Not too fat to fight -The emerging role of macrophage fatty acid metabolism in immunity to Mycobacterium tuberculosis

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Summary

While the existence of a special relationship between *Mycobacterium tuberculosis* (Mtb) and host lipids has long been known, it remains a challenging enigma. It was clearly established that Mtb requires host fatty acids (FAs) and cholesterol to produce energy, build its distinctive lipid-rich cell wall and produce lipid virulence factors. It was also observed that in infected hosts, Mtb constantly resides in a FA-rich environment that the pathogen contributes to generate by inducing a lipid-laden "foamy" phenotype in host macrophages. These observations, and the proximity between lipid droplets and phagosomes containing bacteria within infected macrophages gave rise to the hypothesis that Mtb reprograms host cell lipid metabolism to ensure a continuous supply of essential nutrients and its long-term persistence *in vivo*. However, recent studies question this principle by indicating that in Mtb-infected macrophages, lipid droplet formation prevents bacterial acquisition of host FAs while supporting the production of FA-derived protective lipid mediators. Further, *in vivo* investigations reveal discrete macrophage phenotypes linking the FA metabolisms of host cell and intracellular pathogen. Notably, FA storage within lipid droplets characterizes both macrophages controlling Mtb infection and dormant intracellular Mtb. In this review, we integrate findings from immunological and microbiological studies illustrating the new concept that cytoplasmic accumulation of FAs is a metabolic adaptation of macrophages to Mtb infection, which potentiates their antimycobacterial responses and forces the intracellular pathogen to shift into fat-saving, survival mode.

Introduction

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), has evolved sophisticated strategies to survive intracellularly in lung macrophages and extracellularly within granulomas. High lipid content, including fatty acids (FAs) and cholesterol, is a common denominator of the microenvironments of these two infection sites. The following sections describe how Mtb contributes to their generation and how, in return, host lipids impact on Mtb metabolism and physiology. We then confront these data, mostly generated *in vitro* using cellular models of infection, with the *in vivo* reality of infected animal models. Finally, we highlight current research gaps and the key questions to address in order to progress towards novel anti-tuberculosis (TB) therapies targeting host-lipid metabolism.

I - *Mycobacterium tuberculosis* is constantly exposed to FAs in infected hosts

When aerosols of Mtb penetrate the lungs of a new host, bacilli are phagocytosed by professional alveolar macrophages (AMs) in the lower respiratory tract and start replicating intracellularly. Infected AMs subsequently relocate to the lung interstitium, allowing bacterial dissemination to monocyte-derived macrophages, neutrophils and dendritic cells [1]. Migration of Mtb-infected dendritic cells to the draining lymph nodes initiates adaptive immune responses, leading to the recruitment of monocytes and lymphocytes to the primary site of infection [2]. Immune infiltrates aggregate around Mtb-infected macrophages to form granulomas [3], concentric structures that are pathological hallmarks of mycobacterial diseases. Tuberculous granulomas are characterized by the presence of “foamy” macrophages

harboring dormant bacilli (reviewed in [4]). Their foamy appearance is due to cytoplasmic accumulation of lipid droplets (LDs) that are mainly composed of triacylglycerols (TAGs), a storage form of FAs, and cholesteryl esters [5] (Figure 1).

Tuberculous granulomas often display a central area of caseous necrosis - the caseum - where Mtb bacilli were found to persist extracellularly [6,7]. Rupture of necrotic granulomas releases live Mtb bacilli into the airways, enabling bacterial transmission [8]. Since the lipid composition of TB granulomas [5,6] and sputum from TB patients [9,10] resembles that of LDs, it is believed that the caseum derives from the content of LDs released by dead foamy macrophages. Although Mtb resides primarily within foamy macrophages and the caseous centre of necrotic granulomas, bacilli can also persist in other TAG-rich tissues. Indeed, Mtb was detected in the adipose tissue of patients with active and latent TB, and it was shown that Mtb can infect and survive intracellularly in a non-replicative state within adipocytes [11].

Therefore, whether intracellularly in foamy macrophages or extracellularly within granulomas, Mtb is constantly exposed to a lipid-rich environment during its life cycle in humans. As reviewed in the next section, Mtb contributes to generate this environment by fundamentally reshaping the lipid metabolism of host macrophages and promoting the intracellular accumulation of TAGs.

II - Reprogramming of the metabolism of macrophages by *M. tuberculosis*

Recent advances in gene expression and metabolome profiling have begun to reveal a complex, biphasic remodeling of the metabolism of Mtb-infected macrophages, which is intimately coupled to inflammatory and antimicrobial responses.

a) Reprogramming of energy metabolism

Activation of TLR2/4 by Mtb components was shown to trigger in host macrophages a switch towards aerobic glycolysis within the first 24h of infection (Figure 2). This metabolic shift was associated with inhibition of the tricarboxylic acid (TCA) cycle and reduction in oxidative phosphorylation (OXPHOS) activity [12–14]. Reprogramming of glycolysis involved production of the Hypoxia-Inducible transcription Factor HIF-1α and increased host macrophage ability to restrict intracellular growth of Mtb in both cellular and animal models of infection, *via* a mechanism involving IL-1β [15,16]. Besides, TLR4 activation induces the accumulation of the TCA cycle intermediate succinate, which promotes aerobic glycolysis by boosting HIF-1α activity and the associated production of IL-1β [17]. TLR4 activation also induces a break point in the TCA cycle at isocitrate dehydrogenase (IDH), diverting the cis-aconitate substrate of IDH towards production of itaconate by aconitate decarboxylase, also known as immune-responsive-gene 1 (IRG1) [18]. In macrophages, infection with Mtb downregulated IDH production while increasing expression and activity of IRG1, thus promoting the production of itaconate [19]. Yet, itaconate has anti-inflammatory effects in activated macrophages, through inhibition of the production of inflammatory cytokines and reactive oxygen species (ROS) [20,21]. In addition, itaconate was shown to be a potent inhibitor of isocitrate lyases Icl1/2 in Mtb [22]. Together with malate synthase, mycobacterial Icl1/2 form an anaplerotic pathway called the glyoxylate shunt, which converts the TCA cycle intermediate isocitrate into malate and succinate. Products of the glyoxylate shunt fuel gluconeogenesis in Mtb when FAs are the only available carbon sources, and this metabolic pathway was found critical for bacterial growth *in vivo* [23]. Notably, IRG1-deficient macrophages were defective in their ability to control intracellular Mtb growth [19] and IRG1 expression prevented immunopathology in a mouse

model of Mtb infection [24], demonstrating the importance of this pathway in host defense against Mtb. Reprogramming of aerobic glycolysis and TCA cycle are associated with increased levels of NADPH oxidase and inducible nitric oxide synthase (iNOS), promoting the production of ROS including nitric oxide (NO) with antimycobacterial activity [25,26]. Therefore, the early immunometabolic responses of macrophages to Mtb infection promote Mtb killing (Figure 2).

It is important to note that Mtb has evolved several mechanisms to subvert the immunometabolic response of macrophages to infection (Figure 2). Compared to BCG or dead Mtb, live Mtb decelerated metabolic flux through glycolysis and the TCA cycle in host macrophages, while increasing mitochondrial dependency on exogenous FAs [12]. Unlike the avirulent strain of Mtb H37Ra, infection with virulent H37Rv induced expression of the microRNA (miR-21), which targets a glycolytic enzyme [27]. The resulting defects in glycolysis and production of IL-1β increased Mtb's ability to replicate and persist in resting and IFN-γ-activated macrophages [27]. Of note, overexpression of the cell wall lipids phthiocerol dimycocerosates (PDIMs) by certain multi-drug resistant strains of Mtb impaired the glycolytic reprogramming of host macrophages via an IFN-β-dependent mechanism bypassing the IL-1R signaling pathway [28].

b) <u>Rewiring of lipid metabolism for intracellular accumulation of TAGs</u>

The above-described reprogramming of energy metabolism develops during the first 24h of macrophage infection and resolves thereafter, while a second wave of metabolic changes begins that profoundly modifies the lipid metabolism of macrophages [25]. In addition to its effects on itaconate and succinate (Figure 2), TLR-driven blockade of the TCA cycle favors the accumulation of citrate, whose export from the mitochondria and cytosolic cleavage generates acetyl-CoA that fuels *de novo* synthesis of cholesterol and FAs (Figure 3).

Besides, TLR2 stimulation by mycobacterial cell wall lipoarabinomannan (LAM) was shown to induce macrophage expression of Peroxisome Proliferator-Activated Receptor (PPAR)-γ [29], a transcription factor regulating genes involved in uptake and mobilization of FAs [30,31] (Figure 3). Knocking down PPAR-γ limited the upregulation of low-density lipoprotein receptor CD36 and formation of LDs in infected macrophages, and this coincided with an altered intracellular growth of Mtb [32]. Moreover, PPAR-γ synergized with testicular nuclear receptor 4 (TR4), activated by Mtb-derived ketomycolic acids, to induce LD accumulation in macrophages via an unknown mechanism [33].

Accumulation of TAGs in Mtb-infected macrophages was shown to require autocrine TNF-α receptor signaling, and downstream activation of the caspase cascade and the mammalian target of rapamycin complex 1 (mTORC1) [5]. Although the underlying mechanism was not fully elucidated, TNF-α-mediated accumulation of TAGs was proposed to involve inhibition of autophagy, regulation of transcription factors involved in TAG biosynthesis (e.g. sterol regulatory element-binding proteins, SREBPs) and a mitochondrial dysfunction leading to reduced FA oxidation [5]. Mtb infection also blocked autophagy and associated lipid catabolism in host macrophages by inducing miR-33 via an unknown mechanism [34]. Finally, Mtb decreased LD lipolysis via induction of 3-hydroxybutyrate (3-HB), a metabolite derived from the ketogenesis pathway and generated from acetyl-CoA. Secreted 3-HB activated the G protein-coupled receptor GPR109A, whose signaling resulted in the stabilization of perilipin 1, a coating protein protecting LDs from lipases [35]. The Mtb virulence factor Early-Secreted Antigenic Target (ESAT)-6 was identified as an activator of 3-HB production. ESAT-6 induced glucose uptake as well as the activity of glycolytic enzymes, resulting in accumulation of acetyl-CoA, which was subsequently reoriented towards the generation of 3-HB [35]. Overall, these

studies revealed multiple mechanisms by which Mtb infection promotes LD formation in host macrophages.

Because the presence of foamy macrophages characterizes chronic TB and correlates with pathology, induction of LDs was initially considered a bacterial virulence process ensuring a continuous lipid supply in infected cells [4,35,36]. In support of this hypothesis, intracellular Mtb was shown to import FAs deriving from host TAGs [37]. However, this view was recently challenged by evidence that Mtb loses capacity to acquire host lipids in the presence of IFN-γ induced LDs. In both macrophage and mouse models of Mtb infection, LD formation and maintenance were found to require IFN-γ-driven induction of HIF-1α and its target gene *Hig2*, which inhibits lipolysis [38]. This argues against LDs being a major source of FAs for intracellular Mtb in IFN-γ-activated macrophages, and suggests that LD formation is a host-driven, adaptive immune process of defense. Besides, LDs were found to support the generation of proinflammatory immune responses in mycobacteria-infected macrophages by representing predominant sites of eicosanoid production [4,38,39]. Indeed, Mtb infection triggers rapid mobilization of long-chain FAs from plasma membranes, particularly the poly-unsaturated arachidonic acid (AA), the precursor of eicosanoids [25]. Cyclooxygenases convert AA into prostaglandins and lipoxygenases generate leukotrienes and lipoxins, with distinct immunoregulatory functions. It was shown that prostaglandin E2 promotes mycobacterial control while on the opposite lipoxin A4 decreases mycobacterial resistance, illustrating the dual role of eicosanoids in host response against Mtb infection [40,41]. Finally, recent data indicate that TLR4 stimulation promotes the assembly of host defense proteins (including IFN-inducible effectors and antimicrobial peptides) into LDs [42]. Further, it uncouples LDs from mitochondria, resulting in decreased OXPHOS and increased contacts between LDs and bacteria [42]. While additional studies will be required to determine if such effects occur in Mtb-

infected macrophages, this suggests that LDs may directly participate in innate immunity against Mtb by directly killing intracellular Mtb while mediating metabolic adaptation to infection.

In addition, there is some evidence suggesting that a reduced FA oxidation would enhance host's defense against Mtb. In the cytosol, free FAs released by internalized lipoproteins or by LD lysis are captured by FA binding proteins and thioesterified by the acyl-CoA synthetase. The resulting FA-CoA are conjugated to carnitine by carnitine palmitoyl transferase I (CPT1) and shuttled across the mitochondrial cell wall by a translocase. Within the mitochondria, the carnitine group is removed by CPT2 and FA-CoA are processed through β-oxidation, which generates acetyl-CoA fueling the TCA cycle and reduces flavin adenine dinucleotide FAD (to FADH2) and nicotinamide adeninedinucleotide NAD+ (to NADH). These reduced products directly feed into the respiratory chain to generate ATP. Chemically inhibiting FA oxidation or knocking out CPT2 both restricted Mtb intra-macrophage growth [43,44]. In Mtb-infected macrophages, FA oxidation blockade was associated with an increase in neutral lipid content, suggesting that decreased Mtb growth is not due to limited FA availability. Instead, it was proposed that blockade of FA oxidation generates mitochondrial ROS leading to phagosomal recruitment of NADPH oxidase, thereby promoting xenophagic elimination of Mtb.

Altogether, data presented in this section call into question the notion that foamy macrophages are permissive for Mtb growth. They introduce the new concept that LD formation in Mtb-infected macrophages may benefit the host by preventing Mtb's access to host FAs while supporting the generation of innate immune responses.

III - Effect of the FA environment on Mtb metabolism and biology

The metabolic changes induced by Mtb infection create a lipid-rich environment for the intracellular pathogen. While the mechanisms by which phagosome-contained Mtb gains access to host cell lipids remain largely unknown; *in vitro* and *ex vivo* studies have provided essential insights into how the lipid environment affects the metabolism and physiology of Mtb (Figure 4).

a) FA import and mobilization

Evidence that Mtb's respiration and growth are stimulated by FAs dates back to the 1950s [45,46]. Since then, several studies have characterized the genetic and enzymatic basis of Mtb's ability to import and metabolize FAs [47]. Mtb's uptake of palmitic acid (PA) and oleic acid (OA) was shown to require a dedicated protein machinery named mammalian cell entry (Mce)1, similar in organization to that importing cholesterol (Mce4) [48,49]. The genome of Mtb harbors four *mce* loci (*mce*1-4), all organized as arrays of two *yrbE* genes (A,B) followed by six *mce* genes (A-F), and a variable number of *mam* genes [50]. YrbE proteins are plasma membrane-embedded permeases interacting with a distantly encoded ATPase MceG [51]. MceA-F are cell wall proteins believed to recognize and shuttle lipid substrates across the mycobacterial outer membrane towards YrbE permeases [52]. Mam proteins are putative accessory proteins involved in the formation and/or stability of their respective Mce complexes [47,48]. Interestingly, Mce1 and Mce4 complexes both require the stabilizing protein LucA and the ATPase MceG, indicating that FA and cholesterol imports are coordinated [47,48,53].

Several transcriptome studies reported an increased expression of the bacterial genes involved in FA and cholesterol import during infection of macrophages and mice [54,55], which

collectively suggest that Mtb reprograms its own lipid metabolism during infection to hijack host lipids. In addition to being imported from the environment, FAs can be mobilized by lipolysis of host membrane phospholipids, endogenous or exogenous TAGs reservoirs. Mtb was shown to use host TAGs to synthesize its own TAGs [37]. This process involves LipY, a lipase produced by Mtb upon macrophage infection as both cytoplasmic and secreted forms that hydrolyze bacteria- and host-derived TAGs, respectively [56–58].

Remarkably, Mtb is able to simultaneously co-catabolize different carbon sources including carbohydrates, FAs and cholesterol [59,60]. This metabolic flexibility contrasts with the carbon catabolite repression that enforces a hierarchical use of carbon sources in most bacteria. Metabolic versatility was proposed to facilitate Mtb's adaptation to changing environments, a notion supported by recent metabolomic studies showing the simultaneous utilization of multiple carbon sources by Mtb within macrophages [61]. It should nevertheless be noted that Mtb's import of cholesterol is required for optimal growth and persistence *in vivo* [49].

Notably, cholesterol catabolism generates the highly toxic propionyl-CoA. Accumulation of propionyl-CoA derivatives in Icl1-deficient mutants (unable to metabolize propionyl-CoA, see §b) intoxicated Mtb and inhibited bacterial growth when Mtb was cultivated in a cholesterol-rich medium [62,63]. However, propionyl-CoA toxicity in *Δicl1* mutants was reduced if the culture medium was supplemented with either short-chain (C2-C8), or long-chain saturated FAs (C18-C24), demonstrating that FA uptake can neutralize the deleterious effects of propionyl-CoA excess. Further, incorporation of ^{14}C-FA in PDIMs was increased in mutants grown in propionate-rich media [62]. Since FAs and propionyl-CoA are precursors for the biosynthesis of PDIMs, it was proposed that utilization of host-derived FAs for the synthesis of methyl-branched lipids such as PDIMs provides a sink for toxic propionyl-CoA [62–

[64]. Reversal of cholesterol-dependent growth inhibition could be achieved by providing Mtb with acetate but not glucose as an alternative carbon source, indicating that cholesterol use imposes metabolic constraints [63].

Altogether, these data indicate that the metabolic pressure exerted on Mtb when metabolizing cholesterol can be compensated via complex lipid biosynthesis. The co-dependence of FA and cholesterol metabolism at all levels (uptake, catabolic and anabolic pathways) is likely to be of paramount importance *in vivo*, due to the TAG- and cholesterol-rich environment of Mtb in infected hosts.

b) <u>FAs as carbon and energy sources</u>

β-oxidation of internalized FAs generates acetyl-CoA, while that of cholesterol produces acetyl-CoA and propionyl-CoA [47]. Acetyl-CoA and propionyl-CoA then feed central metabolic pathways (glyoxylate shunt, methyl citrate cycle (MCC) and methylmalonyl pathway) for energy production or biosynthesis of FAs and complex lipids.

In Mtb, β-oxidation of saturated FAs (SFAs) is ensured by the FadAB enzymatic complex [65], with preliminary conversion of monounsaturated FAs (MUFAs) by enoyl CoA isomerases [66]. Expression of genes involved in the glyoxylate shunt was induced in Mtb grown in a culture medium rich in FAs, in alveolar macrophages and in the mouse model of infection [55,67,68]. As described earlier, this metabolic pathway involves the Icl1/2 enzymes (which cleave isocitrate into succinate and glyoxylate) and GlcB malate synthase, which condenses glyoxylate and acetyl-CoA into malate, fueling other metabolic pathways such as gluconeogenesis. The glyoxylate shunt thus constitutes an alternative to the TCA cycle that ensures the metabolization of acetyl-CoA, while avoiding decarboxylation steps and therefore the loss of

carbon in the form of CO_2. It has been suggested that Mtb preferentially uses this pathway *in vivo* due to carbon source limitation [69].

In Mtb, metabolization of propionyl-CoA from the degradation of odd-chain FAs and cholesterol can occur via the MCC, which generates metabolites fueling the TCA cycle [70,71]. This pathway involves 4 specific enzymes including methylcitrate lyase (Icl1-2). The MCC, and more particularly the Icl1-2 bifunctional enzymes, also appear to play a critical role in Mtb survival *in vivo*. The double mutant *Δicl1Δicl2* was unable to grow in both macrophages and mice [23]. Loss of *in vivo* virulence of *Δicl1* mutants was attributed to the accumulation of cholesterol-derived propionyl-CoA, the excess of which is toxic to Mtb [62].

The methylmalonyl pathway is an alternative to the MCC for metabolization of propionyl-CoA [72]. This anaplerotic pathway ensures generation of methylmalonyl-CoA from propionyl-CoA via the action of the propionyl-CoA carboxylase (Pcc) complex, and methylmalonyl-CoA is subsequently converted into succinyl-CoA in the presence of vitamin B12 [72,73]. Succinyl-CoA can then fuel the TCA cycle and interestingly, this pathway is activated when the MCC is non-functional or inhibited [62,72,73].

Taken together, these studies show that Mtb metabolizes lipids for energy production, while limiting the loss of carbon by decarboxylation (glyoxylate shunt) and moderating the risk of intoxication by propionyl-CoA or its derivatives (MCC). FAs, acetyl-CoA and propionyl-CoA can also serve as substrates for the synthesis of mycobacterial cell wall lipids.

c) <u>FAs as precursors of mycobacterial cell envelope</u>

Situated at the host-pathogen interface, the envelope of Mtb not only plays a major role in bacterial physiology, but also in immunomodulation and tolerance to antibiotics. In

mycobacteria, this envelope is particularly thick and impermeable, with a conventional plasma membrane covered by a complex, lipid-rich cell wall and a capsule mainly composed of polysaccharides and proteins [74]. The cell wall itself is a complex structure composed of superposed layers of peptidoglycan, arabinogalactan and mycolic acids, FAs with exceptionally-long chains whose bilayer organization forms the so-called mycomembrane (Figure 4). This outer membrane also contains non-covalently linked lipoglycans and lipids with pathogenic and immunosuppressive properties, such as PDIMs, phenolic glycolipids and lipoarabinomannan [75,76]. By combining data from *in vitro* and *in vivo* transcriptional and lipidomic studies, Dulberger *et al.* proposed that the mycobacterial wall may be dynamically remodeled during infection, displaying a relatively high content of immunostimulatory lipids at early stages (thus promoting granuloma formation) that decreases thereafter, as the proportion of immunosuppressive lipids such as PDIMs augments [77].

FAs are precursors of various mycobacterial cell wall lipids that can either be *de novo* synthetized by Mtb (endogenous FAs), or imported from the host cell (exogenous FAs) (Figure 4). Similar to eukaryotes, Mtb can synthetize medium- to long-chain saturated FAs from acetyl-CoA via malonyl-CoA generation by acetyl-CoA carboxylase ACC, followed by iterative elongation by the FA synthase-I (FAS-I) enzyme [78]. FAS-I is homologous to the eukaryotic FASN system, and generates 16-22 carbon-long saturated SFAs. C16- and C18-SFAs may then be converted into MUFAs through action of stearoyl-CoA desaturase DesA3 (Rv3229c) coupled to an NADPH oxidoreductase (Rv3230c) [79]. DesA3 is essential for long-term persistence of Mtb *in vivo* [80] and is the target of second-line anti-TB drug isoxyl [81]. Two other desaturases (DesA1 and DesA2) were identified in Mtb that contribute to mycolic acid biosynthesis [50,82]. *De novo* synthesized or exogenous SFAs and MUFAs can be incorporated in plasma membrane phospholipids or cytoplasmic TAGs, the major components of mycobacterial LDs. They can

also serve as precursors for the biosynthesis of cell wall methyl-branched lipids like PDIMs by polyketide synthases (Pks) [83].

Unlike FAS-I, Pks preferentially use propionyl-CoA-derived-methylmalonyl-CoA for FA elongation, thereby introducing methyl branches into FA aliphatic chains [84]. Mtb can elongate further endogenous FAS-I products or exogenous long-chain FAs into very long-chain FAs (22 to 62 carbons) named meromycolic acids via the prokaryote-specific FAS-II enzymatic complex [78]. Pks13-mediated condensation of such meromycolic acids with carboxylated forms of FA-CoA deriving from FAS-I reactions and trehalose yields mycolic acids and trehalose mycolates composing the outer membrane of the cell wall [85,86].

FAs being both precursors of the structured lipids composing the mycobacterial cell wall and important energy-storage molecules, FAS-I and FAS-II play a central role in Mtb physiology and virulence. Genetic manipulation of enzymes in the FAS-II system, or those of the meromycolic acid condensation pathway, impacts mycolic acid layer integrity and bacterial viability [87–89]. FAS-I is essential for mycobacterial survival [90] and is the target of first line anti-TB drug pyrazinamide [91]. The enoyl-acyl carrier protein reductase InhA is an enzyme of the FAS-II system that is targeted by the first-line anti-TB drug isoniazid and its structural analog ethionamide [92]. FasR and MabR are transcriptional regulators of the FAS-I and FAS-II operons that are essential for mycobacterial viability [93,94]. Interestingly, a *fasI* conditional mutant in *M. smegmatis* was defective for biosynthesis of FAs and TAGs, and for bacterial growth in axenic cultures [90]. However, suppressing *fasI* expression did not prevent bacterial production of mycolic acids. In fact, it upregulated the expression of genes involved in the FAS-II pathway. The decreased pool of FAS-I-derived FAs that normally fuels mycolic acid synthesis was compensated by the mobilization of FAs from cytoplasmic TAGs. In addition to revealing

the critical importance of FA biosynthesis for maintenance of cell wall lipid homeostasis, this study highlighted a compensatory crosstalk between the two FAS pathways.

d) FAs, TAG accumulation and quiescence

Intra-phagosomal Mtb must adapt its central metabolism to cope with hypoxia, oxidative and nitrosative stress, as well as acidic pH [95,96]. When mimicked *in vitro*, these conditions dramatically decreased Mtb growth rate, inducing a quiescent state (also referred to as dormant) associated with a switch to anaerobic metabolism [97]. This metabolic shift was driven by the nitric oxide (NO)- and hypoxia-sensitive dormancy survival regulator (DosR) [98,99]. DosR-mediated quiescence supports survival in hypoxic conditions and confers Mtb with an increased tolerance to drugs. Quiescent Mtb can re-initiate a replicative state when environmental conditions improve, and the metabolic reprogramming that is associated with dormancy creates conditions anticipating re-activation. Indeed, hypoxia was shown to induce the accumulation of glycolytic and pentose phosphate pathway intermediates deriving from cell wall trehalose mycolates, which provide building blocks for *de novo* synthesis of peptidoglycan upon re-initiation of replication [100]. More generally, Mtb's entry into dormancy redirects carbon fluxes from energy-generating pathways to anabolic pathways dedicated to storage [71]. Hypoxia, oxidative stress and low iron caused TAG accumulation by Mtb *in vitro*, and Mtb deriving from TB patient sputa displayed a 'fat' phenotype [8,101,102]. Stress-driven TAG accumulation was associated with transcriptional upregulation of TAG synthase Tgs1, and Tgs1 was required for TAG biosynthesis both *in vitro* and in a macrophage infection model [101,103]. A Tgs1-deficient mutant of Mtb was unable to arrest growth in response to stress and remained sensitive to antibiotics during infection, showing that TAG synthesis contributes to antibiotic tolerance *in vivo* [97]. Since acetyl-CoA fuels the FAS-I system, thereby generating FAs

for TAG biosynthesis, it was proposed that stress-induced TAG biosynthesis diverts acetyl-CoA away from the TCA cycle.

In contrast to TAGs, production of most bacterial cell wall lipids is downregulated during dormancy [104,105], suggesting that stress signals reprogram FA metabolism of Mtb towards TAG storage. Reciprocally, a sharp drop in the TAG content of dormant Mtb was observed upon re-aeration. TAG degradation was paralleled by transcriptional upregulation of genes involved in β-oxidation and energy metabolism. It correlated with an increased biosynthesis of most phospholipids and mycolic acids, which are necessary for bacterial replication [104,105]. FA oxidation fuels the glyoxylate shunt, generating precursors for the TCA cycle and gluconeogenesis. Both the glyoxylate and gluconeogenic pathways were required for Mtb persistence *in vivo* [23,60,106,107], highlighting the importance of TAG-derived FAs for maintenance of infection.

Notably, host-derived FAs may directly contribute to Mtb's adaptation to host-associated stresses. When grown on long-chain SFAs and MUFAs as their sole carbon sources, Mtb entered a slowly growing and drug-tolerant physiological state resembling stress-induced quiescence. Interestingly, the dormant signature of FA-exposed Mtb was associated with an increased expression of genes related to reductive stress [68]. It was proposed that FAs force β-oxidation, leading to the accumulation of reducing equivalents and therefore redox imbalance in Mtb. Induction of reductive stress by FAs lowered Mtb's susceptibility to hypoxia, suggesting that FAs reprogram the metabolism of Mtb for adaptation to environmental conditions encountered within the host [108]. Importantly, dormancy also makes Mtb phenotypically resistant to drug treatment. Indeed, most of anti-TB drugs target processes essential for growth, such as DNA replication, translation, cell wall formation [109] that are inactive in dormant Mtb.

These studies illustrate several mechanisms of metabolic adaptation that are used by Mtb to promote long term persistence *in vivo*. Mtb's ability to simultaneously co-catabolize different carbon sources facilitates adaptation to the variable host environment. Biosynthesis of methyl-branched lipids (e.g. PDIMs) limits the reductive stress that is generated by β-oxidation of FAs, and the toxicity of propionyl-CoA that is associated with cholesterol assimilation. Dynamic remodeling of the lipid composition of the mycobacterial cell envelope during the course of infection may allow Mtb to manipulate the human immune system and better tolerate antibiotic treatment. Finally, Mtb ensures survival in hostile, nutrient poor, environments by storing TAGs and entering a state of dormancy.

IV - Metabolic profiles of Mtb and host macrophages *in vivo*

While *in vitro* studies using macrophage models generated useful mechanistic information, they do not reflect the heterogeneity of the macrophage populations in Mtb-infected lungs. Studies using non-human primates and mouse models of Mtb infection indeed identified distinct, permissive and controller macrophage phenotypes corresponding to alveolar (AMs) and interstitial (IMs) macrophages, respectively [43,110]. AM and IM have a different ontogenic origin: while AMs are embryonic-derived cells, IMs derive from blood monocytes recruited upon Mtb infection [111,112]. Strikingly, permissive AMs and controller IMs displayed different metabolic signatures (Figure 5). IMs displayed a marked commitment to aerobic glycolysis associated with pro-inflammatory responses, reflected by elevated expression of IL-1β, TNFα, iNOS and HIF1-α, as well as higher NO and ROS levels. IMs also displayed reduced expression of genes involved in lipid catabolism. In contrast, AMs had a transcriptional profile associated

with OXPHOS and lipid uptake (regulated by the PPAR-γ signaling pathway), TAG degradation, lipolysis and FA oxidation, at the expense of glycolysis and pro-inflammatory responses [43,55].

Induction of a DosR-related stress response in Mtb was recently reported in pro-inflammatory, glycolytically-shifted IMs sorted from infected mouse lungs [55]. This transcriptional profile was associated with slowed replication, resembling that of Mtb subjected to hypoxic stress, NO or low-iron conditions. In comparison, Mtb isolated from anti-inflammatory, FA oxidation/OXPHOS-shifted AMs displayed dampened induction of stress-related genes and a higher expression of genes involved in bacterial division, ribosomal protein and cell wall synthesis, a transcriptional profile consistent with high replication rates. Genes involved in FA uptake, mycolic acid biosynthesis, TCA cycle and FA oxidation were up-regulated in AM-derived bacilli, suggesting that FA catabolism is used to fuel bacterial division. These studies suggested for the first time that macrophage ontology may be a major determinant of the innate immune control of Mtb infection.

V - Conclusions and perspectives for TB treatment

Despite the discovery of effective antibiotic treatments and vaccination with Bacillus Calmette-Guérin (BCG), TB remains the leading cause of death by a single infectious agent and it is estimated that 23% of the world's population has a latent TB infection [113,114]. Moreover, with an estimated 4.1% new cases in 2016, multidrug resistant-tuberculosis (MDR-TB) represents a major obstacle to effective care and prevention world-wide [113]. In this context,

host-directed therapies are emerging as promising anti-TB approaches with potential to improve MDR-TB treatment outcomes.

Recent insights into the metabolic interplay between Mtb and host macrophage have changed our conception of successful innate immune responses, bringing the immunometabolic crosstalk into the forefront. The emerging model divides host-pathogen interactions into permissive or restrictive, depending on the metabolic commitment of infected macrophages to either aerobic glycolysis or FA oxidation. Studies by Huang *et al.* indicated that ontogeny dictates the immunometabolic response of macrophages during the first weeks of infection with Mtb [43]. It will be interesting to determine if cytokines produced by cells involved in adaptive immunity (such as IFN-γ), or other immune-driven factors, can rewire the immunometabolism of permissive macrophages *in vivo* to render them more restrictive.

Strikingly, this functional dichotomy not only links the metabolic profile of macrophages with their inflammatory cytokine and antimicrobial responses, but also with the induction of quiescence and drug tolerance programs in Mtb. With regard to FAs, it is interesting to note that intracellular Mtb and host macrophages reprogram their metabolism in duo. 'Controller' macrophages and intracellular quiescent Mtb both direct FAs towards storage within TAGs, while 'permissive' macrophages and intracellularly replicating Mtb actively degrade FAs via β-oxidation. This suggests an interdependent relationship between the FA metabolisms of Mtb and host macrophages, and potentially common mechanisms of regulation. Recent investigations support the view that in macrophages, rewiring of FA metabolism towards storage in LDs potentiates their ability to restrict the intracellular growth of Mtb in several ways: by limiting bacterial acquisition of host FAs, clustering various antimicrobial factors in LDs, promoting the synthesis of protective eicosanoids and limiting

the flux of FAs through β-oxidation. How precisely does FA metabolism reprogramming in host macrophages induce FA storage within intracellular Mtb remains unclear.

Moreover, little is known about how Mtb gains access to host FAs. Knight *et al.* reported that in resting macrophages, Mtb imports FAs in the absence of LDs [38]. This suggests that Mtb has ability to acquire FAs derived from endocytosed lipoproteins, or from lipids composing the phagosomal membrane. Studies of FA uptake by intracellular Mtb have so far used exogenously added palmitate (a SFA) or oleate (a MUFA), whose anabolic pathways are conserved from bacteria to humans. However, mammals synthesize an additional subset of poly-unsaturated FAs (PUFAs), playing important immunoregulatory functions in activated macrophages through generation of eicosanoids. Whether PUFAs synthesized upon infection with Mtb traffic to phagosomes in infected macrophages and can be metabolized by the intracellular pathogen is unknown. In all, a better understanding of the mechanisms regulating FA fluxes at the host-pathogen interface and an increased appreciation of the importance of FA chemistry in co-metabolism, may help identify novel targets within FA metabolic pathways. It could also lead to the design of innovative anti-TB approaches potentiating immune responses and also limiting the development of antibiotic resistance.

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Figure legends

Figure 1: The lipid environment of Mtb in caseous TB granulomas.

Within foamy macrophages, phagosomes containing Mtb bacilli are surrounded by lipid droplets (LDs), mainly consisting of triacylglycerol (TAGs) and cholesteryl esters. Bacilli can persist extracellularly in the caseum, a lipid-rich liquid deriving from LDs released by dead foamy macrophages.

Figure 2: Reprogramming of energy metabolism upon infection with *M. tuberculosis*.

Newly infected macrophages are characterized by enhanced aerobic glycolysis, impaired oxidative phosphorylation (OXPHOS) and increased microbicidal capacity. This immunometabolic shift is triggered by activation of the TLR2/4-NF-κB signaling pathway, leading to upregulation of iNOS, IL-1β and HIF1-α. HIF1-α promotes aerobic glycolysis, which feeds a disrupted tricarboxylic acid (TCA) cycle. Breakpoints in the TCA cycle result in limited OXPHOS, accumulation of succinate (stabilizing HIF1-α) and generation of itaconate, an antimycobacterial compound. Overall, this metabolic reprogramming promotes Mtb killing through production of reactive oxygen species (ROS), nitric oxide (NO) and itaconate, catalyzed by the NADPH oxidase, inducible NO synthase (iNOS) and the immune-responsive gene 1 (IRG1), respectively. Mtb suppresses aerobic glycolysis via induction of microRNA (miR)-21 and interferon (IFN)-β, whose production is triggered by mycobacterial cell wall lipids, namely phthiocerol dimycocerosates (PDIMs).

Figure 3: Rewiring of lipid metabolism and induction of the foamy phenotype.

The foamy phenotype of macrophages is associated with lipid metabolism reprogramming: catabolic pathways (β-oxidation, TAG hydrolysis et autophagy) are inhibited while anabolic pathways are activated, leading to lipid accumulation. Mtb infection leads to activation of several signaling cascades involving Toll-Like Receptor (TLR) 2, Testicular Receptor (TR) 4 and Peroxisome Proliferator-Activated Receptor (PPAR)-γ. Mycobacterial LAM and trehalose dimycolates (TDM) induce the expression of microRNA-33, which negatively regulates FA oxidation and autophagy. Early secreted antigenic target (ESAT)-6 of Mtb induces generation of 3-hydroxybutyrate (3-HB), which is recognized by the GPR109A receptor and mediates inhibition of TAG hydrolysis. Mtb-stimulated production of pro-inflammatory cytokines also promotes LD accumulation: tumor necrosis factor (TNF)-α activates mammalian target of rapamycin complex (mTORC)-1 and the cascade of caspases, which inhibit autophagy effectors and activate lipogenic regulator SRBP (sterol regulatory element-binding protein). The interferon (IFN)-γ pathway activates hypoxia-inducible factor (HIF)-1α, enhancing the production of hypoxia-inducible gene (HIG)2 supporting LD accumulation.

Figure 4: Overview of the FA metabolism of *M. tuberculosis.*

In Mtb, FAs and free cholesterol are imported by mammalian cell entry (Mce) 1 and 4 complexes, respectively. The lipid uptake coordinator A (LucA) and the ATPase MceG are subunits shared by both transporters. Exo- and endogenous TAGs may be hydrolyzed by lipase Y (LipY). β-oxidation of fatty acyl-CoA (FA-acyl-CoA) involves the FadAB complex and generates acetyl-CoA, while cholesterol degradation mainly produces propionyl-CoA. Acetyl-CoA feeds into the TCA cycle or is alternatively assimilated by the glyoxylate shunt, involving isocitrate lyase (icl) 1 or 2. Glycolytic intermediates, such as phosphoenolpyruvate (PEP) and glucose-6-phosphate (Glu-6P) may be regenerated from oxaloacetate (OAA) by gluconeogenesis. Propionyl-CoA enters the methylcitrate cycle (MCC) or is converted into methylmalonyl-CoA, thereby fueling the TCA cycle. Acetyl-CoA may also be used for de novo *FA biosynthesis after conversion into malonyl-CoA. FA synthase I (FAS-I) catalyzes the synthesis of saturated FA (SFA), which can be converted into mono-unsaturated FAs (MUFAs)* via *the action of desaturase (Des) A3.* De novo *synthesized or imported FA can be further elongated by FAS-II to produce mycolic acids. FA may also be incorporated into TAG, phospholipids (PL) and polyketide lipids, such as phthiocerol-dimycocerosate (PDIM). These pathways involve TAG synthase (Tgs1) and several polyketide synthases (Pks), that use propionyl-CoA-derived methylmalonyl-CoA as extender unit.*

Figure 5: Immunometabolic phenotypes of macrophages in *M. tuberculosis*-infected lungs.

In Mtb-infected lungs, interstitial macrophages (IMs) are characterized by enhanced glycolysis, while pathways linked to lipid catabolism and OXPHOS are downregulated. They exhibit a pro-inflammatory profile, involving the nuclear factor (NF)-κB-mediated production of interleukin (IL)-1β, iNOS and hypoxia inducible factor (HIF)-1α. IM thus restrict Mtb growth with higher levels of NO and ROS, which stimulate the expression of stress-induced Mtb genes including the dormancy operon DosR. In contrast, alveolar macrophages (AMs) are engaged in OXPHOS, FA and lipoprotein uptake and breakdown, at the expense of glycolysis and pro-inflammatory responses. AMs are more permissive to Mtb replication and bacteria exhibit a mmarked upregulation of genes involved in cell growth and division.

Figure 1

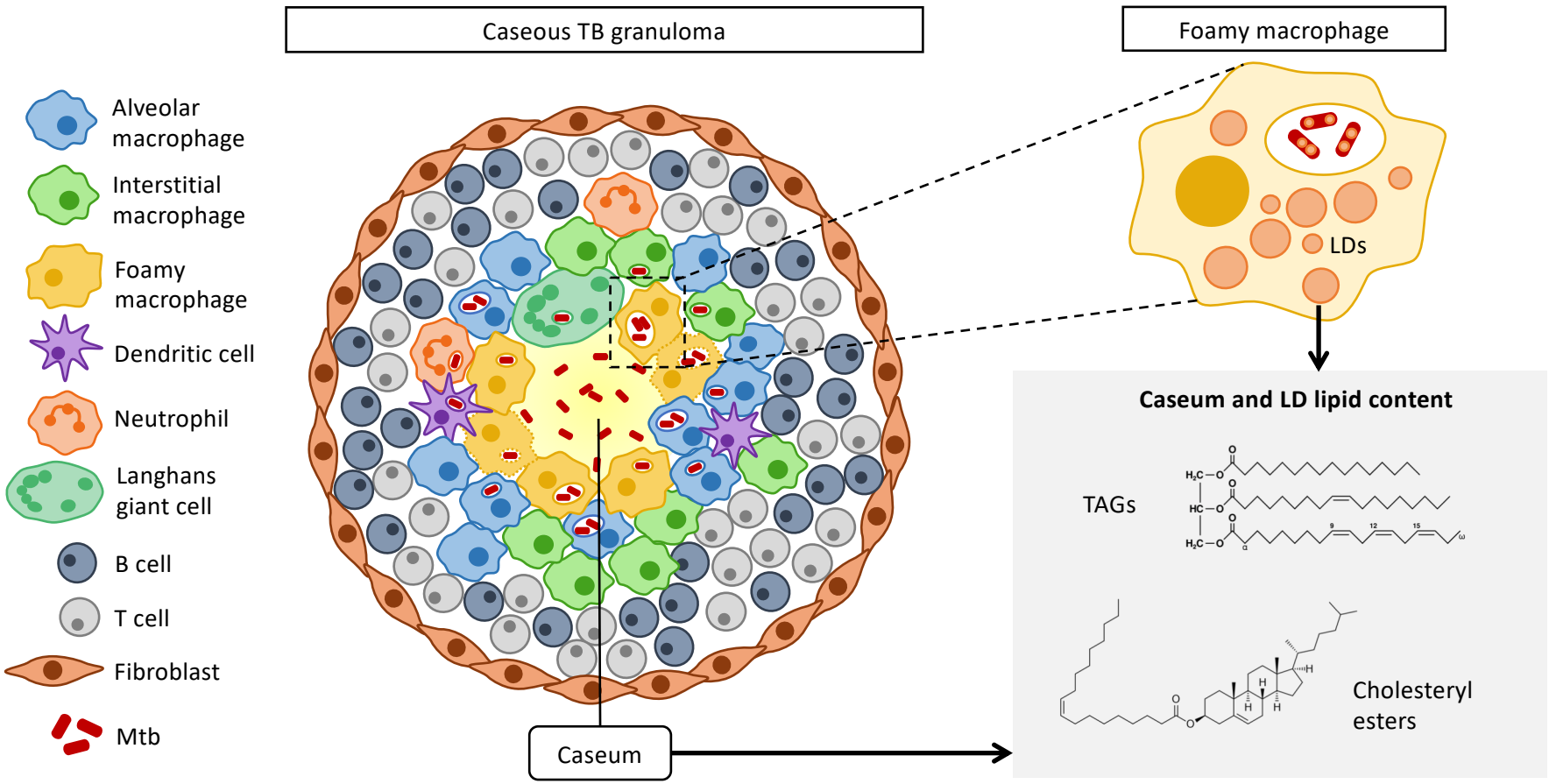

Caseous TB granuloma
Foamy macrophage
Alveolar macrophage
Interstitial macrophage
Foamy macrophage
Dendritic cell
Neutrophil
Langhans giant cell
B cell
T cell
Fibroblast
Mtb
LDs
Caseum
Caseum and LD lipid content
TAGs
Cholesteryl esters

Figure 2

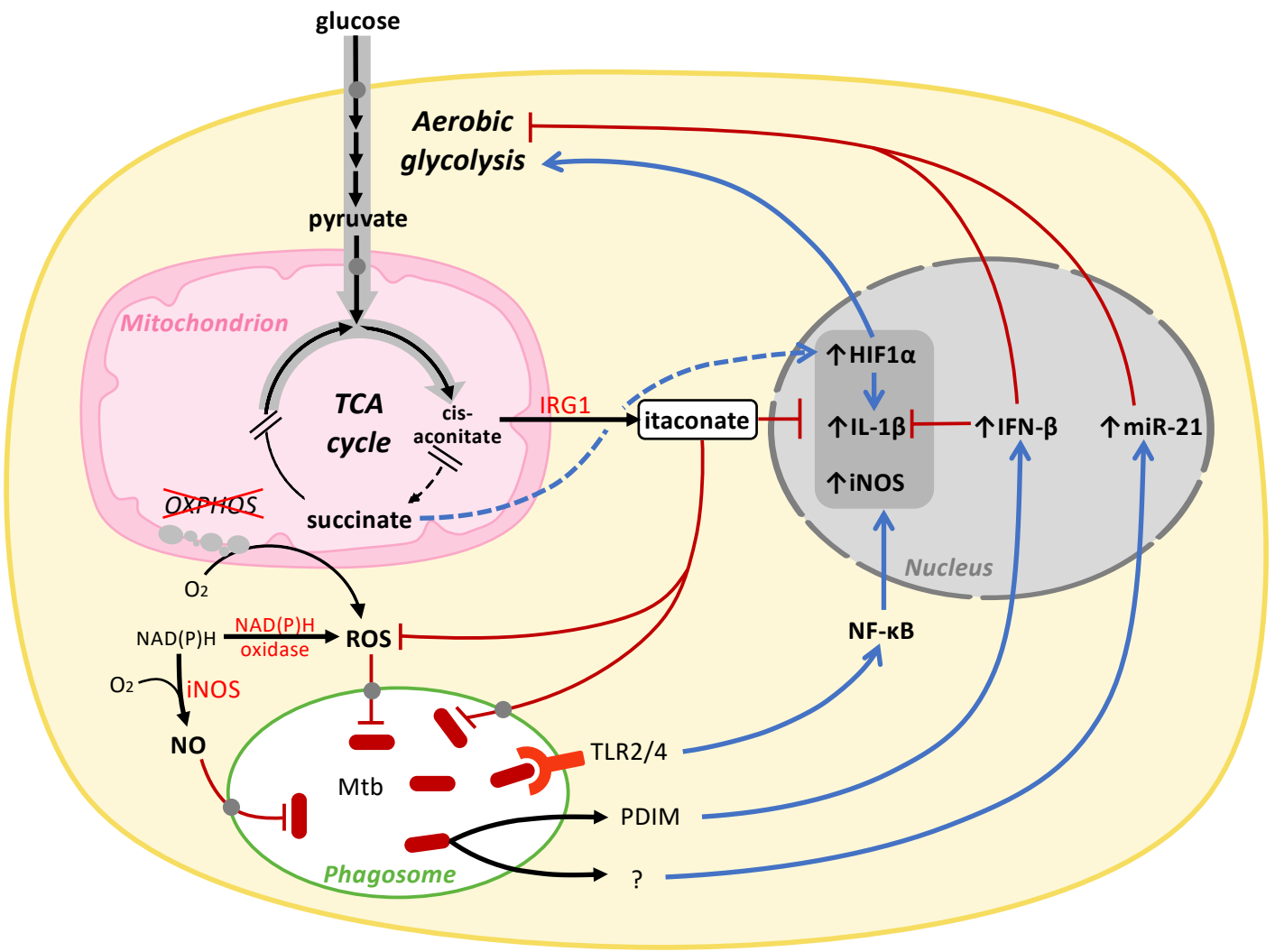
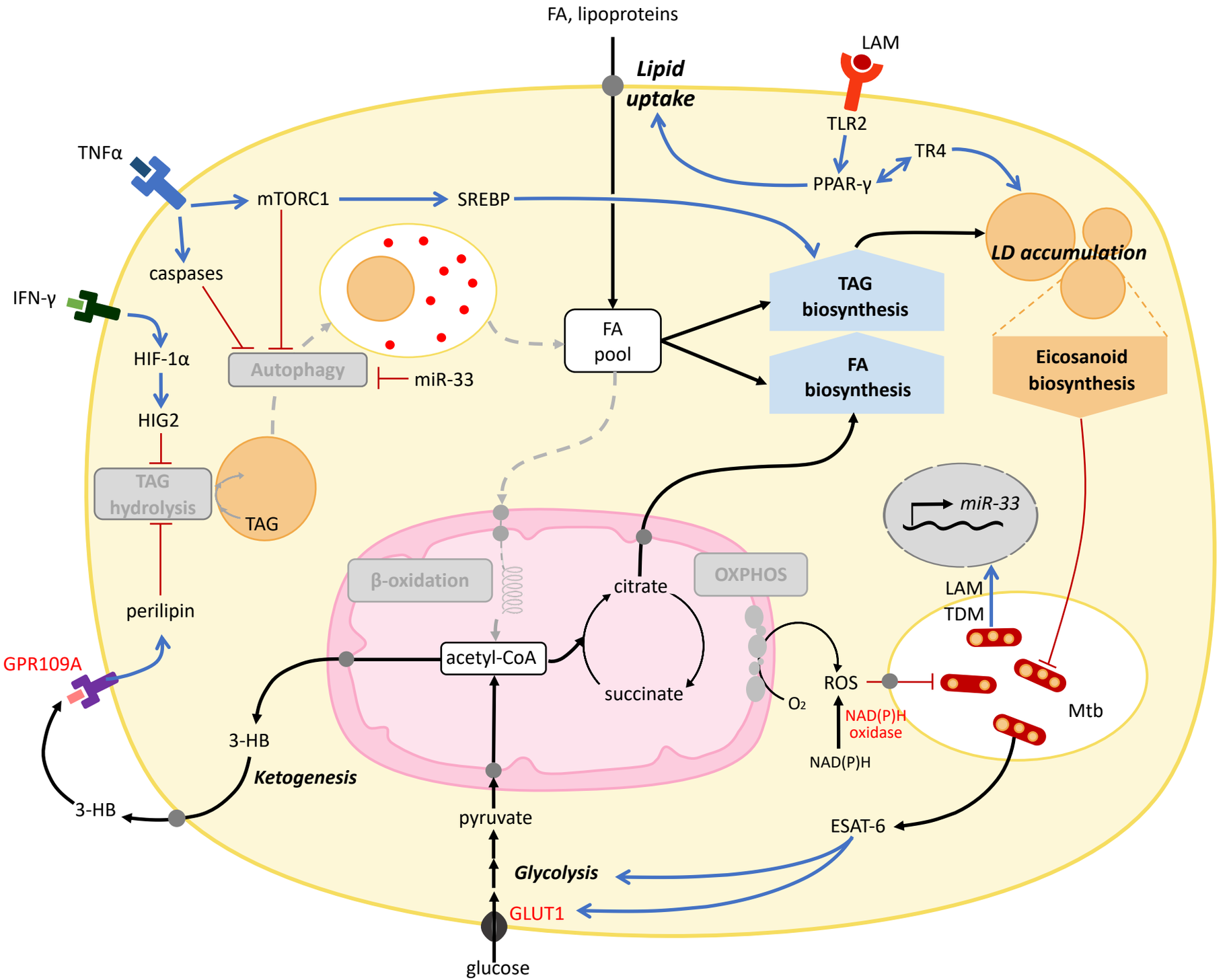
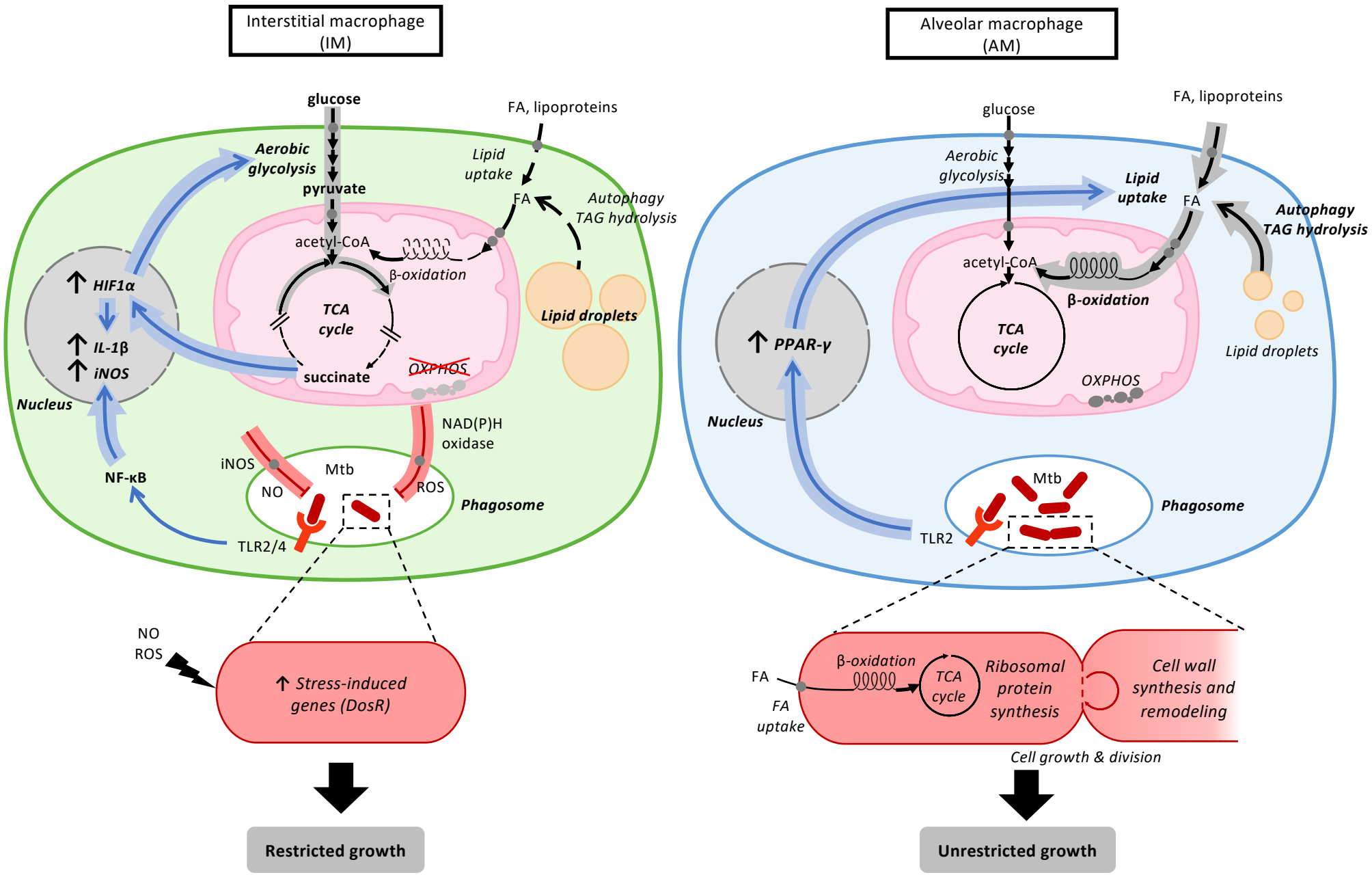

glucose
Aerobic glycolysis
pyruvate
Mitochondrion
TCA cycle
cis-aconitate
IRG1
itaconate
↑HIF1α
↑IL-1β
↑iNOS
↑IFN-β
↑miR-21
Nucleus
OXPHOS
succinate
O2
NAD(P)H
NAD(P)H oxidase
ROS
NF-κB
O2
iNOS
NO
TLR2/4
Mtb
PDIM
Phagosome
?

Figure 3


FA, lipoproteins
Lipid uptake
LAM
TLR2
TR4
PPAR-γ
TNFα
mTORC1
SREBP
caspases
IFN-γ
HIF-1α
HIG2
Autophagy
miR-33
FA pool
TAG biosynthesis
FA biosynthesis
LD accumulation
Eicosanoid biosynthesis
TAG hydrolysis
TAG
perilipin
GPR109A
β-oxidation
citrate
OXPHOS
acetyl-CoA
succinate
O2
ROS
NAD(P)H oxidase
NAD(P)H
miR-33
LAM
TDM
Mtb
3-HB
Ketogenesis
3-HB
pyruvate
ESAT-6
Glycolysis
GLUT1
glucose

glucose
Glu-6P
trehalose
Gluconeogenesis
Glycolysis
NADH
PEP
pyruvate
β-oxidation
FadAB
NADH
FA
Mce1
LucA
MceG
ATP
FA
FA-acyl-CoA
Acetyl-CoA
malonyl-CoA
LipY
LipY
TAG
TAG
OAA
citrate
MCC
TCA cycle
malate
GlcB
glyoxylate
Icl1/2
isocitrate
Methyl-isocitrate
Glyoxylate shunt
NADH
CO₂
succinyl-CoA
Icl1/2
succinate
CO₂
methylmalonyl-CoA
VitB12
cholesterol
LucA
Mce4
MceG
ATP
cholesterol
propionyl-CoA
NADH
OXPHOS
ATP
FA biosynthesis
NEFA
Mce1
ATP
FAS-I
NAD(P)H
SFA (C16-22)
DesA3
Rv3230c
MUFA
FAS-II
NAD(P)H
Tgs1
TAG
meromycolic acids (C22-62)
Desaturations
Modifications
Pks13
Carboxylation
PL
mycolic acids
trehalose
trehalose mycolates
Cell wall lipid biosynthesis
Pks
trehalose
PDIM & other polyketide lipids
Capsule
Outer membrane (mycomembrane)
Arabino-galactan
Peptido-glycan
Periplasm
Inner membrane
LM
LAM
ManLAM
Polysaccharides
Protein
Mycolic acid
Trehalose mycolates
Polyketide lipids
PDIM
PL
Glycolipids

Figure 5


Interstitial macrophage (IM)
glucose
FA, lipoproteins
Aerobic glycolysis
pyruvate
Lipid uptake
FA
Autophagy TAG hydrolysis
acetyl-CoA
β-oxidation
TCA cycle
Lipid droplets
HIF1α
IL-1β
iNOS
Nucleus
succinate
OXPHOS
NAD(P)H oxidase
iNOS
Mtb
NO
ROS
Phagosome
NF-κB
TLR2/4
NO
ROS
↑ Stress-induced genes (DosR)
Restricted growth
Alveolar macrophage (AM)
glucose
FA, lipoproteins
Aerobic glycolysis
Lipid uptake
FA
Autophagy TAG hydrolysis
acetyl-CoA
β-oxidation
TCA cycle
Lipid droplets
PPAR-γ
Nucleus
OXPHOS
Mtb
Phagosome
TLR2
FA
FA uptake
β-oxidation
TCA cycle
Ribosomal protein synthesis
Cell wall synthesis and remodeling
Cell growth & division
Unrestricted growth