

Review Article

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## Host Pathogen Interaction-Mycobacterium and Host Macrophages

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### ABSTRACT

*Mycobacterium tuberculosis* (MTB) is an intracellular parasite that mainly attacks macrophages and establish a niche to survive in macrophages. It can become a long-term infection in humans and animals, causing a series of pathological changes and clinical manifestations. In this article, we summarizes how mycobacterium evades innate immunity and establish a niche in the host cell macrophages. Firstly it evades from the immune detection by masking, it manipulates the TLR response, manipulation of the antigen presentation by MHC, inhibition of phagosomal maturation in which Iron, Hydrogen and Calcium ions down regulation play an important role and phagosomally sosomal fusion, Inhibition of acidification of Phagolysosomes, Inhibition of Oxidative Stress and the Function of Reactive Oxygen and Reactive Nitrogen Intermediates, and inhibition of the apoptosis and autophagy of macrophages. A thorough understanding of this host pathogen interaction and thus immune escape of Mycobacterium an intracellular pathogen is of major importance for the prevention and diagnosis of bovine tuberculosis.

#### Keywords

MTBC, TLR,  
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### Introduction

*Mycobacterium tuberculosis* and *Mycobacterium bovis* are the most commonly encountered members of the *Mycobacterium tuberculosis* complex (MTBC) species worldwide. The former is the main causative agent of TB in humans, and the latter is responsible for the disease mainly in animals (1,2). Transmission of tuberculosis caused by

both pathogens can occur from human to animals and vice versa (3,4). In developing countries like India the dietary habit of people, close physical contact between humans and animals, rise in the incidence of immunosuppressive diseases, and inadequate disease control measures in animals and humans facilitate the transmission of the disease between animals and humans (5). Since both are the intracellular pathogens so

follow same strategies of immune escape. Diagnostic methods such as tuberculin skin test (TST) and cell-mediated immune response-dependent approaches were developed based on the current understanding of the mechanisms that contribute to the establishment of persistent infection (6). The latest developments in understanding the cellular, biochemical, and molecular mechanisms that are employed for the establishment of latent stage by Mtb are discussed in this review.

MTB is the pathogen that causes tuberculosis. Healthy ones can be infected via the respiratory tract, the digestive tract, damaged skin and mucous membranes (7). Inhalation of droplets containing MTB is the main route of infection. So how this pathogen interacts with the host cell macrophages and downregulates the host immune response is discussed in this review.

### **Immune escape mechanism of *Mycobacterium***

#### **Evading immune detection by masking**

*Mycobacteria* adopt multiple strategies to avoid the attack from macrophages its cell membranes contain methyl branched-chain fatty acids that protect them from host enzymes and enable them to escape immune responses (8). They express surface lipids such as phthioceroldimycoserate, which can mask the pathogen-associated molecular patterns (PAMPs), thereby going “unnoticed” by the innate immune system (9). In the upper airway where a constant and heavy recruitment of macrophages occurs due to the presence of TLR stimulating bacteria, Mtb adopts a different immune evasion strategy by forming small infection droplets that allow them to be delivered directly into the alveolar spaces of the lower lung, which anchorages a few microbicidal macrophages (10).

Interestingly, *Mycobacterium* is capable of modulating PPAR $\gamma$  to assist its pathogenesis by manipulating its function in lipid metabolism: mycobacteria use the hosts’ lipids for intracellular survival and replication (11). MTB infection increases the expression of peroxisome proliferator-activated receptor gamma PPAR $\gamma$  via mechanisms including pattern recognition receptor activation, overexpression and activation resulting in increasing lipid droplet formation and downregulation of the macrophage response.

#### **Manipulating the TLR responses**

In the macrophages, which are the crucial niche for replication, Mtb interacts with various receptors to initiate phagocytosis. Despite the bactericidal properties of the macrophages, Mtb employs phagocytosis as a primary mode of gaining entry to establish the niche. Recognition of Mtb through its cell wall glycolipids involves the formation of TLR heterodimers (12). These heterodimers are formed for better ligand and receptor interactions. Exposure of THP-1 cells to Mtb cell wall components results in the de novo synthesis of TLR4, thereby decreasing the production of Th1 cytokines (13). Although Myd88-dependent signaling of TLRs is well established in mycobacterial pathogenesis, recent studies indicate independent roles for Mal (the TLR adaptor) and Myd88. TLR-4 by TRIF/TRAM pathway which is independent of Myd88 signaling increases the production of Th2 cytokines such as TGF- $\beta$ , IL-4, IL-10 which attenuate the defensive cytokine response.

Interaction of Mtb cell wall components with TLRs modulates a number of events that include antigen presentation (14), phagolysosomal fusion (15), apoptosis of macrophages (12), and production of reactive oxygen and nitrogen intermediates (16).

### **Manipulating the antigen presentation by MHC**

The TLR2-dependent surface expression of MHC class II receptor and their antigen-presenting ability was found to be inhibited by either Mtb infected or 19-kDa lipoprotein (LpqH) exposed macrophages (12, 17). Class II transactivator (CIITA), a TLR-2-dependent regulator of MHC class II  $\alpha$ ,  $\beta$ , invariant chains contributes to antigen processing and its expression was found to be decreased during Mtb infection (18,19).Mtb also inhibits the expression of genes involved in MHC class II processing and presentation (20,21) and the posttranslational function of these molecules.

### **Phagosomal maturation and Phagosomal/lysosomal fusion arrest**

Repeated stimulation of TLRs by the Mtb components such as mannosylated-LAM (ManLAM) and PIM causes phagosomal maturation arrest allowing persistence of mycobacteria inside the phagosome (22).Among the successful strategies adopted by Mtb to establish a niche in the host, inhibition of macrophage maturation is best characterized. The mycobacterial products (ManLAM, trehalosedimycolate, and sulfolipids), phosphatase SapM, kinase PknG, and early secretory antigenic target-6 (ESAT-6) have been implicated in the  $Ca^{+}$  surge that modulates the calmodulin- and  $Ca^{2+}$ /calmodulin- dependent kinase II-dependent delivery of early endosomal autoantigen1 (23, 24), EEA1 localizes exclusively to early endosomes and has an important role in endosomal trafficking. EEA1 binds directly to the phospholipid phosphatidylinositol 3-phosphate through its C-terminal FYVE domain.EEA1 is necessary for the delivery of lysosomal hydrolases and vacuolar H<sup>+</sup>-ATPases into phagosomes. ManLAM blocks

ESAT-6 recruitment by inhibiting PI3K hVPS34 to block PIP3 production. SapM also decreases PIP3 production. Phosphatidylinositol 3-phosphate (PI3P) is an important component of the macrophage cell membrane located on the early endosome and phagosome surface. PI3P helps in the fusion between phagosomes and lysosomes. Down regulation of PI3P by mycobacterium suppresses the process of fusion. LAM reduce the levels of Rab5 the gene responsible for EEA1 recruitment and the gene responsible for phagosome and lysosome fusion.Mtb also disrupts the scaffolding of endosomes required for phagosome–endosome interactions leading to delay in phagosomal maturation (25,26).

PknG is a protein similar to protein kinase in eukaryotes. PknG enhances MTB metabolism, growth rate, virulence and drug resistance. PknG secreted by MTB prevents the fusion of phagosomes and lysosomes by enhancing signal transduction in host cells.

### ***M. tuberculosis* inhibits the acidification of phagolysosomes**

MTB inhibits the maturation of phagocytosis by suppressing the acidification of phagosomes and then persists in the relatively lower acidic environment (pH~6.2) (27). First, MTB inhibits phagosome acidification by changing its composition; the structure and specific molecules on the cell wall serve as a barrier, allowing the macrophages to maintain a neutral pH (27). Second, the protein phosphatase A (PtpA) downregulates V-ATPase pump and plays direct role in the acid inhibition (28). Besides, infection of macrophages with MTB leads to the secretion of granulocyte-macrophage colony-stimulating factor, triggering the expression of cytokine-inducible SH2-containing protein (CISH) through mediation by STAT5. V-ATPase catalytic subunit A can also

ubiquitinate and degrade proteasomes by producing CISH.

### ***M. tuberculosis* inhibits oxidative stress and the function of reactive oxygen and reactive nitrogen intermediates**

Oxidative stress is a disorder of pro/antioxidant balance, resulting in potential damage. Underlying the prolonged MTB latency in the host is not only the inhibition of macrophage phagocytosis, lysosome maturation and acidification but also the inhibition of oxidative stress.

ROS is highly toxic to bacteria as it can either directly destroy DNA, protein, and lipids or indirectly damage the nucleic acid via oxidation of the nucleotide pool (29). SigH, mshD, Wag31 gene of mycobacterium protects it from heat, oxidative and nitric oxide stresses. The KatG and TrxB2 enzymes of MTB help to resist ROS (30). TRIM/TRAM pathway leads to the formation of TH2 cytokines which downregulates IL-12. IL-12 is required for the production of interferon- $\gamma$ , iNOS, and NO, a major defense of the host against Mtb. The intracellular pathogen secretes SucB, AhpC, and AhpD, which catalyzes the breakdown of reactive nitric intermediates (RNIs).

### ***M. tuberculosis* inhibits apoptosis and autophagy**

Invasion by MTB and host cell apoptosis require cellular factors, signaling proteins and regulation of the pathways involving TNF- $\alpha$ , IFN- $\gamma$ , transforming growth factors and IL-6, IL-12, IL-4 and IL-10 (31). NKT cells can produce and release IFN- $\gamma$  to inhibit the growth of MTB in macrophages (32). Macrophages infected with Mtb have upregulated IL-6 production, which selectively inhibits IFN- $\gamma$ -induced autophagosome biogenesis. In addition, Mtb possess the “enhanced intracellular survival”

(Eis) gene, which attenuates autophagy and improves Mtb survival. Mtbup regulates the microRNAs miR-23a-5p, miR-20a, and miR-33, which prevent the activation of autophagy. miRNAs are a class of noncoding small single-strand RNA molecules (~22 nucleotides in length) that play a critical role in macrophage function. Overexpression of miR-30A suppresses the elimination of intracellular MTB and this is achieved by inhibiting autophagy.

In the conclusion we can say that upregulation of EIS gene and micro RNA and attenuation of IFN- $\gamma$  induced autophagosome biogenesis block autophagy and leads to *Mycobacterium* survival.

DCs and macrophages are the main components of the first line of defense against MTB and they can also maintain the complementary function of eliminating infectious bacteria (33). Surprisingly, infection by MTB is mainly based on cellular immunity, while the role of humoral immunity is controversial (34).

In conclusions, this review will systematically able us to understand why some genes are more expressed in active tuberculosis and why some of them are more expressed in the latent tuberculosis. Studying the above host pathogen interaction till date we can able to explore the potential of FYVE domain, Rab-5GTPase, STAT-5, CISH, IL-10, MHC-II, MyD88, TLR, mir-30A, iNOS and many of the above discussed cytokines as a biomarker, to distinguish between active and latent tuberculosis in animals. In future we can also target more genes like PPAR $\gamma$  not studied yet for bovine tuberculosis. So in this review we studied the interaction of host macrophages and the pathogens, the immune response of host and its escape by the pathogen this will help us in the bovine tuberculosis diagnosis and treatment.

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