Published in final edited form as:
Nat Rev Immunol. 2015 October ; 15(10): 599–614. doi:10.1038/nri3901.

Regulation of innate immune cell function by mTOR

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Abstract

The innate immune system is central for the maintenance of tissue homeostasis and quickly responds to local or systemic perturbations by pathogenic or sterile insults. This rapid response must be metabolically supported to allow cell migration and proliferation and to enable efficient production of cytokines and lipid mediators. This Review focuses on the role of mammalian target of rapamycin (mTOR) for controlling and shaping the effector responses of innate immune cells. mTOR reconfigures cellular metabolism and regulates translation, cytokine responses, antigen presentation, macrophage polarization and cell migration. The mTOR network emerges as integrative rheostat that couples cellular activation to the environmental and intracellular nutritional status to dictate and optimize the inflammatory response. A detailed understanding of how mTOR metabolically coordinates effector responses by myeloid cells will provide important insights into immunity in health and disease.

Introduction

Rapamycin, the prototypical inhibitor of mammalian target of rapamycin (mTOR), was discovered in soil samples from Easter Island (locally known as Rapa Nui) in the 1970s, and found to have antifungal activity. Now, it is increasingly recognized that mTOR has manifold functions in mammalian cells and acts as a central regulator of cellular metabolism[1,2]. As such, the mTOR pathway is targeted as an immunosuppressive and anti-proliferative therapy for transplantation and cancer. Recent studies have established an important role for mTOR complex 1 (mTORC1) and mTORC2 in regulating the functions of innate immune cell populations. It is now clear that the mTOR signalling network functions as an integrative rheostat that orchestrates a broad network of cellular and metabolic activities that shape immune effector responses. Depending on the type of cell, its target tissue, and its extracellular and intracellular nutrient status, the response of an individual immune cell **OK** is tailored and optimized to its specific needs.

This Review describes the multiple roles of mTOR in monocytes, macrophages, dendritic cells (DCs), neutrophils, mast cells and innate-like natural killer (NK) cells. We discuss the stimuli and signals that activate the mTOR pathway in these cells and the specific effector functions that are controlled by mTORC1 and mTORC2. We review the recent evidence

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showing that the control of cellular energy metabolism by mTOR is central to directing innate effector responses.

Activation of mTOR in myeloid cells

Activated innate immune cells dramatically change their cell morphology, migrate to new tissue sites, and produce large amounts of cytokines, chemokines and lipid mediators, such as prostaglandins and leukotrienes. Many of these metabolically demanding processes are controlled and shaped by the mTORC1–mTORC2 network after cell activation. The mTORC1–mTORC2 network in innate immune cells is activated by various extracellular signals, including growth factors, Toll-like receptor (TLR) ligands and cytokines (Fig. 1). For example, the growth factors granulocyte/macrophage colony-stimulating factor (GM-CSF) and FMS-related tyrosine kinase 3 ligand (FLT3L) induce mTORC1 activation in DCs and neutrophils3–5; TLR ligands activate mTORC1 and mTORC2 in human and mouse monocytes, macrophages and DCs3,6–12, and mTORC1 in mouse neutrophils13; and the cytokine interleukin-4 (IL-4) promotes mTORC1 and mTORC2 activation in mouse macrophages14,15, whereas IL-15 induces mTOR activity in human and mouse NK cells16. Without these activating signals, the mTOR pathway is strikingly inactive in these cells *in vitro*, which is in contrast to resting fibroblasts and other primary cells. Activation of mTORC1 and mTORC2 controls a wide array of basic cellular processes such as translation and protein synthesis, cell growth, metabolism and anabolic processes. For example, mouse macrophages and DCs undergo a massive increase in protein synthesis, peaking after 4 hours of lipopolysaccharide (LPS) stimulation, that is largely dependent on the phosphatidylinositol 3-kinase (PI3K)-mTORC1 pathway17,18. Interestingly, this early increase in protein synthesis seems to be independent of glucose influx and glycolysis19. mTORC1 regulates cap-dependent and cap-independent translation of mRNAs by directly phosphorylating the translation inhibitors eIF4E-binding protein 1 (4E-BP1) and 4E-BP2, which then release eukaryotic translation initiation factor 4E (eIF4E). mTORC1 is particularly efficient in promoting translation initiation of mRNAs containing a 5′ terminal oligopyrimidine tract (5′ TOP) or a pyrimidine-rich translational element (PRTE), which encode many ribosomal and translation-related proteins but also metabolism-related genes2,20. In addition, ribosomal protein S6 kinase 1 (S6K1; also known as RPS6KB1) and S6K2 (also known as RPS6KB2) are two other well-characterized targets of mTORC1-mediated phosphorylation, which in turn induce the phosphorylation and activation of S6 to stimulate protein translation. Thus, one major function of mTORC1 is to stimulate global protein synthesis after innate cell activation, which further induces long term reconfiguration of cellular metabolism. In line with this, a recent report showed that the adaptation of metabolism after LPS stimulation in macrophages mainly occurs through proteome remodelling at the translational level21.

mTOR, metabolism and innate immunity

It is now well known that T cells depend on proper metabolic reprogramming to mount effective responses22. Accumulating evidence suggests that innate immune cells also actively control metabolic processes to adapt and optimize their effector functions23. These various effector functions are supported by an adaptation of energy metabolism to cover

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their metabolic needs and link it to the availability of nutrients. The mTOR network is a central regulator of many core metabolic processes1,24. Activation of mTORC1 usually drives an anabolic response through hypoxia-inducible factor 1α (HIF1α), peroxisome proliferator-activated receptor-γ (PPARγ), sterol regulatory element-binding proteins (SREBPs), and MYC that induces the synthesis of nucleic acids, proteins and lipids1,25. In addition, it drives energy processes such as glycolysis and mitochondrial respiration to provide the cellular energy and building blocks for these responses (Fig. 2). mTORC2 also participates in enhancing glycolytic metabolism by activating AKT and promoting an inactivating phosphorylation of class IIa histone deacetylases24,26. This leads to the acetylation and inactivation of forkhead box protein O1 (FOXO1) and FOXO3, which in turn activates *MYC* transcription.

One key organelle where mTORC1 integrates extracellular growth factor activation with nutrient and energy sensing is the lysosome27. Amino acid sufficiency is sensed on the lysosomal surface by the pentameric Ragulator complex and the vacuolar ATPase. This promotes the accumulation of active heterodimeric GTPases called RAGs, which recruit mTORC1 to the lysosomal membrane. Other organelles such as the Golgi or peroxisomes have also been implicated as places of mTORC1 activation28,29. In addition to amino acids, mTORC1 can sense and integrate the AMP/ATP ratio via AMPK25 and the availability of glucose via glucose-6 phosphate and binding of hexokinase II to mTORC130,31 (Fig. 1)30,31. Moreover, the binding of the lipid metabolite phosphatidic acid (PA) is required for mTORC1 activation on the lysosome32. Hence, mTORC1 can sense and integrate all of the main classes of nutrients and energy sources: amino acids, glucose, lipids and ATP. The current model proposes that mTORC1 activation by extracellular signals requires an intracellular sufficiency of nutrients and energy. In turn, mTORC1 stimulates further glucose uptake through the translation of HIF1α, enhances mitochondrial biogenesis through activation of PPARγ coactivator 1α (PGC1α) and ying yang 1 (YY1), and promotes lipid biosynthesis through activation of SREBPs and PPARγ33 (Fig. 2). Genetic activation of mTORC1 by deletion of tuberous sclerosis 1 (*TSC1*) promotes glycolysis, mitochondrial respiration and lipid synthesis in mouse DCs and haematopoietic stem cells34,35. This metabolic reprogramming in TSC1-deficient DCs occurs partly via mTORC1-mediated expression of MYC, although mTORC2 and AKT are inhibited in these cells35,36. Nevertheless, the specific functions of mTORC1-mediated metabolic reprogramming in innate immune cells are just beginning to be explored.

TLR stimulation of mouse bone marrow-derived DCs, but not primary human myeloid DCs, induces a rapid (after 1 hour) flux of glucose-derived carbon into the tricarboxylic acid (TCA) cycle and pentose phosphate pathway that is dependent on TANK-binding kinase 1 (TBK1), IκB kinase ε (IKKε) and AKT but independent of mTORC1 and HIF1α19,37. This initial increase in glycolysis supports the *de novo* synthesis of fatty acids that are required for the expansion of the endoplasmic reticulum and Golgi necessary to stimulate the production and secretion of pro-inflammatory molecules such as IL-12, tumour necrosis factor (TNF), IL-6 and CD8619. This is followed (hours later) by an mTOR- and HIF1α-dependent metabolic switch from oxidative phosphorylation to aerobic glycolysis in mouse DCs38–41. This switch is due to the induction of nitric oxide (NO) and type I interferon (IFN) production. NO poisons the mitochondrial respiratory chain necessitating an increase

in aerobic glycolysis to promote DC survival39. In these inflammatory mouse DCs, inhibition of mTORC1 inhibits NO production, preserves mitochondrial respiration and extends cell survival40. As human DCs usually produce only low levels of NO, at least *in vitro*, it is currently unclear whether these results can be extrapolated to human DC biology. Autocrine IFN production by mouse splenic DCs activated with polyinosinic–polycytidylic acid also induces the switch from mitochondrial respiration to aerobic glycolysis via activation of HIF1α41. Because the synthesis of NO by inducible NO synthase (iNOS) is HIF1α dependent42, IFN and NO may both promote the switch to aerobic glycolysis via the activation of HIF1α. As mTORC1 positively controls IFN production (see below), mTORC1 regulates metabolic reprogramming in DCs at least via type I IFN and NO production.

mTORC1-mediated reprogramming from oxidative phosphorylation towards aerobic glycolysis represents the metabolic basis of an effect known as trained immunity. Trained immunity refers to the non-specific protection against secondary infections mediated by epigenetic reprogramming of myeloid immune cells to allow a faster and more pronounced secondary immune response43. Low doses of the cell wall component β-glucan of *Candida albicans* activate an AKT-mTORC1-HIF1α-dependent glycolytic response in human monocytes and this response is essential for the exacerbated IL-6 and TNF expression that occurs following restimulation of the monocytes after seven days with TLR ligands44. Deletion of HIF1α in myeloid cells in mice impairs the protective effect of β-glucan priming on survival following a lethal *S. aureus* infection44. In contrast, vitamin D3-induced tolerance in human DCs also requires activation of mTORC1 and aerobic glycolysis45. Interestingly, mTORC1-dependent induction of glycolysis is also important for activation of the NLRP3 inflammasome and maturation of pro-IL-1β in peritoneal macrophages46. Finally, mTORC1-mediated metabolic reprogramming via HIF1α is essential for the proliferation and cytotoxicity in mouse NK cells (see below)47. In conclusion, the current evidence suggests that mTORC1 primes metabolic reprogramming towards enhanced glycolysis via non-mutually exclusive mechanisms in innate immune cells. This can lead to seemingly opposing functional outputs: either enhanced restimulatory potential or tolerance induction; the reasons for these differences are currently unclear. It is important to note that constitutive activation of mTORC1 by deletion of *TSC1* or *TSC2* also enhance mitochondrial respiration and lipid synthesis35. Therefore, although an increase in mTORC1 activity promotes glycolysis, the generation of pyruvate through this pathway can be used for aerobic glycolysis as well as mitochondrial respiration. It will be important to further link the metabolic mechanisms controlled by the mTOR network to distinct effector responses.

mTOR in myeloid phagocytes

mTOR in innate immune cell development

Activation of the PI3K-AKT-mTORC1 pathway is required for the *in vivo* development of normal numbers of mouse and human DCs, particularly pDCs and conventional $CD8^+$ DCs, in the presence of FLT3L4,48,49. Genetic activation of PI3K and mTORC1 by deletion of phosphatase and tensin homologue (*Pten*) in mouse DCs causes a rapamycin-dependent expansion of $CD8^+$ DCs and $CD103^+$ DCs4. However, renal transplant patients treated with

the mTOR inhibitor rapamycin have similar numbers of myeloid DCs and pDCs in their blood compared to control patients, suggesting that the doses of rapamycin used in the patients do not compromise the DC compartment in humans *in vivo*3,49. Deletion of the gene encoding late endosomal/lysosomal adaptor, MAPK and MTOR activator 2 (*LAMTOR2*; also known as p14) in DCs activates mTORC1 and results in a marked expansion of conventional DCs and pDCs in ageing mice50. LAMTOR2 is a component of the ragulator complex and is thought to be essential for amino acid-dependent activation of mTORC1 on lysosomes. However, LAMTOR2 also regulates endosomal biogenesis and therefore decreases FLT3 turnover, leading to increased levels of surface FLT3 and increased responsiveness to FLT3L in LAMTOR2-deficient mouse DCs. This in turn indirectly activates mTORC1 and promotes the expansion of DCs. By contrast, deletion of TSC1 activates mTORC1 but impairs DC development *in vivo* and *in vitro*, and is associated with defective cell survival and proliferation35. Indeed, constitutive activation of mTORC1 might be detrimental as it promotes the generation of reactive oxygen species owing to enhanced mitochondrial biogenesis34. Moreover, deletion of TSC1 is associated with diminished phosphorylation of AKT that usually promotes the survival of various cell types15,34.

DC-specific loss of mTORC1, but not of mTORC2, leads to a progressive decrease in Langerhans cells in the skin of mice51. mTORC1-deficient Langerhans cells have an increased tendency to leave the skin and show increased apoptosis, suggesting that mTORC1 is crucial for the preservation of the Langerhans cell network in mice. Mice with a deletion of LAMTOR2 in $CD11c^+$ DCs display a virtually complete loss of Langerhans cells in the epidermis early after birth due to impaired proliferation and increased apoptosis52. Overall, these results indicate that precisely controlled activation of mTORC1 is crucial for myeloid cell development, whereas constitutive activation of mTORC1 disturbs cell homeostasis.

mTOR-mediated regulation of cytokine production

Treatment of human monocytes or primary myeloid DCs with rapamycin or ATP-competitive mTOR inhibitors (Box 1) enhances their production of IL-12p40 and IL-12p70 after stimulation with TLR ligands3,8,9,11,53,54. Mycobacteria- or LPS-induced production of IL-23 by human monocyte-derived macrophages and monocytes is also enhanced by rapamycin8,55. An immunostimulatory effect of mTOR inhibitors is also seen in human transplant patients *in vivo*9,56,57. Two human studies showed activation of IL-12-induced signalling and increased inflammatory responses by blood leukocytes from rapamycin-treated transplant recipients56,57. Inhibition of the mTOR pathway with rapamycin can also have immunostimulatory effects in mouse cells and models after bacterial infections8,18,58. Inactivation of mTORC1 by genetic deletion of *Rptor* (which encodes regulatory-associated protein of mTOR (RAPTOR)) in mouse intestinal DCs induces a severe inflammatory response to enteric bacteria following treatment with dextran sulfate sodium (DSS)59. Genetic inactivation of PI3K and pharmacological inhibition of mTORC1 promotes IL-12 production by mouse immune cells6,8,58,60, although this seems to be more cell-type dependent than mTORC1-mediated enhancement of IL-12 expression by primary human immune cells. Accordingly, deletion of *Tsc1* reduces IL-12 production by mouse bone marrow-derived DCs, but promotes IL-12 production by mouse macrophages via activation of Janus kinase 1 (JNK1) and/or JNK214,35,36,61. Interestingly, rapamycin treatment or

genetic deletion of *mTORC1* does not prevent and still increases IL-12 production by TSC1-deficient macrophages14, suggesting that some TSC1-mediated effects are independent of mTORC1 and that some of the effects of mTOR inhibitors are mediated by inhibition of mTORC2 (Box 1). Indeed, deletion of the mTORC2 component *Rictor* enhances IL-12 production by macrophages and DCs62–64, potentially through the inactivation of an AKT-dependent inflammatory feedback pathway. After LPS stimulation, mTORC2 directly phosphorylates AKT, which phosphorylates and limits the activation of the pro-inflammatory transcription factor FOXO162. Hence, deletion of mTORC2 activates FOXO1, which augments inflammatory gene expression and IL-12 production62,65. Inactivation of TSC1 and TSC2 could therefore promote inflammatory responses by inhibiting mTORC2, which in turn activates FOXO1.

The immunostimulatory effects of mTOR inhibition can be explained by enhanced activation of nuclear factor-κB (NF-κB) and inhibition of signal transducer and activator of transcription 3 (STAT3) and glycogen synthase kinase 3β (GSK3β)8,54,57 (Fig. 3a). At the same time, pharmacological or genetic inhibition of mTORC1 strongly reduces expression of the anti-inflammatory cytokine IL-10 by human and mouse monocytes and DCs3,7,8,11,53,59,66,67. This occurs through mTORC1-stimulated degradation of programmed cell death protein 4 (PDCD4), which releases twist-related protein 2 (TWIST2) that subsequently activates IL-10 expression through the transcription factor macrophage-activating factor (MAF)68 (Fig. 3a). Rapamycin prevents the LPS-induced degradation of PDCD4 and suppresses IL-10 production by mouse macrophages. Overall, these data show that inhibition of mTORC1 or mTORC2 generally has an immunostimulatory effect in TLR-stimulated primary myeloid immune cells.

Inhibition of mTORC1 in *in vitro*-generated human monocyte-derived DCs (moDCs) during LPS stimulation usually has no immunostimulatory effect3. Rapamycin does not augment NF-κB signalling in these cells and instead inhibits IL-12p40, TNF and IL-6 expression. Interestingly, mTORC1 is constitutively active in moDCs in contrast to human myeloid DCs3,69; however, the molecular reasons for these discrepancies are currently unclear (Box 2). Moreover, *in vitro* differentiation of moDCs with GM-CSF and IL-4 in the continuous presence of rapamycin induces their conversion into tolerogenic DCs49,70,71. These tolerogenic DCs are characterized by an immature phenotype and low expression of pro-inflammatory cytokines and T cell stimulatory molecules70. In contrast to human DCs, rapamycin-conditioned mouse tolerogenic DCs are resistant to LPS- or cytokine-mediated maturation and T cell stimulation70, which has been harnessed for experimental cell-based tolerogenic therapies (see later).

Activating mTORC1-dependent translation can bias the expression towards anti-inflammatory cytokines in mouse macrophages: low abundant mRNAs such as IL-10 are more strongly expressed than high abundant pro-inflammatory cytokine mRNAs18. This is important in the defence against a virulent strain of *Legionella pneumophila*, which induces ubiquitylation and inactivation of mTOR and AKT in macrophages, stimulating a pro-inflammatory cytokine response and bacterial clearance18. Hence, blocking mTOR with rapamycin favours the translation of the more abundant pro-inflammatory cytokines. On the other hand, the proto-oncogene cancer Osaka thyroid oncogene (COT; also known as

MAP3K8 and TPL2) positively controls cap-dependent mRNA translation of TNF and IL-6 through mTORC1 in mouse TLR-stimulated macrophages72. Nevertheless, translational control of mRNA expression can dampen the pro-inflammatory response73. Stimulation of mouse macrophages with LPS activates the translation of many feedback inhibitors such as NF-κB inhibitors and dual specificity protein phosphatase 1 (DUSP1) to limit an excessive inflammatory and self-destructive response73. A potential role of mTORC1 for regulating translation of these inhibitors has not been investigated but might explain some of the immunostimulatory effects of mTORC1 inhibition.

Translational control is also important for the regulation of type I IFN production by myeloid DCs and pDCs. Efficient translation of the transcription factor IFN regulatory factor 7 (IRF7), which stimulates IFNα and IFNβ production in pDCs, is induced by the mTORC1-dependent phosphorylation and inactivation of the translational inhibitors 4E-BPs74 (Fig. 3b). Of note, mouse and human pDCs show constitutive expression of IRF7 in contrast to other DCs, and this correlates with a constitutively active mTORC1 pathway in these cells75. The *Leishmania major* protease GP63 directly cleaves mTOR and maintains translational repression by 4E-BP1. This suppresses the translation of type I IFNs and microbicidal genes and confers an intracellular survival advantage for *L. major*76. Moreover, S6K1 and S6K2 phosphorylate and induce the nuclear translocation of IRF7, which together with IRF5, stimulates type I IFN production10,75,77 (Fig. 3b). Therefore, the mTORC1 downstream effectors 4E-BPs and S6Ks positively control the translation and activation of IRF7 and thus type I IFN expression.

mTOR-mediated control of macrophage polarization

Macrophage function depends on proper activation and polarization into distinct subtypes with individual effector functions, such as the M1 and M2 macrophage subsets (for a discussion see REF.78). Macrophage polarization is thought to be regulated not only by cytokines and growth factors but also by environmental cues, such as the presence of nutrients, fatty acids and other metabolites79,80. Therefore, it is not surprising that PI3K-AKT-mTOR-mediated sensing of environmental and metabolic cues influences macrophage polarization in a complex and incompletely understood manner (Table 1).

Mouse macrophages with constitutively enhanced PI3K and mTOR signalling caused by myeloid cell-specific deletion of *Pten* or *Inpp5d* (which encodes SHIP) express higher levels of M2 markers and have enhanced activation of the STAT6 signalling pathway81–83. Inhibition of mTORC1 with rapamycin in human macrophages enhances M1 macrophage polarization67,84. These results indicate that activation of the PI3K-mTOR network promotes M2 macrophage polarization6,8,59,85. However, increased mTORC1 activity and reduced mTORC2 activity by ablation of *Tsc1* promotes M1 macrophage polarization and reduces M2 macrophage polarization14,15. This seems to be mediated by different pathways that are independent of STAT6 and PPARγ, two well-known M2 macrophage-promoting proteins. *Tsc1* deletion promotes M1 macrophage polarization by activating a RAS GTPase–RAF1–MEK–ERK pathway that is independent of mTORC114. In addition, deletion of *Tsc1* reduces the expression of CEBPβ and M2 macrophage polarization by activation of mTORC114. In contrast, another group has found that deletion of Tsc1 in macrophages

enhances M2 as well as M1 polarization86. Inhibition of mTORC1 by deletion of *Rptor* in myeloid cells protects against inflammation in an obesity model and seems to enhance M2 macrophage polarization87. Moreover, a positive signal of TSC1 to AKT can also drive M2 macrophage polarization15. However, the role of AKT is not straightforward, because the AKT isoforms differentially control macrophage polarization88. Ablation of AKT1 gives rise to M1 macrophages, and deletion of AKT2 promotes a M2 macrophage phenotype in *in vivo* models of colitis and atherosclerosis88,89. *Akt2*-knockout macrophages show enhanced expression of CEBPβ. Deletion of the kinase phosphoinositide-dependent protein kinase 1 (PDPK1) in macrophages that is crucial for AKT activation (Fig. 1) elevates M1 macrophage polarization and promotes insulin resistance90. In line with this, deletion of AMPK reduces AKT activation and promotes M1 macrophage polarization91,92. Finally, mice with a myeloid cell-specific mTORC2 deletion show stronger M1 macrophage polarization potential and reduced M2 macrophage polarization potential63. Overall, these results indicate that macrophage polarization is controlled by the PI3K-AKT-mTOR pathway, however, a complete understanding of the functional roles of the individual pathway members requires further research (Box 2).

There is initial evidence that macrophage polarization might also be regulated by metabolic reprogramming93. In that regard, activation of pyruvate dehydrogenase kinase 1 (PDK1), a positive regulatory enzyme in glucose metabolism, promotes mouse M1 macrophage polarization94. Surprisingly, MYC, a stimulator of aerobic glycolysis, is highly expressed in M2 macrophages and crucial for M2 macrophage polarization by inducing around half of the genes associated with alternative activation95. It is interesting that lactic acid — an end product of aerobic glycolysis — primes M2-like macrophage polarization in mouse macrophages and inhibits IL-12 production but enhances IL-10 production by human monocyte-derived DCs96,97. Hence, one can envision that strongly glycolytic M1 macrophages prime for subsequent M2 macrophage responses. Indeed, the presence of M1 macrophages is a prerequisite for the successive emergence of M2 macrophages and tissue homeostasis during wound healing or *Listeria monocytogenes* infections98,99. Moreover, these results, together with the notion that the AMPK-AKT-mTOR network is a global sensor of nutrients and energy, argue for caution with regard to cell density and cell culture composition in *in vitro* experiments when analysing macrophage polarization or innate immune cell activation. Whether the mTOR network mediates macrophage polarization via metabolic reprogramming is currently unclear, but warrants further investigation.

mTOR-mediated regulation of autophagy

The starvation-induced degradation of cytosolic components known as autophagy is crucial in providing substrates for energy production under catabolic conditions of limited nutrient supply. It is important to note that autophagy in macrophages is not a strictly catabolic process but is actively engaged after macrophage activation by a TLR-TRAF6-Beclin 1-dependent pathway to promote basic effector functions100. Autophagy supports the elimination of intracellular pathogens and phagocytosed materials, and facilitates the synthesis of secretory proteins, such as IL-6 and IL-8, by providing amino acids100–102. mTOR and AMPK control the Ser/Thr kinases ULK1 and ULK2, which are an additional system that controls autophagosomal formation and autophagic flux100. mTORC1 actively

suppresses autophagy by phosphorylating ULK1 and, accordingly, inhibition of mTORC1 strongly induces autophagy100. However, termination of autophagy and reformation of lysosomes requires reactivation of mTORC1103. Autophagy regulation by mTORC1 in mouse macrophages is an important defence mechanism against *Mycobacterium tuberculosis, Salmonella typhimurium* and *Pseudomonas aeruginosa* and limits their intracellular growth104–107. Mycobacterial infection enhances the expression of miR-155, which inhibits the expression of the small GTPase RAS homologue enriched in brain (RHEB) by binding to the 3'-untranslated region of *RHEB* mRNA105. This leads to mTORC1 inhibition, activation of autophagy and enhanced intracellular killing of the mycobacteria *in vitro* and *in vivo*105. *S. typhimurium* encodes genes on the pathogenicity island 2 that activate focal adhesion kinase (FAK). This leads to enhanced AKT and mTOR signalling, which inhibits autophagy and promotes intracellular pathogen survival in mouse macrophages108. To evade immune-mediated control, HIV-1 induces a shutdown of autophagy after infection of human DCs by activating mTOR109.

Defects in macrophage autophagy are linked to the progression of atherosclerosis by regulating cholesterol homeostasis, oxidative stress and inflammation110. Autophagy promotes the degradation and clearance of ingested complex lipids such as low-density lipoprotein (LDL)-cholesterol. Accordingly, activation of mTOR enhances the accumulation of lipids and foam cell formation owing to inhibition of autophagy, whereas genetic or pharmacological inhibition of mTORC1 reduces atherosclerotic disease, macrophage plaque infiltration and systemic inflammation in various animal models111–114. These results suggest that mTORC1 inhibition could be an approach to treat atherosclerosis.

mTOR in antigen presentation and T cell stimulation

mTORC1 is intimately involved in antigen presentation by DCs and thereby able to modulate their T cell stimulatory capacity. As autophagy contributes to the presentation of endogenous and exogenous proteins on MHC class I and class II molecules115, it follows that autophagy induction by mTOR inhibitors enhances antigen presentation116 (Fig. 3c). However, endocytosis of mouse splenic DCs is decreased in rapamycin-treated mice *in vivo*117. Analysis of transplant patients shows that the antigen processing pathway is positively regulated in rapamycin-treated patients, predominantly by triggering antigen presentation56. Accordingly, chronic activation of mTORC1 by deletion of *Tsc1* strongly reduces MHC class II expression by mouse DCs61. In resting wild-type DCs, mTORC1 activity is low, allowing expression of the class II coactivator (CIITA), which stimulates MHC class II mRNA transcription. LPS stimulation or *Tsc1* deletion activates mTORC1 and rapidly shuts off *Ciita* transcription. Thereby, the further transcription of new MHC class II molecules is prevented allowing efficient cell surface expression of existing peptide-MHC class II complexes61. Inhibition of mTOR promotes the expression of the T cell costimulatory molecule CD86, whereas expression of the T cell inhibitory molecule PD-L1 is decreased3,8,37,59 (Fig. 3c). Moreover, inhibition of mTORC1 enhances the life span of LPS-stimulated mouse DCs, increasing the time available to drive T cell activation37,40.

This evidence, together with its IL-12 and IL-10 skewing potential, predicts that inhibition of mTORC1 in DCs promotes T cell activation. Indeed, inhibition of mTORC1 in human or

mouse DCs during TLR stimulation augments the proliferation of $CD4^+$ T helper 1 (T_H1) and T_H17 cells *in vitro*7–9,37,53. Similarly, mTORC2 deficiency in mouse DCs also enhances T_H1 and T_H17 cell responses64. Mice immunized with rapamycin-treated DCs and the tuberculosis-vaccine strain BCG show enhanced T_H1 cell-mediated protection against virulent *Mycobacterium tuberculosis*116. Therapeutic vaccination using DCs treated with TLR agonists plus rapamycin results in improved generation of $CD8^+$ T cells and improved antitumour immunity in a mouse melanoma model37. Treatment of mice with an immunostimulatory agonistic CD40 antibody, together with AZD8055, an ATP-competitive mTOR inhibitor, elicits synergistic antitumour responses in a metastatic renal cell carcinoma model12. Accordingly, constitutive activation of mTORC1 in DCs by deletion of *Tsc1* inhibits T cell responses *in vivo*35,61. Finally, rapamycin-treated transplant patients show increased alloreactive T cell frequencies compared with control patients56. These potent immunostimulatory effects of rapamycin in DCs have to be considered in the context of the immunosuppressive and immunomodulatory effects on T cells on a systemic level118. Nevertheless, it might be possible to design novel human vaccination strategies, in which local administration of mTOR inhibitors together with an antigen-adjuvant complex or with antigen-loaded DCs might allow for an improved immune response. However, a seemingly different outcome with rapamycin can also be generated: as noted above the differentiation of moDCs *in vitro* in the presence of rapamycin generates tolerogenic DCs that inhibit effector T cell activation but maintain the ability to stimulate regulatory T cells71. Tolerogenic DCs generated with the catalytic mTOR inhibitor Torin 1 (Box 1) also promote the differentiation of regulatory T cells119. Injection of such tolerogenic rapamycin-conditioned DCs into mice can prolong graft survival in various transplantation models70,120.

mTOR in innate immune cell migration

mTOR signalling has contrasting effects on innate immune cell migration. On the one hand, mTORC1 inhibition enhances the expression of CC-chemokine receptor 7 (CCR7) by human monocytes and DCs67,121, as well as T cells122, and thereby promotes the migration of DCs and mouse Langerhans cells51,121 to secondary lymphoid nodes and the induction of adaptive immune responses (Fig. 3c). On the other hand, mTORC1 inhibition reduces CC-chemokine ligand 2 (CCL2) secretion by human and mouse monocytes, macrophages and DCs, and thereby reduces chemotactic activity of monocytes and basophils8,113,114,123. This effect of mTORC1 inhibition has been shown to be beneficial in mouse and rabbit models of atherosclerosis113,123. By contrast, AKT-dependent activation of mTORC1 induces a translational switch from the transcription factor isoform CEBPβ long (LAP) to the dominant-negative isoform CEBPβ liver inhibitory protein (LIP) after activation of the bile acid-responsive G protein-coupled receptor TGR5 in mouse macrophages. This diminishes the expression of the chemokines CCL2, CCL3 and CCL4 that have CEBPβ binding sites and the migratory properties of TGR5-activated macrophages124. Adherence of mouse peritoneal macrophages rapidly induces the mTORC1-dependent *de novo* translation of pre-existing mRNAs encoding Rho-associated kinase 1 (ROCK1), which promotes macrophage chemotaxis125. Therefore, the current evidence suggests that mTORC1 inhibition enhances the migration of DCs, whereas it usually inhibits the migratory properties of macrophages.

mTOR in granulocytes

Neutrophil granulocytes are a first line of defence during infections, inflammation and tissue injury. They are rapidly recruited by chemotaxis and migrate to the site of action to kill pathogens by phagocytosis, degranulation and neutrophilic extracellular trap (NET) formation126. Chemotactic migration is controlled by mTORC2 downstream of G-protein-coupled receptors in a dual manner127 (Fig. 4a). mTORC2 regulates actin cytoskeleton organization at the leading edge of migrating cells, and cells that lack the mTORC2 component Rictor exhibit defects in cell polarity and chemotaxis128,129. Small RHO GTPases and AKT2 serve as downstream effectors of mTORC2 to regulate actin assembly at the leading edge in human and mouse neutrophils128,130. In addition, mTORC2 is crucial for tail retraction in human neutrophils via the regulation of myosin II129. mTORC2 phosphorylates PKCβII, which activates adenylyl cyclase 9 to convert ATP into cAMP129,131. cAMP activates RHOA to further induce myosin II phosphorylation and tail retraction129,131. Interestingly, rapamycin inhibits human neutrophil migration very efficiently, indicating that mTORC1 might also have a direct role in cell migration132,133. Indeed, mTORC1 controls F-actin reorganization through S6K1 as well as focal adhesion formation and RHO GTPase activity in other cell types127.

The translation of constitutively present mRNAs by mTORC1 emerges as an important mechanism by which neutrophils rapidly react to inflammatory insults134–136 (Fig. 4b). For example, mTORC1 controls the translation of pre-existing cyclooxygenase 2 mRNA in human neutrophils to induce the rapid expression of prostaglandins that promote inflammation and vasodilation to attract further immune cells134. The soluble IL-6 receptor-α (sIL-6Rα) is synthesized in an mTORC1-dependent manner without the requirement of *de novo* transcription136. sIL-6Rα subsequently signals to endothelial cells and induces a switch from neutrophil recruitment to mononuclear leukocyte recruitment to initiate the resolution of acute inflammation137. Therefore, inhibition of mTORC1 in neutrophils is anti-inflammatory at early phases of inflammation, whereas it can prolong the acute inflammatory phase at later stages. Inhibition of mTOR by rapamycin can differentially regulate NET formation in primary human neutrophils depending on the stimulus138–140. Whereas rapamycin inhibits LPS-mediated NET formation by translational control of HIF1α expression, it enhances formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated NET formation138,139. The reason for these differences is currently unclear but may be due to differential activation of NADPH oxidases and autophagy by LPS and fMLP138,139,141. Finally, mTORC1 positively contributes to the expression of pro-inflammatory IL-8, IL-6 and TNF in human and mouse neutrophils via a transcriptional mechanism *in vitro* and in TLR4-induced lung injury *in vivo*13,142.

So far, relatively little is known about the role of mTOR in eosinophil granulocytes. Rapamycin inhibits the differentiation, proliferation and function of mouse eosinophils *in vitro* and *in vivo*143.

mTOR in mast cells

Mast cells contribute to host defence and also to allergic disorders and asthma144. They migrate into inflamed tissues and release pro-inflammatory mediators by degranulation upon activation of cell surface receptors such as the high affinity IgE receptor (FcεRI), the prostaglandin E_2 (PGE_2) receptor or the receptor for stem cell factor, KIT. In human and mouse mast cells, stimulation of these receptors results in a rapid PI3K-dependent activation of mTORC1 and mTORC2145–147 (Fig. 4c). Activation of mTORC1 is required for human and mouse mast cell survival and the expression of IL-8 and TNF after FcεRI stimulation145,148. mTORC2 activity also contributes to PGE_2-mediated chemotaxis, CCL2 expression, and reactive oxygen species (ROS) production146. However, constitutive activation of mTORC1 by loss of *Tsc1* is detrimental and increases mouse mast cell apoptosis by enhancing mitochondrial ROS production and expression of p53, which reduces the expression of the anti-apoptotic molecule B-cell lymphoma 2 (BCL-2)148. TSC1-deficient mast cells show defective degranulation and histamine production *in vitro* and *in vivo*148. Interestingly, rapamycin is incapable of restoring the impaired degranulation in TSC1-deficient cells suggesting an mTORC1-independent mechanism. Surprisingly, downregulation of Rictor increases FcεRI-induced degranulation independently of mTORC2149. Indeed, about 50% of Rictor is not localized to mTOR in human mast cells149.

mTOR in NK cells

NK cells are group 1 innate lymphoid cells (ILC1s) involved in immune surveillance of cancers and in the control of intracellular infections150. IL-15 is important for the homeostasis, activation and maturation of NK cells. High but not low concentrations of IL-15 activate mTORC1 via PDPK1 and AKT16,151,152 (Fig. 4d). This mTORC1 activation is important for efficient mouse NK cell proliferation and the acquisition of cytotoxicity through the production of IFNγ and granzyme B16,47,151. Rapamycin or genetic deletion of mTOR in NK cells blocks NK cell proliferation, IFNγ and granzyme B expression, and results in elevated viral burdens upon mouse cytomegalovirus infection16,151. Rapamycin inhibits NK cell proliferation and cytotoxicity in humans, and there is a significant decrease in NK cells in rapamycin-treated transplant recipients153. Upon activation, mouse NK cells undergo mTORC1-dependent metabolic reprogramming to upregulate glucose uptake and increase aerobic glycolysis47. Directly limiting the rate of glycolysis is sufficient to block cytotoxic activity by NK cells47, indicating that NK cells are exquisitely dependent on mTORC1-regulated glucose import and use. The finding that rapamycin inhibits IL-5 and IL-13 production by IL-33-stimulated ILC2s suggests a broader role of mTOR in the metabolic regulation of ILC functions154.

Summary and future directions

The past few years have provided us with a wealth of information about how the mTOR signalling pathway operates in innate immunity. A central paradigm emerges suggesting that activation of innate immune cells via pattern recognition or growth factor receptors triggers the pathway, which further integrates the environmental and intracellular nutritional and

energy status to guide and shape the effector response. This occurs partially via the reconfiguration of cellular metabolism to provide energy and building blocks for the subsequent immune response of the cell. In addition, mTOR modulates central transcription pathways, such as NF-κB, STAT3, HIF1α and PPARγ pathways, in a cell-specific manner. While mTOR broadly supports many innate immune functions, a proper timed activation pattern of the mTOR network seems equally essential and might explain some of the unexpected observations in cells, in which mTORC1 is constitutively activated. Moreover, the realization that the environmental nutrient state is sensed by mTOR will have important implications in the design and interpretation of *in vitro* cell culture experiments. Our current knowledge is just the starting point for further studies that will shed new light on the impact of the mTOR signalling network on distinct effector responses. Which extracellular nutrients and metabolites are specifically sensed by mTOR in the context of inflammatory responses? How does mTOR regulate the metabolic system in the context of macrophage polarization and other effector responses? Does the mTOR network control macrophage proliferation as an important determinant for tissue homeostasis? Lysosomes seem to be central intracellular compartments in which mTOR signalling occurs. As lysosomes are essential for phagocytosing cells and the mTORC1-regulated lysosomal transcription factor TFEB is a regulator of host defence155, the intimidate relationship of mTORC1 and lysosome function for bacterial infections or atherosclerosis needs to be further explored. How important is the mTOR-mediated regulation of protein translation for the currently underappreciated role of total protein synthesis for immune effector responses? Answering these and other questions will provide exciting new insight into the regulation of innate immune responses by mTORC1 and mTORC2 and new opportunities for therapeutic approaches.

Acknowledgements

This work is supported by grants from the Austrian Science Fund (FWF) grant FWF-P27701-B20, the Else-Kröner-Fresenius-Stiftung (P2013_A149), and the Herzfelder'sche Familienstiftung. The authors apologize to those colleagues whose work has not been cited owing to space constraints.

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Glossary

OKmTORC1

A complex consisting of: mammalian target of rapamycin (mTOR), which is a serine/threonine kinase; regulatory-associated protein of mTOR (RAPTOR); proline-rich AKT1 substrate of 40 kDa (PRAS40), which is an mTORC1 inhibitor; mLST8 (also known as GβL), which is of unknown function; and DEP domain-containing mTOR-interacting protein (DEPTOR), which is an mTOR inhibitor.

mTORC2

A complex composed of: mTOR, mLST8 and the adaptor proteins rapamycin-insensitive companion of mTOR (RICTOR) and stress-activated MAP kinase-interacting protein 1 (SIN1; also known as MAPKAP1).

Tuberous sclerosis 1

(TSC1). TSC1 forms a heterodimeric complex with TSC2. The TSC1-TSC2 complex actively inhibits mTORC1 in unstimulated cells. Stimulation promotes AKT-dependent phosphorylation and inactivation of the complex. TSC1-TSC2 seems to be a positive regulator of mTORC2.

Pyruvate

The end product of glycolysis, which can be further metabolized to lactate in a process known as aerobic glycolysis (also known as “Warburg effect”). Pyruvate can also oxidized in the mitochondria through the tricarboxylic acid (TCA) cycle followed by oxidative phosphorylation to generate ATP.

Langerhans cells

A specialized subset of dendritic cells that seed the epidermal layer of the skin.

M1 and M2 macrophage subsets

Macrophages display considerable plasticity and can change their function in response to local environmental stimuli. M1 (or classically activated) macrophages mediate defence against various pathogens and tumours and contribute to chronic inflammatory diseases and autoimmunity. M2 (or alternatively activated) macrophages are required for defence against parasitic infections, to promote the resolution of inflammation, and to initiate tissue repair, and they are also implicated in tumour promotion.

STAT6

A transcription factor that is important for M2 macrophage polarization after stimulation with interleukin-4 or interleukin-13. STAT6 regulates many M2-associated genes, such as arginase 1, CD206, resistin-like-α and chitinase-like protein 3 (also known as YM1).

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Box 1

Mechanism of action and clinical use of mammalian target of rapamycin (mTOR) inhibitors

The prototypic mTOR inhibitor rapamycin inhibits mTOR complex 1 (mTORC1) by associating with FK506-binding protein 12 (FKBP12), which then directly binds to mTORC12. mTORC1 recruits substrates through regulatory-associated protein of mTOR (RAPTOR) that are then further aligned to the catalytic cleft of mTOR. Modelling of the rapamycin-FKBP12 complex bound to mTOR suggests that rapamycin does not inhibit initial substrate recruitment, but blocks correct alignment to the catalytic cleft of some substrates2. This could explain why rapamycin is more effective in blocking the phosphorylation and activation of ribosomal protein S6 kinase 1 (S6K1) than eIF4E-binding protein 1 (4E-BP1). In addition, treatment of some cell types and mice with rapamycin can also variably inhibit mTORC2 through a currently unknown mechanism156.

Rapamycin was approved in 1999 by the Food and Drug Administration (FDA) for use in the prevention of kidney allograft rejection. Rapamycin and its derivative everolimus (RAD0001) are currently used as immunosuppressive therapies in solid organ transplantation157. Pro-inflammatory side effects and dyslipidaemia are frequently observed in these patients157. Rapamycin-eluting stents are used in patients with coronary artery lesions to prevent recurrence of stenosis after percutaneous coronary revascularization158. Clinical anticancer effects of mTORC1 inhibitors are mainly restricted to advanced renal carcinoma, advanced oestrogen receptor-positive or human epidermal growth factor receptor 2 (HER2)-negative breast carcinomas, giant cell astrocytoma and neuroendocrine pancreatic tumours159. The limited success of rapamycin in cancer therapy and the recognition that classical mTOR inhibitors do not inhibit some mTORC1 substrates and are largely ineffective against mTORC2 prompted the development of the ATP-competitive catalytic mTOR inhibitor Torin1 that inhibits mTORC1 and mTORC2. Novel catalytic mTORC1 and mTORC2 inhibitors, such as PP242 or AZD8055, have improved anticancer activity *in vitro* and *in vivo* and some candidates have entered clinical trials159. Their clinical efficacy remains to be determined.

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Box 2

Pro-inflammatory versus anti-inflammatory effects of mammalian target of rapamycin (mTOR)

There are seemingly contrasting roles of mTOR in individual cells or models, which mirror contradictory data from the phosphatidylinositol 3-kinase (PI3K) pathway160. These observations might be a consequence of different experimental conditions, such as the analysis of primary versus *in vitro* generated cells or cell lines. Alternatively, species-specific differences between mice and humans concerning the importance of the arginine and nitric oxide system, which is regulated by PI3K-mTOR might explain these results. Importantly, the analysis of the investigated cytokines also influences the researcher's perception. Inhibition of mTOR usually promotes interleukin-12 (IL-12) expression and blocks IL-10 expression, suggesting a pro-inflammatory (or immunostimulatory) role for mTOR inhibitors. However, tumour necrosis factor (TNF) and IL-6 are often blocked by rapamycin, leading to the conclusion that mTOR inhibitors are anti-inflammatory. The complexity of the pathway itself could also account for differential outputs. For example, inhibition of mTORC1 activates the PI3K-AKT pathway through a feedback loop via ribosomal protein S6 kinase 1 (S6K1). AKT acts upstream of mTORC1 and downstream of mTORC2, and individual AKT isoforms promote distinct macrophage polarization states and AKT phosphorylation status changes substrate specificity161. Tuberous sclerosis 1 (TSC1) and TSC2 have distinct and not completely overlapping functions that are not always mTOR dependent162. Constitutive activation of the mTOR network by *TSC1* or *TSC2* deletion may change manifold biological processes that differ from short-term activation that occurs after Toll-like receptor stimulation, for example. Deletion of *TSC1* or *TSC2* consistently reduces activation of mTORC2 and AKT (following serine 473 phosphorylation), which provide feedback to dampen forkhead box O1 (FOXO1)-induced innate immune activation. Incomplete inhibition of mTOR complex 1 (mTORC1) and mostly undefined inhibition of mTORC2 by classical mTOR inhibitors complicate the interpretation of results using inhibitors. Moreover, depending on the *in vivo* model used, the relative importance of the mTOR network for cell migration and chemotaxis versus cell-intrinsic immunomodulation will dictate the "inflammatory" outcome. These observations suggest that the PI3K-AKT-mTOR network is not a simple linear pathway that transmits a single signal when activated but integrates diverse intracellular and extracellular cues to regulate a diverse set of basic biological processes within the cell to adapt a given effector response to the environment.

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Key points

- The mammalian target of rapamycin (mTOR) is an evolutionary conserved serine/threonine kinase that is present in two complexes, mTORC1 and mTORC2. mTORC1 is the main energy and nutrient sensor of the cell: it senses the presence of amino acids, glucose, lipids and ATP to allow efficient activation of the network in response to growth factors, Toll-like receptor ligands and cytokines.
- Activation of the mTOR pathway usually promotes an anabolic response that induces the synthesis of nucleic acids, proteins and lipids. In addition, it stimulates glycolysis as well as mitochondrial respiration. Emerging data suggest that this metabolic reconfiguration is required for specific effector functions in myeloid cells.
- Translational control of gene expression in myeloid immune cells emerges as one way in which mTORC1 controls cellular processes such as migration, interferon and pro- or anti-inflammatory cytokine expression as well as metabolic reprogramming.
- Counterintuitively, inhibition of mTORC1 during TLR triggering generally promotes IL-12 production and inhibits IL-10 and type I interferon expression by dendritic cells (DCs) and augments their T cell stimulatory capacity. Inhibition of mTORC2 enhances a pro-inflammatory response and IL-12 production in DCs.
- Inhibition of mTORC1 in macrophages promotes autophagy, which is important for intracellular pathogen killing and clearance of ingested complex lipids such as LDL cholesterol.
- mTORC2 is especially important for cell polarity and chemotaxis in neutrophils and mast cells. mTORC2 controls the leading edge as well as tail retraction during chemotactic migration.
- Activation of mTORC1 in NK cells by IL-15 triggers a glycolytic response, which is important for their proliferation and acquisition of cytotoxicity.

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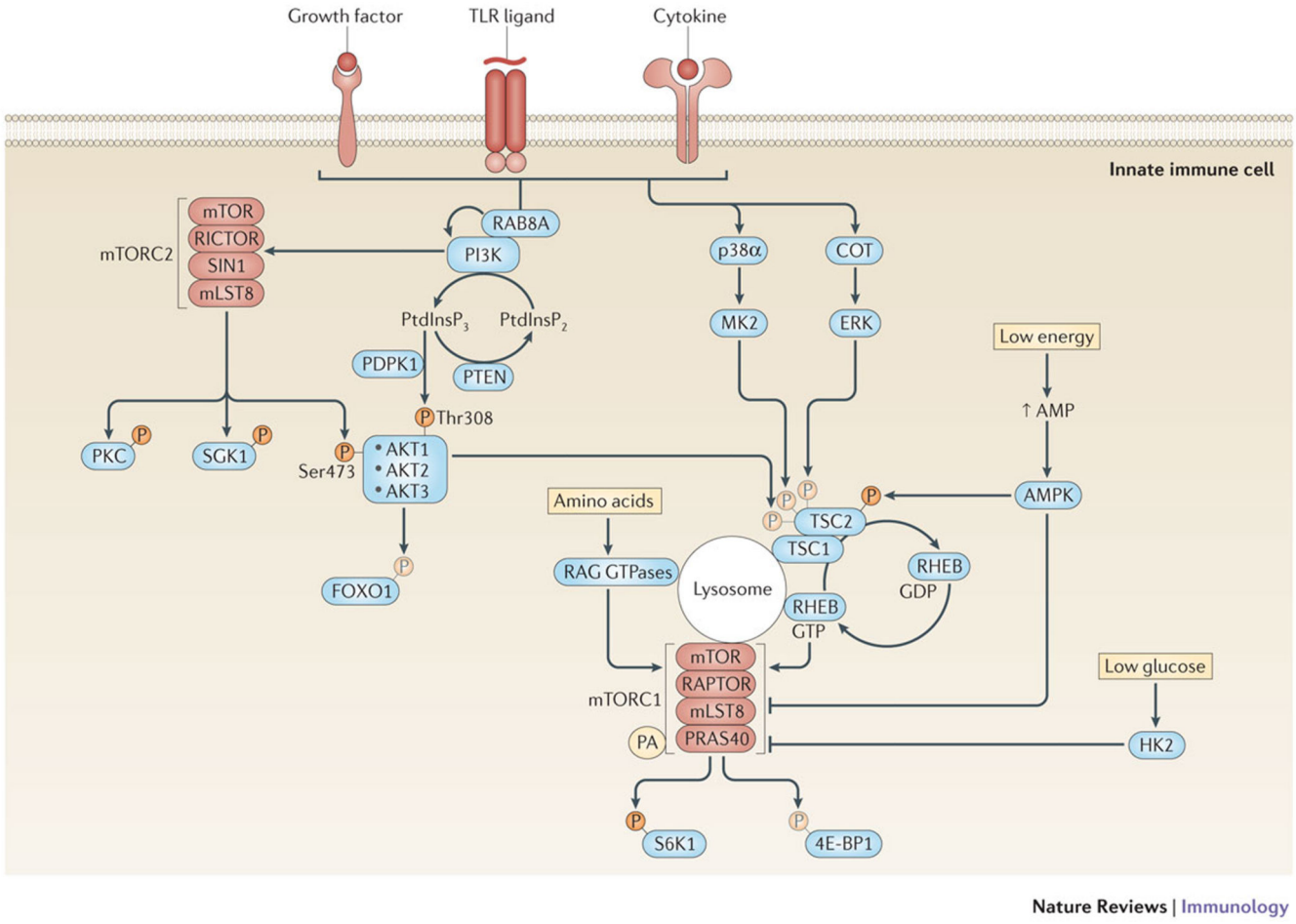

Figure 1. The mTOR pathway in innate immune cells.

In innate immune cells, the mammalian target of rapamycin (mTOR) network can be activated via multiple signals. Growth factors, Toll-like receptor (TLR) ligands or cytokines activate mTOR complex 1 (mTORC1) and mTORC2 through their cognate receptors. Receptor activation leads to the recruitment of class I phosphatidylinositol-3 kinases (PI3Ks) to the receptor complex by different adaptors including the small GTPase RAB8A in macrophages60. PI3Ks generate phosphatidylinositol-3,4,5-trisphosphate ($PtdInsP_3$) as a second messenger to recruit and activate the serine-threonine kinases AKT1, AKT2 and AKT3 via phosphorylation on threonine 308 by phosphoinositide-dependent protein kinase 1 (PDPK1). This process is negatively regulated by phosphatase and tensin homologue (PTEN), which dephosphorylates $PtdInsP_3$2. mTORC2 phosphorylates AKT on serine 473, which seems to be required for full activation and substrate specificity of AKT. In addition, mTORC2 phosphorylates protein kinase C (PKC) and serum and glucocorticoid-regulated kinase 1 (SGK1) to regulate important cellular processes such as cytoskeleton reorganization. Two main effectors of AKT are forkhead box O1 (FOXO1) and tuberous sclerosis 2 (TSC2)1. TSC2 forms a heterodimeric complex with TSC1 and inhibits mTORC1. Phosphorylation of TSC2 at threonine 1462 (Thr1462) by AKT inhibits its GTPase-activating protein (GAP) activity for the small GTPase RAS homologue enriched in brain (RHEB), which therefore remains in a GTP-bound active state and activates mTORC1

on the lysosome. In parallel to the PI3K-AKT pathway, the mitogen-activated protein kinases (MAPKs) p38α163,164 and COT also activate mTORC1 via MK2- and ERK-mediated phosphorylation of TSC2, respectively72,165. AMP-activated protein kinase (AMPK) detects low cellular energy by sensing the AMP levels and inhibits mTORC1 by phosphorylating regulatory-associated protein of mTOR (RAPTOR) at serine 792 and TSC2 at serine 1387 that promotes the inhibitory function of the TSC1-TSC2 complex. mTORC1 senses amino acid sufficiency on the lysosome via RAG GTPases and the ragulator complex, which seems to be a prerequisite for mTORC1 activation by growth factors. Moreover, low levels of glucose-6-phosphate inactivate mTORC1 by inducing binding to hexokinase 2 (HK2). Binding of the lipid metabolite phosphatidic acid (PA) to mTORC1 is also a prerequisite for mTORC1 activation. mTORC1-dependent phosphorylation of a variety of downstream effectors, such as eIF4E-binding protein 1 (4E-BP1), 4E-BP2 and ribosomal protein S6 kinase 1 (S6K1), promotes protein synthesis.

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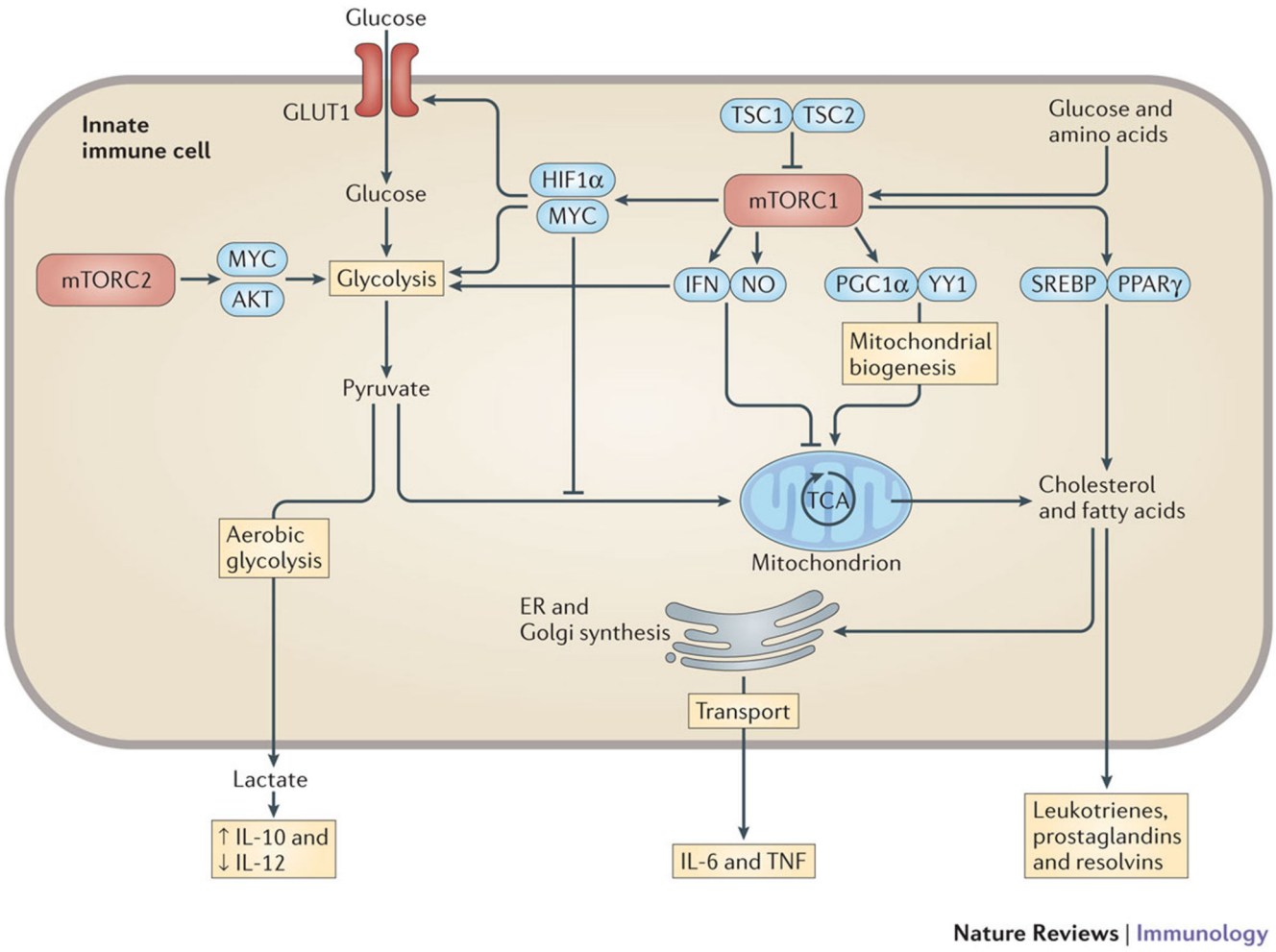

Figure 2. Metabolic control by mTOR in innate immunity.

The mammalian target of rapamycin complex 1 (mTORC1) promotes glycolysis through hypoxia-inducible factor 1α (HIF1α) and MYC, which enhances glucose import by increased expression and surface translocation of glucose transporter 1 (GLUT1) and increased expression of glycolytic genes. Activation of mTORC1 induces mitochondrial biogenesis through the transcription factors PPARγ coactivator 1α (PGC1α) and ying yang 1 (YY1), and promotes cholesterol and fatty acid synthesis from the tricarboxylic acid (TCA) cycle by sterol regulatory element-binding proteins (SREBPs) and peroxisome proliferator-activated receptor-γ (PPARγ). Fatty acids are further metabolized to lipid mediators such as leukotrienes, prostaglandins and resolvins. Cholesterol and fatty acids are also used as building blocks for endoplasmic reticulum (ER) and Golgi synthesis, which can promote the secretion of pro-inflammatory cytokines. This is especially important in lipopolysaccharide-activated dendritic cells, which promote ER and Golgi synthesis predominantly through AKT. mTORC1 can also have a negative effect on mitochondrial respiration by inducing the expression of interferon (IFN) and nitric oxide (NO), which subsequently promote aerobic glycolysis. mTORC2 promotes metabolic reprogramming by activating MYC and AKT, which enhance the expression of various glycolytic enzymes24.

Lactate, the end product of aerobic glycolysis, can directly reprogramme macrophages and dendritic cells and reduce the expression of interleukin-12 (IL-12), while enhancing the production of IL-10.

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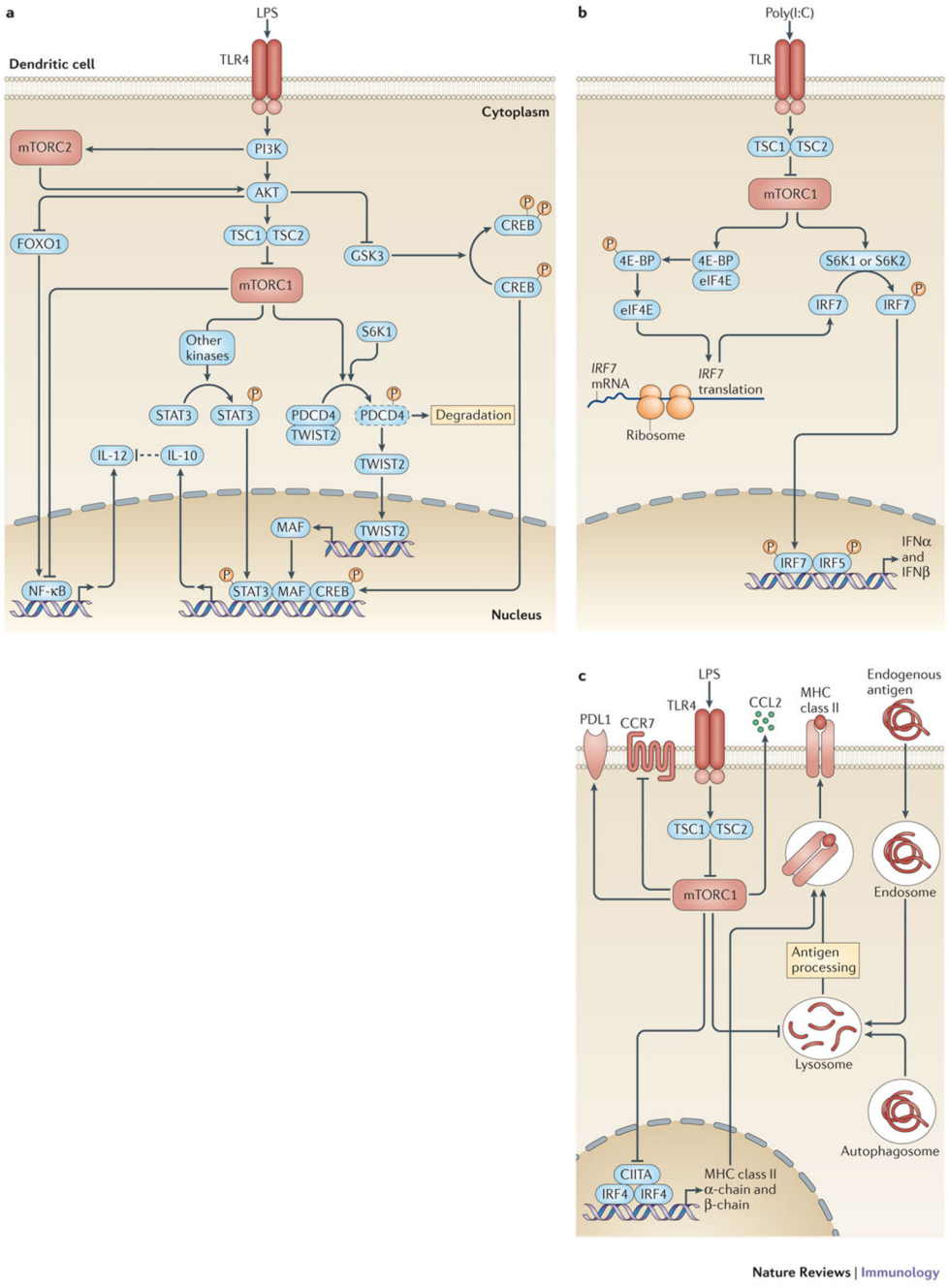

Figure 3. mTOR in dendritic cells (DCs) and its impact on T cell activation.

a| Toll-like receptor (TLR) stimulation regulates interleukin-10 (IL-10) and IL-12 production in a complex manner through mTOR complex 1 (mTORC1) and mTORC2. The AKT-mTORC1 network positively regulates IL-10 expression by at least three non-mutually exclusive mechanisms. mTORC1 induces the phosphorylation of signal transducer and activator of transcription 3 (STAT3) via currently unknown kinases. mTOR or ribosomal protein S6 kinase 1 (S6K1) phosphorylate programmed death cell protein 4 (PDCD4), which subsequently gets degraded by the proteasome. The transcription factor twist-related protein

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2 (TWIST2) is released from PDCD4 and translocates to the nucleus to induce the expression of MAF. In addition, AKT phosphorylates and inhibits glycogen synthase kinase 3 (GSK3), which is then unable to phosphorylate and inhibit the transcriptional activity of cAMP response element-binding protein (CREB). Hence, mTORC1 promotes the expression of IL-10 via STAT3, MAF and CREB. mTORC1 decreases the activation of nuclear factor-κB (NF-κB) and the expression of IL-12. In addition, mTORC2 negatively regulates IL-12 production by inducing an AKT-dependent inactivation of forkhead box O1 (FOXO1), which usually promotes NF-κB activation. **b|** The expression of type I interferon production by DCs is regulated by S6Ks and eIF4E-binding proteins (4E-BPs) downstream of mTORC1. Phosphorylation of 4E-BP1 and 4E-BP2 by mTORC1 releases the eukaryotic translation initiation factor 4E (eIF4E), which promotes the translation of IFN regulatory factor 7 (*IRF7*) mRNA. In addition, S6K1 and S6K2 phosphorylate IRF7 to activate IFNα and IFNβ production, which is supported by IRF5. **c|** Lipopolysaccharide (LPS) activates mTORC1 and inhibits the transcription of new MHC class II mRNAs mediated by IRF4 and class II coactivator (CIITA). mTORC1 activation also limits antigen processing by decreasing autophagy, which contributes to the presentation of endogenous and exogenous antigen loading on MHC class I and class II molecules. mTORC1 promotes the expression of programmed cell death ligand 1 (PDL1), which limits T cell activation. mTORC1 positively regulates the production of CC-chemokine ligand 2 (CCL2) and negatively regulates signalling by CC-chemokine receptor 7 (CCR7), thereby affecting the migration of DCs to tissues and secondary lymphoid organs.

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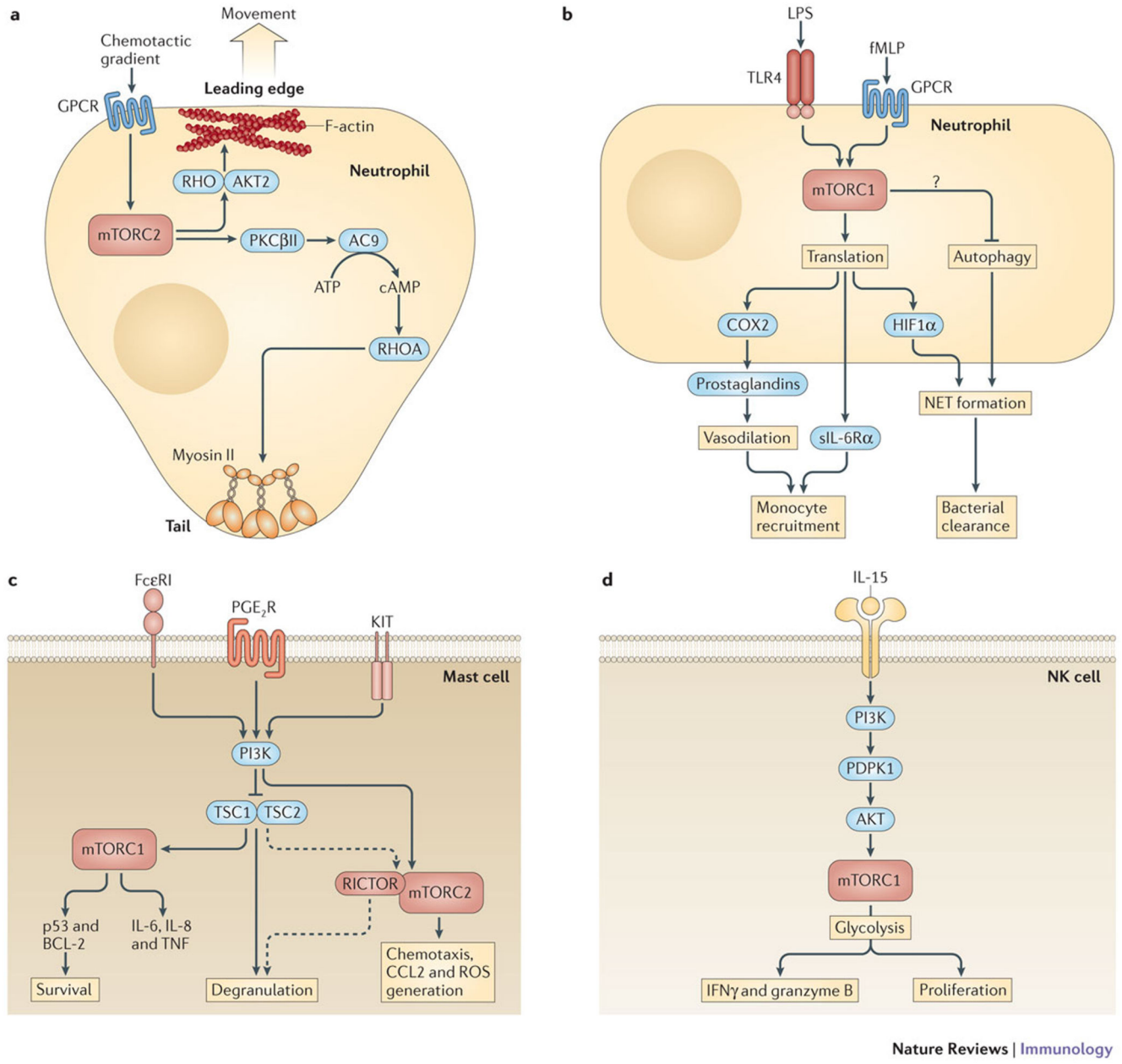

Figure 4. mTOR in neutrophils, mast cells and natural killer (NK) cells.

a| Chemotactic migration of neutrophils and mast cells is controlled by G-protein coupled receptor (GPCR)-mediated activation of mTOR complex 2 (mTORC2). mTORC2 activates RAS homologue (RHO) GTPases and AKT2 to regulate F-actin assembly at the leading edge. mTORC2 is also critical for tail retraction by phosphorylating protein kinase C βII (PKCβII). PKCβII then activates adenylyl cyclase 9 (AC9), which converts ATP into cAMP to activate RHOA. RHOA induces myosin II phosphorylation and tail retraction. **b |** mTORC1 controls the translation of preformed cyclooxygenase 2 (*COX2*) mRNA in LPS- or formyl-methionyl-leucyl-phenylalanine (fMLP)-activated neutrophils to induce the expression of prostaglandins that promote vasodilation. Soluble interleukin-6 receptor-α

(sIL-6Rα) is synthesized via mTORC1. sIL-6Rα translocates to endothelial cells and induces a switch from neutrophil to mononuclear leukocyte recruitment to initiate the resolution of inflammation. mTORC1 promotes LPS-mediated neutrophil extracellular trap (NET) formation by translational control of hypoxia-inducible factor 1α (HIF1α) expression. Activation of mTORC1 can also negatively influence fMLP-stimulated NET formation by inhibiting autophagy. **c |** Activation of the high affinity receptor for IgE (FcεRI), prostaglandin E_2 receptor (PGE_2R) or the KIT receptor for stem cell factor (SCF) results in rapid phosphatidylinositol 3-kinase (PI3K)-dependent activation of mTORC1 and mTORC2 in mast cells. mTORC1 is required for mast cell survival and the expression of IL-8 and tumour necrosis factor (TNF) following FcεRI stimulation. However, constitutive activation of mTORC1 by loss of tuberous sclerosis 1 (TSC1) is detrimental and increases apoptosis by enhancing the expression of p53, which reduces the expression of B cell lymphoma 2 (BCL-2). mTORC2 promotes PGE_2-mediated chemotaxis, CC-chemokine ligand 2 (CCL2) expression and reactive oxygen species (ROS) production. TSC1 promotes mast cell degranulation and histamine production independent of mTORC1. RICTOR contributes to FcεRI-induced degranulation independent of mTORC2. **d |** IL-15 activates mTORC1 through PI3K, phosphoinositide-dependent protein kinase 1 (PDPK1) and AKT in NK cells. mTORC1 promotes NK cell proliferation and the acquisition of cytotoxicity through the induction of interferon-γ (IFNγ) and granzyme B production. mTORC1-mediated cytotoxicity requires metabolic reprogramming and enhanced glycolysis.

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Table 1

Genetic deletion of mTOR network components in macrophages and their phenotypes

Target gene (protein encoded)	Genetic deletion	Number of M1 macrophages (relative to controls)	Number of M2 macrophages (relative to controls)	Polarization evidence	Biochemical features	*In vivo* phenotype	Refs.
Pten (PTEN)	LysM-Cre mediated	decreased	increased	*in vivo*	AKT enhanced; STAT6 and CEBPβ increased; polarization mediated by PI3K	Increased protection from ischaemia reperfusion injury and EAE	82,83
Inpp5d (SHIP)	Full gene knockout	no change	increased	*in vitro*	STAT6 increased; polarization mediated via PI3K	No obvious phenotype	81
Pdpk1 (PDPK1)	LysM-Cre mediated	increased	decreased	*in vitro; in vivo*	AKT reduced; enhanced migration via FOXO1 and CCR2	Enhanced insulin resistance	90
Prkaa1 (AMPKα1)	Full gene knockout	increased	no change	*in vitro*	mTORC1, AKT, PI3K and SOCS3 reduced	No obvious phenotype	91
Prkab1 (AMPKβ1)	Deficient bone marrow transplanted into wild-type mice	increased	decreased	*in vivo*	AKT reduced; JNK enhanced	Enhanced insulin resistance and inflammation	92
Akt1 (AKT1)	Full gene knockout	increased	decreased	*in vivo*	STAT6 no significant change	Increased sensitivity to LPS-induced shock and DSS-induced colitis	88
Akt2 (AKT2)	Full gene knockout	decreased	increased	*in vivo*	CEBPβ increased; STAT6 no significant change	Increased resistance to LPS-induced shock and DSS-induced colitis	88
Akt2 (AKT2)	Deficient bone marrow transplanted into LDLR- mice	decreased	increased	*in vivo*	Impaired migration	Reduced atherosclerosis	89
Tsc1 (hamartin or TSC1)	LysM-Cre mediated	increased	decreased	*in vitro, in vivo*	mTORC1 enhanced, AKT reduced (causing decreased M2 polarization); STAT6 no significant change	reduced M2 polarization in response to IL-4 and chitin	15
Tsc1	LysM-Cre mediated	increased	decreased	*in vitro; in vivo*	mTORC1 enhanced, AKT, reduced; enhanced M1 polarization due to RAS-ERK; reduced M2 polarization due to mTORC1-CEBPβ	Increased sensitivity to LPS; resistant to OVA-induced asthma	14
Tsc1	LysM-Cre mediated	increased	increased	*in vitro*	Impaired migration and T cell activation	Spontaneous lymphoproliferative disorder	86

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Target gene (protein encoded)	Genetic deletion	Number of M1 macrophages (relative to controls)	Number of M2 macrophages (relative to controls)	Polarization evidence	Biochemical features	*In vivo* phenotype	Refs.
Rptor (RAPTOR)	LysM-Cre mediated	decreased	increased	*in vivo*	mTORC1 reduced; AKT enhanced; reduced JNK and NF-κB	Increased protection against insulin resistance and inflammation	87
Prr5 (Rictor 1)	LysM-Cre mediated	increased	decreased	*in vitro*	AKT reduced; MAP and NF-κB no significant change	Increased susceptibility to LPS-induced shock	63

PTEN, phosphatase and tensin homologue; SHIP, SH2-domain containing inositol-5-phosphatase; PDPK1, phosphoinositide-dependent protein kinase-1; AMPK, AMP-activated protein kinase; TSC1, tuberous sclerosis 1; Raptor, regulatory associated protein of mTOR; Rictor, rapamycin-insensitive companion of mTOR; LysM, lysozyme 2; LDLR, low density lipoprotein receptor; STAT6, signal transducer and activator of transcription 6; Pi3K, phosphatidylinositol-3 kinases; Foxo1, forkhead box O1; CCR2, CC-chemokine receptor 2; SOCS3, suppressor of cytokine signaling 3; CEBPβ, CCAAT/enhancer-binding protein β; MAP, mitogen-activated kinase; EAE, experimental autoimmune encephalomyelitis; DSS, dextran sulfate sodium