Mycobacterium tuberculosis and the host macrophage: maintaining homeostasis or battling for survival?

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*Mycobacterium tuberculosis* is endowed with the ability to persist within its intracellular niche for years, often tilting the balance of the host–pathogen interaction in its favour. The host resists infection by releasing damaging free radicals, pushing the pathogen towards lysosomal degradation, releasing an arsenal of cytokines for triggering adaptive immunity and facilitating apoptosis for effective T cell antigen presentation. The bacterium counters these mechanisms and also metabolically reprograms the macrophage to its own benefit. Recent advances in these areas are reviewed here.

**Keywords:** Autophagy, cell death, innate immunity, *Mycobacterium tuberculosis*, macrophage.

**Introduction**

*Mycobacterium tuberculosis* (*Mt*) is one of the most successful intracellular pathogens responsible for chronic infection. It infects approximately one third of the human population. For the purpose of this review, our focus will be on pulmonary tuberculosis. Following inhalation, bacilli are internalized within the macrophages followed by an early proinflammatory response accompanied by recruitment of the NADPH complex at the phagocytic cup and the generation of reactive oxygen species (ROS) facilitate an antimicrobial response at the cell surface dependent on the superoxide-generating NADPH oxidase (NOX) family proteins, including the catalytic subunit NOX2/gp91phox. *Mt* actively avoids the detrimental effects of the transient superoxide burst using its superoxide dismutase and cell surface glycolipids that possibly scavenge oxygen radicals. Studies with the zebrafish model of infection by *M. marinum* suggest that the oxidative burst of the neutrophil could play a protective role in containing infection. Signals from dying infected macrophages within the granuloma facilitate recruitment of neutrophils, which then kill the internalized mycobacteria through NADPH oxidase-dependent mechanisms.

Household contacts of TB patients produce high amounts of bactericidal NO, suggesting a likely protective role of NO. However, while NO is strongly induced in murine macrophages during infection, isolated human macrophages fail to do so. It is important to emphasize that for want of a better method of analysing the initial events of *Mt* infection, cultured macrophages have been the tools of choice. Nonetheless, the macrophage in culture is not the equivalent of the tissue macrophage in its

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milieu. Further, the differences in behaviour between human and mouse macrophages may indeed reflect differences in macrophage immune mechanisms between the two species.

Among the mechanisms used by Mycobacterium spp. to persist in the host is to exploit the production of arginase 1 by macrophages. Mice deficient in Arg1 control TB more efficiently than wild type mice15,16. MyD88-dependent production of the cytokines IL-6, IL-10 induces STAT3-dependent autocrine–paracrine synthesis of arginase 117. Recent studies demonstrate that NO suppresses IL-1β production by inhibiting the NLRP3 inflammasome18. NO-dependent S-nitrosylation of NLRP3 is independent of the antimicrobial function of NO.

**Arrest of phagosomal maturation**

The bacterium must arrest phagosomal maturation in order to escape lysosomal degradation. Mycobacterial lipoarabinomannan (LAM) plays a prominent role in this process. Mannose-capped LAM (ManLAM) modulates protein trafficking pathways associated with phagosome maturation arrest19. It incorporates into lipid rafts20 and inhibits phagosomal maturation in macrophages21. Phosphatidylinositol 3-phosphate (PI3P) recruits the endosomal tethering molecule EEAA1 to the endocytic organelles22,23, whereas ManLAM inhibits the recruitment of EEAA1 to phagosomal membranes through a block in [Ca²⁺] rise24.

Pathogenic mycobacteria counter phagosomal acidification25 by excluding the proton pump from mycobacterial phagosomes26. The notion that Mtb remains within intracellular phagosomes has been challenged. In dendritic cells (DCs), Mtb resides in a compartment that is positive for the lysosome-associated membrane proteins LAMP-1, LAMP-2 and CD63, and the lysosomal aspartic protease cathepsin D27. The ESX-1 secretion system is required for phagosomal escape28,29. Recent studies show that inhibition of the Abl tyrosine kinase by imatinib reduces lysosomal pH and limits the multiplication of Mtb in macrophages30,31.

**Metabolic reprogramming as a result of interplay between Mtb and the macrophage**

The ‘foamy’ phenotype of macrophages infected with Mtb is characterized by accumulation of intracellular lipid bodies32, which serve as a reservoir of nutrients in the form of fatty acids33. Exemplary of metabolic reprogramming of the Mtb-infected macrophage is the accumulation of triacylglycerol (TAG). Mtb utilizes host TAG-derived fatty acids to synthesize mycobacterial lipids34. Reduction in intra-phagosomal lipolysis correlates with increase in the retention of host lipids in the infected macrophage35. Utilization of host TAG therefore helps in establishment of a persister population of Mtb. ESAT-6 triggers a metabolic pathway activating a G protein coupled receptor (GPR109A) leading to reduction in cellular cAMP levels and culminating in reduced turnover of TAG and enhanced lipid body formation. Mycobacteria released into these lipid bodies are protected from the host microbicidal pathways36. Mtb reprograms its own metabolic pathways to relieve the pressures arising out of the generation of propionyl CoA during utilization of cholesterol and fatty acids by the bacterium. The bacterium exploits pathways which allow incorporation of propionyl CoA into methyl-branched lipids in the cell wall37.

**Pattern recognition receptors and their mycobacterial ligands**

Central to the innate immune system are the germline-encoded pattern recognition receptors (PRRs) which are expressed on innate immune cells and sense pathogen-associated molecular patterns (PAMPs)38,39. The membrane-bound receptors include the mannose receptor (MR or CD206), dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) (CD209) [expressed predominantly on dendritic cells], Dectin-1, Toll-like receptors (TLRs) and complement receptor 3 (CR3, CD11b/CD18). The MR binds to ManLAM of Mtb and creates an immunosuppressive environment partly through upregulation of PPARγ signalling40. Dectin-1, a β-glucan collaborates with TLR2 in the induction of cytokines in response to Mtb41.

When Mtb is internalized by alveolar macrophages or other innate cells, it encounters the TLRs42,43. TLR2 partners TLR1 or TLR6 to recognize bacterial components (of which the best studied ones are the lipoproteins) to trigger canonical NF-κB and MAPK signalling. Among its ligands are the mycobacterial 19-kDa lipoprotein, gangliosides like lipomannan (LM), 38-kDa antigen, LprG lipoprotein and phosphatidylinositol mannoside (PIM)44. TLR signalling is required for activation of the vitamin D receptor by 1,25-dihydroxyvitamin D3 (converted from vitamin D3 by CYP27B1) and synthesis of the antimicrobial peptide cathelicidin (or LL-37)45. The transcription factor NFAT5 is activated by TLR signalling and the co-infection of HIV-1 in tuberculosis accelerates an increase of viral load through expression of NFAT5 (ref. 46). TLR4 is activated by heat shock protein 60/65 and 38-kDa antigen. TLR9 recognizes unmethylated CpG motifs of mycobacterial DNA47. In mouse models, TLR9 knockout mice display defective mycobacteria-induced interleukin (IL)-12p40 and interferon IFN-γ responses48. However, neither TLR2 nor TLR9 knockout mice showed substantial changes in resistance to low dose pathogen challenge. TLR2/9 knockout mice displayed markedly enhanced susceptibility to infection and altered pulmonary pathology.
Figure 1. Overview of the regulation of macrophage signalling pathways by M. tuberculosis through pattern recognition receptors. Mycobacteria or its effectors bind to a variety of cell surface as well as intracellular receptors and induce signalling pathways leading to activation of transcription factors including NF-κB, PPARγ or IRF. Regulators like IRAK-M negatively regulate the activation of NF-κB in order to control time-dependent activation of cytokine release. Mtb also induces NLRP3 mediated activation of caspase 1 and subsequent conversion of pro-IL-1β to IL-1β.

Trehalose dimycolate (TDM) an important cell surface component of Mtb is tethered to several receptors, including TLR2, the class A scavenger receptor MARCO, Fc receptor-γ (FcRγ) and macrophage-inducible C-type lectin (Mincle). TDM has been reported to trigger MARCO/TLR2/CD14-dependent signalling to produce proinflammatory cytokines. It also activates macrophages and dendritic cells via FcRγ-Syk-Card9 pathway by signalling through Mincle.

Other than the activating signals arising out of ligation of TLRs, Mtb is endowed with the ability to dampen TLR signals using a variety of mechanisms. ManLAM suppresses TLR4-driven IL-12p40 induction by virtue of its ability to induce IRAK-M, a kinase-dead variant of the IRAK family which negatively regulates the classical NF-κB pathway. Basu and coworkers have demonstrated that exogenous ESAT-6 downregulates MyD88-dependent TLR signalling. ESAT-6 and CFP-10 also downregulate LPS-induced ROS production. The zinc metalloprotease Zmp1 and the cell envelope-associated serine hydrolase Hip1 dampen activation of the inflammasome. These are a few of the examples of mycobacterial effectors that dampen proinflammatory signalling. An overview of mycobacterial regulation of some innate immune signalling pathways through PRRs is summarized in Figure 1.

The mycobacterial genome is characterized by the presence of the unique PE/PPE family of proteins which have highly conserved proline–glutamate (PE) and proline–proline–glutamate (PPE) residues near the N-termini. The pep/ppe families have coevolved with the esx genes. Several of these proteins are surface-localized and could also be localized to the cell envelope as part of a secretory system. It is likely that the surface localized PE/PPE proteins interact directly with macrophages. For example, Rv1759c has fibronectin-binding properties. Other members of the family, Rv1818c, Rv1787 and Rv3018 influence virulence and survival of Mtb in macrophages. Some of the PE/PPE family members elicit B- and T-cell responses. The PPE18 protein signals through TLR2 to activate IL-10 production and dampen NF-κB signalling by upregulating SOCS3. It is likely that many more members of the family fulfill varied roles in mycobacterial pathogenesis.
The NOD-like receptors (NLRs) and RIG-like receptors (RLRs) cooperate with the TLRs in innate immunity. The NLRs NOD1 and NOD2 respond to peptidoglycan fragments to activate NF-κB. The NLRs NLRP1 and NLRP3 respond to bacterial products to activate caspase-1. The NLRs NOD1 and NOD2 respond to peptidoglycan to activate NF-κB. The RLRs detect viral nucleic acid and activate interferon-regulated factor (IRF) family members. Type II IFNs (IFN-α and IFN-β) negatively regulate host resistance to Mtb in mice. The transcription factor IRF3 which is activated by phosphorylation by TBK1 is required for IFN-β induction. The Cox group suggests that the ESX-1 secretion system promotes escape of Mtb from the cytosol, followed by recognition of bacterial DNA by IFI204 (ref. 66). This stimulates the Sting/TBK1/IRF3 signalling axis leading to IFN-β induction. These results are in conflict with the findings of Pandey et al. which suggest that the type I interferon response depends on the recognition of mycobacterial peptidoglycan by NOD2 and NOD2/RLP2/IRF5 signalling. The role of DNA-dependent activation of STING/TBK1 signalling is shown in Figure 2.

Brooks et al. have shown that pre-treatment of human monocyte-derived and alveolar macrophages with the NOD2 ligand muramyl dipeptide enhances production of TNF-α and IL-1β in response to Mtb and BCG in a RIP2-dependent fashion. NOD2 controls the growth of both Mtb and BCG in human macrophages. Using a high-throughput shRNA-based screen NLRs and CARDs important for IL-1β secretion upon Mtb infection have been identified. NLRP3, ASC and caspase-1 form an infection-inducible inflammasome complex that is dependent on ESAT-6.

The protein CARD9 is important for NOD2-mediated activation of p38 and JNK. Macrophages from CARD9-deficient mice activate NF-κB normally in response to MDP, but p38 and JNK activation is inhibited. Card9 mice succumb early after aerosol infection.

In vivo studies showed somewhat discordant results in susceptibility of mice deficient in several TLR-related genes, including TLR2, TLR4, TLR6 or MyD88, in Mtb infection. However, genetic studies suggest a link between TLR signalling and susceptibility to disease in humans. I602S is a frequent single-nucleotide polymorphism of human TLR1 that greatly inhibits cell surface trafficking, confers hyporesponsiveness to TLR1 agonists and protects against leprosy and tuberculosis. rs352139, an SNP located in the intron of TLR9, is associated with tuberculosis susceptibility in Indonesian and Vietnamese populations. The TLR2 variant R753Q influences the progression of infection to TB disease in children. A reported role of the nonsynonymous SNP S180L (975C/T) in TIRAP in protection against TB results from attenuation of TLR2 signal transduction. Another polymorphic variant (558C/T) in TIRAP, discovered in the Vietnamese population, showed an association with TB meningitis but not pulmonary TB. An association of TLR8 with pulmonary TB has also been suggested. These associations strengthen the role of TLRs in innate immunity against TB.

Arachidonic acid metabolites and innate immunity

The arachidonic acid metabolites, eicosanoids, lipoxins and leukotrienes play crucial roles in the inflammatory response associated with mycobacteria-induced necrosis in macrophages. Prostanoids such as PGE2 induce plasma membrane repair and prevent mitochondrial damage; promoting apoptosis rather than necrosis. On the other hand, products of 5-lipoxygenase (5-LO) such as LXA4 inhibit cyclooxygenase 2 (COX2) production, shutting down prostaglandin synthesis. 5-LO knockout mice show resistance to Mtb infection. Activation of the 5-LO pathway inhibits Mtb-induced apoptosis, prevents cross-presentation of antigens by dendritic cells and therefore compromises the adaptive immune response.

LXA4 a product of the 5-LO reaction, is produced by macrophages after infection with virulent Mtb. Tobin et al. have shown that zebra fish lacking the leukotriene
A4 (LTA4) hydrolase enzyme show decreased transcription of TNF-α and an anti-inflammatory phenotype. SNPs in the leukotriene A4 hydrolase (ita4h) gene are associated with protection against tuberculosis, arguing in support of a role of ita4h in the human disease.

**Autophagy and Mtb infection**

During autophagy, cytoplasmic components are sequestered by double membranous structures which subsequently fuse to lysosomes for degradation generating substrates for energy metabolism and protein synthesis. Autophagy plays a role in defense against intracellular pathogens. Autophagy can be induced exogenously via starvation, treatment with IFN-γ or vitamin D3 or genetic depletion of inhibitors of autophagy resulting in decreased bacterial replication. A recent system-level analysis has identified the tyrosine kinase Src as a major regulatory hub which restricts phagosomal acidification and autophagy during infection. The prevalent view is that Mtb inhibits autophagy. This has been challenged in recent times by the Cox group, suggesting that the ESX-1 secretion system plays a role in eliciting autophagy leading to the targeting of Mtb to lysosomes. Recognition of cytosolic bacterial DNA by STING leads to the LC3 phagophore around the bacilli, a process requiring the binding adaptors p62 and NDP52 collaborating to form a double membranous structure which subsequently fuse to lysosomes for degradation generating substrates for energy metabolism and protein synthesis. Autophagy plays a role in defense against intracellular pathogens.

**Cellular death pathways triggered by Mtb**

*Mtb* can trigger both apoptotic and necrotic forms of cell death in macrophages. Mice with resistant *sst1* (super-susceptibility to tuberculosis-1) locus undergo apoptosis in response to *Mtb* infection. Apoptosis is associated with mycobacterial killing. Apoptotic vesicles enhance T cell responses via the ‘detour pathway’ of antigen presentation. At low multiplicities of infection (MOI), virulent *Mtb* strains undergo less apoptosis than attenuated strains. An ASK1/p38 MAP kinase driven pathway regulates caspase-8 dependent apoptosis in macrophages. Factors such as ManLAM, nuoG, secA2 (ref. 117), the OppABCD peptide transporter of *Mtb* and Rv3654c-Rv3655c, inhibit apoptosis. There are reports in favour of apoptosis-inducing factors of *Mtb* such as ESAT-6 (ref. 120) and apoptosis induced by *Mtb* is associated with mitochondrial membrane disruption.

At higher MOIs, virulent *Mtb* also triggers necrosis in macrophages. Chen et al. have proposed that cleavage of annexin-1 induced by *Mtb* leads to the inefficient formation of the apoptotic envelope and progression to necrosis. Wong and Jacobs have linked the ESX-1 secretion system and its substrate ESAT-6 to necrotic death in THP1 human macrophages in a process dependent on the Syk tyrosine kinase and NLRP3. At a high MOI of 10, ESAT-6 dependent, caspase-1 and cathepsin B-independent necrosis was also observed in human monocyte-derived macrophages.

**The role of microRNAs**

MicroRNAs (miRNAs) are single-stranded RNAs of 22 nucleotides that are processed from approximately 70 nucleotide precursors. Circulating miRNAs have been explored as potential biomarkers of pulmonary tuberculosis. The expression profile of miRNAs under PPD challenge of peripheral blood mononuclear cells (PBMCs) isolated from active TB patients and healthy controls, showed the specific upregulation of miR-155 and miR-155* in PBMCs from active TB patients, again suggesting a potential diagnostic value of miRNAs in TB infection. There are at present, a handful of studies showing that miRNAs regulate the response of host macrophages to *Mtb* challenge. Rajaram et al. present evidence that *M. tuberculosis* lipomannan signals through TLR2 to express miR-125b. miR-125b production lowers the stability of the TNF-α protein thereby limiting TNF-α production. The authors show that, in human macrophages, pathogenic mycobacteria skew the balance of miRNA production towards a high miR-125b and low miR-155 production, thereby favouring subversion of the host innate immune response. On the other hand, using the murine macrophage as a model system, Kumar et al. presents evidence that miR-155 production is higher in the virulent *M. tuberculosis* H37Rv strain compared to *M. bovis* BCG, and that miR-155 production is dependent on the virulence factor ESAT-6. The authors argue that by suppressing the miR-155 target SHIP1 and activating Akt, the balance of signalling is tilted in favour of macrophage survival thereby preserving the intracellular niche of the pathogen. Further, miR-155 inhibits expression of the transcriptional repressor Bach1 which enhances the activation of heme oxygenase-1 thereby favouring the production of carbon monoxide which activates the DosR regulon of *Mtb*. This too augments survival of the pathogen. In addition, *Mtb* induces miR-99b in dendritic cells. Inhibition of miR-99b production enhances production of IL-6, IL-12 and IL-7. Taken together, these results highlight the ability of pathogenic mycobacteria to exploit miRNAs of the host to the advantage of the pathogen. The existing literature point to a need to carefully elucidate the responses of human and murine
Concluding remarks

In conclusion, this review tries to bring into focus some of our current understanding of the interplay between \textit{Mtb} and the macrophage and the likely outcome of this interplay on the course of infection. It touches upon some of the important findings of recent years which have shed new light on the fine tuning of the innate immune response, modulation of cellular survival or death pathways (to the benefit of either the host or the pathogen), and metabolic reprogramming of the macrophage often to the benefit of the pathogen. At this juncture it appears increasingly likely that the immune response may be manipulated in a manner that could augment existing strategies of intervention without the associated risk of developing antimicrobial resistance.

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