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Immunometabolism during *Mycobacterium tuberculosis* Infection

Nicole C. Howard¹, Shabaana A. Khader^{1,*}

¹Department of Molecular Microbiology, Washington University School of Medicine, Saint Louis, MO 63110, USA

Abstract

Over a quarter of the world's population is infected with *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB). Approximately 3.4% of new and 18% of recurrent cases of TB are multidrug-resistant (MDR) or rifampicin-resistant. Recent evidence has shown that certain drug-resistant strains of *Mtb* modulate host metabolic reprogramming, and therefore immune responses, during infection. However, it remains unclear how widespread these mechanisms are among circulating MDR *Mtb* strains and what impact drug-resistance-conferring mutations have on immunometabolism during TB. While few studies have directly addressed metabolic reprogramming in the context of drug-resistant *Mtb* infection, previous literature examining how drug-resistance mutations alter *Mtb* physiology and differences in the immune response to drug-resistant *Mtb* provides significant insights into how drug-resistant strains of *Mtb* differentially impact immunometabolism.

Introduction

Over a quarter of the world's population is infected with *Mtb*, the causative agent of TB. The increasing emergence of drug-resistant strains of *Mtb* significantly limits our efforts to reduce the global TB burden [1]. TB is primarily a pulmonary disease, although disseminated disease can occur [2]. The outcome of infection is likely determined by the first interactions between *Mtb* and lung immune cells. After entry into the host's airway, *Mtb* initially infects [3–5] alveolar macrophages (AMs) and subsequently infects recruited interstitial macrophages [6]. However, *Mtb* is also able to reside and thrive in a number of other cellular niches, including dendritic cells (DCs), neutrophils, and non-myeloid cells such as respiratory epithelial cells, fibroblasts, lymphatic endothelial cells, adipocytes, and hematopoietic stem cells [7,8]. *Mtb* may also be able to persist within extracellular niches, although the duration of extracellular persistence is unclear during pathogenesis [9]. *Mtb* interaction with the host cells it encounters can be crucially important for determining the outcome of disease progression [10].

After phagocytosis of *Mtb*, macrophages initiate a signaling cascade to recruit other immune cells to the lung. This aggregate of immune cells surrounds the site of infection and forms the tubercle granuloma. *Mtb* utilizes a variety of strategies to avoid or inhibit mechanisms of

*Correspondence: sakhader@wustl.edu (S.A. Khader).

host defense in order to survive and replicate within the macrophage and manipulate the cytokine response to infection. The balance of *Mtb* and macrophage interactions can influence the outcome of the granuloma, which may either constrain the infection or promote its systemic dissemination [11,12]. When a macrophage is infected by *Mtb* it can trigger different transcriptional programs that determine how the macrophage responds. Depending on the stimuli they are exposed to, macrophages polarize into either classically activated ‘M1’ or alternatively activated ‘M2’ macrophages, or on the spectrum in between these two activation states [13,14]. Macrophage polarization is driven by metabolic reprogramming. M1 macrophages are characterized by reliance on glycolysis for energy production, which rapidly generates energy and the accumulation of metabolic intermediates required for the production of proinflammatory cytokines, reactive oxygen species (ROS), nitric oxide (NO), and prostaglandin. By contrast, M2 macrophages commit to utilizing oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO), and produce anti-inflammatory cytokines such as interleukin (IL)-10 [15].

The pathogenicity of *Mtb* may thus depend on its ability to modulate host metabolism. During *Mtb* infection, the metabolic phenotype of the macrophage can be modulated by the infecting strain of *Mtb* [16], macrophage ontogeny [6], as well as the proximity of other activated immune cells [17]. The macrophage response to *Mtb* likely occurs in waves, beginning with the tissue-resident AMs which are among the first cells to be infected [3–6], followed by lung interstitial monocyte-derived macrophages, which start to accumulate around 2 weeks postinfection [6]. In recent years, our understanding of how *Mtb* infection drives metabolic reprogramming of these different types of macrophage, and the consequences this has for disease outcome, has expanded.

Most of our knowledge thus far comes from studies using drug-susceptible strains of *Mtb*. However, although it is recognized that *Mtb* strains of different lineages may drive distinct macrophage metabolic reprogramming, there remains a large gap in our understanding of how drug resistance can impact the host response. Recently, it is becoming clear that drug-resistance mutations in *Mtb* can modulate host–pathogen interactions and drive differential metabolic reprogramming [18]. Thus, in this review, we summarize previous literature on how *Mtb* infection modulates host macrophage metabolism and examine how drug-resistance mutations may alter the ability of *Mtb* to drive metabolic reprogramming and survive in host cells.

Macrophage Activation and Metabolism

Macrophage activation requires shifts in energy metabolism to accommodate the needs of the cell as it responds to pathogens or inflammatory stimuli. Immune cells typically utilize OXPHOS as their primary energy source when quiescent, but stimulation from Toll-like receptors (TLRs) or proinflammatory cytokines can induce metabolic reprogramming to support activation and downstream effector functions [19,20]. Depending on the inflammatory/infection stimulus they receive, macrophages may polarize towards a classically activated proinflammatory M1 phenotype, an alternatively activated immunomodulatory M2 phenotype, or somewhere in between [15,21]. During *Mtb* infection, recruited monocyte-derived macrophages often express an M1-like phenotype by switching

to aerobic glycolysis for energy production [22]. This dependence on glycolysis for energy is similar to what is termed the 'Warburg effect' in tumor cells – the utilization of glycolysis and production of lactate while in the presence of oxygen [23]. The central regulator of aerobic glycolysis is hypoxia-inducible factor 1 α (HIF-1 α), a transcription factor regulating the expression of many glycolytic enzymes [23–25]. TLR activation or signaling by inflammatory mediators, including IL-1 β [26], tumor necrosis factor (TNF)- α [27,28], and NO [29,30], induces nuclear factor kappa B (NF- κ B) signaling, which then upregulates the expression of HIF-1 α [23,31]. In macrophages, the upregulation of HIF-1 α expression after *Mtb* infection is TLR2-dependent, and is mediated in part by the activation of the protein kinase B (AKT)-mechanistic target of the rapamycin (mTOR) pathway [32]. In quiescent or M2 macrophages, the end product of glycolysis, pyruvate, is converted into acetyl coenzyme A (CoA) in the mitochondria and enters the tricarboxylic acid (TCA) cycle [33]. During aerobic glycolysis, however, pyruvate can instead be converted into lactate and secreted from the cell [24,34,35]. Lack of the substrate acetyl-CoA, as well as the downregulation of critical TCA cycle and OXPHOS enzymes during *Mtb* infection, further drives the commitment to glycolysis while also allowing immunomodulatory TCA cycle intermediates such as succinate and citrate to accumulate [36].

Macrophage polarization has been suggested to drive the outcome of TB disease in the non-human primate (NHP) model. Having a high proportion of alternatively activated macrophages is associated with enhanced bacterial growth and loss of granuloma control in this model [37]. Thus, glycolytically active M1 macrophages are thought to be more effective at controlling *Mtb* infection [6,22] as they are able to rapidly, if inefficiently, produce ATP as well as metabolic intermediates that support the production of proinflammatory cytokines and antimicrobial peptides [38].

Glycolysis

To support increased rates of glycolysis, macrophages require greater import of glucose into the cell. Activation by inflammatory cytokines or various TLRs, including TLR2, stimulates the upregulation of glucose transporters. Glucose transporters are highly upregulated in macrophages infected with *Mtb* [39,40]. Glucose transporter (GLUT)6 is upregulated in murine bone marrow-derived macrophages (BMDMs) infected with *Mtb* [39], while GLUT1 and GLUT3 are upregulated following *Mtb* infection in the human monocytic cell line THP-1 cells or primary human monocyte-derived macrophages (MDMs) [40]. ESAT-6, the 6 kDa early secretory antigenic target protein of *Mtb*, has been shown to mediate increased glucose uptake in THP-1 cells, although the exact mechanism of action is unclear [41]. There is some evidence that ESAT-6 also interacts with TLR2 [42–45], which could be related to its role in stimulating increased glycolysis. However, ESAT-6 has also been implicated in other critical aspects of pathogenesis that may influence the metabolic state of the macrophage, including *Mtb* escape into the cytosol [46–48]. Therefore, *Mtb* infection drives increased glucose transport into macrophages through upregulation of glucose transporters (Figure 1). (See Tables 1 and 2.)

However, there may be a decoupling of glucose uptake and glycolysis during *Mtb* infection. The upregulation of the enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3

encoded by the gene *Pfkfb3* is critical for the accumulation of fructose-2,6-bisphosphate (F-2,6-BP) and maintaining glycolytic flux. Expression of *Pfkfb3* can be differentially regulated by infection with different *Mtb* strains in murine BMDMs, although GLUT6 is upregulated by all strains [36] (Figure 1). This suggests that some *Mtb* strains do not allow macrophages to fully induce the Warburg effect, which coincides with low induction of proinflammatory responses. This may also be indicative of dysregulated host lipid metabolism, and may represent the induction of an improper immune response that allows for continued *Mtb* replication [16].

Long-term metabolic changes and epigenetic modifications in innate immune cells represent a process called ‘trained immunity’. Blockade of glucose and/or glutamine metabolism in innate cells has been shown to inhibit their ability to undergo this process after BCG vaccination. The establishment of trained immunity after BCG vaccination is reminiscent of the metabolic shift seen during virulent *Mtb* infection, requiring the classical Warburg effect and activation of the AKT-mTOR pathway to undergo epigenetic changes. The establishment of trained immunity in innate cells like monocytes and macrophages has been shown to increase their responsiveness upon later challenge with different pathogens [33]. This shift could impact the ability of *Mtb* to further modulate the metabolic state of host innate immune cells.

Recent work has tracked the metabolic state of macrophages over several days postinfection in murine BMDMs, and has suggested that cellular metabolism is biphasic, with an initial glycolytic proinflammatory stage, followed by a resolution phase characterized by an upregulation of OXPHOS and downregulation of glycolysis. The resolution phase, while allowing for cellular survival, also fails to inhibit bacterial replication as effectively [49]. This may be specific to murine BMDMs as the same was not seen in THP-1 cells or human MDMs after *Mtb* infection. In human macrophages, there is some evidence that *Mtb* may decelerate both glycolysis and OXPHOS [50]. Others have found that, in THP-1 cells, the upregulation of glycolysis after *Mtb* infection was maintained until 48 h postinfection (hpi). Treatment of *Mtb*-infected THP-1 cells with chemical inhibitors of glycolysis like 2-deoxy-D-glucose (2-DG) instead led to a decline in ATP production and subsequent apoptosis once the cell’s energy was depleted [40]. Apoptosis is thought to be an important mechanism of host defense; macrophage apoptosis has been found to decrease the viability of *Mtb* [51–53], and proapoptotic *Mtb* mutants drive greater T cell responses and enhanced host control [54]. This could suggest that while glycolysis is important for driving M1 polarization and an inflammatory macrophage response, the continued survival of the macrophage also provides a niche for *Mtb* growth.

In murine BMDMs, where glycolysis is transiently induced, the critical glycolysis transcription factor HIF-1 α is also only upregulated for a short time after *Mtb* infection, peaking 4 hpi (Figure 1). Expression of HIF-1 α was not required for control of bacterial replication during an *in vitro* infection model [55]. This is surprising, since the inhibition of glycolysis by 2-DG in murine BMDMs [6], THP-1s, or human MDMs impairs the ability of macrophages to control *Mtb* infection, as shown by increased intracellular bacterial colony-forming units (CFU) [22]. The induction of glycolytic enzymes and increased glucose consumption in macrophages after *Mtb* infection has been shown to be HIF-1 α -dependent

[25,29,56], suggesting that it would also be required for bacterial control. Additionally, it has been shown that HIF-1 α ^{fl/fl} LysM^{cre} mice were found to be susceptible to *Mtb* infection. However, HIF-1 α was found to be required in macrophages for control of *Mtb* infection in the context of interferon (IFN)- γ stimulation. IFN- γ is a critical mediator of antimycobacterial immunity, and IFN- γ stimulation of macrophages has been shown to stimulate metabolic reprogramming [57], as the addition of exogenous IFN- γ induces sustained and robust expression of HIF-1 α [55]. This does not fully explain the discordant results for the role of HIF-1 α , and it bears further study (Figure 1).

The transcription factors NF- κ B and HIF-1 α undergo extensive crosstalk in immune cells such as macrophages [58,59]. In *Ikk*^{-/-} BMDMs, which cannot activate NF- κ B, HIF-1 α protein failed to accumulate and HIF-1 α target genes were not expressed after infection with Gram-negative or Gram-positive bacteria [31]. This suggests that NF- κ B is required for HIF-1 α expression and stabilization during macrophage infection. NF- κ B can canonically be induced in macrophages by various stimuli signaling through cytokine receptors, pattern-recognition receptors (PRRs) [60], and TNF superfamily receptors [61] during *Mtb* infection. NF- κ B is further regulated by various factors associated with modulating the metabolic state of the cell. One particularly important mediator is NO, which is known to be critical for controlling *Mtb* infection through its bactericidal activity [62–64]. NO is also required for HIF-1 α stabilization during IFN- γ stimulation, and *Nos2*^{-/-} macrophages fail to upregulate GLUT1 or *Pfkfb3* to the same extent as wild-type macrophages [29]. NO also suppresses NF- κ B activity, preventing a hyperinflammatory response to *Mtb* infection [29]. In addition to upregulating HIF-1 α to enhance the rate of glycolysis, NO also helps to shift macrophage metabolism by inhibiting mitochondrial respiration [65]. Interestingly, a correlation has been found between reduced NO susceptibility and drug resistance in *Mtb* [66]. Additionally, the anti-inflammatory cytokine IL-10 serves as another layer of regulation of glycolytic commitment by inhibiting NO and thus mediating the level of OXPHOS suppression in the macrophage [67].

NF- κ B signaling is also regulated by sirtuins (SIRT), important molecules that bridge inflammation and metabolism [68]. SIRT1 is a major metabolic regulator that deacetylates NF- κ B to promote an anti-inflammatory phenotype and mitochondrial biogenesis. SIRT1 expression is downregulated during *Mtb* infection in macrophages, which may contribute to increased levels of NF- κ B signaling that are important for establishing a proinflammatory, glycolytically active M1 phenotype. Enhancing SIRT1 expression in THP-1 cells has been shown to improve control of *Mtb* growth [69]. In murine peritoneal macrophages and peripheral blood mononuclear cells (PBMCs) taken from human TB patients, SIRT1 was found to be downregulated in a TLR2-dependent manner. It also functioned as a negative regulator of proinflammatory cytokine induction after *Mtb* infection through decreased signaling through the NF- κ B and MAPK pathways [70]. SIRT1^{+/-} mice were more susceptible to *Mtb* infection and developed increased immunopathology, suggesting that SIRT1 has a role in both controlling *Mtb* and preventing tissue destruction. Like SIRT1, SIRT6 is a well-studied regulator of glycolysis and lipid metabolism, but it is upregulated during *Mtb* infection. SIRT6 negatively regulates NF- κ B (Figure 1) and aerobic glycolysis; deletion of SIRT6 in macrophages results in enhanced M1 polarization and increased glucose metabolism [71]. Blockade of SIRT6 results in reduced blood sugar through

increased GLUT expression and glycolysis. However, it is not known whether SIRT6 plays a protective or pathologic role in *Mtb* infection.

TCA Cycle

Integration of glycolysis with the TCA cycle is mediated through oxidation of pyruvate to acetyl-CoA. *Mtb* infection drives the downregulation of several critical TCA cycle enzymes [36] as well as the conversion of pyruvate to lactate [22]. This results in an accumulation of TCA cycle intermediates, particularly the metabolites succinate and citrate, which are important substrates for the generation of immunomodulatory molecules (Figure 2).

Succinate inhibits HIF prolyl hydroxylases to increase HIF-1 α stabilization [72], and it also promotes production of the proinflammatory cytokine IL-1 β [23], which has been shown to be crucial for controlling *Mtb* infection in macrophages [22,73,74]. Accumulation of succinate relies on the inhibition of succinate dehydrogenase (SDH) activity; this enzyme has been found to be downregulated during *Mtb* infection in macrophages [36]. Succinate oxidation by SDH in the electron transport chain (ETC), and increased membrane potential as a result of glycolysis, can also lead to mitochondrial production of ROS [75]. Mitochondrial ROS is produced by the NADPH-dependent NOX2 oxidase complex, and ROS can kill microbes with a variety of mechanisms. Mice deficient in NADPH oxidase are highly susceptible to *Mtb* infection, and *Mtb* has many different mechanisms of maintaining redox balance in order to survive ROS exposure and oxidative stress [76]. The downregulation of SDH by *Mtb* may be another such mechanism for avoiding excessive ROS generation. Glutathione (GSH) is an important antioxidant that can help to maintain cellular redox balance. Its synthesis is increased in *Mtb*-infected macrophages, as is xCT (SLC7A11) expression, which imports cysteine, the GSH substrate. xCT deficient mice have increased ability to control *Mtb* growth and have reduced pathology [77], suggesting this as another potential mechanism by which *Mtb* survives oxidative stress in host cells.

During virulent *Mtb* infection, there is still increased acetyl coenzyme-A (Ac-CoA) synthesis. However, rather than cycling Ac-CoA through the TCA cycle and utilizing OXPHOS for energy generation, the TCA cycle intermediate citrate is instead exported from the mitochondria to the cytoplasm [40]. Citrate accumulation is likely mediated by downregulation of the enzyme isocitrate dehydrogenase 2 (IDH2) [78], which represents a breakpoint where the metabolite isocitrate can be diverted from the TCA cycle. It is exported from the mitochondria to the cytosol where it is metabolized to Ac-CoA, resulting in a cytoplasmic pool of this important precursor to various inflammatory mediators in macrophages, such as NO, ROS, and prostaglandin E₂ (PGE₂) [79–81]. Like succinate, citrate also inhibits HIF hydroxylases, thus contributing to HIF-1 α stabilization and activity [72].

SDH is also regulated by itaconate, which is generated from citrate by the decarboxylation of cisaconitate by the enzyme aconitate decarboxylase 1 (ACOD1). While itaconate is important for the inhibition of SDH it also plays many other critical roles during *Mtb* infection. It mediates an anti-inflammatory response that prevents excess immunopathology; when LysM⁺ myeloid cells lack ACOD1, they succumb rapidly to *Mtb* infection as a result of neutrophil-mediated inflammation [82]. In macrophages, itaconate suppresses the

production of ROS as well as proinflammatory cytokines like IL-1 β , IL-6, and IL-12p70. It also has a role in increasing mitochondrial respiration rates, even after murine BMDMs undergo M1 polarization [83]. Therefore, the downregulation of TCA cycle flux and subsequent accumulation of metabolic intermediates may represent a protective host response to prime macrophages to release ROS and other proinflammatory molecules (Figure 2).

OXPHOS

The TCA cycle reduces nicotinamide adenine dinucleotide (NAD⁺) to NADH, which is then fed into the OXPHOS pathway. During *Mtb* infection and M1 polarization of macrophages, there is an increased requirement for NAD⁺ and decreased ability of mitochondria to oxidize NADH. *Mtb* also secretes an NAD⁺ glycohydrolase known as tuberculosis necrotizing toxin that contributes further to the depletion of NAD⁺ to potentially trigger necroptosis in infected cells [84]. Macrophages attempt to maintain homeostasis by increasing NAD synthesis. Accordingly, expression of nicotinamide phosphoribosyltransferase (Namp1), the first enzyme in the NAD synthesis pathway, is increased in *Mtb*-infected murine macrophages. Namp1 is regulated by SIRT6, which is also upregulated by *Mtb* infection [36]. SIRT6 deacetylates Namp1, resulting in increased activity [85]. SIRT6 may therefore help to maintain balance during *Mtb* infection, preventing an overly inflammatory response and prolonging macrophage survival.

Resting and M2 macrophages are characterized by their reliance on OXPHOS for ATP production. During *Mtb* infection, the enzymes involved in OXPHOS are downregulated [39]. During the early phase of infection, decreased OXPHOS in the mitochondria leads to ROS and reactive nitrogen species (RNS) production. NOS2 is highly upregulated by *Mtb* has been shown to be critically important for protection in murine macrophages, though its role in human infection is more controversial. RNS also inhibit the ETC and mitochondrial function, leading to reduced redox and increased ROS [86,87] (Figure 2). Elevated oxidative stress may be a mechanism by which *Mtb* drives macrophage necrosis in order to better facilitate bacterial replication [88].

However, oxidative metabolism may be upregulated at later time points during *Mtb* infection of murine BMDMs, as glucose uptake and glycolysis is downregulated and the TCA cycle/OXPHOS are upregulated after 24 h postinfection. This switch back to OXPHOS may be regulated by the late induction of a member of the PPAR γ coactivator-1 (PGC-1) family, *Pgc1b*, which is involved in mitochondrial biogenesis. This may represent a resolution phase of infection, meant to avoid immunopathology, while allowing *Mtb* to better survive in the host cell [36]. There is also evidence that, in human monocytes and MDMs, *Mtb* may decelerate both glycolysis and OXPHOS, leading to increased dependency on FAO for energy generation [50].

Fatty Acid and Lipid Metabolism

Citrate exported from the mitochondria is cleaved to generate cytoplasmic Ac-CoA, which is subsequently converted to mevalonate (MVA) and malonyl-CoA (Ma-CoA) to support the synthesis of cholesterol or free fatty acids (FAs), respectively [40]. During macrophage

infection, *Mtb* prevents phagolysosome maturation and escapes into the cytosol, where cholesterol and FAs, contained in lipid droplets, are accessible as a nutrient source [89]. In human MDMs, mycobacteria-containing phagosomes even migrate towards lipid bodies, leading to the incorporation of host lipids in the bacteria [90]. Several models have been described for increased lipid biogenesis in macrophages upon *Mtb* infection. The mycobacterial surface is enriched in nonpolar lipids, and these lipids are proposed to promote the emergence of neutral lipid-rich macrophages. Alternative models of increased *de novo* FA synthesis and inhibition of lipolysis mediated by perilipin 1A have also been suggested [91]. Necrosis induced by *Mtb* infection may also facilitate the differentiation of bystander macrophages to foamy macrophages [91]. Host factors also play an important role, including HIF-1 α and TNF α . HIF-1 α expression has also been shown to decrease the rate of β -oxidation and increase accumulation of the neutral lipid triacylglycerol (TAG), which supports the formation of lipid droplets during *Mtb* infection of macrophages [92]. Lipid-laden macrophages can develop into foamy macrophages, which are associated with necrotic granulomas [90,93]. The proinflammatory cytokine IL-17 downregulates HIF-1 α and negatively regulates the formation of hypoxic, necrotic granulomas in mice, suggesting that HIF-1 α contributes to the formation of foamy macrophages [94]. During *in vitro* infection of human MDMs, MDMs that did not harbor intracellular *Mtb* still accumulated lipid droplets, but blockade of TNF α or its receptor TNFR inhibited lipid droplet formation in *Mtb*⁺ and *Mtb*⁻ cells [95]. Inhibition of cholesterol or FA results in reduced lipid body accumulation in macrophages infected with *Mtb*, as well as reduced intracellular CFU [40]. This is somewhat controversial, as new evidence suggests that *Mtb* is restricted from acquiring host lipids in IFN- γ -activated macrophages [96].

Ketone body synthesis is an alternate pathway for utilizing mitochondrial Ac-CoA, particularly when Ac-CoA is produced in greater quantities than can be used in the TCA cycle [97], such as when the TCA cycle is inhibited during *Mtb* infection. During *Mtb* infection, there is increased expression of the ketone body 3HB, an agonist of the GiPCR GPR109a, which reduces cellular cAMP and decreases perilipin phosphorylation to protect lipid bodies from lipolysis [41] and drive lipid droplet formation. Blockade of GPR109a has been shown to effectively lower *Mtb* CFU *in vivo*, even during MDR *Mtb* infections [41], suggesting that DS and DR *Mtb* strains need lipid bodies as a nutrient source (Figure 2).

The increased synthesis and turnover of long-chain fatty acyl-CoAs can also increase PGE production, a lipid mediator important for controlling *Mtb* infection. However, lipid metabolism can be manipulated by the infecting *Mtb* strain, with different strains inducing different patterns of gene expression. The hypervirulent lineage 2 *Mtb* strain HN878 drives fatty acid synthase (FASN) expression, while other *Mtb* strains, like the laboratory-adapted H37Rv, do not [16,98] (Figure 2). While FASN does drive the expression of proinflammatory cytokines, like IL-1 β and TNF α , and contributes to macrophage activation, it also drives the synthesis of cholesterol [99], which is an important nutrient source for *Mtb* [100]. Mycolic acids have also been found to have a role in the accumulation of cholesterol inside alveolar macrophages, which may therefore contribute to foam cell formation. MAs are a major type of lipid in the mycobacterial cell wall, and are critical for maintaining the structure of the wall [101].

HN878 also generates more Acyl-CoA synthetase long-chain family member 4 (*Acsl4*), which favors arachidonate and eicosapentaenoate production [16,102], while the lineage 4 strain CDC1551 induces expression of the calcium-sensitive cytosolic phospholipase A2 (*cPla2*), which results in amino acid (AA) liberation from cellular phospholipids. Expression of *cPla2* also generates increased PGE2 induction during CDC1551 infection [16,53]. *Mtb*-induced apoptosis relies on *cPla2* [103] through PGE2 and other lipid mediators and subsequently the cyclooxygenases COX1 and 2 [104]. PGE2 is balanced by lipoxins such as lipoxin A4 (LXA4), which have anti-inflammatory effects [105] (Figure 2). Modulating the balance between these two eicosanoids may be a potential mechanism by which *Mtb* skews the macrophage towards necrosis or apoptosis. PGE2 suppresses necrosis, while LXA4 promotes it [53].

Together, these studies demonstrate that metabolic regulation of host cells, particularly macrophages, is a critical component of both host defense and pathogenesis during drug-susceptible *Mtb* infection. Host macrophages have highly coordinated programs of inflammatory responses established through metabolic reprogramming following infection, which primes them to effectively control invading pathogens. However, *Mtb* may be able to perturb cellular metabolism in order to modulate the immune response and establish its own survival and persistence. Although our understanding of immunometabolism during *Mtb* infection has greatly increased in recent years, recent evidence suggests that MDR *Mtb* strains differentially regulate these responses [18] and require further study.

MDR TB

The role that drug resistance may play in altering *Mtb* physiology, and subsequently altering its interactions with host cells, has been highly understudied. There is a growing body of evidence that, despite highly conserved genomes between *Mtb* strains [106], the genetic background of the *Mtb* strain can still induce differential responses during infection [5,94,107–110], and some of this has been linked back to differences in the cell-wall composition between these strains [94,111,112]. The cell wall of *Mtb* is critically important for modulating the immune response of the host, and changes in *Mtb* cell wall lipid composition can drastically change the immune response during infection. Many drug-resistance mutations in *Mtb* are associated with perturbations in the cell wall lipid composition [113,114], and particularly in the case of rifampicin-resistance-conferring *rpoB* mutations, cell-wall biosynthesis and cellular metabolism pathways may be heavily impacted [18,115]. Recent evidence suggests that *rpoB* SNPs may mediate differential induction of host immune responses during infection, as a consequence of changes in cell-wall-associated lipid expression [18]. Thus, drug resistance mutations could potentially change the environment that *Mtb* must survive in in the macrophage, while also altering the bacilli's ability to respond to and process the nutrients available in this niche. The impact this has on disease pathogenesis is unclear. Thus, there is a critical need to better understand the physiology and pathogenesis of drug-resistant strains of *Mtb* and interactions with its host in order to develop novel effective therapeutic options.

In particular, very little has been done to directly investigate macrophage metabolic reprogramming after drug-resistant (DR) or MDR *Mtb* infection. In order to bridge this gap,

it may be possible to link the literature examining how drug-resistance mutations alter *Mtb* physiology with the known differences in the immune response to DR *Mtb*, and provide insights into how drug-resistant strains of *Mtb* drive metabolic reprogramming during infection.

Glycolysis

One of the initial steps in mediating macrophage reprogramming is TLR stimulation, especially TLR2, which is required to drive the upregulation of HIF-1 α and commit the macrophage to aerobic glycolysis. Interestingly, there is some indication that drug resistance in *Mtb* may mediate how much TLR2 is stimulated during infection. When assessing an extensively drug-resistant (XDR) and DS *Mtb* strain of the same family, it was found that the XDR *Mtb* stimulated TLR2 to a lower extent than the DS *Mtb* in THP-1 cells, leading to reduced induction of both pro- and anti-inflammatory cytokines, which suggests an altered metabolic state of the macrophage [116]. MDR TB patients have an altered ratio of IFN- γ /IL-10 in serum in comparison with DS TB patients, with MDR TB patients having high levels of IL-10 and lower levels of IFN- γ [117,118]. Like TLR2 stimulation, IFN- γ is critical for the sustained upregulation of HIF-1 α . However, ESAT-6, which has also been implicated in increased glucose uptake to support aerobic glycolysis, has been shown to be upregulated in particular MDR *Mtb* isolates [119], suggesting that MDR *Mtb* strains may strongly induce glucose uptake during macrophage infection. Thus, DR *Mtb* may have increased expression of ESAT-6 but decreased TLR2 stimulation and IFN- γ signaling, making it unclear if the infected macrophages are able to commit to aerobic glycolysis to support M1 polarization after infection (Figure 3).

TLR2 signaling is also required for expression of the inflammatory eicosanoid PGE2 [120], while immunoregulatory signals, like type I IFN and IL-10, both downregulate PGE2 expression through their inhibition of IL-1 signaling [121]. Both type I IFN [18] and IL-10 [117,118] signaling is increased during MDR *Mtb* infection, while TLR2 signaling is decreased [116], which would suggest that PGE2 is likely downregulated. However, this has not been demonstrated during MDR *Mtb* infection. It has been suggested that MDR TB results in increased pulmonary cavitation in patients [122,123], a process thought to be induced through necrosis [49], but there may be many mechanisms driving that response.

Like PGE2, SIRT6 is strongly regulated by the type I IFN response [124]. The role of SIRT6 during *Mtb* infection is not entirely understood, although it is known to be upregulated by DS *Mtb* infection in macrophages, and negatively regulates NF- κ B signaling, glucose metabolism, and M1 polarization [71]. As some DR *Mtb* strains more strongly induce type I IFN [18], it is possible that they also more strongly induce SIRT6. The role type I IFNs play during DR *Mtb* infection is still unclear, but they may in fact be beneficial for the host; type I IFN administration has been beneficial in the treatment of MDR TB patients [125,126].

Chemical inhibition of glycolysis after *Mtb* infection exacerbates disease and raises intracellular CFU in macrophages, suggesting that glycolysis is necessary for macrophage activation. It has been hypothesized that this could be due to defective induction of IL-1 β , which is known to be critical for controlling *Mtb*. Macrophages treated with the inhibitor 2-DG were unable to produce IL-1 β and, as a result, were unable to control bacterial

replication as treatment of *IIIrI*^{-/-} macrophages with 2-DG had no effect, suggesting that glycolysis was upstream of IL-1 signaling [22]. Human macrophages infected with MDR *Mtb* from various lineages produced variable amounts of IL-1 β in comparison with H37Rv infection [127], which could suggest that drug resistance is modulating the metabolic state of the cell, or inhibiting expression at a different stage. While differential IL-1 β induction in murine BMDMs has also been shown for some rifampicin-resistant *Mtb* strains in comparison with DS *Mtb* of the same background, the strains used by Chakraborty *et al.* [127] were from multiple lineages. Therefore, differences in IL-1 β production could likely be lineage-dependent rather than their drug resistance profiles (Figure 3).

During aerobic glycolysis, pyruvate can be converted into lactate rather than entering the TCA cycle as Ac-CoA. *Mtb* has also recently been shown to metabolize lactate as a carbon source. Mutants that lack the L-lactate dehydrogenase gene *lldD2* are unable to utilize lactate and have impaired replication in human macrophages [128]. Interestingly, *lldD2* expression has been found to be significantly upregulated in isoniazid (INH)-resistant *Mtb* strains in comparison with closely related DS strains [129] (Figure 3), which may affect their growth in glycolytically active cells during infection.

OXPHOS

While there is limited direct evidence that DR strains of *Mtb* also shut down OXPHOS to a similar extent as DS *Mtb* strains [18], MDR and XDR *Mtb* strains have been found to induce high levels of oxidative stress in macrophages, even in bystander uninfected cells, when compared with H37Rv [130], which suggests that OXPHOS is likely being downregulated (Figure 3). While the MDR and XDR strains were not of the same lineage as H37Rv, they were from four distinct lineages and consistently produced similar results in comparison with H37Rv, suggesting that it is unlikely to be a lineage-dependent effect. Interestingly, DR *Mtb* strains may also be more resistant to ROS than drug-susceptible strains. Mutations in the gene encoding alkyl hydroperoxide reductase subunit C (*ahpC*) frequently occur to compensate for the loss of fitness associated with INH resistance [131]. However, they are also associated with resistance to ROS [132], which may contribute to why DR *Mtb* strains have some resistance to RNS stress as well. A correlation has also been found between reduced NO susceptibility and drug resistance in *Mtb* [66]. However, it is not known if this is related to the direct antimicrobial effects of RNS or if it indicates an improved ability of drug-resistant *Mtb* to reside in M1 glycolytically active macrophages. Additionally, IL-10 serves as another layer of regulation of glycolytic commitment by inhibiting NO and thus mediating the level of OXPHOS suppression in the macrophage [67]. Given the enhanced levels of IL-10 in the inflammatory milieu of MDR TB [117,118], it is possible that MDR *Mtb* strains drive M2-like rather than M1 polarization of macrophages through this mechanism. Accordingly, the M2-like polarization rate of macrophages is significantly higher in granulomas from resected lungs of MDR/XDR TB patients, when compared with granulomas from DS TB patients, although there was no difference in the M1-like polarization rate between the groups [133]. M1 and M2 polarization states were defined by the expression of iNOS and arginase-1 surface markers respectively. However, as these results were generated from patients with severely progressed disease, they may not be broadly representative, and thus these results should be confirmed in other TB disease

models and *Mtb* strains. Within many of these human studies it also remains unclear if differences in the lineage background or growth rate of DR isolates, compared with DS isolates, could be confounding factors in the study results.

FA Oxidation

FA and cholesterol are nutrients available to intracellular *Mtb* that are important for *Mtb* survival and virulence, but their degradation generates propionyl-CoA, a byproduct toxic to the bacteria. Therefore, *Mtb* has multiple mechanisms for removing propionyl-CoA, including incorporating it into methyl-branched lipids in the cell wall. The proteins PDIM, SL-1, PAT, and LOS are commonly used as a sink for propionyl-CoA [134]. This is particularly interesting in the context of drug resistance since it has been shown by multiple groups that *rpoB* mutations can induce up-regulation of some of these methyl-branched lipids [18,113,135] – which may better allow DR *Mtb* to reside in host macrophages and mitigate stress. Conversely, failing to induce FA accumulation may be especially detrimental to the survival of DR *Mtb* strains as lacking substrates to incorporate into methyl-branched lipids may cause additional stress due to nutrients being inappropriately allocated during infection.

The observed increase in central metabolism may affect the fitness of drug-resistant strains during infection. In INH-resistant clinical and laboratory-generated strains, there was an increased abundance of enzymes in the TCA cycle, including *lld2*. Additionally, there was an increased abundance of genes involved in FA β -oxidation, which together may suggest increased ATP production. Unlike *rpoB* mutants, there was not an increase in methyl-branched lipids in the cell wall, suggesting that different drug-resistance mutations differentially influence *Mtb* physiology how it reacts to the macrophage environment [136]. These findings also highlight one of the challenges of understanding MDR *Mtb*, when drug-resistance-conferring mutations may induce opposing effects.

Mycolic acids, which contribute to triggering foam-cell formation, have been found at an increased abundance in some DR *Mtb* strains in comparison with DS *Mtb* strains [137] (Figure 3). This suggests that some DR *Mtb* strains may have an enhanced ability to accumulate FA nutrient sources in macrophages through the increased development of lipid bodies. Other DR *Mtb* strains, particularly mono-INH-resistant isolates, have been found to have lower levels of mycolic acids in their cell walls, as well as a downregulation of the enzymes involved in their synthesis. However, these strains are also associated with lower virulence and survival during *in vivo* infection in comparison with closely related DS *Mtb* strains [136,138], with no notable differences during growth in media, perhaps in part because of this defect or other underlying fitness defects.

TAG accumulation in human macrophages also requires TNF- α signaling after *Mtb* infection [95]. TNF- α signaling is closely linked to IL-1R signaling, which upregulates TNFR expression [139], and IL-1 signaling is perturbed by certain MDR *Mtb* strains [18]. It is unclear how TNF- α cytokine expression is affected, as some DR TB patients have lower expression [140] and others have enhanced expression in the serum in comparison with DS TB patients [117], which suggests that some DR strains may differentially accumulate TAG if TNF signaling is modulated. Infection of MDMs isolated from MDR TB patients by an

MDR *Mtb* strain strongly induced TNF- α production, significantly more so than infection of MDMs isolated from DS TB patients or healthy controls. This correlated with increased bacterial CFU in the MDR TB patients MDMs [141], suggesting that enhanced TNF- α expression was playing a pathogenic role, perhaps through lipid accumulation and foam cell biogenesis.

Metabolic Drugs/Therapies

TAG and neutral lipid accumulation leads to necrosis and facilitates escape of bacilli from host cells to spread infection. Thus, extracellular glucose concentrations influence the balance between apoptosis and necrosis, and consequently *Mtb* spread. Models that modulate the availability of extracellular glucose therefore impact *Mtb* pathogenesis. Diabetes models, where glucose levels are high, result in increased *Mtb* CFU. However, in a hypoglycemia model, the opposite result was found where macrophages were better able to control *Mtb* infection [40]. Type 2 diabetes has been found to be associated with increased likelihood of DR *Mtb* infection [142–144]. Additionally, diabetes is associated with an increased risk of both treatment failure and death during MDR TB disease [145]. T2D alters macrophage activation and their ability to respond to *Mtb* infection [146]. In particular, IL-1 β and IL-10 secretion was altered in the T2D macrophages, and those pathways are known to be modulated by DR *Mtb* infection in comparison with DS *Mtb* infection as well. Enhanced oxidative stress may also have an impact on comorbidities like diabetes, as increased oxidative stress may result in poor treatment outcomes [147]. The antidiabetic drug metformin (MET) reduces the intracellular growth of *Mtb* in an AMPK (adenosine monophosphate-activated protein kinase)-dependent manner. MET is typically prescribed to aid in lowering excess levels of blood glucose. In the context of *Mtb* it was found to increase the production of mitochondrial ROS and facilitate phagolysosome fusion to control the growth of DS and DR *Mtb* strains [148]. Unfortunately, a retrospective cohort study showed that MET treatment failed to significantly improve TB control in TB patients who also had diabetes. While some patients also had DR TB, they were not analyzed separately [149].

Concluding Remarks and Future Perspectives

Our understanding of how host macrophage metabolism can influence the outcome of *Mtb* infection has grown considerably over the past years. While it is increasingly clear that appropriate metabolic activation of macrophages is critical for controlling *Mtb* infection, it remains understudied how drug-resistant *Mtb* strains may modulate the paradigms being uncovered. In particular, drug-resistance mutations in *Mtb* seem to mediate changes in cell-wall biosynthesis and bacterial metabolism, which suggests that DR *Mtb* modulates the host response to infection in two ways: (i) by altering the expression of lipids and proteins that are sensed by host cells, and (ii) by altering their own need for nutrients or a host environment. These changes could be detrimental or beneficial to pathogenesis, but an enormous amount of work remains to fully understand the effect of drug-resistance mutations on bacterial physiology, both singly and in combination, and then to examine their subsequent role in mediating an immune response. Based on the literature reviewed here, it is likely that many of the currently circulating MDR *Mtb* strains modulate host immune

responses, driven by changes in immunometabolism. Future studies are needed to elucidate the relationship between drug resistance in *Mtb* and host metabolism during infection.

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Highlights

Mtb infection drives metabolic reprogramming for induction of protective immune responses.

Drug resistance in *Mtb* is associated with changes in lipid and protein expression that can mediate differential host–pathogen interactions.

Rifampicin-resistant *Mtb* strains carrying the H445Y mutation modulate host metabolic reprogramming and alter downstream immune responses.

Outstanding Questions

What drug-resistance mutations are associated with altered immunometabolism?

What is the effect of multiple drug-resistance mutations on modulating host metabolism and downstream immune responses?

Mycobacterial metabolism is linked to host cellular metabolism. *Mtb* is able to metabolize various host metabolites (including lactate, pyruvate, and cholesterol) as a nutrient source. Given the strong evidence that drug-resistance mutations impact cell-wall biosynthesis, the nutrient requirements for drug-resistant *Mtb* may differ from those of drug-susceptible *Mtb*. What is the relationship between drug-resistant *Mtb* and host cellular metabolism?

What impact does drug resistance in *Mtb* have on disease outcome in patients?

Figure 1. Drug-Susceptible (DS) *Mycobacterium tuberculosis* (*Mtb*) Infection Drives Increased Glycolysis.

DS *Mtb* infection drives the Warburg effect: increased glycolytic flux driving production of lactate and decreased mitochondrial oxidative metabolism. Following Toll-like receptor (TLR)2 stimulation, increased glycolysis is mediated by upregulation of key genes, including those encoding glucose transporters GLUT1/3/6, although certain genes, like *Pfkfb3*, are differentially regulated by *Mtb* strains of different lineages. Activation of various pattern-recognition receptors (PRRs) and cytokine receptors drives the induction of the nuclear factor kappa B (NF- κ B) pathway, which drives hypoxia inducible factor (HIF-1 α) expression, leading to further upregulation of glycolytic genes and the production of critical inflammatory mediators like interleukin (IL)-1 β . Sirtuins SIRT1 and SIRT6 downregulate NF- κ B and are differentially regulated by *Mtb* infection. Green indicates upregulation, red indicates downregulation, and purple indicates differential expression that varies between *Mtb* strains. Abbreviations: IFN, interferon.

Figure 2. Drug-Susceptible (DS) *Mycobacterium tuberculosis* (*Mtb*) Infection Modulates the tricarboxylic acid (TCA) Cycle, Oxidative Phosphorylation (OXPHOS), and Fatty Acid (FA) Metabolism.

While glycolysis is upregulated, the TCA cycle and OXPHOS are downregulated in the acute phase of *Mtb* infection. Various TCA cycle enzymes are downregulated, including ACO2, isocitrate dehydrogenase 2 (IDH2), and succinate dehydrogenase (SDH), to drive reduced oxidative metabolism. Accumulation of TCA cycle intermediates like citrate and succinate increase the expression and stability of hypoxia inducible factor (HIF)-1 α and drives production of the metabolite itaconate. Decreased OXPHOS also drives increased reactive oxygen species (ROS) and reactive nitrogen species (RNS). Acetyl coenzyme-A (Ac-CoA) generated in the mitochondria is converted into the ketone body 3HB rather than fed into the TCA cycle. 3HB signals through GPR109a to drive formation of lipid bodies. Citrate export and the stabilization of HIF-1 α may also drive increased lipid droplet (LD) formation. Citrate conversion to Ac-CoA in the cytoplasm feeds into FA metabolism, which is differentially regulated by DS *Mtb* strains of different lineages. Green indicates upregulation, red indicates downregulation, and purple indicates differential expression that varies between *Mtb* strains. Abbreviations: AA-CoA, CAC, CIT, FUM, fumarate; α -KG, LXA4, lipoxin A4; NOS2, nitric oxide synthase 2; NOX2, OAA, oxaloacetate; PGE2, prostaglandin E2; SUCC, TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor.

Figure 3. Drug-Resistant (DR) *Mycobacterium tuberculosis* (*Mtb*) Infection Modulates Metabolic Reprogramming.

DR *Mtb* strains have altered cell wall lipid expression and drive differential immune responses during infection in comparison with drug susceptible (DS) strains. Reduced Toll-like receptor (TLR)2 stimulation by some DR *Mtb* strains may impair optimal glycolytic flux, although upregulation of ESAT-6 expression by DR *Mtb* strains may enhance it. Lactate is secreted from the cell as a by-product of aerobic glycolysis; some DR *Mtb* strains have increased expression of the *Ildl2* gene that encodes an enzyme that allows *Mtb* to metabolize lactate as a nutrient source. The increased ratio of interleukin (IL)-10:interferon (IFN)- γ in multidrug-resistant (MDR) tuberculosis (TB) patients may suggest that hypoxia inducible factor (HIF)-1 α expression is not strongly upregulated, which correlates with lower IL-1 β production during DR *Mtb* infection. Critical tricarboxylic acid (TCA) cycle enzymes are downregulated by DR *Mtb* infection, leading to the accumulation of metabolic intermediates and maintaining production of reactive nitrogen species (RNS) and reactive oxygen species (ROS). However, increased induction of immunoregulatory molecules like IL-10 may interfere with RNS/ROS generation and downstream protective inflammatory responses. Lipid droplet (LD) formation may be modulated by altered mycolic acid (MA) expression in the cell wall of DR *Mtb*, and/or differential induction of tumor necrosis factor (TNF)- α may additionally drive lipid body formation. Green indicates upregulation, red indicates downregulation, and purple indicates differential expression that varies between DR *Mtb* isolates. Abbreviations: CAC, CIT, FUM, fumarate; IDH2, isocitrate dehydrogenase 2; α -KG, NOS2, nitric oxide synthase 2; NOX2, OAA, oxaloacetate;

OXPHOS, oxidative phosphorylation; SDH, succinate dehydrogenase; SUCC, TNFR, tumor necrosis factor receptor.

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Table 1.

Host Gene Products Related to Immunometabolism^a

Pathway	Host gene/protein	Abbreviation	Function	Response to DS <i>Mtb</i>	Response to DR <i>Mtb</i> (known or proposed)
Glycolysis	6-Phosphofructo-2-Kinase/ Fructose-2,6-Biphosphatase 3	Pfkfb3	Synthesis and degradation of fructose-2,6-bisphosphate during glucose metabolism	Upregulated to varying extent by DS <i>Mtb</i> strains; <i>Mtb</i> CDC1551 drives higher expression than <i>Mtb</i> HN878 [36]	Weakly upregulated by certain MDR <i>Mtb</i> ; MDR <i>Mtb</i> W_7642 drives lower expression than <i>Mtb</i> HN878 [18]
	Glucosetransporter 1	Glut1	Transport of glucose into the cell	Upregulated by DS <i>Mtb</i> in human macrophages [40]	No direct evidence of changes in
	Glucose transporter 3	Glut3	Transport of glucose into the cell	Upregulated by DS <i>Mtb</i> in human macrophages [40]	expression compared to DS <i>Mtb</i> ; increased ESAT-6 expression may suggest increased glucose uptake
	Glucose transporter 6	Glut6	Transport of glucose into the cell	Upregulated by DS <i>Mtb</i> in murine BMDMs [39]	
	Hypoxia Inducible factor 1 subunit alpha	HIF-1 α	Transcriptional regulator of metabolism and the response to hypoxia	Upregulated by DS <i>Mtb</i> infection to mediate shift to aerobic glycolysis [25,29,32,55]	No direct evidence of changes in expression compared to DS <i>Mtb</i> ; impaired IFN- γ response may prevent sustained HIF-1 α expression [117,118]
	Hypoxia Inducible factor prolyl- hydroxylases	PHD1 –3	Hydroxylate HIF-1 α to target it for degradation	Inhibited [25,29,32,55]	Unknown
	Peroxisome proliferator-activated receptor gamma coactivator 1-beta	Pgc1b	Stimulates several transcription factors; it is involved in glucose metabolism	Upregulated during later stages of infection [36]	Unknown
Cytokines and their receptors	Interleukin 10	IL-10	Immuno-regulatory cytokine that limits immune responses to prevent damage to the host and maintain tissue homeostasis	Induced by DS <i>Mtb</i> infection to assist in immune evasion	MDR TB patients have elevated IL-10 in plasma [116,117]; increased type I IFN may also drive more IL-10 [18]
	Interleukin 1 beta	IL-1 β	Proinflammatory cytokine	Expression is induced by DS <i>Mtb</i>	Variable induction in comparison with H37Rv by different MDR strains [118]; may have lower induction due to certain DR mutations [18]
	Tumor necrosis factor alpha	TNF- α	Proinflammatory cytokine	Expression is induced by DS <i>Mtb</i>	Lower [140] or higher [117] expression in the serum of DR TB patients in comparison with DS TB patients, depending on study population
	Interferon gamma	IFN- γ	Proinflammatory cytokine	Expression is induced by DS <i>Mtb</i>	Lower levels of IFN- γ in the serum of MDR TB patients in comparison with DS TB [117,118]
	Tumor necrosis factor receptor	TNFR	Receptor for TNF α	Upregulated by DS <i>Mtb</i> [139]	Unknown

Pathway	Host gene/protein	Abbreviation	Function	Response to DS <i>Mtb</i>	Response to DR <i>Mtb</i> (known or proposed)
Switch to aerobic glycolysis	Toll-like receptor 2	TLR2	Drives proinflammatory cytokine production, expression of co-stimulatory molecules, and activation of antigen presenting cells; important for initiating shift of cellular metabolism to aerobic glycolysis	Can be stimulated by various <i>Mtb</i> bacterial products, potentially including ESAT-6 [42–45]	Some XDR <i>Mtb</i> strains may induce less TLR2 stimulation [116]; some MDR <i>Mtb</i> strains have increased expression of ESAT-6 that may drive more TLR2 stimulation [119]
	Protein kinase B	PLB/Akt	Key component of the Akt signaling pathway that promotes cell metabolism, growth, proliferation, and survival	Activated by DS <i>Mtb</i> to drive metabolic reprogramming to aerobic glycolysis[32]	Differential activation by DR <i>Mtb</i> of these particular genes is unclear; impaired aerobic glycolysis induction by certain DR <i>Mtb</i> strains [18]
	Mammalian target of rapamycin	mTOR	Central regulator of cell growth		
Sirtuins and related proteins	Sirtuin 1	SIRT1	NAD-dependent deacetylase that impacts a variety of cellular functions, including energy metabolism, cell survival and stress responses	Downregulated by DS <i>Mtb</i> [68]; increased expression may aid <i>Mtb</i> control but exacerbate immunopathology [69,70]	Unknown
	Sirtuin 6	SIRT6	NAD-dependent deacetylase that impacts a variety of cellular functions, including energy metabolism, cell survival and stress responses	Upregulated by DS <i>Mtb</i> [36]	
TCA cycle	Nicotinamide phosphoribosyltransferase	Nampt	Regulator of the intracellular NAD pool	Upregulated by DS <i>Mtb</i> [36]	
	Aconitase 2	ACO2	Part of the TCA cycle where it converts citrate into isocitrate		
	isocitrate dehydrogenase 2	IDH2	Part of the TCA cycle where it converts isocitrate into alpha-ketoglutarate		No direct evidence of changes in expression compared with DS <i>Mtb</i> ;
	Pyruvate dehydrogenase complex	PDC	Complex of three enzymes that acts as part of the TCA cycle to convert pyruvate into acetyl-CoA		however, there is evidence for downregulation of OXPHOS [18,131]
	Succinate dehydrogenase	SDH	Part of the TCA cycle where it converts succinate to fumarate		
Proteins related	Nitric oxide synthase 2	Nos2	Production of NO	Expression is induced by DS <i>Mtb</i>	Unknown
	Solute carrier family 7 member 11	xCT (SLC7A11)	Antiporter that imports cysteine and exports glutamate	Increased expression [76]	

^a The known function(s) of critical host genes and proteins related to immunometabolism are annotated. If known, the impact of drug-susceptible (DS) or drug-resistant (DR) *Mtb* infection on the function or expression of these genes/proteins is included.

Table 2.**Mycobacterial Gene Products Related to Immunometabolism^a**

Pathway	<i>Mtb</i> gene/protein	Abbreviation	Function	Expression in DR <i>Mtb</i> strains
Secreted proteins	6 kDa early secretory antigenic target	ESAT-6	Abundantly secreted virulence factor	Upregulated by some MDR <i>Mtb</i> isolates [119]
	Tuberculosis necrotizing toxin	TNT	NAD ⁺ glycohydrolase which induces necrosis by known mechanism	Unknown if expression differs from DS <i>Mtb</i>
Involved in drug resistance	Beta subunit of RNA polymerase	rpoB	Subunit of RNA polymerase, which catalyzes transcription of RNA from DNA	Rifampicin-resistant <i>Mtb</i> strains frequently carry mutations in the 81-bp 'rifampicin resistance determining region' of rpoB
	Catalase peroxidase	katG	Enzyme with broad catalase and peroxidase activity; processes INH to its active form	Mutations within katG can give rise to INH resistance
	Alkyl hydroperoxide reductase C	ahpC	Peroxidase	Upregulated in INH-resistant mutants [131]
Bacterial metabolism	L-lactate dehydrogenase (cytochrome)	lld2	Conversion of lactate to pyruvate	Upregulated in INH-resistant mutants [129]
Cell wall associated	Phthiocerol dimycocerosates	PDIM	Various proposed roles related to virulence and immune responses; the toxic intermediate propionyl-coa can also be incorporated into these multimethyl branched FAs	May be upregulated in some DR <i>Mtb</i> strains [18,113,135]
	Sulfolipid-1	SL-1		
	Polyacyltrehalose	PAT		
	Lipooligosaccharide	LOS		
	Mycolic acid	MA	Major component of the <i>Mtb</i> cell wall; may also drive foam cell formation	Variable amounts of MAs have been found in DR strains in comparison with DS <i>Mtb</i> , perhaps due to differences in which DR mutations are carried [137,138]

^aThe known function(s) of *Mtb* genes and proteins are annotated as they relate to immunometabolism, as is any differential expression associated with drug resistance.