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Control of macrophage metabolism and activation by mTOR and Akt signaling

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Abstract

Macrophages are pleiotropic cells that assume a variety of functions depending on their tissue of residence and tissue state. They maintain homeostasis as well as coordinate responses to stresses such as infection and metabolic challenge. The ability of macrophages to acquire diverse, context-dependent activities requires their activation (or polarization) to distinct functional states. While macrophage activation is well understood at the level of signal transduction and transcriptional regulation, the metabolic underpinnings are poorly understood. Importantly, emerging studies indicate that metabolic shifts play a pivotal role in control of macrophage activation and acquisition of context-dependent effector activities. The signals that drive macrophage activation impinge on metabolic pathways, allowing for coordinate control of macrophage activation and metabolism. Here we discuss how mTOR and Akt, major metabolic regulators and targets of such activation signals, control macrophage metabolism and activation. Dysregulated macrophage activities contribute to many diseases, including infectious, inflammatory, and metabolic diseases and cancer, thus a better understanding of metabolic control of macrophage activation could pave the way to the development of new therapeutic strategies.

Keywords

mTOR; Akt; macrophage activation; macrophage metabolism; immunometabolism

1. Introduction

Here we review the role of mTOR and Akt in control of macrophage activation and metabolism. We begin with an overview of mTOR and Akt signaling, followed by a discussion of their roles in macrophage activation as revealed by genetic models in which their activities are perturbed. The metabolic underpinnings of their control of macrophage activation are beginning to be unraveled and is a new and exciting area of research in the field, thus in the last sections, we discuss metabolic control of macrophage activation, and the potential role of the mTOR and Akt signaling in this process.

2. Overview of mTOR and Akt signaling

The serine threonine kinase mTOR is a key regulator of cellular metabolism that is conserved from yeast to man(1, 2). In mammals, mTOR exists in two complexes, mTORC1 and mTORC2 (Fig 1). Other subunits are unique to and define the two complexes, such as Raptor and Rictor in mTORC1 and mTORC2 respectively, and serve to regulate complex stability, activation, and/or activity. mTORC1 couples nutrient availability to major anabolic processes (Fig 1). In growing/proliferating cells, mTORC1 promotes the synthesis of macromolecules (e.g. lipids, proteins, and nucleotides), while in metabolic tissues like the liver, mTORC1 facilitates nutrient storage while inhibiting catabolic metabolism (e.g. autophagy). mTORC2 phosphorylates and activates Akt and other kinases of the AGC superfamily to control cellular metabolism, survival, and cytoskeletal organization (Fig 1). Interestingly, the activities of mTORC1, mTORC2, and Akt are intricately intertwined in some contexts. This includes growing and proliferating cells, in which Akt is a critical activator of mTORC1 and activated mTORC1 mediates feedback inhibition to shut down mTORC2 and Akt activation. Therefore, mTORC1, mTORC2, and Akt constitute a key metabolic signaling network that coordinates many metabolic processes that are best characterized in growing/proliferating cells and metabolic tissues (Fig 1)(1, 2).

3. Activation of mTOR and Akt by macrophage polarizing signals

In contrast, the role of mTOR and Akt signaling in macrophages is much less well-understood. Importantly, emerging studies indicate that macrophage activities require the support of metabolic processes (see below). This suggests that polarizing signals that trigger macrophage activation should also induce metabolic shifts that support the acquisition and execution of relevant effector activities. Consistent with this idea, recent studies show that macrophage polarizing signals regulate mTOR and Akt signaling. We will discuss these studies below, which focus on M1 and M2 activation, but it is likely that most/all macrophage polarizing signals will impinge on major metabolic pathways to coordinate macrophage metabolism and activation.

Macrophage activation or polarization to the M1 (classical) and M2 (alternative) states has long served as a paradigm for studying macrophage activation(3, 4). During microbial infection, microbial stimuli such as LPS trigger M1 activation, which is characterized by increased production of pro-inflammatory cytokines and antimicrobial activity. M2 macrophages upregulate pro-fibrotic and tissue repair activities to coordinate Type 2 immunity, and are activated by stimuli such as IL-4 and IL-13 present during parasite infections (Fig 2). Induction of new effector activities by activated macrophages is regulated transcriptionally. Stat6 is the master regulator for M2 activation, while NF- κ B and IRFs are key transcriptional regulators of the M1 program(3, 4).

In addition to these transcription factors, LPS and IL-4 signaling also target metabolic pathways such as mTOR and Akt to trigger metabolic shifts and metabolic reprogramming (Fig 2). In the case of IL-4, activation of Jak1 and Jak3 by ligation of the IL-4R allows for phosphorylation and activation of Stat6, as well as recruitment of the adaptor protein IRS2 (Fig 2A). IRS2 engages PI3K, which phosphorylates PIP2 at the plasma membrane to

generate PIP3. PIP3 recruits Akt and mTORC2 to the plasma membrane, allowing mTORC2 to phosphorylate and activate Akt. Activated Akt phosphorylates and inactivates the TSC complex. This complex, which contains TSC1 and TSC2, is a GAP for the small GTPase Rheb, so TSC inactivation results in Rheb activation. Although the underlying mechanism is not known, the GTP-bound form of Rheb activates mTORC1. Therefore, the IL-4R activates canonical signaling (e.g. Stat6) as well as the Akt-mTORC1 axis (Fig 2A)(5, 6). Of note, the Akt-mTORC1 axis is also engaged by growth factor signaling to regulate anabolic metabolism(1), and it is likely that this signaling module may have similar activities downstream of IL-4.

In the case of LPS-mediated M1 activation, TLR4 engages PI3K through an adaptor protein called BCAP (7), followed by Akt and mTORC1 activation (Fig 2B). The MEK/ERK(8) and IKK β (9) pathways have also been implicated in mTORC1 activation downstream of LPS, through their inactivation of the TSC complex. Moreover, a recent study in dendritic cells (DCs) suggests that Akt can be activated by a non-canonical mechanism, leading to Akt-mediated but mTORC1-independent regulation of aerobic glycolysis at acute time points after LPS stimulation. Such activation of Akt is independent of PI3K but dependent on TBK and IKK ϵ (Fig 2B)(10). These findings indicate that regulation of Akt and mTORC1 downstream of TLR4 may be more complex compared to growth factor receptors and IL-4R, where an Akt-mTORC1 axis can be more clearly delineated.

4. Control of macrophage activation by mTOR and Akt signaling

In the next sections, we review studies indicating that Akt and mTOR play key roles in macrophage activation. Most studies to date have focused on how the mTORC1 and Akt pathways control either canonical signaling (e.g. JNK, NF- κ B) or metabolic processes (e.g. HIF1 α , glycolysis), thus we will discuss these topics separately, but future studies are expected to illuminate how control of the two processes are integrated.

Many studies have implicated a role for Akt in macrophage activation. Here we will briefly discuss some salient points, and the reader is referred to (11, 12) for additional reading. Akt appears to promote M2 polarization, since pharmacological inhibition of Akt attenuates induction of M2 genes(5, 13). Interestingly, only some M2 genes are regulated by Akt, and how specificity is achieved would be interesting to pursue in future studies. The role of Akt in M1 polarization is not quite as clear, with many studies implicating Akt and its upstream regulator PI3K as negative regulators, but others reporting positive regulation of M1 activation(12). Perhaps contributing to the discrepancy, the mammalian genome encodes 3 Akt proteins, of which Akt1 and Akt2 are thought to be expressed in macrophages. In one study, use of genetic models specifically deficient in one or the other Akt protein suggests that Akt1 inhibits while Akt2 promotes M1 activation, since loss of Akt1 and Akt2 augments and reduces M1 activation respectively (Table 1)(14). It is possible that depending on the experimental models used, preferential expression and/or activation of Akt1 versus Akt2 underlies the different results obtained with pharmacological Akt inhibitors (which are expected to target both proteins). Further analysis of macrophage activation using Akt1- and Akt2-deficient macrophages is warranted.

Akt is likely to control macrophage activation through multiple downstream effectors. For example, Foxo1, a transcription factor inhibited by Akt signaling, is implicated in proinflammatory gene induction in M1 macrophages(15). C/EBP β is a transcription factor implicated in both M1 and M2 activation, and its expression has been shown to be controlled by Akt2 (Table 1)(14). Akt has also been implicated in positive as well as negative regulation of the activity of NF-kB(12), a master regulator of M1 activation.

Our discussion of control of macrophage activation by mTOR will be somewhat selective. Conflicting findings with respect to macrophage activation have been obtained with rapamycin, the small molecule inhibitor of mTORC1. In the mTOR field, it is well known that there are caveats associated with the use of rapamycin to inhibit mTORC1(16). Rapamycin is not a catalytic site inhibitor of mTOR and allosterically regulates mTOR activity in the mTORC1 complex through poorly understood mechanisms; such inhibition is selective in that some (e.g. S6K1) but not other (e.g. 4EBP1) targets of mTORC1 are affected. Additionally, while acute rapamycin treatment selectively inhibits mTORC1, prolonged treatment leads to mTORC2 disassembly and Akt inhibition. Catalytic site inhibitors of mTOR are available (e.g. Torin), but these will inhibit both mTORC1 and mTORC2. Therefore, analysis of mTORC1 activity solely by pharmacological blockade is problematic (16), and here we focus on studies where mTORC1 activity is perturbed by genetic models.

Several reports have addressed macrophage activation in macrophages genetically deficient in the TSC complex. Genetic models lacking Tsc1 or Tsc2, key subunits of the TSC complex, have been used to interrogate the consequences of aberrantly increased mTORC1 activity in multiple tissues and cell types, including liver, pancreas, and T cells. Mice with myeloid specific Tsc1 deficiency (LysM-Tsc1^{-/-}) are born normally, but spontaneously develop inflammatory disorders, including inflammation in the liver and lung, and enlargement of the spleen and some lymph nodes(17). TSC-deficient bone marrow derived macrophages (BMDMs) appear to develop and differentiate normally in response to MCSF, but have elevated mTORC1 activity at basal state, as indicated by increased phosphorylation of its downstream targets S6K and 4EBP1. This contrasts with WT BMDMs, which have low basal mTORC1 activity that can be induced in a signal-dependent manner, for example by LPS or IL-4(5, 18). Importantly, and consistent with the phenotype of LysM-Tsc1^{-/-} mice, TSC-deficient BMDMs mount a hyperinflammatory response to LPS(5, 18) but are deficient in IL-4-induced M2 activation (Table 1)(5, 17). Such divergence in M1 versus M2 activation is also observed with other genetic models (e.g. deficiency of Stat6 or KLF4, transcription factors implicated in M2 activation), and reflects the opposing regulation of these two biological programs.

In terms of M2 activation, TSC-deficient BMDMs are attenuated in IL-4-mediated induction of multiple M2 genes, including Arg1, Fizz1, and Ym1, as well as arginase activity(5, 17). In response to injection of IL-4 or chitin (which induces M2 activation in a IL-4-dependent manner), peritoneal macrophages in LysM-Tsc1^{-/-} mice are impaired in the induction of M2 genes. Likewise, these mice are resistant to OVA-induced allergic asthma—bronchoalveolar lavage fluid macrophages from LysM-Tsc1^{-/-} mice express lower levels of M2 markers, correlating with reduced leukocyte infiltration into the lung and tissue

pathology. Multiple mechanisms may underlie diminished M2 activation in TSC-deficient BMDMs. One is attenuated Akt signaling(5), a consequence of mTORC1-mediated feedback inhibition of receptor proximal signaling events. While such negative feedback likely evolved to maintain inducibility of the Akt-mTORC1 axis, increased mTORC1 activity in genetic models of TSC deficiency severely blunts Akt activity. Consistent with a critical role for attenuated Akt signaling in TSC-deficient BMDMs, ectopic expression of a constitutively active Akt mutant is able to restore some features of M2 activation. Additionally, reduced expression of the transcription factor C/EBP β may underlie the polarization defect of TSC-deficient BMDMs. Akt activity has been shown to regulate C/EBP β expression(14), and ectopic expression of C/EBP β rescues the expression of some M2 genes in TSC-deficient BMDMs (Table 1)(17).

With regard to M1 activation, TSC-deficient BMDMs produce higher levels of pro-inflammatory cytokines like TNF- α , IL-6, and IL-12 but lower levels of the anti-inflammatory cytokine IL-10, at the mRNA and protein levels(5, 18). They have higher basal and LPS-inducible levels of the costimulatory molecules CD80 and CD86, and increased production of NO. The enhanced inflammatory phenotype is also observed with other TLR ligands, including PolyIC and Pam3Cys which stimulate TLR3 and TLR2 respectively, and with LPS and IFN γ . In a LPS-induced endotoxin shock model, LysM-Tsc1 / mice produce higher levels of inflammatory cytokines correlating with increased susceptibility (Table 1)(18). Although there is agreement on the exaggerated inflammatory response of TSC-deficient BMDMs, distinct and in some cases inconsistent underlying mechanisms have been proposed. Pan et al show that TSC-deficient BMDMs have increased JNK activity and that a JNK inhibitor reverses the enhanced NO production(18). Byles et al proposed that attenuated Akt activity contributes to increased inflammatory responses in TSC-deficient BMDMs(5). (As discussed above, constitutive mTORC1 activity leads to feedback inhibition of receptor proximal signaling events that reduces Akt activity.) Consistently, in some studies LPS-mediated induction of several pro-inflammatory cytokines is augmented by pharmacological Akt inhibition(11). Thus enhanced JNK and attenuated Akt activity may both contribute to the M1 phenotype of TSC-deficient BMDMs, and/or in a selective manner depending on the M1 effector response. Pan et al report that rapamycin treatment and/or deficiency of the mTORC1 subunit Raptor attenuates the hyperinflammatory phenotype of TSC-deficient BMDMs, indicating that the phenotype is a consequence of aberrant mTORC1 activity(18). In contrast, Zhu et al show that rapamycin treatment and deletion of mTOR do not reverse the phenotype of TSC-deficiency, and implicate Ras as a new TSC target(17). However, there are caveats associated with rapamycin treatment, and mTOR deletion would ablate mTORC2 as well as mTORC1 such that consequent reduction of Akt activity would lead to the observed increase in inflammatory gene expression; thus additional studies are warranted to definitively establish mTORC1 independency (Table 1). In contrast to a hyperinflammatory response in TSC-deficient macrophages, TSC1-deficient DCs generated from the same mice have a more complex phenotype characterized by increased production of some inflammatory cytokines but a reduction in MHC class II expression and CD4 T cell priming(19). This may suggest cell-type specific differences in the consequences of TSC-deficiency(19, 20) or alternatively, complexity in regulation of the M1 program that should be addressed in future

studies (Table 1). It is also noteworthy that in CD8+ T cells, the degree of mTORC1 activity has been linked to quantitative and qualitative differences in T cell responses(21, 22). Therefore, the onset, duration, and strength of mTORC1 activity is likely to determine effects on macrophage metabolism and activation.

Importantly, the enhanced M1 but diminished M2 activation of TSC-deficient macrophages could be relevant to macrophage function in obesity. In the white adipose tissue, polarization of adipose tissue macrophages (ATMs) to a M2-like state, mediated by IL-4 and IL-13, is thought to be critical for maintaining tissue insulin sensitivity; during obesity, ATMs switch to a M1-like phenotype, which directly promotes tissue inflammation, insulin resistance, and metabolic derangement(23). Since the mTORC1 pathway is a major metabolic node that integrates diverse metabolic inputs, metabolic signals may impinge on this pathway to modulate ATM activation. Chronic nutrient excess may aberrantly activate mTORC1, leading to feedback inhibition of Akt signaling and impaired responsiveness to IL-4 and IL-13; increased mTORC1 but decreased Akt activation in the ATM-containing stromal vascular fraction of mice on high fat diet is consistent with this idea (A.C. and T.H., unpublished data). In contrast, how activation of mTORC1 by *physiological* increases in metabolic signaling impacts M2 activation is less clear, and it is possible that the consequence is to *support* certain aspects of ATM M2 activation. This is also consistent with the role of the Akt-mTORC1 axis in insulin signaling—while postprandial increases in insulin act in an Akt-mTORC1-dependent manner to stimulate nutrient storage in the healthy liver, chronic nutrient excess leads to aberrant increases in mTORC1 signaling, downregulated Akt activity, and hepatic insulin resistance(2). Likewise, physiological and pathophysiological Akt-mTORC1 signaling may have divergent outcomes in control of macrophage activation. Integration of the Akt-mTORC1 pathway into IL-4 signaling may allow this pathway to calibrate metabolic input to certain aspects of M2 activation, while corruption of the Akt-mTORC1 axis by chronic nutrient excess contributes directly to impaired macrophage polarization and loss of metabolic homeostasis. Additional studies are warranted to test this idea, which has implications for metabolic control of macrophage function in many contexts, most notably obesity-associated diseases like type 2 diabetes, atherosclerosis, and nonalcoholic steatohepatitis(23).

Studies analyzing macrophages deficient in Raptor or Rictor (and thus mTORC1 or mTORC2) are more limited. One study showed that mice with macrophage specific Raptor deficiency fared better on a high fat diet, with reduced liver and WAT inflammatory gene expression and increased systemic insulin sensitivity(24). Although not directly examined, this phenotype may be associated with increased M2 but decreased M1 gene expression in liver and WAT macrophages. Another study showed that Raptor deficiency in the myeloid compartment protected LDLR^{-/-} mice from developing atherosclerosis on a western diet, as indicated by reduced lesion size and macrophage infiltration into plaques(25). These studies suggest that in the context of nutrient excess, Raptor signaling promotes pathophysiological M1 activation. Rictor deficient BMDMs and DCs have exaggerated M1 activation, expressing higher levels of inflammatory cytokines in response to LPS stimulation(26, 27). Consistently, myeloid specific deletion of Rictor leads to increased susceptibility to sepsis(26). Rictor deletion ablates mTORC2 assembly and Akt activation, thus the

underlying defect may be due to reduced Akt activity. As mentioned above, Akt appears to antagonize many aspects of LPS signaling, and in Rictor deficient BMDMs, Akt-mediated inhibition of FoxO1 is alleviated, allowing the transcription factor to promote induction of inflammatory genes (27)(Table 1).

In summary, the studies above focused on regulation of “canonical” signaling (e.g. JNK and NF- κ B) by mTOR and Akt in control of macrophage activation. Later in the review, we discuss emerging studies that highlight the metabolic aspects of mTOR- and Akt-regulated macrophage polarization.

5. Overview of macrophage activation and metabolism

As alluded to above, macrophages are pleiotropic cells that assume diverse functions depending on the context. M1 macrophages upregulate pro-inflammatory and antimicrobial activities, while M2 macrophages coordinate tissue repair and Type 2 immunity. Tissue-resident macrophages mediate tolerance to the gut microflora, insulin sensitivity in white adipose tissue, and thermogenesis in brown adipose tissue(3, 4). Emerging studies indicate that cellular metabolism and function are intricately intertwined, thus these diverse macrophage functions are likely to be supported by distinct metabolic programs. Here we present an overview of macrophage activation and metabolism. We discuss the types of signals that regulate macrophage metabolism and activation, and general principles underlying control of macrophage activation by metabolism.

In broad terms, there are two types of signals that regulate macrophage metabolism and function. First, metabolic signals, either systemic or derived from the tissue or microenvironment, can directly modulate macrophage activation and function. For example, fatty acids can play either a biosynthetic or regulatory role in stimulation of M2 activation. Fatty acids can engage β -oxidation, which supports M2 activation through unclear mechanisms, or activate nuclear receptors such as PPAR γ and PPAR δ , which synergize with Stat6 for transcriptional control of M2 activation(23, 28, 29). Another interesting example is regulation of two alternative macrophage activities in TB granulomas by availability of the essential nutrient oxygen. Macrophage iNOS uses oxygen as a substrate to produce NO and kill bacteria(30), but hypoxia in the necrotic core of the granuloma can upregulate macrophage expression of Arginase. Arginase activity limits lung granuloma pathology, perhaps by local depletion of its substrate Arginine and consequent suppression of T cell proliferation and activation(31). Finally, lactate production by tumor cells undergoing Warburg metabolism skews tumor-associated macrophages (TAMs) to an M2-like phenotype. Lactate stabilizes HIF1 α expression in the TAMs, allowing for induction of Arginase and pro-tumoral activities, perhaps via Arginase-dependent production of polyamines that are essential for cellular proliferation(32).

Second, signals that are not strictly of metabolic nature can control macrophage metabolism and activation. These signals include the prototypical polarizing signals such as LPS and IL-4. As discussed above, macrophage activation requires metabolic support, hence the need for polarizing signals to engage and regulate specific metabolic pathways. The example of growth factor-mediated cellular proliferation is informative for illustrating why a biological

signal would need to trigger particular metabolic changes. Cellular proliferation requires an accumulation of biomass in the form of proteins, lipids, and nucleotides. In unicellular organisms like yeast, TORC1 couples nutrient availability to nutrient uptake and macromolecule synthesis. In contrast, cellular proliferation in multicellular organisms is regulated by growth factors, which allows proliferation to be coordinated with the needs of the tissue and organism(1). Importantly, growth factors engage mTORC1 signaling such that mTORC1 activity is controlled by both nutrients and growth factors; apparently growth factor signaling co-opted the ancestral TORC1 pathway for regulating anabolic metabolism during the evolution of multicellularity. Similarly, the signals that regulate macrophage activation engage key metabolic pathways to trigger the metabolic shifts necessary to support the associated functional reprogramming.

How might metabolic shifts control macrophage activation? First, metabolic shifts may enable bioenergetic support of macrophage activities. Indeed, it has been suggested that the distinct metabolism of M1 and M2 macrophages is necessary to meet their unique bioenergetic requirements (33). M1 macrophages upregulate glycolytic metabolism, which allows for the rapid production of ATP that may be needed during infection by fast-replicating microbes. M2 macrophages augment β -oxidation, which is much more energy efficient (i.e. yielding more ATP) and thus more compatible with host defense to slow-growing and endemic parasites.

Second, metabolic shifts lead to corresponding changes in metabolites, both qualitative and quantitative. For example, increased glycolytic flux in M1 macrophages promotes the accumulation of lactate and succinate (resulting from aerobic glycolysis and pyruvate oxidation)(34, 35). In general, such changes to metabolite profiles can have a regulatory or biosynthetic role in controlling functional activities. Regulatory roles include modulation of signaling pathways, gene expression, and other cellular processes, since metabolites serve as essential cofactors or allosteric regulators of enzymatic activity. For example, succinate inhibition of prolyl hydroxylase (PHD) controls the stability of HIF1 α , a transcription factor implicated in M1 activation(34) (see below). In a biosynthetic capacity, metabolites are substrates for protein modifications. This includes acetyl-CoA and S-adenosylmethionine, which are substrates for histone acetylation and methylation and thus control gene expression and epigenetics(36, 37). The biosynthetic roles of metabolites also support diverse macrophage effector activities. For example, ROS- and NO-mediated killing of intracellular bacteria is key to the antimicrobial activity of M1 macrophages. NADPH serves as an electron donor for both complexes, thus M1 macrophages boost production of this metabolite. As another example, LPS-stimulated DCs and macrophages produce high levels of inflammatory cytokines like TNF α , IL-6, and IL-12. This challenge to cellular secretory capacity is met by increased production of phospholipids, allowing for expansion of the ER and Golgi compartments(10).

In conclusion, macrophage activation is associated with metabolic shifts and metabolic reprogramming that enable bioenergetic and biosynthetic support as well as regulatory control of macrophage activities.

6. Metabolic control of macrophage activation

Here we discuss examples from recent studies indicating how metabolic shifts and metabolic reprogramming sustain macrophage activation. Because this topic has been comprehensively reviewed(38–40), we focus on the literature most relevant to mTORC1 and Akt. Diagrams of major metabolic pathways discussed in the text (Fig 3), as well metabolic control of M1 and M2 activation (Fig 4), are included for the reader's reference.

In M1 macrophages, LPS stimulation triggers a rapid increase in glycolytic flux via post-translational mechanisms (Fig 3). This is mediated, at least in part, by phosphorylation of hexokinase by activated Akt. Phosphorylated hexokinase associates with the mitochondria, which is thought to give the glycolytic enzyme privileged access to mitochondrial ATP to rapidly augment its activity and glycolytic flux(10). In other cell types, Akt upregulates endosomal recycling of Glut1 to augment cell surface expression of the glucose transporter(41), and it is likely that macrophage Akt mediates increased glucose utilization via multiple mechanisms. Transcriptional regulation underlies delayed, long-term boosts in glycolytic metabolism. Examples include induction of the glucose transporter Glut1 and glycolytic enzymes like Pfkfb3 and Ldha(35, 42). In some cases, alternative isoforms of metabolic enzymes are induced, apparently because their distinct activities play key roles in altering metabolic shifts (e.g. expression of the phosphofructokinase 2 isoform Pfkfb3 instead of Pfkfb1(42)). Transcriptional induction of many glycolytic enzymes is mediated by HIF1 α , a master regulator of glycolysis that is activated by multiple mechanisms downstream of LPS signaling(35). One is via isoform switch to PKM2, the enzymatically less active isoform of Pyruvate kinase that doubles as a HIF1 α -regulator, promoting its nuclear localization and transcriptional activity(43). A second mechanism for HIF1 α regulation is via increased oxidation of glucose-derived pyruvate, which enhances TCA cycle activity leading to accumulation of the TCA cycle metabolite succinate. Succinate inhibits the enzymatic activity of PHD, which mediates steady-state degradation of HIF1 α , so succinate accumulation stabilizes HIF1 α (34, 43). In this way, the initial, Akt-mediated increase in glycolytic flux is sustained by HIF1 α -mediated transcriptional upregulation of the glycolytic program (Fig 3, 4A).

What are the consequences of increased glycolytic flux? As discussed above, one is HIF1 α stabilization, leading to transcriptional induction of glycolytic genes and a bona fide reprogramming of the M1 macrophage to glycolytic metabolism. The gene encoding pro-IL-1 β is also regulated by HIF1 α . Consistently, HIF1 α -deficient macrophages are impaired in multiple aspects of M1 activation, including induction of glycolytic genes and glucose uptake/metabolism as well as IL-1 β production(34, 35). Citrate is another TCA cycle metabolite that accumulates upon LPS stimulation as a result of increased pyruvate oxidation in the mitochondria (Fig 3). This enables citrate transport to the cytosol, where it serves as a precursor for fatty acid and phospholipid synthesis, needed for expansion of the ER and Golgi compartments to accommodate the increased secretory demand of M1 macrophages. Consistently, inhibition of fatty acid synthesis reduces ER and Golgi expansion and secretion of inflammatory cytokines like IL-6 and TNF α (10). Increased glycolysis in M1 macrophages also drives flux through the PPP, which provides NADPH to fuel respiratory burst(44). Therefore, enhanced glycolysis in M1 macrophages supports

inflammatory cytokine production as well as respiratory burst. As in the other examples above, transcriptional changes complement glycolytic flux in promoting these metabolic processes, e.g., via transcriptional control of the genes encoding mitochondria-to-cytosol transport of citrate (45) and flux through the PPP(44) (Fig 3, 4A).

As noted above, the LPS-stimulated increase in glycolytic flux initially promotes pyruvate oxidation in the mitochondria (enabling accumulation of TCA metabolites and enhanced oxidative phosphorylation), in addition to aerobic glycolysis (Fig 3). However, macrophages and DCs downregulate mitochondrial oxygen consumption after ~12 hours, due to damage of the electron transport chain by iNOS-mediated NO production(46). Related to this defect in oxidative phosphorylation, a recent study showed that M1 macrophages harbor “breaks” in the TCA cycle. One break is mediated by LPS-triggered downregulation of the TCA cycle enzyme isocitrate dehydrogenase(47). This leads to accumulation of the precursor isocitrate, which is used to produce itaconic acid, a metabolite with antimicrobial activity (Fig 4A)(48). Therefore, a broken TCA cycle in M1 macrophages appears to be critically linked to antimicrobial activity.

In addition to an increase in glucose utilization, M1 macrophages upregulate glutamine metabolism(34, 47). Glutamine is metabolized to the TCA cycle metabolite α -ketoglutarate via glutaminolysis. Such replenishing of the TCA cycle (“anaplerosis”) stimulates succinate accumulation and HIF1 α activation (Fig 3, 4A)(34). Pharmacological inhibition of glutamine metabolism reduces production of IL-1 β in M1 macrophages, as well as serum IL-1 β production and mice survival during Salmonella infection and sepsis(34, 47).

Relatively less is known about the IL-4-mediated metabolic shifts that occur in M2 macrophages. As indicated above, these macrophages are thought to be dependent on β -oxidation, since pharmacological block of this process with etomoxir attenuates transcriptional induction of the M2 program (33). Although little is known regarding how it supports M2 activation, β -oxidation is upregulated via IL-4-mediated transcriptional induction of the nuclear receptors PPAR- γ and PPAR- δ , and their coactivator PGC1 β . In macrophages lacking these master regulators of fatty acid oxidation and mitochondrial biogenesis, IL-4-inducible β -oxidation and M2 gene expression are deficient (28, 29, 33). An important source of fatty acids for M2 macrophages is exogenous lipoproteins, which are taken up by the scavenger receptor CD36 and broken down in the lysosome by lysosomal acid lipase (LAL). In support, deletion or pharmacological ablation of LAL or CD36 reduces mitochondrial oxygen consumption and M2 activation (Fig 4B)(49). Enhanced β -oxidation is linked to augmented spare respiratory capacity(49), indicating an increased ability to make ATP by oxidative phosphorylation. Such increase in SRC may be important when macrophages are challenged with energy-intensive tasks, or alternatively has been linked to longevity.

M2 macrophages also increase glutamine utilization(47). This enables biosynthesis of UDP-GlcNAc, the substrate for N-glycosylation, in the hexosamine pathway (Fig 4B). Consistently, block of N-glycosylation or the hexosamine pathway reduces cell surface expression of N-glycosylated M2 markers. Glutamine utilization also fuels TCA cycle activity via anaplerosis and thus presumably oxidative phosphorylation(47) (Fig 4B).

Finally, glutamine consumption supports optimal induction of multiple M2 genes, although the underlying basis is not known. Finally, a recent study indicates that Myc is upregulated by IL-4 stimulation and controls M2 activation(50). Myc is key regulator of oxidative metabolism and other metabolic processes in many contexts, warranting further scrutiny of its role in macrophage activation.

7. Established and likely nodes of metabolic control by mTOR and Akt

Little is known regarding control of macrophage metabolism by mTORC1 and Akt. Here we review their major effector activities in tumor cells and proliferating cells, before discussing established as well as potential nodes of mTORC1- and Akt-mediated control of macrophage metabolism (Fig 4, 5).

As mentioned above, mTORC1 promotes the synthesis of proteins, lipids, and nucleotides in growing and proliferating cells(1) (Fig 1). Its control of protein synthesis is mediated by 4EBP and S6K, key regulators of translation, and lead to ribosome biogenesis and a profound increase in protein synthetic capacity. mTORC1 triggers lipogenesis by activating the transcription factors Srebp1 and Srebp2, which regulate expression of key enzymes in fatty acid and cholesterol synthesis respectively. In addition to rate-limiting enzymes in fatty acid synthesis, Srebp1 upregulates expression of enzymes in the pentose phosphate pathway (PPP), a glycolytic shunt. Increased flux through the PPP provides reducing power during fatty acid synthesis, justifying co-regulation of PPP and lipid synthesis enzymes by Srebp1 (Fig 5). mTORC1 stimulates nucleotide synthesis by increasing the activity of CAD (via S6K1), which catalyzes the first steps of pyrimidine synthesis. mTORC1-mediated activation of Srebp1 also contributes to nucleotide synthesis because ribose-5-phosphate, a building block in nucleotide synthesis, is produced in the PPP (Fig 1, 3). In this way, mTORC1 coordinates the synthesis of the major macromolecules needed in growing and proliferating cells(1).

In M1 macrophages, de novo lipogenesis supports expansion of the ER and Golgi compartments, which has been linked to secretion of high levels of cytokines. Mechanistically, this requires citrate production as well as activation of Srebp1, the transcriptional regulator of lipogenesis (Fig 4A, 5). Consistent with its role in this setting, Srebp1 accumulates upon LPS signaling and its absence reduces fatty acid synthesis(51). As in tumor cells, Srebp1 is activated by mTORC1 in macrophages, as indicated by accumulation of activated Srebp1 in TSC-deficient BMDMs (T.H., unpublished data). Another macrophage effector activity likely to be critically fueled by increased lipid synthesis is phagocytosis. Phagocytic activity, which is augmented in activated macrophages to accommodate killing of microbial pathogens and dead cell clearance, requires an increase of cellular membranes. Lipid synthesis may also sustain production of pro-inflammatory and anti-inflammatory lipid mediators like prostaglandins and lipoxins respectively(45). Lipid mediators are synthesized and stored in lipid bodies, cytosolic organelles that can be formed in response to macrophage polarizing signals in an mTORC1-dependent manner (52). It would be interesting to further explore the role of the mTORC1-Srebp axis in these macrophage effector activities. In addition, mTORC1-mediated increases in protein synthetic capacity is likely to be important for supporting the production of cytokines,

chemokines, and other highly induced factors in activated macrophages. Whether nucleotide levels may be rate-limiting for transcription is not known, but mTORC1-mediated increases in nucleotide synthesis is thought to bolster protein synthesis in proliferating cells, because rRNA is an abundant component of ribosomes(1). Therefore, the ability of mTORC1 to promote protein and nucleotide synthesis may coordinate production of high levels of cytokines, chemokines, and other factors in activated macrophages, while lipid synthesis may support secretory capacity, phagocytosis, and production of lipid mediators (Fig 5).

mTORC1 and Akt regulate glycolysis in tumor cells and proliferating cells(1, 41), and apparently also in M1 macrophages. In response to LPS signaling, Akt mediates an initial increase in glycolytic flux (apparently independent of mTORC1), which is reinforced by HIF1 α -mediated induction of the glycolytic program (Fig 4A, 5). In addition to the mechanisms described above for regulation of HIF1 α activity (succinate accumulation and PKM2 induction), mTORC1 is likely to exert critical control. HIF1 α levels are increased in TSC-deficient BMDMs (T.H., unpublished data), consistent with TSC-deficient MEFs where mTORC1 activity promotes translation of the HIF1 α mRNA (Fig 5)(1). As discussed above, Srebp1 activation by mTORC1 is critical for lipid synthesis and boosts inflammatory cytokine production. Srebp1 also controls NADPH production in the PPP pathway(1), so the activity of the mTORC1-Srebp axis may underlie respiratory burst in M1 macrophages (Fig 4A, 5). Consistent with this discussion, TSC1-deficient DCs have increased lipid biosynthesis and glycolytic metabolism at steady state, although perturbed development and survival in this genetic model complicates analysis of responses to polarizing signals like LPS(53). Finally, mTORC1 has been linked to glutamine metabolism through its control of glutamine hydrolysis to glutamate, the rate-limiting step in glutamine utilization(54). As discussed above, M1 macrophages couple glutamine consumption to anaplerotic refilling of the TCA cycle, which supports citrate production and HIF1 α stabilization (Fig 4A, 5).

mTORC1 and Akt could also support the metabolism of M2 macrophages. Here it is worth reiterating that decreased M2 activation in TSC-deficient BMDMs is a consequence of aberrantly increased mTORC1 activity, and that such mTORC1 hyperactivity is seen in conditions of chronic nutrient excess and cancer(1). In our view, *physiological* mTORC1 signaling in macrophages may not antagonize M2 activation; rather, IL-4 signaling may engage the Akt-mTORC1 pathway to support some aspects of metabolic reprogramming. For example, Akt and mTORC1 regulate glucose utilization in other contexts and may stimulate UDP-GlcNAc synthesis and N-glycosylation of lectins in M2 macrophages (Fig 4B). Another possible node of control is glutamine metabolism, which is increased in M2 macrophages as discussed above and is regulated by mTORC1 (Fig 4B). Additional studies are needed to test these hypotheses.

A recent study has shed light on a new aspect of macrophage metabolism and activation that is regulated by mTORC1 and Akt(55). Macrophages primed with a TLR ligand can be rendered more responsive to a subsequent encounter, a process called “training”. Macrophage training may contribute to the ability of a primary challenge to protect against secondary infection, for example during vaccination. Training is mediated by the Akt-mTORC1-HIF1 α pathway and a sustained upregulation of glycolysis that enable heightened responsiveness upon the second challenge; blocking either glycolytic flux or the Akt-

mTORC1-HIF1 α pathway during the initial challenge inhibits training. Importantly, training is reflected in epigenetic changes at genes encoding glycolytic enzymes, and it would be important to determine how the Akt-mTORC1-HIF1 α axis coordinates such chromatin remodeling in future studies. Since metabolites serve as cofactors of chromatin modifying enzymes and substrates of chromatin modifications, how regulation of metabolite levels by this signaling pathway would bear on chromatin regulation would be of interest. Such epigenetic control by mTORC1 and Akt signaling is likely critical to modulating macrophage functional states in vivo, given the long-lived nature of major macrophage populations in many tissues(55, 56).

8. Concluding remarks and future directions

As highlighted by the discussion above, mTORC1 and Akt (and metabolic processes more generally) can exert context-dependent effects in regulation of macrophage activation. M1 and M2 macrophages both increase glutamine utilization, but the metabolite is channeled into different metabolic pathways to support either HIF1 α stabilization and IL-1 β production or N-glycosylation of cell surface receptors. Likewise, mTORC1-dependent increases in lipid synthesis may support production of lipid mediators in all macrophages, but expression of Cox1 in M2 versus Cox2 in M1 macrophages allows for synthesis of anti-inflammatory and pro-inflammatory mediators respectively(40). A key determinant of these differences is differential expression of key metabolic enzymes, which is specified by the Jak-Stat pathway in M2 macrophages, or alternatively TLR signaling and downstream transcriptional regulators like NF- κ B and IRFs in M1 macrophages. Therefore, mTORC1 and Akt work coordinately with the “canonical” pathway activated by the macrophage polarizing signal; canonical signaling provides specificity while the mTORC1 and Akt have a permissive but critical role, and together they ensure optimal macrophage activation and execution of effector activities (Fig 2).

Many questions remain outstanding in the emerging field of macrophage metabolism and activation and their control by mTORC1 and Akt signaling. A non-exhaustive list includes the following:

- How do polarizing factors other than LPS and IL-4 (e.g. IL-10, butyrate) regulate cellular metabolism to control macrophage activation? In addition to mTOR and Akt, what other metabolic regulators are engaged by polarizing factors?
- mTORC1 is a major metabolic hub that integrates many stimuli. The TSC complex is regulated by cytokines, inflammatory stimuli, hypoxia, and energy levels, while amino acids control mTORC1 activity through an independent pathway. Do these diverse inputs converge on mTORC1 to regulate metabolic aspects of macrophage activation?
- Since macrophages assume a variety of functions depending on the tissue and context, it is logical to suggest that in vivo populations of macrophages are supported by distinct metabolic programs. It would be important to examine various macrophage populations in this regard, ideally complementing ex vivo metabolic profiling with in vivo analyses.

- Many pathogens exploit macrophages as a replicative niche, and host and pathogen modulate macrophage metabolism for antagonistic purposes during intracellular infection. While the macrophage seeks metabolic support for antimicrobial activities, the goal of the pathogen is to subvert host defense and to co-opt macrophage metabolism for its own survival and replication(57). Although the activities of mTORC1 and Akt underlie multiple aspects of host defense, macrophages inactivate mTORC1 during *Shigella* and *Salmonella* infection; mTORC1 inhibits autophagy, so this enables autophagic clearance of the bacteria. However, mTORC1 inactivation is transient during *Salmonella* infection, because the pathogen recruits mTORC1 to its vacuole to stimulate mTORC1 reactivation(58). Another example of bacterial modulation of macrophage metabolism is the M2-like metabolism enforced by *Salmonella typhimurium* and *Brucella abortus*, apparently to spare glucose for their own consumption(59, 60). Future studies will no doubt highlight pathogen exploitation of macrophage metabolism as a key component of host-pathogen interactions.
- Manipulation of metabolic pathways is being explored for therapeutic control of cancer and T-cell mediated diseases(61–64), and there is increasing interest in extending this to diseases where macrophages (and DCs) play a critical role. It is appealing to speculate that modulating macrophage metabolism can be used to enforce a switch in macrophage polarization that would be beneficial in sepsis, IBD, or cancer, for example. While specificity, efficiency, and safety are challenges associated with targeting macrophages by drugs or gene delivery, ongoing studies exploring the use of liposomes, nanoparticles, and other delivery systems may lead to viable therapeutic options(65, 66).

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Fig 1.

Overview of mTOR and Akt regulation and function. mTOR exists in two distinct complexes, mTORC1 and mTORC2. mTORC1 couples nutrient availability to anabolic processes such as cell growth and proliferation and nutrient storage, by promoting the synthesis of macromolecules such as proteins, lipids, and nucleotides. mTORC2 phosphorylates Akt and other kinases of the AGC superfamily to regulate cell metabolism, survival, and cytoskeletal organization.

Fig 2.

Integration of Akt and mTORC1 signaling into the IL-4R and TLR4 pathways. **A)** In parallel to the canonical Jak-Stat pathway, the IL-4R activates the PI3K-Akt-TSC-mTORC1 signaling pathway. **B)** TLR4 engages the adaptor protein BCAP to activate the PI3K-Akt-TSC-mTORC1 signaling pathway in parallel to the canonical pathway that culminates in activation of NF- κ B and IRFs. Independent of its role in the PI3K-Akt-TORC1 pathway, Akt can be activated by canonical TLR4 signaling via TBK/IKK ϵ . Additionally, other kinases activated by TLR4 signaling can regulate mTORC1 activity via phosphorylation of the TSC complex (e.g. Erk and IKK β). Such integration of Akt and mTORC1 signaling into canonical signaling allows for coordinate control of cellular metabolism and function during M1 and M2 activation.

Fig 3.

Overview of major metabolic pathways discussed in the text. Glucose is metabolized in the cytosol via glycolysis, yielding pyruvate which can be converted to lactate (Warburg metabolic or aerobic glycolysis). Alternatively, pyruvate can be further oxidized in the TCA cycle. The pentose phosphate pathway is a glycolytic shunt that produces ribose 5-phosphate and NADPH to support nucleotide synthesis and lipid synthesis. Glucose and glutamine also fuel the hexoamine pathway, which produces UDP-GlcNAc and other amino sugars that are used for the generation of glycoproteins, glycolipids, and proteoglycans. Fatty acids are catabolized via β -oxidation in the mitochondria, yielding Ac-CoA which enters the TCA cycle. Glutamine is metabolized via glutaminolysis to produce α -ketoglutarate. Entry of α -ketoglutarate into the TCA cycle ("anaplerosis") replenishes the cycle, which may be important in conditions where citrate is diverted to the cytosol for Ac-CoA production and lipogenesis. The TCA cycle is a major metabolic hub that produces cytosolic Ac-CoA (via citrate) for lipogenesis, amino acids, and other key metabolites. In addition, NADH and

FADH₂ generated by TCA cycle activity fuels ATP generation by oxidative phosphorylation; alternatively, ATP can be produced by aerobic glycolysis (not shown).

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M1 macrophage

Fig 4B

Fig 4.

Control of metabolic nodes in M1 and M2 macrophages by mTORC1 and Akt. A summary of the major metabolic shifts that occur in M1 (A) and M2 (B) macrophages. Metabolic shifts/processes are shown in boxes (uppercase font), as well as the macrophage effector activity that is supported (*italics*). Nodes of regulation by mTORC1 and Akt and their downstream effectors (HIF1 α and Srebp1) are indicated (red). Question marks indicate likely rather than established nodes of regulation.

Fig 5.

Schematic representation of control of macrophage activation by mTOR and Akt. Key metabolic regulators (ovals), metabolic pathways/processes (white rectangles), and macrophage effector activities (gray rectangles) are shown. In some cases, a regulatory link is inferred based on findings in other cell types (e.g. mTORC1-mediated translational control of HIF1 α). See text for detailed discussion.

Table 1

Macrophages with genetic ablation of mTORC1 and Akt reveal the critical roles of these metabolic regulators in control of macrophage activation.

	Macrophage polarization phenotype	Underlying mechanism	Metabolic phenotype	In vivo phenotypes
TSC-deficient BMDMs	Increased M1 (5, 17, 18)	Increased JNK activity (18), reduced Akt activity (5), increased Ras activity (17)	Defect in IL-4-inducible fatty acid oxidation(5)	Increased susceptibility to sepsis (18); spontaneous development of inflammatory disorders (17)
	Reduced M2 (5, 17)	Decreased Akt (5), decreased C/EBP β (17)		Reduced IL-4 and chitin induced M2 activation (5); resistant to asthma (17)
TSC-deficient DCs	Decreased M1 (20); Mixed phenotype (19)	Reduced NF- κ B activity (20)	Increased steady-state lipid synthesis and glycolytic metabolism(53)	Reduced T cell priming (19)
Rictor-deficient DCs and BMDMs (mTORC2 deficiency)	Increased M1 (26, 27)	Relief of FoxO1 inhibition by Akt (27)		Increased susceptibility to sepsis (26)
Myeloid specific deficiency in Raptor (mTORC1 deficiency)	N.D.	N.D.		Resistance to obesity-associated complications (24) and atherosclerosis (25)
Akt1-deficient peritoneal macrophages	Increased M1(14)	N.D.		Better survival in cecal ligation puncture (attributed to enhanced bacterial killing) but reduced survival in DSS colitis (14)
Akt2-deficient peritoneal macrophages	Reduced M1(14)	Increased expression of C/EBP β (14)		Better survival in septic shock (attributed to decreased proinflammatory cytokines) and DSS colitis (14)

N.D., not determined.