosome permeabilization, so the PAMP of *M. tuberculosis* (N-glycolyl-muramyl dipeptide) will be exposed to NOD2 receptors (nucleotide oligomerization domain 2) in the cytoplasm, which will induce IFN type 1. Phagosome permeabilization also will make DNA *M. tuberculosis* exposed to the DNA-sensing pathway in the cytosol, activating autophagocytosis.[18,19]

M. tuberculosis employs an additional strategy to manipulate the innate immune system indirectly. *M. tuberculosis* recruits TACO1/CORO1A, a host protein, to its phagosome, thereby preventing phagolysosome fusion. Coronin-1 is evenly distributed in the cytosol and membranes of non-infected macrophages and is specific to alveolar macrophages. Coronin-1 is recruited exclusively to the phagosome membrane encircling *M. tuberculosis* that has survived within macrophages. Coronin-1 is initially co-internalized in macrophages containing dead *M. tuberculosis* but swiftly dissociates from the phagosome. Mycobacteria are degraded due to the uncoated phagosome fusing with the lysosome. The activation of Ca2+-dependent phosphatase and calcineurin requires coronin-1. Activation of calcineurin during *M. tuberculosis* internalization inhibits phagolysosome fusion through an unknown mechanism.[14,17-21]

EVASION IN THE PHAGOLYSOSOME OF MACROPHAGES

Phagolysosomes have an acidic pH (less than 4.5) due to the transport of H^+ protons in large numbers into the lumen mediated by V-ATPase in the phagosome membrane. Phagolysosomes also contain the NADPH oxidase complex (NOX), which generates ROS, superoxide anion ($O2^{*-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^*). In addition, the phagolysosome

compartments also have antimicrobial peptides active at low pH, such as hydrolytic enzymes, glycosides, DNAse, cathepsin, protease, lysozyme, and lipase play a role in the destruction of microbes in phagolysosomes.[16,22]
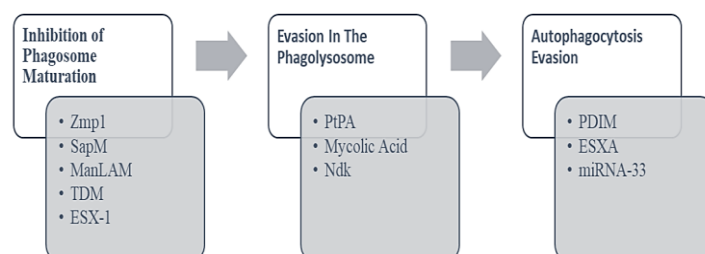

DIAGRAM 1. MECHANISM IMMUNE EVASION OF M.TUBERCULOSIS

PtpA inhibits the recruitment of vacuolar proton-ATPase into phagosome membranes containing mycobacteria upon activation. PtPA binds covalently to the H subunit of V-ATPase, preventing lysosomal acidification despite lysosomal membrane fusion-related molecular markers (CD63, LAMP-1, LAMP-2, transferrin receptor, and Rab5) on the membrane surface.[14,16] *M. tuberculosis* is intracellular bacteria that also survive in an acidic environment in phagolysosomes due to its intrinsic characteristics. Mycolic acid creates a thick, waxy cell wall as a physical barrier against proton entry in acidic environments. Genes that regulate cell wall structure, such as the mymA operon, are upregulated when exposed to an acidic environment. In addition, the production and storage of triacylglycerol increase when *M. tuberculosis* is stressed in an acidic environment. Mycolic acid creates a thick, waxy cell wall that offers protection. During the latent phase, triacylglycerol can also serve as an energy source for bacilli, which is an essential survival strategy.[14]

M. tuberculosis also inhibits NADPH oxidase (NOX_2) in phagolysosomes. NOX_2 will produce reactive oxygen species (ROS), which can kill intracellular pathogens. Two transmembrane proteins, four cytosolic subunits, and a GTPase make up the NOX_2

complex. NOX_2 requires membrane translocation of these subunits to function correctly. The interaction of these NOX subunits activates gp91^{phox}, resulting in the formation of superoxide in the phagosome. Nucleoside diphosphate kinase (Ndk) of Mycobacterium tuberculosis interacts with Rac1 and deactivates it, thereby preventing the joining of the NOX_2 sub-protein; this also inhibits apoptosis.[23]

The entry of *M. tuberculosis* to macrophages via different receptors will activate pathways that inhibit the replication of various bacteria. Recognition of mycobacterial ManLAM by TLR-2 activates the transcription genes NF-kB and NOS2, leading to nitric oxide (NO) production as an anti-mycobacterial. However, *M. tuberculosis* has catalase-peroxidase, KatG, which can inactivate reactive oxygen in the phagosome. *M. tuberculosis* also has a proteasome that mediates resistance to nitrosative stress.[4]

Through the interaction of tyrosine phosphatase, PtpA, with the ubiquitin system, *M. tuberculosis* has an additional intracellular survival mechanism. PtpA inhibits innate immunity by interacting with TRIM27, a host protein. According to reports, this protein has an inverted RING domain and typically functions as an E3 ubiquitin ligase that promotes apoptosis in macrophages. [14]

AUTOPHAGOCYTOSIS EVASION IN MACROPHAGES

M. tuberculosis's cell wall contains lipids contributing to its pathogenesis, specifically phtiocerol dimycocerosates (PDIM). The transcription repressor Rv3167c inhibits the expression of PDIM in M. tuberculosis. This gene deletion increases phagosomal escape, autophagocytosis, and host cell necrosis compared to wild-type tuberculosis cells. PDIM is synthesized in the cytosol and transported to the outer membrane by the lipid transporter mml7 in *M. tuberculosis*. Compared to the *M. tuberculosis* wild type, deletions of this transporter-encoding gene significantly reduced cell mortality. PDIM contributes to phagosomal escape, which is also related to the activity of the ESX-1 substrate EsXA. PDIM and EsXA cooperate to assist *M. tuberculosis* in escaping the phagosome. EsXA creates a pore in the phagosome membrane, while PDIM may weaken the phagosome membrane, allowing microbes to escape more easily.[17]

Cytoplasmic constituents are degraded through autophagocytosis. The function of autophagocytosis within the anti-mycobacterial immune system is well understood. There is evidence that autophagocytosis is a host response to *M. tuberculosis* phagocytosis, which initiates with the recognition of *M. tuberculosis* DNA via STING-dependent cytosolic sensing and induction by IFN-γ. *M. tuberculosis* is also reported to have a strategy to avoid this autophagocytosis. It has been reported that *M. tuberculosis* expresses microRNA-33, inhibiting autophagocytosis and regulating intracellular lipid metabolism, which benefits bacterial replication.[4,23,24]

III. CONCLUSION

M. tuberculosis is an intracellular pathogen transmitted by inhaling aerosol droplets containing bacteria. *M. tuberculosis* can evade the body's immune system, including against macrophages. *M. tuberculosis* can avoid inhibition of phagolysosome maturation so that fusion between phagosomes and lysosomes does not occur. *M. tuberculosis* also can prevent phagosome acidification to the ability to avoid autophagocytosis. The ability of *M. tuberculosis* to evade the immune system, especially macrophages, can be used as a reference in approaches to developing drugs and vaccines. Further research is needed to determine the best approach to fight the ability of *M. tuberculosis*.

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