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Targeting immunometabolism in host defence against *Mycobacterium tuberculosis*

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Summary

In the face of ineffective vaccines, increasing antibiotic resistance and the decline in new antibacterial drugs in the pipeline, tuberculosis (TB) still remains pandemic. Exposure to *Mycobacterium tuberculosis* (*Mtb*), which causes TB, results in either direct elimination of the pathogen, most likely by the innate immune system, or infection and containment that requires both innate and adaptive immunity to form the granuloma. Host defence strategies against infectious diseases are comprised of both host resistance, which is the ability of the host to prevent invasion or to eliminate the pathogen, and disease tolerance, which is defined by limiting the collateral tissue damage. In this review, we aim to examine the metabolic demands of the immune cells involved in both host resistance and disease tolerance, chiefly the macrophage and T-lymphocyte. We will further discuss how baseline metabolic heterogeneity and inflammation-driven metabolic reprogramming during infection are linked to their key immune functions containing mycobacterial growth and instructing protective immunity. Targeting key players in immune cellular metabolism may provide a novel opportunity for treatments at different stages of TB disease.

Keywords: Mycobacterium tuberculosis; macrophage; lymphocyte; alveolar macrophage; T-cell; disease tolerance; host resistance; immunometabolism; glycolysis; oxidative phosphorylation; fatty acid oxidation; ATP; metabolism; Trained immunity; vaccine.

CELLULAR RESPONSES TO *Mtb*

1.5 million people died because of infection with *Mycobacterium tuberculosis* (*Mtb*) in 2018[1], mainly in sub-Saharan Africa and South-East Asia, although tuberculosis (TB) cases remain a global pandemic with 1 in 4 people worldwide estimated to be infected with the disease-causing pathogen, *Mtb*. Moreover, when this old TB pandemic collides with a novel threats (e.g. HIV or COVID-19), this silent killer will only get worse as a result of syndemic and the reduced accessibility to health

Abbreviations: TB, Tuberculosis; *Mtb*, *Mycobacterium tuberculosis*; BCG, bacille Calmette–Guérin; AM, alveolar macrophage; IMMs, interstitial monocyte-derived macrophages; DCs, dendritic cells; AEC, alveolar epithelial cell; BMDMs, bone marrow-derived macrophages; MDMs, monocyte-derived macrophages; HSCs, haematopoietic stem cells; TCA, tricarboxylic acid cycle; OXPHOS, oxidative phosphorylation; FAO, fatty acid oxidation; NO, nitric oxide; T2D, type 2 diabetes; TST, tuberculin skin test; PD1, programmed cell death

care[2,3]. In spite of staggering numbers in infection rate, illness and mortality, we face significant challenges, both politically and scientifically, which we must overcome to curb the development of this global health problem.

The heterogeneity in infection outcomes ranging from elimination, inactive TB or active disease manifests as a result of the complex evolution and interaction between *Mtb* and the human immune system[4]. This involves multiple cell types including resident pulmonary and recruited immune cells, each of which display plasticity and heterogeneity in their ability to eliminate or contain infection[5]. Recent scientific advances have allowed us to more precisely profile the multiple cell types at various stages of infection[6], ranging from initial host–pathogen interaction, subsequent immune cell recruitment and granuloma formation. However, our understanding of the inherent functional differences in these cells and the systemic factors in the host that impinge upon their function is very limited.

One of the most sophisticated strategies of *Mtb* is that the bacteria have adapted to specifically infect the lung-resident alveolar macrophage (AM), which plays a dual role as both the effector against and reservoir for TB infection[7]. Following internalization of the bacteria via various phagocytic receptors[8,9], pattern recognition receptors trigger cytokine and chemokine production in the alveolar and interstitial space[10], causing the recruitment of blood-borne immune cells via underlying blood vessels. These include monocyte-derived macrophages classified as interstitial monocyte-derived macrophages (IMMs)[11] and multinucleated granular neutrophils[12]. Both classes of phagocytic myeloid cells play key roles containing bacteria through various intracellular mechanisms, as well as orchestrating the inflammatory response. However, similar to the AM, they also can act as intracellular niches for *Mtb* replication and the balance between these processes impacts the outcome of infection and the extent of bacterial dissemination. Following pulmonary infection, *Mtb*-infected dendritic cells (DCs) or monocyte-derived macrophages migrate to the thoracic draining lymph nodes where they present antigens to naïve T cells, which leads effector T cells to traffic to the lung[4]. Interestingly, *Mtb* hijacks this process by delaying it up to 3 weeks post-infection[13]. B cells also play a role in TB immunity by providing *Mtb*-specific antibodies to promote opsonization, as well as producing cytokines to modulate macrophage and T-cell function[14,15]. Where complete sterilization fails, the extracellular inflammatory milieu and recruited immune cells form a granuloma, with infected macrophages at the centre, surrounded by layers of uninfected macrophages of various phenotypes and ultimately T cells with some follicular B cells and fibroblasts to limit infection and prevent subsequent dissemination.

Although the induction of inflammatory mediators within a granuloma is required for preventing *Mtb* dissemination, overly intense pro-inflammatory responses lead to reduced disease tolerance, including the destruction of granulomas via necrosis, enhanced lung parenchymal damage, lung cavitation and transmission that results in the onset of active disease[4,16,17]. Indeed, targeting TNF in immunodeficient patients with advanced inflammatory mycobacterial disease, including tuberculosis, actually improved outcome[18]. Furthermore, studies in animal models of TB and in humans have elegantly demonstrated that inflammatory signalling is highly organized within the granuloma as pro-inflammatory signalling is mainly found at the core of the granuloma, while anti-inflammatory signalling predominates in the periphery[19]. This spatial compartmentalization of pro- and anti-inflammatory signalling determines the granuloma's function in controlling both disease tolerance and host resistance to *Mtb* (Figure 1). Thus, the optimal host response may be a balance of inflammatory and anti-inflammatory signalling that leads to the regulation of inflammation within and around the granuloma and reduced frequency of active disease[20].

Considering both the pro- and anti-inflammatory phenotypes of immune cells are linked to their cellular metabolism, it is imperative to delineate the molecular mechanisms that dictate their metabolism. The granuloma itself is a complex metabolic environment with areas of hypoxia, lipid accumulation and necrosis and is completely dependent on cytokine instruction to maintain integrity[21]. With obesity now recognized as a metabolic disease that alters homeostatic immune function[22] and TB–diabetes now an increasing comorbidity observed in South-East Asia[23], changes in whole-body metabolic signalling impact immune cells' ability to contain *Mtb*. Thus, metabolic disease should be considered a key factor that determines susceptibility to active TB. In this review, we will dissect the metabolic phenotypes observed in two hallmark cell types of the immune response to *Mtb* – macrophages and T lymphocytes – and overlay how recent knowledge on immunometabolism in these cells may dictate host resistance or disease tolerance in TB.

RESIDENT ALVEOLAR MACROPHAGES

The AM response to *Mtb*

It is now well recognized that AMs, like many tissue-resident macrophages, is not originated from blood-borne monocytes but rather from embryologically distinct progenitors (yolk sac), which can proliferate locally[24]. AMs were first described as 'dust cells', due to the large amounts of darkly staining particulate matter indicative of their key phagocytic function surveying the external environment and maintaining homeostasis[25]. These features however predispose these cells to *Mtb* infection, as they express a wide array of phagocytic scavenger receptors (SRs) used by *Mtb* to enter phagocytes,[4,10]. The

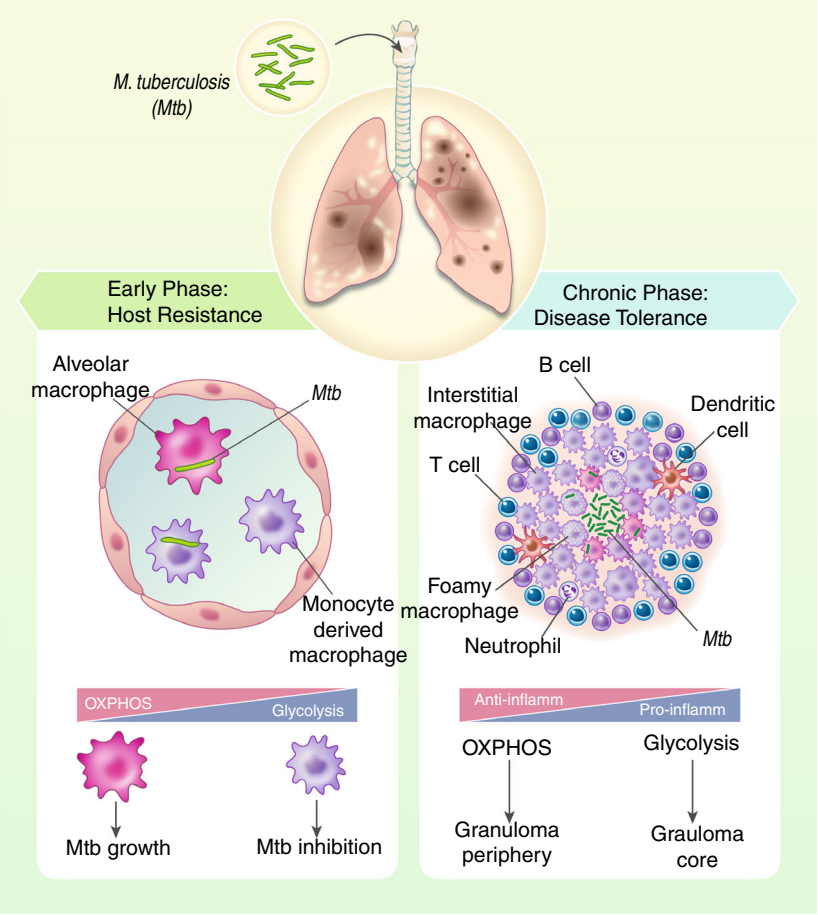

Figure 1. Immune cell metabolism in host defence against *Mtb*. The early phase of host defence against *Mtb* is dominated by macrophage attempts to resist the infection. However, resident alveolar macrophages that rely on fatty acid oxidation (FAO) in the lipid-rich lung microenvironment to fuel oxidative phosphorylation (OXPHOS) provide a niche for *Mtb* replication. Recruited interstitial monocyte-derived macrophage (IMM) up-regulate glycolytic activities to promote pro-inflammatory activities to eliminate the bacteria. If the bacteria persist by migrating into the lung interstitial tissue that leads to the granuloma formation, the host defence mechanism switches to disease tolerance to containing infection and limiting tissue damage. This is driven mainly by activated T cells, B cells, phagocytic cells such as DC, and interstitial macrophages. As the granuloma develops, pro-inflammatory glycolytic macrophages attempt to restrict mycobacterial growth in the centre, while anti-inflammatory interstitial macrophages balance the inflammatory response and limit bacterial dissemination. Foamy macrophages in which lipid metabolism is remobilized have been also associated with both bacterial growth and pro-inflammatory antimicrobial activities. While the centre of the granuloma appears to be pro-inflammatory, the periphery is anti-inflammatory. This spatial compartmentalization of pro- and anti-inflammatory signalling that regulates by cellular metabolism determines the capacity of the granuloma to prevent bacterial dissemination

immunotolerant environment of the lung also favours *Mtb* infection, promoted by both AM and alveolar epithelial cell (AEC)-derived TGF-β and vitamin A metabolite all-trans retinoic acid driving anti-inflammatory[26] and regulatory T-cell signalling[27]. Pulmonary surfactant, the lipoprotein mixture that regulates lung compliance[28], also affects AM phenotype[29]. This combination of lung features promotes disease tolerance, and thus, AMs possess a phenotype to maintain the lung immunotolerant environment at the steady state. However, AMs express many surface and endosomal TLRs, which recognize mycobacterial epitopes and pro-inflammatory activation[30]. *Mtb* evolved exquisite strategies to evade and inhibit this, the most crucial of which is inhibiting phagolysosomal fusion to escape destruction and allow an intracellular niche for survival[9]. Although this responsiveness of AMs is seemingly dictated by their expression of surface receptors and interactions with the immunotolerant pulmonary environment, investigators are now examining the metabolic activity of AM, which may explain their failure to efficiently contain *Mtb* during the initial phase of infection.

Macrophage metabolism

We now know that the flux, fate and substrate preference of central C-metabolism pathway can be altered to

support T-cell energy and biosynthetic demands, in response to changes in the tissue microenvironment, which uniquely affects migrating and patrolling immune cells with varying functional demands[31]. The predominant view in macrophage biology has been that macrophages exist on 2 metabolic spectrums, in line with broad in vitro classifications used to distinguish opposing functional programmes[32]. Classically activated (M1) macrophages, generated in vitro by treating naïve human blood or mouse bone marrow-derived macrophages (hMDM or BMDM) with the TLR ligand LPS and IFN-γ, adopt a glycolytic phenotype with impaired tricarboxylic acid cycle (TCA) dynamics, even in the presence of oxygen[33,34]. Through the up-regulation of glycolytic rates, this metabolic reprogramming provides rapid ATP from cellular glycolysis without the need for mitochondrial oxidative phosphorylation (OXPHOS). TCA metabolites and glycolytic intermediates are syphoned into alternative pathways including citrate-derived fatty acid synthesis (FAS) and increased pentose–phosphate pathway (PPP) activity to support the biosynthetic demands of activation[33,35–37], which in cancer cells that adopt similar Warburg metabolism or in activated T cells, is easily explained by the proliferative demand[12]. It has been hypothesized that in macrophages this anabolic metabolism supports the antimicrobial oxidative burst, as well as the increased transcriptional and translational activity required for cytokine production[38,39]. In contrast, alternatively activated (M2) macrophages, generated in vitro through anti-inflammatory cytokine stimulation, chiefly IL-4, maintain high levels of OXPHOS with an intact TCA cycle[33]. While M1 and M2 are the example of two extreme spectrum of cellular metabolism, nonetheless these are useful benchmark to define the differences observed in macrophage metabolism and phenotype during the course of *Mtb* infection.

AM immunometabolism

At baseline, AMs represent M2-like macrophages, showing similar levels of oxidative metabolism as IL-4-treated human MDMs[40]. This aligns with their phagocytic function, which is an energetically demanding activity. Although rapid cellular proliferation requires anabolic metabolism, long-lived self-renewal cells such as AM rely heavily on OXPHOS to maintain viability[41]. The lung is a lipid-rich environment due to surfactant, and therefore, a diversity of fuels is available for AM. While glucose is the preferential fuel, its availability is often limited in microenvironments[42], and therefore, immune cells have adapted to undertake fatty acid metabolism. Indeed, lung glucose levels are thought to be lower than serum levels[43], which may force alternative metabolism in immune cells engaged in the granuloma as an adaptation to the new environment. Increased fatty acid oxidation (FAO) is a feature of M2 macrophages[33]. Similar up-regulation of FAO has also been observed in mouse AMs ex vivo[44]. Up-regulation of CD36 expression by IL-4 drives FA uptake and lipolysis to feed FAO in M2 macrophages and is linked to M2 marker up-regulation and anti-inflammatory cytokine production[45]. Thus, it is possible that a similar switch occurs in the lung with surfactant lipoprotein SP-A known to drive CD36 expression and activity[46], which could skew towards an M2-like AM phenotype forcing both FAO and anti-inflammatory gene expression. Although *Mtb* can utilize glucose, multiple studies suggest FA as the preferred C-source for *Mtb* during infection[44], while *Mtb* does not possess enzymatic machinery for cholesterol biosynthesis[43]. However, it has been postulated that the lipid-rich environment of the AM favours *Mtb* by providing triacylglycerols, cholesterol and FA as a C-source for mycobacterial growth. CD36-mediated uptake of surfactant lipoproteins by AMs may in fact provide a double hit for the bacteria, limiting inflammation and providing access to nutrients for growth. Indeed, ex vivo studies suggest that mouse AM represent a more favourable niche for *Mtb* replication than observed in BMDM or recruited lung IMs[44,47], in a manner completely dependent upon FAO[44].

A major question that remains to be answered is the functional and metabolic plasticity of AMs during *Mtb* infection. While in vitro infection of both human and mouse AMs with attenuated or inactivated forms of *Mtb* allows glycolysis, live virulent *Mtb* abolishes the glycolytic capacity and activity of these cells[44,48]. More extensive bioenergetic analysis reveals a strong dependence on OXPHOS for glucose metabolism and ATP production in LPS-treated AM ex vivo[49]. These findings are supported by in vivo analysis of influenza infection and profiling of lung populations[49]. Extensive transcriptomic profiling of both uninfected and infected AMs reveals increased expression of FAO and lipid metabolism genes[44,50]. Although glycolysis could be driven in AMs through the stabilization of HIF-1α[49], this does not significantly contribute to LPS responses observed. Impaired IL-4 responses in AM in the lung were also found to be due to environmental suppression of glycolysis and were restored to similar levels as peritoneal macrophages when AMs were treated ex vivo[50]. AMs therefore have adapted their immunometabolic pathways to circumvent the low availability of glucose and lipid-rich environment in the lung. This would suggest that following *Mtb* infection, the threshold for AM activation via glycolytic metabolism should be higher than IMMs. Thus, identifying the regulatory mechanism involved in AM metabolism can map out targeted therapy for switching AM metabolism from OXPHOS towards the glycolytic pathway and therefore halting the initial phase of *Mtb* infection.

At the interface of the external environment, AMs are uniquely susceptible to factors that can impinge upon immune function. Smoking increases the risk of developing TB disease by twofold[51]. Recent evidence suggests smokers'

AMs are defective at multiple cellular compartments, with increased expression of oxidative stress genes[52], mitochondrial impairment leading to defective autophagy[53] and lysosomal storage disorders[54]. These intriguing findings link mitochondrial reprogramming in these cells with antimicrobial mechanisms and could explain the observed increased bacillary burden in smokers' AMs[51]. In our model of ex vivo AM activation with non-viable *Mtb* or LPS treatment, AMs from smokers were unable to shift towards glycolytic metabolism after stimulation, although basal glycolytic levels were higher[40]. This was also noted in a separate study examining smokers' AM and AM from COPD patients against healthy AM[52]. Together, these data suggest cigarette smoke exposure triggers basal reprogramming of AM metabolism, impairing normal oxidative phosphorylation required for AM homeostatic activities, which triggers compensatory glycolysis. This low-level induction of glycolysis exhausts the AM-limited capacity for glycolysis, such that appropriate immunometabolic-mediated inflammatory activation to pathogens is lost.

RECRUITED MACROPHAGES

Metabolic reprogramming supports *Mtb* host defence

Most studies to date examining the immunometabolic effects of *Mtb* infection have been performed in macrophages, which model naïve, newly recruited macrophages at sites of inflammation (hMDM, BMDM and THP1 cells), and for the most part, the results are consistent with what is observed in M1 macrophage models, with a profound up-regulation of glycolytic activity[55–59]. Encouragingly, these cells contain *Mtb* growth in a superior fashion and their ability to do so is completely dependent upon the up-regulation of glycolysis[44,57]. The landmark study by Huang et al characterized the recruited IMMs in mouse models of *Mtb* infection, and targeting glycolysis in these mouse models completely abolished the ability to contain infection[44], a feature also observed in vitro when glycolysis is poisoned in mouse BMDM or hMDM[44,57,60] or in BMDM from mice that lack a key regulator of the glycolytic response, HIF-1α[58,61]. While multiple groups have now reported the up-regulation of glycolysis in *Mtb*-infected macrophages, a recent extensive metabolic screen in hMDM and THP1 cells infected with various forms and strains of *Mtb* found virulent *Mtb* can actually decelerate flux through this pathway[55], supporting the notion that during successful infection, this pathway is attenuated to promote immune evasion. This, coupled with the finding that one of the chief functions of Th1-derived IFN-γ is to drive glycolysis in *Mtb*-infected macrophages[48,58], supports an important pro-inflammatory role for the glycolytic response in host defence.

Although initial work suggested a mechanism whereby maintenance of glycolysis supports pro-inflammatory IL-1β to regulate eicosanoid PGE_2 production and bacterial containment[57,62], the existence of other glycolysis-linked pathways to host defence has emerged. We and others have observed production of nitric oxide (NO) linked to increased macrophage glycolysis[48,58,61]. This could not only occur as a consequence of increased IL-1β signalling, but may also result from NADP generation through glycolysis-linked PPP[37]. This glycolysis-associated NO also acts as an important immunomodulatory signal by feeding back and enhancing glycolysis via HIF-1α activation, while simultaneously promoting inflammatory resolution through inhibition of NF-kB[61]. Although NO production is undoubtedly an important component of the host response to *Mtb*, not all models support a direct role of NO as antimycobacterial. In fact, recent studies suggest that NO production not only does represent an important immunomodulatory signal impacting on magnitude of inflammation, but may also regulate subsequent immunometabolic responses[63,64]. It has been elegantly demonstrated that nitric oxide (NO) inhibits NLRP3 inflammasome-mediated IL-1β production to prevent neutrophil-dependent pulmonary tissue damage[65]. A recent study has shown that the role of NO in host resistance to *Mtb* acts via the recruitment of neutrophils, which are permissive to *Mtb* growth[63]. Importantly, this immunoregulatory function of NO is co-ordinated with the initial recruitment of IFN-γ-producing T cells into the lung, which leads to granuloma formation and perhaps the transition from host resistance to disease tolerance. In a mouse model of influenza viral infection, accumulation of IMM in the lung tissue compromises disease tolerance that leads to pulmonary dysfunction. Interestingly, the production of IFN-α via leukotriene B_4 signalling was required to minimize IMM-mediated lung tissue damage[66]. Much of our understanding of the role of NO comes from both targeting and measuring its production in mouse models. Human macrophages, including AM, however produce limited amounts of NO in comparison[67], and therefore, it is important to confirm its role in the immunometabolic response in these systems. However, these studies collectively indicate that the regulation of IMM by various pro-inflammatory mediators is required for maintaining disease tolerance in lung tissue.

Regulation of IMM metabolism

While examining the potential of antibiotic-resistant strains of *Mtb* for the modulation of macrophage metabolism that limits IL-1β responses[68], Howard et al identified mycobacterial lipids, which when mutated in MDR-TB strains were unable to drive glycolysis in BMDM. This aligns with the work of Lachmandas and colleagues who identified the bacterial lipoprotein sensor TLR2 and subsequent PI3/AKT kinase signalling as a key axis through which recognition of *Mtb* drives metabolic

reprogramming[56,69]. This intersects with IFN-γ signalling to activate pro-glycolytic HIF-1α[58]. The up-regulation of glycolytic genes by HIF-1α is a key step supporting enhanced glycolytic flux during *Mtb* infection[59]. Intriguingly, tracing studies suggests that only a small amount of glucose-derived carbon makes it to the pyruvate and lactate steps of later glycolysis in *Mtb*-infected cells[55]. This metabolic consequence of increased glycolytic flux in *Mtb*-infected macrophages may do more than promote a pro-inflammatory and antimicrobial environment through the regulation of inflammation, but, in itself, can deprive replicating *Mtb* of essential nutrients required for intracellular growth. Osaka-Oka et al, 2019, demonstrate that IFN-γ treatment restores *LdhA* expression and activity in *Mtb*-infected macrophages and *LdhA*-deficient BMDMs allow uncontained intracellular mycobacterial growth[70]. De Carvalho et al recently demonstrated that pyruvate and lactate are in fact superior carbon sources for *Mtb* over glucose and fatty acids but only when oxygen is plentiful[71], which may not be entirely reflective of the situation in a developing TB granuloma.

Although a feature of classically studied LPS-activated macrophages[33,34,72], impaired TCA cycle activity has not been well documented in *Mtb*-infected macrophages. This is consistent with TLR2-mediated metabolic reprogramming, which preserves oxidative metabolism to maintain phagocytic capacity[69]. Although less virulent mycobacterial strains drive glycolysis without significantly altering TCA activity, the thorough analysis performed by Cumming et al in hMDM revealed that virulent *Mtb* reduces total bioenergetic activity, including both glycolytic metabolism and oxidative metabolism[55]. Another key observation is that although oxidative metabolism remains intact in *Mtb*-infected macrophages, anaplerotic pathways feed TCA cycling and infected macrophages display a profound fuel preference for fatty acids as their carbon source[55]. This fits with the long-held observation of foamy macrophage appearance following *Mtb* infection both in vitro and in vivo[73,74], discussed in the following section.

FOAMY MACROPHAGES

Lipid droplet-laden foam cell macrophages are hallmarks of the developing TB granuloma[83]. These differ from those observed in systemic lipid disorders such as obesity and atherosclerosis[22] both quantitatively (numbers of lipid droplets per cell and foamy cells per granuloma) and quantitatively (lipid composition). Additionally, the source of lipids fuelling this remains unclear. It has been shown, however, that *Mtb* can use host cholesterol as a carbon source in long-term murine models of infection[75]. Thus, it is plausible that the lipid-rich pulmonary environment provided by surfactant is a source of exogenous lipids, both FA and cholesterol, which are taken up by unregulated SR activity in *Mtb*-infected macrophages to drive foamy macrophage appearance, although this has not been formally examined. While atherosclerotic foam cells are cholesterol-dominated, *Mtb* granulomas are triglyceride-rich[76], with mycobacterial ligand signalling through macrophage receptors to alter cellular triglyceride content[77]. This indicates a dynamic remobilization of intracellular lipids in *Mtb*-infected macrophages that can favour bacterial growth[78]. In fact, using FA-loaded macrophages, Ouimet et al observed strong colocalization of *Mtb* with lipid droplets in a pathway regulated by microRNA-33-mediated control of the autophagy machinery[79].

Although hyperlipidaemia often accompanies type 2 diabetes (T2D) – an increasing risk factor for TB[23] – it is unclear whether changes in systemic lipoprotein levels affect intracellular lipids in recruited or alveolar macrophages to predispose to *Mtb* infection. Recently, oxLDL, the modified form of LDL cholesterol that accumulates subendothelially in atherosclerotic plaques and that drives unregulated SR uptake and pro-inflammatory activation[80], was found to both accumulate in guinea-pig granuloma models[81] and circulate in serum from T2D patients[82]. Vrieling et al demonstrated that oxLDL-loaded hMDMs display higher mycobacterial burden consistent with lysosomal cholesterol accumulation and subsequent dysfunction[82].

How *Mtb* infection drives lipid droplet formation in macrophages remains unclear, although a role for the lipid-responsive nuclear receptor PPARγ has been demonstrated [83–85]. While this process may serve as another immunometabolic evasion mechanism to co-opt the intracellular environment towards bacterial growth, recent evidence suggests that the appearance of a foamy macrophage phenotype may in fact serve as a key host defence mechanism. Knight et al profiled the lipid content of *Mtb*-infected macrophages with and without IFN-γ treatment. Remarkably, while both treatments induced the appearance of lipid droplets, the lipid species in IFN-γ-treated macrophages were qualitatively different from those measured in *Mtb* foamy macrophages with more apparent triglycerides and cholesterol esters[86]. They demonstrate that IFN-γ drives metabolic reprogramming through HIF-1α, which alters the macrophage lipidome to promote the production of host bioactive lipids prostaglandin E2 and leukotriene B4, which are protective in *Mtb* infection[87], and reprogramming lipid metabolism away from species that would favour *Mtb*.

Finally, it is must be noted that lipids themselves represent a key feature of the TB granuloma, with an area of extracellular lipid deposition apparent in developed granuloma, termed the caseum[88]. This is thought to originate from cell death of infected and bystander foamy macrophages, suggesting that although these cells may play a role containing *Mtb*, over time the metabolic stresses

caused by exhausted glycolysis, extensive FAO and mitochondrial repurposing may eventually become too much for the cell and loaded with the added burden of pathogen growth, eventually commits to cell death. The form of cell death and the way in which surrounding phagocytes recognize and respond to this can determine the fate of the granuloma and the response to TB[89]. Regulated apoptosis driven by functional healthy mitochondria can lead to efferocytosis and successful degradation of its infectious cargo. Unregulated macrophage necrosis indicative of dysfunctional, repurposed mitochondria will release the cell metabolic contents alongside extracellular *Mtb* and eventually lead to granuloma breakdown and dissemination. Although the impact of various *Mtb* strains and macrophage backgrounds on cell death pathways has been well documented[90], how they overlay with the metabolic stresses triggered by *Mtb*-induced metabolic reprogramming and impinge upon mitochondria function may in fact determine the outcome of infection as shown in Figure 2, and therefore is a fruitful area for further investigation.

T-CELL RESPONSES IN TB

T-cell and *Mtb* containment

During a nascent *Mtb* infection, a robust T-cell response can be detected by 6–8 weeks after infection. Epidemiological observations indicate that this immune response is not associated with clearance of the pathogen. Rather, this response is used in clinical medicine as a proxy for ongoing infection, with tuberculin skin test (TST)-positive individuals offered antibiotic therapy, precisely because they are thought to harbour live *Mtb*. In the minority of infected contacts (5%–10%) who progress to active pulmonary TB disease, the size of their TST response is indistinguishable from infected contacts without disease[91], again providing evidence that this immune response does not correlate with pathogen killing. In fact, in studies that have enumerated interferon-gamma-producing cells that respond to *Mtb*-specific antigens, a stronger T-cell response if anything correlates with disease, rather than

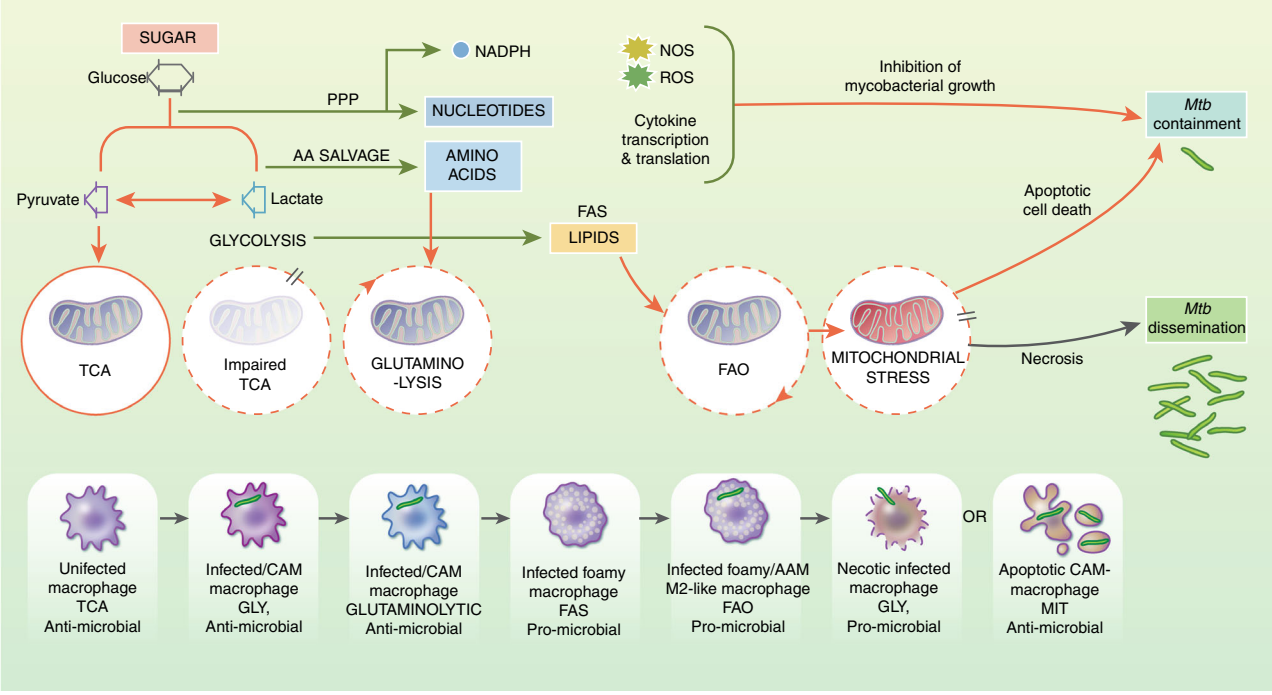

Figure 2. Metabolic reprogramming in the macrophage response to *Mtb*. 6-C Sugar is metabolized via cellular glycolysis to 3-C pyruvate, reduced to 2-C acetyl-CoA to feed mitochondrial TCA and associated oxidative phosphorylation (OXPHOS). After infection or classical activation (CAM), macrophages adopt glycolysis and up-regulate 3-C lactate, which attenuates TCA cycling but supports pro-inflammatory and antimicrobial mechanisms. In *Mtb*-infected macrophages, AA derived from glycolytic salvage pathways or endogenously can support TCA via glutaminolysis and support pro-inflammatory activities. TCA repurposing supports fatty acid synthesis (FAS) and is associated with foamy macrophage appearance after *Mtb* infection. De novo fatty acids can generate acetyl-coA via b-oxidation to support TCA (FAO), as observed in altenatively-activated macrophages. Distinct foamy macrophage phenotypes are associated with host defence or *Mtb* growth. Finally, if infection is not contained by the induction of pro-inflammatory cytokines and antimicrobial species (ROS and NO), macrophages commit to cell death. Metabolic reprogramming and associated glycolysis and defective TCA could lead to mitochondrial repurposing and induction of apoptosis, which supports host defence through restricting niches for bacillary replication. However, if other metabolic signals support mitochondrial activities, the bacteria can preferentially drive host T-cell lysis and necrosis, leading to enhanced bacterial dissemination. Thus, the metabolic output of the macrophage has a major effect on host cell fate and bacterial containment

protection[92,93]. Among patients with TB disease, a significantly lower proportion of individuals have a measurable TST response in disseminated forms of TB, such as miliary TB and meningeal TB[94]. Consistent with this, in CD4 T-cell-deficient HIV-positive individuals who have been exposed to *Mtb*, extrapulmonary TB is more frequent[95]. Thus, while there is epidemiologic evidence supporting an association between T-cell responses and containment of infection, there is no evidence that these responses are associated with *Mtb* elimination.

Mtb has coevolved with humans to achieve an evolutionary trade-off that infrequently compromises host fitness for survival. Usually, rapid changes in the genome of a pathogen (e.g. influenza virus) provide an evolutionary strategy to outpace host immunity. However, the mutation rate in *Mtb* is low[96] and human T-cell epitopes of *Mtb* reveal no more sequence variation than genes essential for pathogen survival, indicating that *Mtb* genomes are not being selected for evasion of T-cell recognition[97]. T cells have in fact been proposed to contribute to TB transmission by participating in the induction of cavitary lung disease[98,99]. Furthermore, a mouse strain (C3HeB/FeJ) that generates robust T-cell responses rapidly succumbs to experimental *Mtb* infection due to necrotic granulomas[100,101]. In addition to the studies above, increasing T-cell responses above the normal generation of natural immunity may not provide enhanced protection. A TB vaccine candidate, called MVA85A (recombinant vaccinia Ankara-expressing Ag85A), generates enhanced T-cell mediated immunity, yet, in a phase IIb infant clinical trial, there was no demonstrable protection against TB disease[102]. Similarly, a large randomized adult clinical trial of comparison of BCG revaccination with H4:IC31 (a candidate subunit vaccine contains both Ag85B and TB10.4 *Mtb* antigens) showed a significant protection in the BCG group [103]. However, genetic investigations have revealed that prominent T-cell antigens of *Mtb* (e.g. ESAT-6, CFP-10, MPT64, MPT70 and MPT83) are missing from BCG strains used in clinical trials[104], consistent with the clinical observation that in BCG trials, there was no correlation between the proportion of subjects that converted their TST and subsequent protection against TB[105–107]. Furthermore, in human studies that monitored T-cell responses, while it was shown that BCG induced increased T-cell responses, there were no data linking these responses to protection[108,109].

Although the basis for using adjuvants in non-live vaccines (e.g. M72 in TB) is their ability to promote effector T- and B-cell differentiation, the direct impact of adjuvants on innate immune cells via pattern recognition receptors (PRR) and innate immunity has so far been neglected. Indeed, adjuvants containing diverse PRR agonists have significantly improved the efficacy of several human vaccines. For instance, the ASO1 adjuvant is common to the current M72 TB vaccine with 54% efficacy among individuals with prior *Mtb* infection[110], the shingles vaccine (Shingrix) with more than 95% efficacy among older adults (>50 years old)[111], and the RTS,S malaria vaccine with 50% efficacy[112]. AS01 is a liposome-based adjuvant containing MPLA (TLR4 agonist) and the purified saponin fraction QS-21 (NLRP3 agonist)[113,114]. Thus, in M72-TB vaccine, the direct impact of ASO1 in boosting innate immunity and protection versus *Mtb* antigen-specific T-cell-mediated protection remains to be determined. It is also important to indicate that the preclinical trials of subunit vaccines with *Mtb* antigens provide modest protection against *Mtb*[115,116,117]. In addition, people previously diagnosed and treated for TB retain strong T-cell responses, yet remain at high risk for developing disease again[118]. Similarly, in mice cured of primary pulmonary *Mtb* infection, North and colleagues demonstrated that the secondary T-cell responses to subsequent *Mtb* challenge do not prevent and resolve the infection[119]. This argues that although the primary infection has elicited a robust *Mtb* antigen-specific T-cell responses, which helps to contain the infection, this was not sufficient to prevent additional episodes of disease. Whether the generation of enhanced T-cell-mediated responses is detrimental, or merely not beneficial, remains to be determined.

For a long time, immunologists have studied host resistance to infections, which focuses on detection and destruction of pathogens. However, it appears that tolerating an infection, as well as controlling tissue damage, is also critical for host defence against pathogens[120]. While the concept of disease tolerance is well established in plants[121], its contribution to mammalian host defence has only recently been appreciated. We have also recently shown (unpublished data) that a mitochondrial protein known as cyclophilin D is a key molecular mediator of T-cell-mediated host tolerance to TB[122,123]. Thus, one possibility is that T cells contribute to containing *Mtb* and tolerating its presence, to mitigate immunopathology and maximize host fitness (Figure 1). As T cells have not been shown to generate sterilizing immunity, efforts to generate a TB vaccine based on T cells might exceed their intrinsic functional capacity, which is potentially containment of *Mtb*.

Contribution of T cells to disease tolerance

The identification of mutations in the IL-12/IFN-γ/STAT1 axis that lead to disseminated mycobacterial infections, termed Mendelian susceptibility to mycobacterial disease (MSMD), along with the susceptibility of T-cell-deficient hosts to mycobacterial infections established the dogma that IFN-γ-producing T cells play a crucial role in host resistance against TB. However, there is no direct evidence of T cells/IFN-γ in protection against *Mtb*, but rather contains infection[92,93,95], via regulation of the

inflammatory response. For instance, extrapulmonary TB is associated with individuals having lower measurable tuberculin skin test (TST) responses[94], as well as with HIV-positive individuals with very low $CD4^+$ T-cell counts[95]. In addition, IFN-γ has been shown to inhibit pulmonary neutrophilic inflammation to prevent lung tissue damage during the chronic phase of *Mtb* infection[124,125]. High levels of neutrophils generate a strong inflammatory response that results in increased pulmonary pathology and mortality. Importantly, neutrophil depletion in IFN-$\gamma R^{-/-}$ mice prolonged their survival[124]. The contribution of neutrophils to immunopathology during *Mtb* infection has been well established in mice[126], NHP[127,128] and humans[129]. These studies collectively indicate that the IFN pathway is critical in the regulation of inflammatory signals and disease tolerance rather than host resistance.

Furthermore, dysregulated T-cell responses appear to be detrimental for the host by inducing overt immunopathology. It has been well documented that during chronic viral infection, constant exposure of T cells to antigens and inflammatory cytokines leads to loss of T-cell function, a process termed 'T-cell exhaustion'[130]. One of the well-defined pathways in T-cell exhaustion is programmed cell death (PD1). The interaction between PD1, which is expressed on antigen-experienced T cells, and its ligands PDL-1 and PDL-2 prevents T-cell proliferation and cytokine production. Thus, it was thought that the inhibition of PD1 signalling should promote protection via 'reviving' T-cell-mediated immunity to chronic *Mtb* infection. However, while disruption of PD1 signalling through either genetically or neutralizing antibodies significantly enhanced T-cell-mediated immunity to *Mtb* infection, this was associated with increased bacterial growth, massive pulmonary immunopathology and reduced survival[131,132]. Thus, the regulatory mechanisms involved in the expansion and contraction of T-cell responses become a critical determinant of the outcome of TB infection. While the surface expression of some of these markers (e.g. PD1 or KLRG) on T cells appears to be critical for dictating their functional role during infection, the intrinsic immunoregulatory mechanisms of T cells are poorly understood.

T-cell immunometabolism

To meet the metabolic demands of active cells, mitochondria rapidly switch from a state of catabolism to anabolism to provide the biosynthetic intermediates that are particularly important for lymphocyte function. Naïve T cells have a low rate of metabolic activity, characterized by minimal nutrient uptake and biosynthesis. These cells procure ATP from the energetically efficient processes (OXPHOS) and fatty acid oxidation (FAO)[133]. Upon TCR activation however, dramatic metabolic reprogramming occurs to generate the increased energy needed for T-cell proliferation, differentiation and cytokine production. To ensure adequate metabolic resources are available, activated T cells increase nutrient uptake and switch from OXPHOS and FAO to aerobic glycolysis[133]. While energetically inefficient, glycolysis enables the cells to rapidly produce ATP and other biosynthetic precursors essential for cell growth and proliferation. This switch from predominantly OXPHOS to aerobic glycolysis, despite the presence of abundant oxygen, is known as the 'Warburg effect'. Metabolic shift from OXPHOS to glycolysis or vice versa is also highly associated with the inflammatory and anti-inflammatory role of immune cells[134].

The metabolic condition also controls the cell fate determination[135]. Th17 cell differentiation relies on glycolysis, whereas blocking glycolysis inhibits Th17 development and promotes regulatory T-cell (Treg) differentiation. Th17 cells are important in host resistance to *Mtb*, but uncontrolled production of IL-17 induces inflammation via recruitment of neutrophils and increases the mortality of *Mtb*-infected mice[124]. Higher susceptibility of TLR-2-KO mice to *Mtb* has been linked to reduced accumulation of Treg cells and concomitant increased inflammation[136]. Thus, a balance is required for Th17/Treg development in TB. These findings suggest that the metabolic state determines the fate of immune cells, which is critical in promoting or dampening inflammation.

An equally important function of mitochondria is their role in the cell death programme. Cyclophilin D (CypD), a member of the cyclophilin protein family, is a conserved protein located in the mitochondrial matrix[137]. It has been previously shown that CypD plays a key role in necrosis by regulating the mitochondrial permeability transition pore (MPTP), which allows the passage of solutes and water from the cytoplasm into the mitochondria[138,139]. Necrosis of macrophages represents an exit mechanism for *Mtb*[13,140–142]. Remold and colleagues initially demonstrated that the pharmacological inhibition of CypD in human macrophages leads to the inhibition of necrosis and reduction of *Mtb* growth in vitro[143]. This observation has been recently extended to the zebrafish and mouse models of tuberculosis where the genetic blockade of CypD prevented macrophage necrosis and enhanced their antimycobacterial capacity[144,145]. Based on the role of CypD in macrophage immunity to *Mtb* infection, we initially hypothesized that CypD-deficient mice ($CypD^{-/-}$) are resistant to *Mtb* infection. Surprisingly, $CypD^{-/-}$ mice were highly susceptible to *Mtb* infection compared with control animals, despite similar numbers of bacteria in both groups. We further identified that this susceptibility was related to an enhanced T-cell response that promoted lung immunopathology independent of host resistance. We could show that CypD intrinsically regulates T-cell metabolism and critically regulates disease

tolerance in TB[146]. Similarly, the C3HeB/FeJ mouse strain that generates a profound T-cell response to *Mtb* infection quickly succumbs to death due to the overgrowth of necrotic granulomas[147]. Although it is still unclear why the functional role of CypD is different in macrophages versus T cells, we envision that as T cells are intrinsically programmed to proliferate, the functional role of CypD in these cells may be wired to regulate the metabolism and proliferation rather than cell death. Collectively, these data indicate that, similar to granulomas, T cells are a double-edged sword: while they are crucial to initiate granuloma formation during the early phase of *Mtb* infection and prevent the dissemination of disease, they also play an important role in transmission of *Mtb* by promoting granuloma necrosis during the active phase of the disease[2]. Thus, the function and location of these effector immune cells are critical determinants of host resistance and disease tolerance in TB.

TARGETING INNATE IMMUNITY AND CELLULAR METABOLISM AGAINST *MTB*

In TB, while there is evidence to support a role for T cells in disease tolerance during infection, the evidence that they can contribute to host resistance is limited. We envision that the translocation of *Mtb* from the airways into the lung interstitial tissue signals the transition of host defence mechanisms from resistance to tolerance[148]. However, the remarkable efficacy of innate immunity to eliminate a significant numbers of pathogens from plant to invertebrates and vertebrates, as well as their memory-like capacity (trained immunity), indicates that the power of innate immune cells, particularly macrophages, can be harnessed against pathogens. The finding that BCG vaccination provides protection against *C. albicans* in SCID mice lacking both B and T cells, which depends on macrophages[149], along with human studies indicating that BCG protection in volunteers challenged with yellow fever or malaria was associated with inflammatory monocytes[150,151] supports the concept of trained immunity, which can be used to promote host resistance against TB. A variety of live vaccine (e.g. BCG or oral polio vaccine) and adjuvants (e.g. β-glucan or TLR agonists) have shown to induce trained immunity, which drive metabolic and epigenetic changes in target cells, including innate immune cells (e.g. monocytes, macrophages, NK cells) or stromal cells (e.g. epithelial cells). While direct training of fully differentiated innate immune cells has a limited efficacy for generating long-term memory response, bone marrow haematopoietic stem cells (HSCs) with self-renewing capacity can overcome this limitation[152]. For instance, we have recently demonstrated that exposure of HSCs to bacille Calmette–Guérin (BCG) or β-glucan in the BM results in their reprogramming to promote myelopoiesis and protective trained immunity in macrophages against *Mtb* infection in type II IFN- or IL-1-dependent manner, respectively[153,154]. Activation and proliferation of HSCs by β-glucan appears to be dependent on IL-1β and glycolytic metabolism[155]. Up-regulation of glycolysis alongside a broken TCA cycle provides acetyl and methyl groups required as both cofactors and substrates for histone remodelling at inflammatory genes[156]. In addition to up-regulated glycolytic metabolism, increased lipid biosynthesis is also a feature of Training[154,155,157]. Mevalonate is a key metabolic intermediate in the cholesterol biosynthesis pathway, and

Table 1. Select examples of targeting immunometabolism for improved TB host defence

Strategy	Metabolite	Immune Pathway	Outcome	Ref
Micronutrient supplementation	Vitamin D Vitamin A	(AMPS) cathelicidins Autophagy, lipid metabolism	Promote resistance	163,164,165
Macronutrients supplementation	Glutamine Arginine	Metabolic reprogramming, NO production	Promote resistance Modulate tolerance	166,167
Macronutrient depletion	Tryptophan	Mycobacterial metabolism, granuloma formation	Promote tolerance	168
Microbial metabolites	SCFA IPA	Anti-inflammatory	Modulate tolerance Promote resistance	173,174
Repurposing small molecule	Rapamycin analogues (everolimus)	mTOR/AKT1 glycolytic reprogramming	Modulate tolerance	169,56
Targeting whole-body metabolism	Metformin	Modulate hyperglycaemia, autophagy, cytokine production	Modulate tolerance	170,171,172
	Statins	Macrophage foam cells	Promote resistance	
Promote immune training	BCG Beta-glucans	Promote bone marrow myeloid expansion	Increased resistance	153,154

Abbreviations: AMPK, AMP protein kinase; AMPs, antimicrobial peptides; BCG, bacille Calmette–Guérin vaccine; HMGCR, HMG-CoA reductase; IPA, indole-3-propionic acid; NO, nitric oxide; SCFA, short-chain fatty acids.

mevalonate treatment can drive trained immunity[158]. Finally, both BCG and β-glucan-trained macrophages differ from classically primed macrophages as not only they increase glycolytic metabolism but also they manage to retain some oxidative capacity through anaplerotic feeding of TCA, particularly using amino acids as alternative carbon sources, chiefly through glutaminolysis[156,159]. This preserves the viability of trained cells and allows the replenishment of TCA mediators including α-keto-glutarate production, to provide the substrates and cofactors for epigenetic remodelling. Collectively, these studies suggest that targeting trained immunity can be a novel approach for developing vaccine against TB. Additionally, the use of trained immunity inducers to drive metabolic reprogramming in immune progenitor cells can be a useful strategy to promote disease resistance (Table 1).

CONCLUDING REMARKS

Mtb has coevolved with humans for ~70,000 years[160,161] and achieved an evolutionary trade-off that infrequently compromises host survival. This trade-off has been conventionally considered to be dependent on host resistance for limiting the growth of *Mtb*. This ultimately led to the discovery of extensive cellular and molecular mechanisms that were thought to be only engaged in host resistance to TB. However, recent studies indicate an equally important arm of immunity to TB is disease tolerance that limits the collateral tissue damage caused by *Mtb* or immune responses maintaining the physiological function of the lung. Thus, understanding the entire landscape of both innate and adaptive immunity and their contributions to host resistance and disease tolerance against *Mtb* is required for developing novel vaccine or therapy. In TB, while there is evidence to support a role for T cells in disease tolerance during infection, the evidence that they can contribute to host resistance is limited. We envision that the translocation of *Mtb* from the airways into to the lung interstitial tissue, which leads to granuloma formation, signals the transition of host defence mechanisms from resistance to tolerance[148]. Therefore, it becomes essential to dissect the functional capacity of T cells, which is regulated by their cellular metabolism and dictated by the complex microenvironment of the granuloma, preventing dysregulated T-cell responses and overt immunopathology.

Although we know a great deal about the immune response to chronic pulmonary *Mtb* infection, our understanding of *Mtb*-Mφ interactions and its contribution to the chronicity of disease is extremely limited. Throughout the tug of war between macrophages and *Mtb* that includes lysosomal function and ultimately the mitochondria metabolism, the fate of the macrophages is decided to either become a permissive host for *Mtb* and die of necrosis promoting infection or induce apoptosis terminating the niche of infection[162,140,13,89]. Thus, strategies targeting trained Immunity and cellular metabolism (Table 1) are novel approaches to enhance host defence against TB.

TB was known as 'consumption' as this chronic infection causes dramatic cachexia (wasting). Interestingly, a study examining *Mycobacterium marinum* infection in the fruit fly *Drosophila melanogaster* showed that the increased mortality is independent of bacterial load and is mediated by altered host metabolism and increased body wasting. Thus, the fitness of the host during infection can not only be evaluated by the ability to eliminate the pathogen, but also by the ability to survive chronic infection. Unravelling the cellular and molecular mechanisms involved in the regulation of metabolism in different population of Mφ and T cells is essential for a complete understanding of the pathogenesis of TB.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Not applicable.

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