Regulation of Immune Responses by mTOR

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Abstract

mTOR is an evolutionarily conserved serine/threonine kinase that plays a central role in integrating environmental cues in the form of growth factors, amino acids, and energy. In the study of the immune system, mTOR is emerging as a critical regulator of immune function because of its role in sensing and integrating cues from the immune microenvironment. With the greater appreciation of cellular metabolism as an important regulator of immune cell function, mTOR is proving to be a vital link between immune function and metabolism. In this review, we discuss the ability of mTOR to direct the adaptive immune response. Specifically, we focus on the role of mTOR in promoting differentiation, activation, and function in T cells, B cells, and antigen-presenting cells.

Keywords

CD4; CD8; Tregs; antigen-presenting cells; B cells; metabolism

INTRODUCTION

The mammalian target of rapamycin (mTOR) is now appreciated to be a central regulator of immune responses. Specifically, mTOR appears to function as a central node in a signaling cascade that directs the integration of diverse environmental inputs in the immune microenvironment. Furthermore, in light of the ability of mTOR activation to regulate metabolism, this kinase provides a critical link between metabolic demands and cellular function. mTOR plays a role in regulating diverse immune cells, including neutrophils, mast cells, natural killer cells, γδ T cells, macrophages, dendritic cells (DCs), T cells, and B cells (1–4). The focus of this review is on mTOR's ability to sense signals from the environment in order to direct the outcome of antigen recognition. We focus on the role of mTOR in T cell, B cell, and antigen-presenting cell (APC) differentiation, activation, and function (Figure 1).

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mTOR SIGNALING

A Kinase Named After a Drug

In an effort to identify potential new antibiotics, scientists from pharmaceutical companies bring back soil samples for lab tests. One compound identified in this fashion was rapamycin, which was extracted from *Streptomyces hygroscopicus* found in soil from Easter Island (the local name for the island is *Rapa nui*) (5). Rapamycin was found to inhibit the growth of yeast as well as to have immunosuppressive and antitumor properties (6). In yeast, rapamycin complexes with an immunophilin, FK-binding protein 12 (FKBP12) (7). The target of rapamycin (TOR) was subsequently found to be a 289-kDa serine/threonine kinase. Subsequent studies led to the identification of the mammalian TOR [mTOR, also known as FKBP-rapamycin-associated protein (FRAP) or rapamycin- and FKBP12-associated protein (RAFT)] (8).

mTOR is an evolutionarily conserved serine/threonine protein kinase that is a member of the phosphoinositide 3-kinase related kinase (PIKK) family (9, 10). It is constitutively expressed, and regulation of mTOR occurs predominantly post-translationally. Structurally, the N terminus of mTOR is characterized by a cluster of huntingtin, elongation factor 3, a subunit of protein phosphatase 2A, and TOR1 (HEAT) repeats that play a role in protein-protein interactions (Figure 2). Next, there is a FRAP, ATM, and TRRAP (FAT) domain, followed by the FKBP12 rapamycin–binding (FRB) domain. Adjacent to the FRB is the kinase domain responsible for mTOR's serine/threonine kinase activity and also the binding site for mTOR kinase–specific inhibitors. The C terminus consists of the FATC domain that is believed to play a role in maintaining structural integrity. In mammals, mTOR is encoded as a single gene product. Downstream, mTOR signaling proceeds via two distinct complexes: mTOR complex 1 (mTORC1) and mTORC2 (11). mTORC1 is composed of regulatory-associated protein of mTOR (RAPTOR), mammalian lethal with Sec13 protein 8 (mLST8), the proline-rich Akt substrate 40 kDa (PRAS40), and DEP domain–containing mTOR-interacting protein (DEPTOR) (11). mTORC2 also contains mLST8 and DEPTOR in addition to the scaffolding protein RAPTOR-independent companion of TOR (RICTOR), mSIN1 proteins, and the protein observed with RICTOR (PROTOR) (11). The genetic deletion of these components has provided important insight into the function of mTORC1 and mTORC2 signaling.

The assembly of mTOR with various adapter proteins to form mTORC1 and mTORC2 has functional consequences, as different adapter proteins confer substrate specificity to the mTOR kinase. There are pharmacologic consequences as well. Rapamycin and other rapalogs bind to FKBP12 and, by binding to the FRB site on mTOR, are believed to block the ability of RAPTOR to bind to mTOR, thus inhibiting mTORC1 (12). However, it has become clear that prolonged treatment with rapamycin for some tissues and cell types can lead to the inhibition of mTORC2 signaling as well (13). Although the mechanism is not precisely clear, it may involve the sequestration of mTOR, resulting in a lack of availability of this core component for mTORC2 assembly. Importantly, particularly for naive T cells, rapamycin can potently inhibit mTORC2 signaling at relatively modest doses without the necessity for prolonged exposure (14).

Upstream Regulation of mTOR Activity

mTOR is regulated by environmental cues in the form of nutrients, growth factors, energy, and stress. Although much is known about how these inputs regulate mTORC1 signaling, there are surprisingly few data connecting mTORC2 to these stimuli (Figure 3) (15–17). Immediately upstream of mTORC1 is the Ras homolog enriched in brain (RHEB). RHEB is a small GTPase that is a crucial regulator of mTORC1 signaling (18–20). RHEB is

controlled by the GTPase-activating protein (GAP) activity of a complex consisting of tuberous sclerosis complex 1 (TSC1) and TSC2. When this complex is phosphorylated by Akt or ERK1/2, its GAP activity is inhibited and RHEB is active, leading to the activation of mTORC1. For example, growth factors such as insulin or insulin-like growth factor lead to the activation of phosphatidylinositol 3-kinase (PI3K) that through the production of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) will activate 3-phosphoinositide-dependent protein kinase-1 (PDK1), which in turn will activate Akt, leading to subsequent mTORC1 activation.

Several immunologically relevant accessory molecules and cytokine/growth factor receptors promote the activation of mTOR. Notably, CD28 is a potent activator of PI3K activity and thus an important activator of mTORC1 in T cells (21, 22). Alternatively, the coinhibitor PD-1 ligand 1 inhibits mTOR activity by binding to PD-1 on the surface of T cells (23). IL-2 and IL-4 are also potent activators of mTORC1 activity via PI3K activation (24, 25). In CD8^{+} T cells, IL-12 and IFN-γ prolong the activation of mTOR upon stimulation (26). Likewise, the ability of IL-1 to promote Th17 development is due in part to its ability to stimulate mTORC1 (27). Finally, the adipokine leptin influences regulatory T cell (Treg) function through its ability to induce mTOR activation (28). In addition to growth factors, regulation of mTORC1 activity via TSC can be modulated through numerous other diverse extracellular cues. For example, AMP-activated kinase (AMPK) is turned on in the setting of a decreased ATP:AMP ratio, indicative of low intracellular energy stores (29, 30). AMPK in turn phosphorylates TSC. However, unlike Akt, which inactivates TSC, phosphorylation of TSC by AMPK promotes its ability to inhibit mTORC1. Likewise, phosphorylation of TSC by glycogen synthase kinase 3β (GSK3β) also inhibits mTOR (31). Wnt signaling inhibits GSK3β and thus promotes mTORC1 activity. Finally, the hypoxia-induced factor protein regulated in the development of DNA damage response 1 (REDD1) can inhibit mTOR by promoting the assembly and activation of TSC in the setting of low oxygen (32).

Although growth factor–induced activation of RHEB is essential for mTORC1 signaling, mTORC1 activation requires the presence of amino acids (33). Indeed, amino acids play an essential role in promoting the interaction between RHEB and mTORC1, thus enhancing mTORC1 activity. This is accomplished by the ability of amino acids to activate the Rag GTPases, which physically interact with RAPTOR and promote the localization of mTORC1 with RHEB on the surface of late endosomes and lysosomes (33). Furthermore, increased concentrations of amino acids or growth factors will lead to increased mTORC1 activity. Branch chain amino acids (BCAA) such as leucine are among the most potent activators of mTORC1. The immunologic relevance of this pathway is demonstrated by reports that Tregs can promote infectious tolerance in part by depleting BCAA, leading to mTOR inhibition and further Treg generation (34).

Downstream mTOR Signaling

mTORC1 and mTORC2 activity is routinely measured by the phosphorylation of their canonical substrates (Figure 4). For mTORC1, the phosphorylation of S6K1 and 4E-BP1 is a standard marker of mTORC1 activity (35). S6K1 plays a critical role in promoting translation of mRNA and is activated by mTORC1, whereas 4E-BP1 is an inhibitor of eIF-4E, a cap-binding protein, and its phosphorylation by mTOR leads to its inactivation (36). For example, the translations of cyclin D3 and of VEGF are tightly regulated by mTORC1-controlled 4E-BP1 (37). Additionally, hypoxia inducible factor (HIF) is controlled by mTORC1 in a 4E-BP1-dependent manner (15). Data are emerging that HIF and mTORC1-activated sterol regulatory element–binding protein 1 (SREBP1) play critical roles in regulating glucose and lipid metabolism (38). Specifically, it has recently been shown that SREBP1 promotes the upregulation of genetic programs involved in lipid biosynthesis and the pentose phosphate pathway. In addition to its role in metabolism,

mTORC1 also plays a role in mitochondrial biosynthesis and autophagy. Indeed, mTORC1 inhibition leads to an increase in autophagy, whereas mitochondrial biosynthesis is enhanced in the setting of increased mTORC1 activation (39, 40). In the latter case, mTORC1 was shown to promote the transcriptional activity of PPARγ coactivator 1 (PGC1a), a factor that is necessary for mitochondrial biogenesis and oxidative metabolism.

Investigators have shown that, in general, mTORC2 plays various roles in cell survival, metabolism, proliferation, and cytoskeleton organization (41). Often, mTORC2 activation is determined by measuring the phosphorylation of Akt at serine 473. This mTORC2-dependent phosphorylation (and activation) of Akt is distinct from PDK1-dependent phosphorylation of Akt at threonine 308 that is upstream of mTORC1 activation. Indeed, our group (looking at T cells) and other groups (examining different tissues) have shown that upstream Akt activation as measured by threonine 308 phosphorylation is robust even in the absence of mTORC2 activity (14, 42). Of note, some studies (including that of the Boothby lab working with T cells) have noted slight inhibition of Akt phosphorylation at threonine 308 in the setting of the deletion of RICTOR (43). In this regard, the process of specifically defining mTORC2-dependent and -independent functions of Akt activity is evolving. However, several studies have clearly demonstrated that Akt's ability to regulate Forkhead box protein O1 (FOXO1) and FOXO3 is mTORC2 dependent (42). Akt activation leads to the phosphorylation of these transcription factors, which results in their sequestration in the cytoplasm, thus inhibiting their activity.

A second downstream target of mTORC2 is the serum glucocorticoid-regulated kinase 1 (SGK1). SGK1 is similar to Akt and S6K1 in that it is an AGC (cAMP-dependent, cGMP-dependent, and protein kinase C) kinase (44). Its activity has been associated with the regulation of the membrane expression of sodium channels in renal cells (45). Like Akt, SGK1 also phosphorylates and inhibits FOXO family members (46). Finally, protein kinase C alpha (PKCα) is phosphorylated and activated by mTORC2, leading to actin reorganization (47). The ability of mTORC2 to regulate PKC activation has important immunologic consequences, given that mTORC2 can activate PKCθ to promote Th2 differentiation, as recently demonstrated (43).

THE REGULATION OF T CELL DIFFERENTIATION, ACTIVATION, AND FUNCTION BY mTOR

mTOR, Proliferation, and Anergy

Rapamycin proved to be a poor antibiotic but was found to have potent immunosuppressive properties. Similar to FK506, rapamycin's action depends on its ability to bind to the immunophilin FKBP12 (48). However, unlike FK506 (and cyclosporin A, which binds to the immunophilin cyclophilin), rapamycin does not inhibit calcineurin and hence does not inhibit T cell receptor (TCR)-induced NF-AT activation. As a result, rapamycin does not acutely block the expression of many TCR-induced genes (Figure 5). This includes the genes for cytokines such as IL-2, IFN-γ, and TNF-α, as well as the genes for NF-AT-dependent inhibitory proteins such as cbl-b, GRAIL, and DGK-α and the transcription factors Egr-2 and Egr-3 (49). Initially, investigators believed that the immunosuppressive effects of rapamycin were due to its ability to inhibit T cell proliferation. Indeed, mTOR activity leads to the degradation of the cell cycle inhibitor p27, as well as to the increased expression of cyclin D3 (15).

Initial models proposed that T cell anergy was the result of TCR engagement in the absence of proliferation and that IL-2-induced proliferation could reverse anergy (50). Because rapamycin inhibited proliferation, it was hypothesized that mTOR's immunosuppressive properties were partially due to its ability to promote anergy. Indeed, rapamycin can

promote T cell anergy even in the presence of costimulation (22, 51–53). Interestingly, concomitant inhibition of calcineurin by cyclosporin A prevented the induction of rapamycin-induced anergy (52). Such findings highlight the fact that although calcineurin inhibitors are potent suppressors of T cell activation, they also inhibit the induction of T cell tolerance. However, subsequent studies demonstrated a disassociation between the ability of rapamycin to block cell cycle progression and anergy (51). It was shown that cell cycle arrest in G1 in the absence of mTOR inhibition did not induce anergy. Likewise, in other experiments, investigators found that inducing T cell proliferation in the presence of rapamycin was unable to overcome anergy (22). The interpretation of these observations was that rapamycin promoted anergy not by inhibiting proliferation, but rather by inhibiting mTOR. Such studies provided the initial insight in terms of the ability of mTOR to regulate T cell fate.

Linking T Cell Function and Metabolism

In yeast and mammalian cells, TOR serves to link nutritional availability with cellular functions. When oxygen, energy, amino acids, and growth factors are readily available, mTOR is active and coordinately promotes cellular processes that facilitate growth such as translation, lipid synthesis, and mitochondrial biogenesis (10, 15). Alternatively, when there is a dearth of nutrients, mTOR is inhibited, leading to a decrease in biosynthesis and increase in autophagy. Interestingly, a regulatory feedback loop exists whereby the amino acids generated from autophagy can ultimately lead to increased mTOR activation and subsequent inhibition of autophagy (39).

In the presence of oxygen, most differentiated cells will employ the TCA cycle and mitochondrial respiration because these pathways are the most efficient means to generate energy in the form of ATP (54). However, for lymphocytes (and cancer cells), such is not the case. Instead, lymphocytes utilize oxidative glycolysis, the so-called Warburg effect, to generate ATP (55). Lymphocyte activation and cancer growth demand markedly increased protein, nucleotide, and lipid biosynthesis. Researchers have proposed that, although glycolysis is less efficient at generating ATP, the by-products of this metabolic pathway provide the substrates necessary for biosynthesis (56). mTOR's central role in regulating metabolic programs makes it an important link between metabolism and immune function.

In the resting state, lymphocytes are catabolic, employing autophagy to derive molecules required for protein synthesis and energy. Interestingly, the quiescent state in lymphocytes is actively maintained by the expression of numerous regulatory transcription factors. For example, Krüppel-like factor 2 (KLF2) and the FOXOs, both of which are inhibited by mTORC2 activation, promote the expression of inhibitory proteins (57–59). Upon activation, T cells become anabolic and switch to glycolysis to derive energy and create biosynthetic substrates. That is, the transition from a resting T cell to an active T cell requires the upregulation of the metabolic machinery involved in nutrient uptake and glycolysis. This switch is intimately linked to immunologically derived activation signals. For example, CD28-induced PI3K activation leads to Akt activation, which in turn promotes the surface expression of glucose transporters (60–62). Furthermore, activation of mTORC1, acting via HIF, promotes the expression of proteins involved in glycolysis and glucose uptake, whereas mTORC1-dependent activation of SREBP leads to the upregulation of proteins critical for the pentose phosphate pathway as well as fatty acid and sterol synthesis (38). The requirement for the metabolic pathways in lymphocyte function is demonstrated by the fact that blocking these pathways can inhibit T cell activation. For example, the drugs metformin and AICAR, which mimic energy depletion and activate AMPK, an inhibitor of mTORC1, inhibit IL-2 production and can promote T cell anergy (63–65). Likewise, the glucose analog and mTORC1 inhibitor 2-deoxyglucose (2-DG) also blocks T cell cytokine production and can promote anergy (65). Recall that, despite adequate growth factor

stimulation, mTORC1 activity requires the presence of amino acids. As such, the leucine antagonist N-acetyl-leucine-amide can inhibit mTORC1 activity even in the presence of strong mTORC1 activators and thus can lead to the inhibition of T cell function (66).

Thus, the specialized metabolic demands of T cell activation represent a node at which T cell function can be regulated. That is, the inhibition of immune cell metabolism can lead to the inhibition of immune cell function. In this regard, the work of Mellor, Munn, and colleagues (67, 68) has shown that T cell activation can be controlled by the ability of APCs to modulate tryptophan availability. Currently, it is not clear whether this form of regulation intersects with mTOR. However, the depletion of BCAA availability by Tregs regulates T cell function (34). BCAA depletion leads to the inhibition of mTOR, which in turn promotes infectious tolerance via the generation of more Tregs. Finally, anergic T cells have provided insight in terms of how metabolism can regulate T cell function. Anergic T cells are hyporesponsive when rechallenged under otherwise fully activating conditions. Such cells demonstrate decreased mTOR activation upon rechallenge, leading to decreased glycolysis and amino acid transporter expression (65). These findings suggest that the inability to upregulate the metabolic machinery sufficiently upon rechallenge is one mechanism that maintains the anergic phenotype.

mTOR AS SIGNAL 2

The two-signal model provides the foundation for our understanding of adaptive immune responses. Based on the ideas of Lafferty & Cunningham (168) and Bretscher & Cohn (169), this model posits that the outcome of antigen recognition is determined by the presence of a second, APC-derived costimulatory signal. In the presence of costimulation, antigen recognition leads to activation, whereas in the absence of costimulation, recognition leads to tolerance (170). At the molecular level, costimulation consists of a plethora of both activating and inhibitory accessory signals (171). Likewise, a greater appreciation for the plasticity of the CD4+ T cell effector response highlights the diversity of outcomes that may occur upon antigen stimulation (69). We propose that by integrating costimulatory, cytokine, and metabolic environmental cues, mTOR acts as Signal 2, dictating the outcome of antigen recognition. TCR recognition (Signal 1) in the absence of mTOR activity leads to anergy or Treg generation. Alternatively, Signal 1 in the setting of various skewing conditions leads to the selective mTORC1- and mTORC2-promoted effector differentiation.

mTOR Regulates CD4+ T Effector Cell Differentiation

It has become increasingly clear that there is great plasticity among CD4+ T cells with regard to their ability to differentiate into unique effector subsets (69). Interestingly, in spite of its exquisite specificity, TCR recognition of antigen provides little guidance in terms of effector lineage commitment. Rather, the immune microenvironment plays a critical role in directing the outcome of antigen recognition. In vitro, naive CD4+ T cells are readily skewed to specific effector populations through the use of high concentrations of cytokines combined with antibodies to antagonizing cytokines. In vivo, however, T cells encounter more subtle concentrations of cytokines, often with opposing effects. Likewise, although lineage-specific transcription factors such as T-bet, RORγt, and GATA-3 are critical for generating Th1, Th17, and Th2 cells, respectively, initial activation of naive T cells can result in the simultaneous expression of these transcription factors (70). Thus, T cells must integrate diverse and sometimes opposing signals to derive instruction for effector lineage commitment. Given that (*a*) mTOR has evolved to sense and integrate diverse signals from the environment and (*b*) mTOR plays a critical role in regulating metabolism, which is essential for T cell activation, researchers proposed that mTOR activity might play a central

role in integrating cues from the immune microenvironment to instruct helper cell differentiation.

To address this hypothesis, mice were generated in which mTOR was selectively deleted in T cells by crossing CD4-cre mice with floxed mTOR mice (71). For the most part, these mice had normal complements of T cell subsets in the periphery. Importantly, because mTOR deletion occurs at the double-positive stage, no real conclusions could be made concerning the role of mTOR during thymocyte development. Because full mTOR deletion is embryonic lethal, hypomorphic mTOR mice have also been generated (72). These mice have decreased overall body mass and demonstrate decreased mTOR activity in a diversity of cells. Interestingly, although the total number of thymocytes was decreased, there were no overt defects in T cell development in these particular mice. Thus, the role of mTOR in regulating thymocyte development awaits more incisive models to address this question.

Functionally, TCR-induced signaling was intact in mTOR-deficient T cells, as IL-2 production upon initial stimulation was normal (71). Investigators also found that mTOR was not required for CD4^{+} T cell proliferation, although the mTOR-deficient T cells proliferated more slowly than wild-type cells. Strikingly, the mTOR-deficient CD4^{+} T cells failed to differentiate into Th1, Th17, or Th2 effector cells in vitro or in vivo under strongly polarizing conditions. Instead, stimulation of naive CD4^{+} mTOR-deficient T cells led to the generation of Foxp3^{+} T cells even under normally activating conditions. Mechanistically, the inability to become CD4^{+} effector cells was associated with a decrease in STAT4, STAT3, and STAT6 activation in response to the skewing cytokines IL-12, IL-6, and IL-4, respectively. This decrease in STAT activation was associated with decreased expression of the lineage-specific transcription factors T-bet, RORγt, and GATA-3. Interestingly, for GATA-3, recent studies demonstrate the ability of mTOR to regulate expression of this lineage-specific transcription factor at the level of translation (73). Consistent with their propensity to become Foxp3^{+} T cells, the mTOR-deficient T cells demonstrated hyperactive SMAD3 in the absence of transforming growth factor-β (TGF-β).

mTOR Inhibition Promotes CD4^{+}Foxp3^{+} Regulatory T Cells

These genetic studies are consistent with a series of previous studies demonstrating the ability of rapamycin to promote the generation of Foxp3^{+} Tregs (74–76). As previously described, when Th1 T cells are activated in the presence of rapamycin, they are rendered anergic (52). However, researchers observed that, when they employed freshly isolated primary T cells, activation in the presence of rapamycin led to an increase in Foxp3^{+} Tregs (74), the result of both the de novo generation of such cells and the selective expansion of Foxp3^{+} T cells (77). Studies have demonstrated that Foxp3^{+} T cells display a selective advantage to proliferate in the absence of mTOR activation (78, 79). In the presence of rapamycin, Tregs are more resistant to apoptosis (79). This resistance to mTOR inhibition appears to be mediated by the upregulation of Pim-2 kinase (80). This serine/threonine kinase has similar signaling attributes to Akt with regard to its ability to control activation, growth, and survival. Unlike Akt, however, the activation of Pim-2 kinase is mTOR independent (81). Upon mTOR inhibition, Pim-2 kinase expression increases in part due to Foxp3-induced transcription. Consistent with this finding, Tregs have been noted to have less mTOR activation upon stimulation, partly because of an increase in the PI3K inhibitor phosphatase and tensin homolog (PTEN) (78). As such, IL-2 stimulation results in decreased mTOR activation but, interestingly, enhanced STAT5 phosphorylation. These findings suggest that Tregs employ alternative pathways for proliferation and function. The fact that Tregs do not require mTOR activity is consistent from a metabolic perspective as well (21). Unlike T effector cells, the metabolic demands of Tregs are more modest.

Interestingly, one study reported that Foxp3^{+} T cells from humans display increased mTOR activity when compared with effector cells (28). In this study, transient inhibition of mTOR with rapamycin led to increased proliferation of the Tregs in vitro. However, addition of leptin to rapamycin-expanding Tregs inhibited their proliferation, thereby promoting Treg anergy. This correlated with enhanced mTOR activity in the leptin-treated cells. On the basis of these findings, the authors suggest that leptin keeps Treg proliferation in check by promoting mTOR activation, whereas loss of mTOR activation by rapamycin treatment enables Treg proliferation.

T cells lacking mTOR display constitutively phosphorylated SMAD3, indicative of TGF-β activation (71). This is not due to an increase in the expression of TGF-β but rather to the T cells' increased responsiveness to the baseline levels of TGF-β present in the cultures. In the absence of exogenous TGF-β, neutralizing antibodies to TGF-β lead to a decrease in Tregs, suggesting that TGF-β signaling is contributing to the generation of such cells in the absence of mTOR. These findings highlight the ability of mTOR to integrate external cues to regulate T cell responses. That is, although baseline levels of TGF-β found under normally activating conditions do not promote the generation of Tregs, in the absence of mTOR, T cells are hypersensitive to these relatively low concentrations of TGF-β. Alternatively, high concentrations of TGF-β can dominate and promote Treg generation, even in the setting of robust mTOR activity. This model is supported by recent findings demonstrating that the ability of rapamycin to promote Tregs is in part TGF-β dependent (82). In addition to regulating TGF-β sensitivity, the inhibition of mTOR with rapamycin also induces epigenetic changes at the Foxp3 promoter. Specifically, treatment of T cells with rapamycin leads to histone H3K4me2 and 3 methylation (which promotes transcription) at the Foxp3 promoter (83). Additionally, the rapalog everolimus enhances the generation and stability of TGF-β-induced Foxp3^{+} cells by reducing the activation of DNA methyl transferase 1 (DNMT1) (84). DNMT1-induced promoter methylation of the Foxp3 promoter inhibits expression of Foxp3 in effector cells.

Although inhibition of mTOR promotes Treg generation, activation of the Akt-mTOR axis by the forced expression of a constitutively active Akt blocks the generation of Foxp3^{+} cells in the thymus (85). Inasmuch as Akt lies both upstream of mTORC1 and downstream of mTORC2, the ability of constitutively active Akt to block Treg generation may be multifactorial. Along these lines, a robust link between mTORC2 inhibition and the expression of Foxp3 has been revealed. Both FOXO1 and FOXO3a promote Foxp3 transcription (86). mTORC2 activates both Akt and SGK1, which in turn phosphorylate and inhibit the activation of these two transcription factors. Thus, inhibition of mTORC2 leads to FOXO1 and FOXO3a activation that in turn promotes the expression of Foxp3.

mTORC1 and mTORC2 Selectively Regulate CD4^{+} T Effector Cell Development

Observations employing mTOR null T cells suggested a central role for mTOR in facilitating T helper cell differentiation. Furthermore, such studies implied that the default pathway for antigen recognition in the absence of mTOR activation is down a Treg pathway. In an effort to further dissect the signaling pathways downstream of mTOR that are responsible for regulating T helper cell differentiation, our group selectively inhibited mTORC1 activity by deleting RHEB in T cells (14). Like the mTOR null T cells, the RHEB^{−/−} T cells failed to differentiate into Th1 or Th17 cells under appropriate skewing conditions. Surprisingly, the RHEB^{−/−} T cells still maintained their ability to become Th2 cells. Consistent with this phenotype, these mice were relatively resistant to the development of experimental autoimmune encephalomyelitis (EAE). Interestingly, instead of developing an ascending paralysis in response to myelin oligodendrocyte glycoprotein (MOG) peptide, the RHEB^{−/−} T cell mice developed ataxia characterized by immunologic infiltrate of the cerebellum (87). This syndrome, known as nonclassical EAE, had previously been observed

in mice in which MOG-specific IFN-γ null T cells were employed. In the absence of mTORC1 activity, instead of developing into Th1 and Th17 T cells in response to MOG peptide, the RHEB$^{-/-}$ T cell mice developed into Th2-type MOG-specific T cells. In other words, the environmental cues that would have normally led to Th1 and Th17 development, in the absence of mTORC1 led to an antigen-specific Th2 response.

Consistent with their ability to differentiate into Th2 cells, STAT6 signaling in response to IL-4 remained intact in RHEB-deficient T cells (14). However, RHEB$^{-/-}$ T cells exhibited decreased STAT4 and STAT3 signaling in response to IL-12 and IL-6, much like the mTOR null T cells. The ability of mTOR to regulate STAT activation has been described, but the differential contribution of mTORC1 and mTORC2 to regulating the STATs was previously unappreciated. Some reports have suggested a direct interaction between mTOR and the STATs (88–90). One hypothesis proposed that, in the RHEB$^{-/-}$ T cells, the decrease in STAT phosphorylation was due to increased activity of the suppressor of cytokine signaling 3 (SOCS3). SOCS3 inhibits STAT6 and STAT3 and mitigates Th1 and Th17 differentiation, and indeed, we found an increase in the levels of SOCS3 in RHEB$^{-/-}$ T cells (91, 92). Concomitant with decreased responsiveness to IL-12 and IL-6 in the RHEB$^{-/-}$T cells, there was a decrease in the Th1- and Th17-specific transcription factors T-bet and RORγt. Thus, a model has emerged in which mTORC1 activation promotes Th1 and Th17 differentiation by enhancing STAT activation in response to cytokine stimulation, leading to the upregulation of lineage-specific transcription factors. We hypothesize, however, that this pathway only partially represents the mechanism by which mTORC1 integrates signals to promote Th1 and Th17 differentiation. For example, the ability of mTORC1 to regulate translation as well as various metabolic pathways will most likely prove to be involved in an overall program of differentiation controlled by mTOR.

To dissect the role of mTORC2 signaling in T helper cell differentiation, Lee et al. (43) as well as our own group (14) selectively deleted RICTOR in T cells. Observations from both groups revealed that, in the absence of mTORC2 signaling, CD4$^+$ T cells fail to differentiate into Th2 cells under appropriate in vitro and in vivo polarizing conditions. In the Lee paper, the inability to generate Th2 cells in the RICTOR null T cells could be overcome by the expression of PKCγ. In our model, the lack of Th2 differentiation was attributed in part to a decrease in IL-4-induced STAT6 activation. This was associated with an increase in SOCS5.

The selective deletion of either mTORC1 or mTORC2 signaling did not result in the enhanced generation of Foxp3$^+$ Tregs. Such findings suggest that inhibition of both pathways is required for enhanced generation of Tregs under normally activating conditions. In this regard, simultaneous inhibition of mTORC1 and mTORC2 by employing a kinase inhibitor results in the potent generation of Foxp3$^+$ Tregs (14). However, numerous reports demonstrate the ability of rapamycin (which initially was described as a specific inhibitor only of mTORC1) to promote the generation of Tregs (74–76, 78, 93). Under prolonged culture conditions, rapamycin may also inhibit mTORC2. We have found that in T cells (particularly naive T cells) mTORC2 is exquisitely sensitive to inhibition with rapamycin (14). Picomolar concentrations of rapamycin that do not inhibit mTORC2 and thus do not promote Treg generation in wild-type T cells can generate Foxp3$^+$ T cells in RICTOR null T cells. That is, selective pharmacologic inhibition of mTORC1 with rapamycin can promote the generation of Tregs in cells in which mTORC2 has been genetically inhibited. Alternatively, complete inhibition of mTORC1 and mTORC2 is not required for the generation of Tregs. For example, TGF-β promotes the generation of Foxp3$^+$ Tregs even under conditions in which both mTORC1 and mTORC2 are active (94, 95). Likewise, the selective inhibition of mTORC1 can facilitate the generation of Tregs in cultures that contain concentrations of TGF-β that are otherwise insufficient to enhance Treg generation in the absence of rapamycin (82). Additionally, under certain culture conditions in vitro

activation of T cells in the presence of rapamycin can promote the generation of Th2 cells (96). Presumably, the selective inhibition of mTORC1 facilitates Th2 differentiation in these studies. Other studies have observed that culturing human T cells in the presence of Th1-skewing cytokines and rapamycin yields T cells resistant to apoptosis that are potent mediators of xenogeneic graft-versus-host disease (97).

In summary, a model has emerged on the basis of the genetic studies in which mTORC1 activation facilitates the generation of Th1 and Th17 cells, whereas mTORC2 activation promotes the generation of Th2 cells (Figure 6). The absence of both mTORC1 and mTORC2 activity leads to the generation of Tregs. Consistent with these models are several studies employing pharmacologic mTOR inhibitors that inhibit T helper cell differentiation and promote the generation of Foxp3$^+$ T cells. However, dose, timing, and culture conditions clearly can alter the outcome of rapamycin exposure on T cells in vitro.

Regulation of CD8$^+$ T Cells by mTOR

Quiescence in naive CD8$^+$ T cells is actively maintained by transcription factors such as Schlafen-2 (Slfn2), KLF2, KL4, and E74-like factor 4 (ELF4) (98). Upon TCR engagement, the expression of these molecules is inhibited. Recently, investigators demonstrated that mTOR plays a role in reversing the quiescent state by inhibiting ELF4 and KLF4 expression (99).

Antigen recognition leads to a marked increase in proliferation and subsequent increase in the frequency of antigen-specific cells (100). As with CD4$^+$ T cells, the activated CD8$^+$ cells switch from catabolism to anabolism and oxidative glycolysis (101). Thus, from a metabolic perspective, one might expect that mTOR activity is linked to the generation of CD8$^+$ effector cells. Indeed, we have found that CD8$^+$ T cells lacking mTORC1 signaling fail to become effector cells (J.D. Powell & K.N. Pollizzi, unpublished observations). Alternatively, by genetically deleting the mTORC1 inhibitor TSC2, we have been able to generate CD8$^+$ T cells with hyperactive mTORC1 activity. Such cells demonstrate enhanced effector generation. Along these lines, IFN-γ and IL-12 lead to prolonged mTORC1-dependent T-bet expression in CD8$^+$ T cells (26). Also, consistent with the fact that CD8$^+$ effector generation requires increased protein synthesis is the observation that antigen recognition in CD8$^+$ T cells leads to mTOR (and MAP-kinase signaling)-induced ribosomal S6 phosphorylation (102). These observations link TCR engagement and CD8$^+$ effector generation through mTOR. However, it remains to be determined which TCR-induced proteins are specifically dependent on mTORC1.

Following the initial expansion of effectors, there is a contraction phase, leading to the development of a pool of memory cells (103). Such cells express the IL-7 receptor (CD127) and the IL-2 receptor beta chain (CD122), reflecting the role of IL-7 and IL-15 in promoting the generation of memory cells. In addition, the development of memory is associated with the reexpression of CD62L and the upregulation of the chemokine receptor CCR7, both of which regulate trafficking to the lymph nodes. At the transcriptional level, the switch from effector to memory cells is facilitated by a transition from T-bet-mediated gene expression to eomesodermin-mediated gene expression (104, 105). Also, this contraction phase and transition to a memory phenotype are associated with a metabolic switch back from anabolism to catabolism. Thus, from a bioenergetics perspective, CD8$^+$ effector cells resemble CD4$^+$ effector subsets, and CD8$^+$ memory cells resemble Foxp3$^+$ Tregs.

Inasmuch as mTOR promotes effector generation, several groups have addressed whether inhibition of mTOR can actually promote the effector-to-memory transition. By examining CD8$^+$ T cell responses during lymphocytic choriomeningitis virus (LCMV) infection, Araki et al. (106) demonstrated the ability of low-dose rapamycin to promote the generation of

memory T cells. Rapamycin administered during the initial expansion phase as well as during the contraction phase led to increased memory cell generation. This phenomenon appeared to be both T cell intrinsic and dependent upon mTORC1 signaling. Targeting RAPTOR in the antigen-specific T cells increased memory responses, similar to rapamycin treatment. Furthermore, memory cells generated in the presence of rapamycin appeared to be more robust in terms of their recall response in the LCMV model of infection. In a similar system, culturing LCMV-specific T cells with rapamycin and then adoptively transferring them in vivo led to a marked increase in long-lived memory cells (107). Metabolically, the rapamycin-treated cells demonstrated an increase in oxidative phosphorylation when compared with untreated T cells.

Pearce et al. (108) approached the issue of how metabolism regulates memory development using a different genetic model in which TNF receptor–associated factor 6 (TRAF6) was specifically deleted in T cells. $CD8^+$ effector generation in these mice was unaffected; however, the effector-to-memory transition was markedly impaired. Metabolically, this failed transition was associated with an inability to switch to catabolism relating to fatty acid oxidation. Specifically, AMPK activity was markedly decreased in the TRAF6-deficient T cells upon growth factor withdrawal. Activating AMPK with metformin, which in turn also inhibits mTOR activation, restored the generation of memory cells. Additionally, treating the mice with rapamycin also enhanced memory cell generation in this model.

Although mTOR can clearly regulate the metabolic programs induced in effector and memory cells, as is the case for $CD4^+$ T cells, mTOR can also direct expression of transcriptional programs that define immune function. Rao and colleagues (26) were able to demonstrate that IL-12 stimulation led to prolonged costimulatory molecule-induced mTOR activation that in turn led to prolonged T-bet expression. Blocking mTOR with rapamycin promoted eomesodermin-associated memory cells even in the presence of high concentrations of IL-12. As in the previously described models, the rapamycin-promoted memory cells were superior effectors upon rechallenge and displayed robust antitumor immunity. Having demonstrated a role for mTOR inhibition in promoting antigen-induced memory formation, this group has also addressed the role of mTOR in regulating the clonal expansion and memory generation that occurs via homeostatic proliferation (109). In this model, IL-7-induced mTOR activation promotes T-bet expression, which in turn leads to the upregulation of CD122. IL-15 activation (which employs the IL-2Rβ for signaling) promotes the transition to memory T cells in part through the up-regulation of eomesodermin. Rapamycin, by inhibiting T-bet expression, promoted eomesodermin expression independently of IL-15. Therefore, by blocking mTOR, memory cells can be generated by the upregulation of eomesodermin in an IL-15-independent manner. Consistent with these findings is a recent report demonstrating that constitutively active Akt represses IL-7 and IL-15 receptor expression. Such cells display decreased memory $CD8^+$ T cell survival (110). These studies also revealed that constitutive STAT5 activation augments effector and memory CD8 T cell survival and homeostatic proliferation.

The ability of mTOR inhibitors to promote $CD8^+$ memory T cell formation has important implications for vaccine development. Treatment with rapamycin and metformin can enhance memory formation in antitumor responses (26, 106, 108). This has been extended to a vaccine model in which tumor antigens are delivered with hsp-110 as an adjuvant (111). Vaccination given concomitantly with the rapalog temsirolimus resulted in enhanced memory formation as well as antitumor immunity. Similar findings were made in nonhuman primates (112). Treatment of rhesus macaques with sirolimus during vaccinia virus vaccination led to enhanced central and effector memory $CD8^+$ T cell development. These findings suggest that the strategic administration of an mTOR inhibitor during vaccination might lead to more robust and long-lasting responses. In light of the fact that rapamycin is

currently employed as an immunosuppressive agent, such a strategy may seem paradoxical. In an attempt to address this paradox, Ferrer et al. (113) examined the effect of rapamycin on T cell–mediated antibacterial responses and graft rejection. They found that rapamycin selectively enhanced responses to an antigen when it was in the context of a bacterial infection but failed to do so when the same antigen was presented as part of a transplant. Indeed, a mouse model of skin transplantation demonstrated that rapamycin can promote CD8^{+} suppressor cells that home to the graft and prevent rejection (114). Taken together, these observations suggest that mTOR inhibitors can be successfully employed to prevent transplant rejection without compromising (in fact perhaps enhancing) the ability of CD8^{+} T cells to fight infection.

The Link Between mTOR and T Cell Trafficking

In T cells, mTOR provides a link between regulation of metabolism and function. Interestingly, events downstream of mTOR signaling also play an important role in the regulation of T cell trafficking. In this way, metabolic status, functional status, and trafficking are coordinately regulated by mTOR (115).

Naive T cells must circulate through secondary lymphoid tissue, and these trafficking patterns are facilitated by the expression of several cell surface receptors, including CD62L and the chemokine receptor CCR7 (115). Upon recognition of their cognate antigen, CD8^{+} T cells activate mTOR and switch to anabolic metabolism. The expression of their homing receptors also changes: Namely, both CD62L and CCR7 are downregulated, resulting in the diversion of the now activated effector cells out of the lymph nodes and into the tissues. Concomitant with mTOR's ability to promote effector generation, the PI3K-mTOR axis plays a role in the downregulation of CD62L and CCR7 (116). In CD8^{+} T cells that lack the PI3K inhibitor PTEN and thus have hyperactive mTOR, CD62L and CCR7 remain down-regulated. Conversely, cells treated with rapamycin display persistent expression of these molecules on their cell surface.

Upon resolution of an infection, the number of antigen-specific CD8^{+} T cells contracts as the cells transition from anabolism to catabolism, from high mTOR activity to low mTOR activity, and from effector to memory cells (103). These transitions are associated with the reexpression of CD62L and CCR7, thus allowing the newly formed memory cells to continue surveillance by trafficking in and out of secondary lymph nodes.

Mechanistically, the expression of CD62L, CCR7, and the memory marker CD127 has been linked to the FOXOs and KLF2 (117, 118). For example, mTORC2 activates Akt, leading to the inactivation of the FOXOs and thus decreased KLF2 expression. Because KLF2 positively regulates the transcription of these trafficking molecules, the expression of CD62L and CCR7 declines upon mTOR and Akt activation. In this regard, a critical role for Akt in the regulation of CD8^{+} T cell trafficking has been described (119). We have observed persistent CD62L expression in activated T cells that lack mTORC2 activity due to deletion of RICTOR (J.D. Powell & K.N. Pollizzi, unpublished observations). Additionally, Akt can regulate trafficking in an mTORC1-dependent fashion (119). This pathway involves Akt-mediated activation of mTORC1 and does not appear to involve the FOXOs.

In addition to CD62L, CCR7, and CD127, the G protein–coupled receptor sphingosine 1-phosphate receptor 1 (S1P1) is also regulated by KLF2 (120). S1P1 plays a critical role in promoting T cell egress from lymph nodes (121). The immunosuppressive agent FTY720 acts on S1P1, enforcing sequestration of T cells in the lymph nodes. Interestingly, S1P1 signaling leads to increased mTOR activation (122), which enhances Th1 development and antagonizes Treg differentiation (123). Likewise, transgenic expression of S1P1 on Foxp3^{+} T cells impairs their ability to suppress. That is, S1P1-induced mTOR activity blocks Treg

function. Alternatively, rapamycin treatment inhibits S1P1-mediated attenuation of Treg function.

REGULATION OF B CELLS BY mTOR

Compared with the data regarding mTOR and T cells, there is a relative paucity of data concerning the role of mTOR in regulating B cell differentiation and function. Several studies have addressed the role of mTOR in B cell lymphomas, but this work is outside the scope of this review.

In the mTOR hypomorph mouse, B cell development appeared to be more adversely affected than T cell development (72). These mice had a partial block in the large pre-B to small pre-B stages of development. For the B cells that made it to the periphery, there was an increase in mature B cells with a concomitant decrease in transitional and marginal zone cells. Functionally, B cells from the mTOR hypomorph mice demonstrated defective proliferation in response to B cell mitogens. Specifically, signaling through the B cell receptor (BCR) and CD40 was more compromised than Toll-like receptor (TLR) signaling. In addition, antibody production to both T-dependent and T-independent antibodies was decreased. Recently, investigators deleted TSC1 in B cells, which led to hyperactive mTORC1 activity (124). In these mice, there was impairment of B cell maturation with a significant reduction in marginal zone B cells. These defects in B cell differentiation were partially reversed by treatment with rapamycin. Similar to the mTOR hypomorph mice, T-dependent and -independent antibody production in these mTOR hyperactive mice was also decreased. That is, antibody production was compromised both in mice in which mTOR activity was decreased and in mice in which mTOR was hyperactive. These observations highlight the need to evaluate the role of mTOR signaling in regulating B cell maturation and function.

Recently, Lazorchak et al. (125) examined B cell development in B cells lacking mSIN1, which is a component of mTORC2. Because mSIN1 null mice are embryonic lethal, these studies were performed by transferring knockout fetal livers into lethally irradiated congenic mice. In the absence of mTORC2 activity, an increase in IL-7R expression and recombinase activating gene (RAG) activity was observed. Mechanistically, this increase was attributed to mTORC2's ability to activate Akt2, leading to inhibition of FOXO1 and thus decreased expression of IL-7R and RAG expression. Previous studies had demonstrated the ability of PI3K activity to inhibit RAG expression, but this work identified mTORC2 as the specific mediator of this effect (126). Thus, these studies place mTORC2 and FOXO1 as playing a critical role in B cell development.

The role of the PI3K-mTOR axis in promoting B cell survival and function has also been studied using pharmacologic inhibitors of the mTOR pathway. BCR signaling leads to enhanced activation of mTOR (127). For T-independent antigens, clonal proliferation and survival require prolonged and robust antigen exposure. The necessity for prolonged activation was linked to the generation of robust mTOR signaling. In the setting of limited antigen exposure, mTOR was only minimally activated, and expansion was limited. Only in the setting of prolonged exposure could T-independent antigens generate sufficient mTOR activation to promote B cell function. That is, in the absence of T cell help, antigen titration can modulate B cell activation by regulating mTOR activity. Such a mechanism might prevent inappropriate responses to transient antigen exposure. In this regard, engagement of inhibitory Fc receptors leads to the inhibition of PI3K signaling and presumably decreased mTOR activity (128). Thus, enhanced mTOR activation by BCR stimulation and suppressed mTOR activation by Fc receptor signaling demonstrate a pivotal role for mTOR in integrating signals that affect B cell function. Similarly, the Ig receptor–binding protein α4

binds to and inhibits the inhibitory phosphatase PP2a (129). mTOR regulates PP2a by inhibiting its function. Upon BCR stimulation, α4 associates and hence inhibits PP2a, leading to enhanced B cell activation. Blocking mTOR activity with rapamycin abrogates the ability of α4 to enhance B cell function.

In addition to being activated by antigen receptor–mediated signaling, the PI3KmTOR axis is also activated by TLR and CD40 stimulation in B cells. For splenic B cells, rapamycin inhibits anti-CD40-mediated proliferation as well as the ability of anti-CD40 to prevent apoptosis (130). Likewise, LPS-induced B cell proliferation and differentiation are markedly inhibited by rapamycin (131). The observation that LPS-induced ribosomal S6 phosphorylation is mTOR-dependent but PI3K-independent suggests an alternative mode of mTOR activation. Furthermore, unique B cell subsets are apparently differentially controlled by mTOR. For example, marginal zone B cells maintain high levels of mTOR activity in response to nutrients in the absence of mitogens. Conversely, follicular B cells display lower basal levels of mTOR activity and are less sensitive to rapamycin inhibition. These observations reflect the necessity for future studies of mTOR's role in specific B cell subsets.

As is the case for T cells, the resting state in B cells must be actively maintained (132). B lymphocyte stimulator-dependent (BLyS) is a growth and survival factor that helps to maintain the pool of resting B cells. Interestingly, BLyS mediates its functions by activating both mTOR and Pim-2 kinase (133). For example, Pim-2-deficient B cells can survive in the presence of BLyS; however, this survival is abrogated by treatment with rapamycin. Downstream of these two pathways, the antiapoptotic protein Mcl-1 is critical for executing these survival signals.

Upon activation, B cells rapidly increase glucose uptake and glycolysis, as do T cells. These metabolic changes depend on the PI3K pathway. The precise role of mTOR in this process remains to be determined. However, the dependence on glycolysis and the pentose phosphate shunt suggests a role for mTOR-induced HIF and SREBP1 (38). Indeed, a role for HIF in the upregulation of the glycolytic pathway has been described for B cell development (134).

THE ROLE OF mTOR IN REGULATING ANTIGEN-PRESENTING CELL DIFFERENTIATION AND FUNCTION

We propose that mTOR plays a fundamental role in sensing the immune microenvironment to dictate the outcome of antigen recognition in lymphocytes. Because APCs are central to the establishment of the adaptive immune response, one might predict that mTOR plays a critical role in their function as well. Indeed, recent studies suggest that, in APCs, mTOR integrates cues from the immune microenvironment to regulate differentiation and function. However, as is the case for T cells and B cells, the precise outcome of mTOR activation (and inhibition) is complex and cell type dependent.

mTOR Activation Promotes the Induction of Type I Interferons

Plasmacytoid DCs play a critical role in the antiviral immune response through the rapid elaboration of type I interferons (135). This response is principally mediated through TLR7 and TLR9 (136). Inhibition of mTOR with rapamycin abrogates the ability of TLR7 and TLR9 agonists to induce IFN-α and -β (Figure 6) (137, 138). Mechanistically, inhibition of mTOR disrupts the complex of TLR9 and MyD88, leading to decreased nuclear translocation of IRF7 (137). Furthermore, the mTORC1 target 4E-BP1 plays a role in the translation of IRF7. 4E-BP1 and 4E-BP2 double-knockout mice demonstrate increased

translation of IRF7, leading to increased expression of type I interferons. Indeed, such mice are resistant to vesicular stomatitis virus infection (138).

mTOR Regulates Dendritic Cell Maturation

DCs play a central role in directing adaptive immune responses. Resting DCs act as sentinels, sampling the environment and presenting antigen on their surface (139). Upon activation, DCs express costimulatory molecules and cytokines that induce robust T cell responses. Alternatively, when T cells encounter antigen presented by resting DCs that do not express costimulatory molecules, this interaction can lead to antigen-specific tolerance (140). A role for mTOR in antigen uptake and processing is suggested by the observation that rapamycin inhibits macropinocytosis in APCs (141).

Several subsets of DCs are derived from bone marrow precursors and mature in response to specific growth factor combinations (142). For example, Flt3 ligand, acting on the Flt3 receptor, promotes the generation of conventional DCs as well as CD8$^+$ DCs and plasmacytoid DCs (143). This process is mTOR dependent, as rapamycin inhibits Flt3 ligand–induced DC maturation (144). Alternatively, targeted deletion of PTEN leads to hyperactive mTOR activity, resulting in excessive expansion of the DC compartment, specifically CD8$^+$ DCs. This latter finding suggests that the regulation of mTORC1 signaling plays a role in maintaining DC subset composition in vivo.

Maturation of bone marrow–derived cells into DCs in vitro using GM-CSF and IL-4 is inhibited by rapamycin, demonstrating the necessity of mTOR for this process (145). Interestingly, bone marrow–derived DCs cultured in the presence of rapamycin demonstrate decreased upregulation of MHC and costimulatory molecules. As such, DCs matured in the presence of rapamycin not only are poor stimulators of T cells, but also actually promote T cell tolerance (146). Indeed, T cells stimulated by the rapamycin-matured DCs are rendered anergic. Furthermore, such DCs can promote the generation of Foxp3$^+$ Tregs (147). In vivo, rapamycin-derived allogeneic DCs can induce tolerance, leading to the prevention of graft rejection in mouse models of solid organ transplantation (146, 148). Thus, DCs matured in the presence of rapamycin can promote T cell tolerance by inducing deletion and anergy of effector cells, as well as by promoting the generation of regulatory cells.

Somewhat surprisingly, when the rapamycin-matured DCs are stimulated with LPS, they actually express increased amounts of IL-12 (149). This increased IL-12 is associated with increased GSK-3β activity. However, in spite of increased IL-12 expression, the LPS-stimulated, rapamycin-matured DCs do not promote Th1 responses. This may be because they still express low levels of costimulatory molecules. Accordingly, these cells still induce Foxp3$^+$ Tregs.

mTOR Inhibits IL-12 and Enhances IL-10 in Mature DCs and Monocytes

mTOR activation promotes IFN-α and -β in plasmacytoid DCs. In monocytes, macrophages, and primary DCs, mTOR signaling inhibits the expression of IL-12 and enhances the production of the anti-inflammatory cy-tokine IL-10 (150, 151). When monocytes, macrophages, and peripheral DCs are stimulated with LPS in the presence of rapamycin, there is an increase in IL-12 production that is due to an increase in NF-κB activation (150). Simultaneously, LPS-induced IL-10 production is decreased in a STAT3-dependent fashion. Alternatively, deletion of TSC2 (leading to activation of mTOR) promotes IL-10 production while concomitantly suppressing IL-12 expression. Peripheral DCs stimulated in the presence of rapamycin proved to be potent inducers of Th1 and Th17 cells. A separate study also found that mTOR inhibition leads to increased LPS-induced IL-12 expression and a decrease in IL-10 (151). In this model, the ability of rapamycin to

enhance IL-12 was correlated with its ability to inhibit IL-10. That is, IL-12 enhancement was not seen in IL-10 null cells. This group also found that GSK-3β could positively regulate IL-12 expression in an IL-10-independent fashion. Importantly, increased IL-12 production resulted in an increase in Th1 differentiation in this model. Finally, the regulation of monocyte-derived DCs by mTOR has also been examined (152), and the generation of DCs from monocytes was found to be mTOR dependent. The presence of rapamycin during this process led to apoptosis and the generation of tolerogenic DCs. When monocyte-derived DCs were stimulated via TLR ligation in the presence of rapamycin, they displayed decreased expression of IL-12, IL-6, and IL-10. Such cells also expressed decreased levels of costimulatory molecules and were poor T cell activators.

Thus, in plasmacytoid DCs, mTOR signaling heralds active proinflammatory immune responses (Figure 7). Likewise, mTOR is required for the maturation of fully competent myeloid-derived DCs. In monocytes, macrophages, and mature primary DCs, mTOR signaling appears to mitigate the production of proinflammatory cytokines such as IL-12 and enhance the elaboration of anti-inflammatory cytokines such as IL-10. The precise role of this dynamic in vivo remains to be determined. On the one hand, pretreatment of BALB/c mice with rapamycin prior to *Listeria* infection promoted increased immunity to this bacterial infection, suggesting that DCs generated under these conditions promote a protective Th1 response (150). On the other hand, the targeted delivery of mTOR inhibitors to macrophages and DCs using microencapsulated rapamycin led to decreased IFN-α and -β and an attenuated response to yellow fever virus vaccine (137). Taken together, these two experiments demonstrate that mTOR signaling serves to mitigate antibacterial immune responses while enhancing antiviral immune responses (153). It will be interesting to determine the relationship between metabolic demands, mTOR activation, and APC function. Furthermore, determining the precise regulators of mTOR for APCs in vivo will be important. For example, does the local depletion of BCAA, which inhibits mTOR and promotes infectious tolerance in T cells, also inhibit mTOR in APCs and thus enhance IL-12 production? In this regard, it is intriguing that in at least one study the enhanced production of IL-12 by rapamycin-derived DCs was associated with increased generation of Foxp3+ T cells (149). Recent studies have suggested that Th1-specific Tregs generated in the presence of IL-12 can inhibit Th1 responses (154). Perhaps these seemingly opposing effects of mTOR inhibition on APCs and T cells actually serve to coordinate the generation of such effector-specific Tregs.

THE USE OF mTOR INHIBITORS AS IMMUNOSUPPRESSIVE AGENTS

Rapamycin, other rapalogs, and more recently mTOR kinase inhibitors have proven to be exceedingly useful in dissecting mTOR signaling pathways (48). Only recently, however, the use of mTOR inhibitors has begun to meaningfully penetrate immunosuppressive protocols for solid and bone marrow transplantation. In part this is because of the transformative use of calcineurin inhibitors in transplantation. As such, initial use of rapalogs was in the context of adjuvants to calcineurin inhibitors or as a means of stopping treatment with calcineurin inhibitors (155). Indeed, some of the major side effects of calcineurin inhibitors include nephrotoxicity, accelerated atherosclerosis, and post-transplant malignancies, but these adverse events are not inherent to mTOR inhibition and in some cases are actually improved by this treatment.

The most appealing aspect of mTOR inhibitors in transplantation, however, is the prospect of inducing long-term tolerance (156). Indeed, from an immunologic perspective, in spite of their potent immunosuppressive properties, calcineurin inhibitors prevent T cell anergy and the induction of Tregs (52, 76). mTOR inhibitors thus offer the prospect of inducing long-term tolerance in the absence of long-term immunosuppression. Importantly, calcineurin

inhibitors block the ability of mTOR inhibitors to promote tolerance. Therefore, tolerance-inducing regimens need to avoid the simultaneous use of these inhibitors. Along these lines, calcineurin inhibitor–free regimens for transplantation have been evaluated in clinical trials (157, 158). In this regard, a regimen that employs CAMPATH (humanized monoclonal antibody directed against CD52), low-dose radiation, and rapamycin has been used to induce bone marrow chimerism to treat sickle cell disease in the setting of nonmyeloablative hematopoietic stem cell transplantation (159). This regimen (which did not block TCR signaling) resulted in long-term bone marrow chimerism, and for a number of patients immunosuppression was completely stopped, indicating the induction of tolerance. Other promising approaches include using a combination of costimulatory blockade and mTOR inhibition, as well as employing mTOR inhibitors to generate Tregs (160–162). It is crucial to remember, however, that mTOR inhibition does not block the acute expression of inflammatory cytokines. Therefore, future regimens may include the strategic use of calcineurin inhibitors during the initial transplant period to prevent acute rejection, followed by prolonged mTOR inhibition to promote long-term tolerance.

Transplantation offers a unique opportunity to promote immunological tolerance clinically. In the case of autoimmunity, the challenge remains both in terms of interrupting an insidious ongoing process and then reestablishing tolerance. Nonetheless, such strategies are currently being investigated for type I diabetes, for example (163). In this regard, many approaches have focused on employing mTOR inhibition to generate Tregs ex vivo. For example, studies have demonstrated the ability of mTOR inhibition to generate Foxp3$^+$ Tregs from patients with type I diabetes (164). This modality is also being tested for treatment and prevention of graft-versus-host disease (162, 165). In addition, interesting experimental approaches have employed mTOR inhibitors to enhance the generation of Tregs in vivo. Administration of IL-2-anti-IL-2 immune complexes, which accentuate IL-2 receptor signaling, under the cover of mTOR inhibition can promote the in vivo generation of Tregs that can confer resistance to EAE and promote long-term acceptance of islet grafts in the absence of immunosuppression (166). It remains to be determined if the tolerance-inducing properties of mTOR inhibition will be translated into human autoimmune disease.

CONCLUSION

The immunosuppressive properties of rapamycin were originally ascribed to its ability to inhibit proliferation. Since that time, it has become clear that mTOR plays a central role in regulating the outcome of antigen recognition in sculpting the outcome of the adaptive immune response. Just as yeast TOR senses environmental cues to direct cellular function, mTOR also senses the immune microenvironment to influence the differentiation and maturation of T cells, B cells, and APCs. In doing so, mTOR provides an important link between metabolism and immune cell function. Although there have been more studies focused on elucidating the role of mTOR in T cells, it appears that future studies will identify an equally important role for mTOR in regulating B cells and APCs. As more cell type–specific genetic models become available, the precise role that mTOR plays in B cell and APC differentiation and function should be clarified. For example, does mTOR play a role in regulating M1 and M2 macrophage differentiation (167)? In this regard, it will be interesting to determine the ability of mTOR to coordinately regulate function and metabolic demands in these cell types. Additionally, the field awaits further insight into the ability of mTOR inhibition to enhance inflammation in mature APCs while promoting tolerance in T cells. Finally, in light of the new insight regarding the ability of mTOR inhibitors to regulate (rather than suppress) immune responses, one can imagine strategic integration of mTOR inhibitors into transplantation protocols as well as into the treatment of autoimmunity, and the emerging observations concerning mTOR inhibition and memory T cell generation suggest that these agents may also soon be employed to actually enhance vaccine responses.

Acknowledgments

Glossary

Mammalian target of rapamycin (mTOR)	a serine/threonine protein kinase involved in regulating growth, survival, function, metabolism, and differentiation
mTOR complex 1 (mTORC1)	a complex of proteins associated with mTOR, distinguished by the presence of RAPTOR and PRAS40
mTOR complex 2 (mTORC2)	a complex of proteins associated with mTOR, defined by association with RICTOR, PROTOR, and mSIN1
Regulatory-associated protein of mTOR (RAPTOR)	a scaffolding protein required for the assembly of mTORC1
Akt	a serine/threonine kinase important for survival, proliferation, and metabolism; it is found both upstream of mTORC1 and downstream of mTORC2
RAPTOR-independent companion of TOR (RICTOR)	a scaffolding protein required for the assembly of mTORC2
mSIN1	protein associated with mTORC2 that may localize mTORC2 to membranes
Rapalog	a compound, similar to rapamycin, that inhibits mTORC1 activity by blocking RAPTOR and mTOR interaction
Ras homolog enriched in brain (RHEB)	a small GTPase that, when bound to GTP, activates mTORC1
Tuberous sclerosis complex 2 (TSC2)	a negative regulator of mTORC1 activity
Phosphatidylinositol 3-kinase (PI3K)	a signal transducer protein that, upon activation, phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2), leading to Akt/mTOR activation
Phosphatase and tensin homolog (PTEN)	a tumor suppressor protein that functions by negatively regulating PI3K

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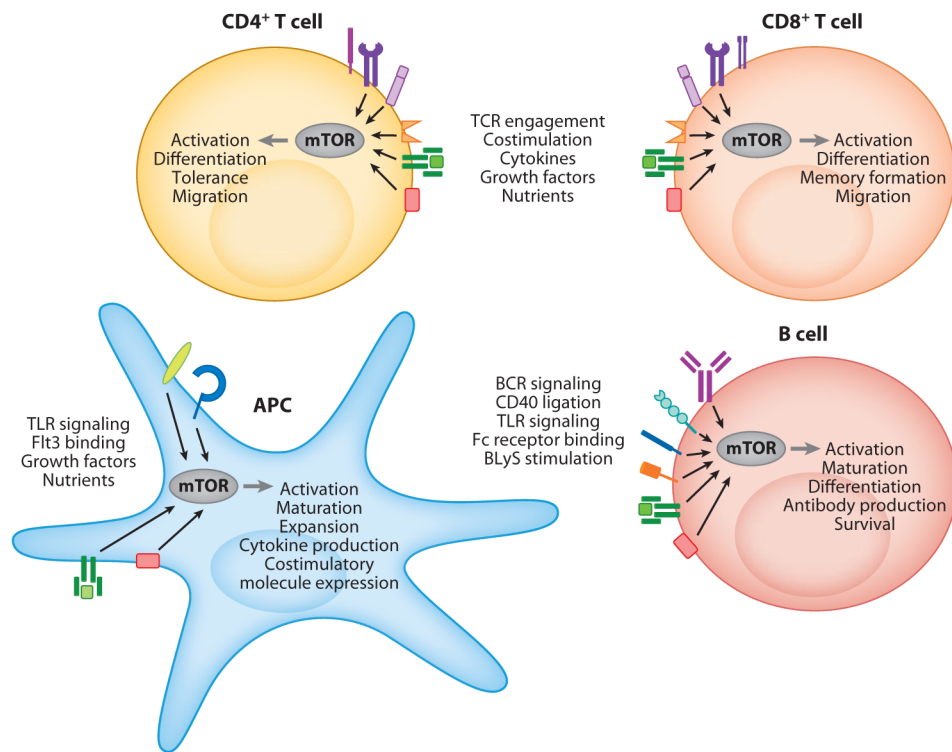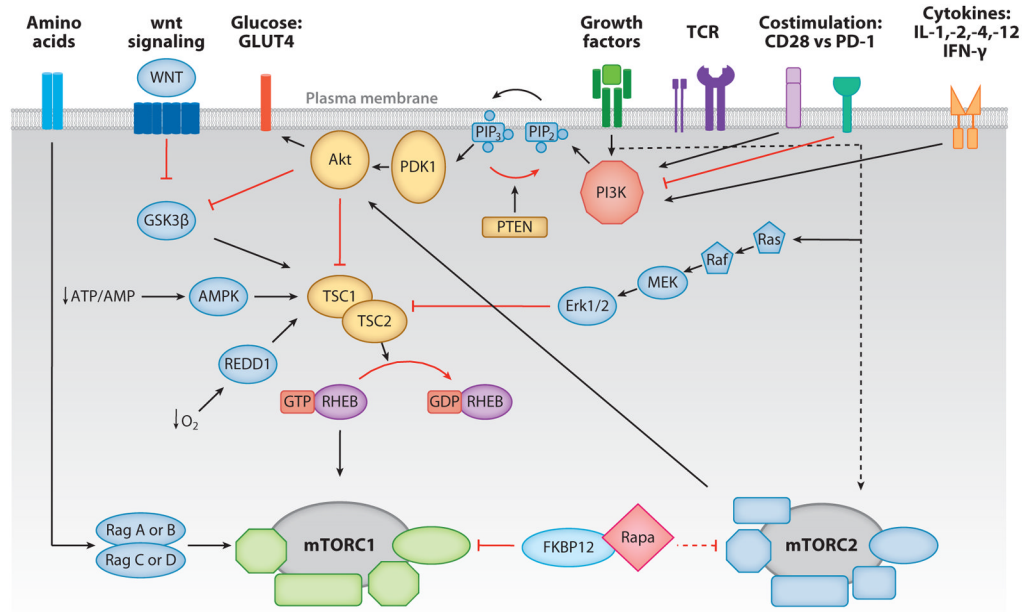
Figure 1.
mTOR integrates environmental cues to direct the adaptive immune response. Antigen-presenting cells (APCs), T cells, and B cells play central roles in the adaptive immune response. This figure highlights the diverse environmental cues integrated by mTOR, which are discussed in this review.
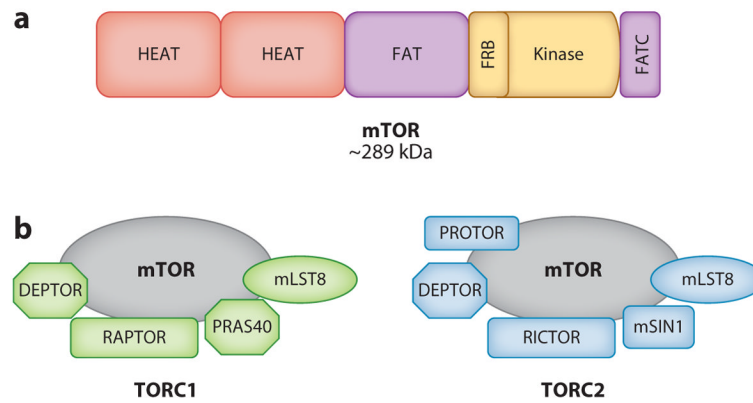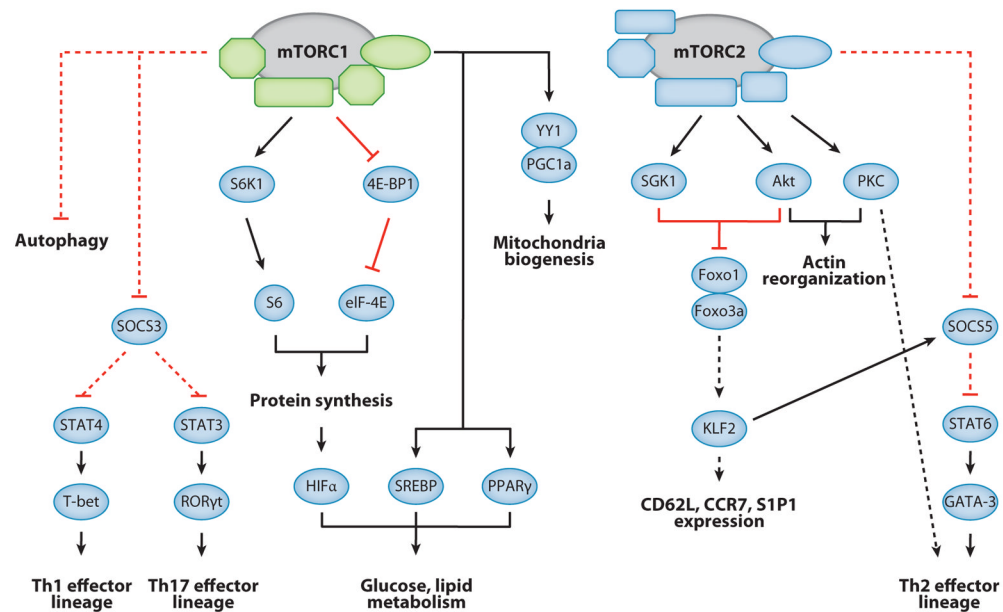
Figure 2.
Structure of the mTOR signaling complex. (*a*) mTOR is an evolutionarily conserved 289-kDa serine/threonine protein kinase that is composed of two N-terminal HEAT (huntingtin, elongation factor 3, subunit of PP2A, and TOR) domains, which mediate protein-protein interactions, adjacent to a FRAP, ATM, and TRRAP (FAT) domain. The FRB domain is where the small 12-kDa FK506-binding protein (FKBP12) bound to the macrolide drug rapamycin binds to mTOR to inhibit its activity. The mTOR kinase catalytic domain lies C-terminal to the FRB site, and this is where mTOR kinase inhibitors bind. The carboxy FAT (FATC) domain maintains the structural integrity of this large protein kinase. (*b*) mTOR associates with two distinct sets of adapter proteins to form two intracellular signaling complexes with unique substrate specificity. The TORC1 signaling complex is composed of the regulatory-associated protein of mTOR (RAPTOR) and mammalian lethal with Sec13 protein 8 (mLST8), which are both adapter proteins that mediate protein-protein interactions via their WD-40 domains. The proline-rich Akt substrate 40 kDa (PRAS40) and DEP domain–containing mTOR-interacting protein (DEPTOR) inhibit mTORC1 activity. The mTORC2 complex can also associate with DEPTOR and mLST8, but this complex is distinguished by the adapter protein RAPTOR-independent companion of TOR (RICTOR) and protein observed with RICTOR (PROTOR). Another unique component of mTORC2 is mSIN1, which contains a pleckstrin homology domain that is thought to target TORC2 to the membrane, where it can activate myristoylated Akt.
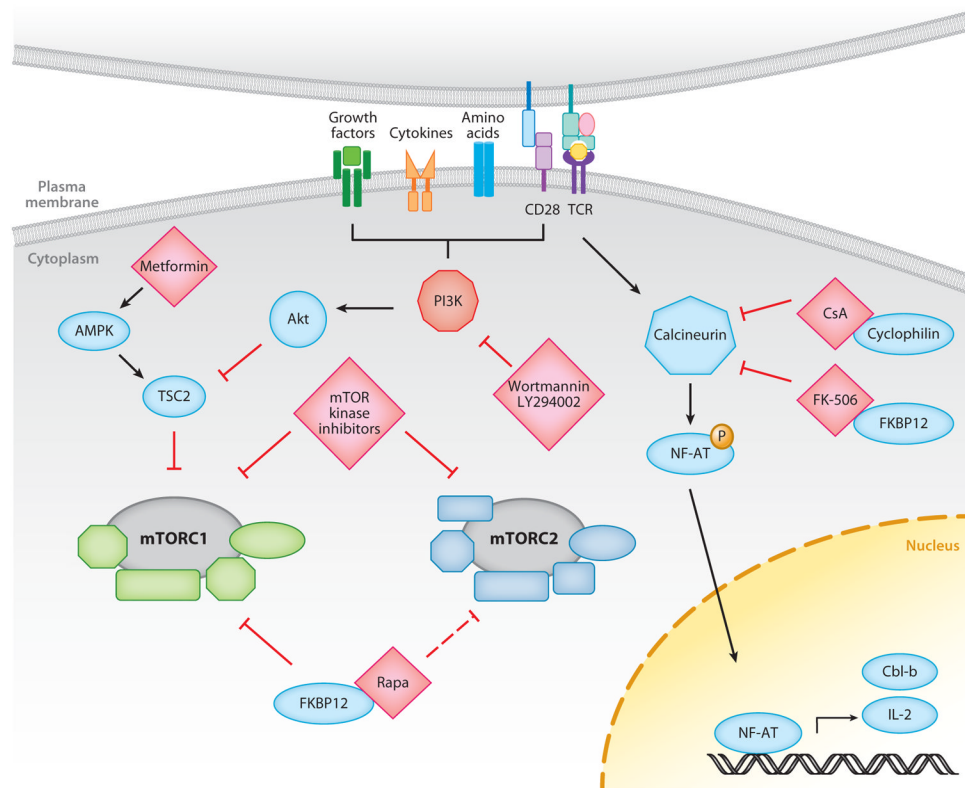
Figure 3.
The upstream signaling cascade leading to mTOR activation in an immune setting. Growth factor stimulation leads to the recruitment of PI3 kinase (PI3K), which phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) at the 3′ position to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3). This leads to the recruitment of Akt to the membrane to be phosphorylated (at position T308) by PDK1. Activated Akt phosphorylates TSC2 in an inhibitory manner, yielding a separation of the TSC1/TSC2 complex and loss of GAP activity for RHEB-GTP. This leads to an accumulation of RHEB-GTP, which promotes mTORC1 function. Similarly, Erk can phosphorylate TSC2 in an inhibitory manner. Alternatively, the following phosphorylate TSC2 in an activating way, thereby enhancing its GAP activity: AMPK, in response to low levels of energy; REDD1, in response to low oxygen tension; and GSK3β, which is regulated by WNT and Akt. In the presence of amino acids, Rag proteins bind to Raptor and promote the relocalization of mTORC1 with Rheb-GTP, leading to activation. Rapamycin bound to FKBP12 allosterically inhibits mTORC1 activity. Additionally, many immunologic inputs also play a role in regulating mTORC1 activity. Positive costimulation (such as CD28 engagement) as well as cytokine signaling lead to recruitment of PI3K activity. Conversely, PD-1 ligation (co-inhibition) inhibits PI3K function. The upstream regulation of mTORC2 is poorly understood. Growth factor stimulation activates this complex, whereas high doses or prolonged exposure to rapamycin will disrupt the mTORC2 complex. Dashed lines indicate that the exact mechanism is unknown. Black lines show activating signals, red lines show inhibitory signals, and red arrows indicate signals that indirectly lead to inhibition of mTORC1 activity.
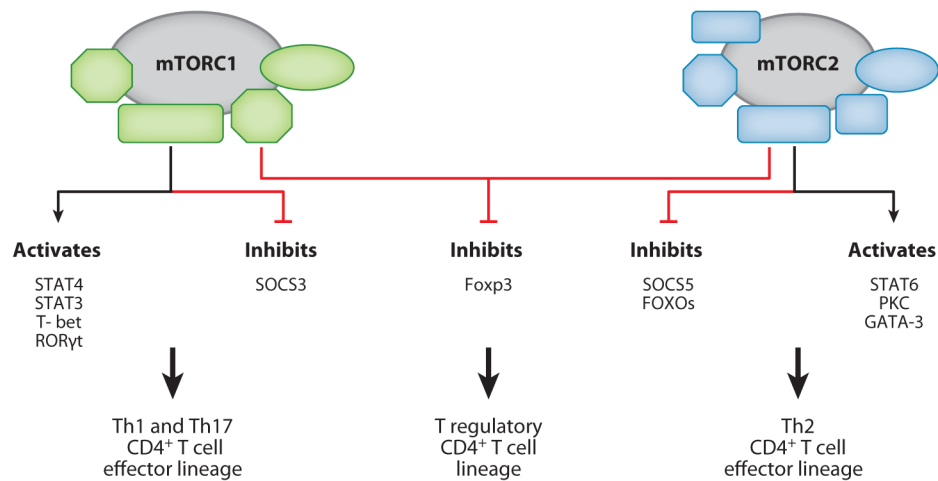
Figure 4.
Downstream mTORC1 and mTORC2 signaling. Upon activation of mTORC1, mTOR phosphorylates S6K1, leading to the phosphorylation of ribosomal S6 protein, which allows for enhanced protein translation. Phosphorylation of 4E-BP1 by mTOR releases eIF-4E to participate in the translation-initiation complexes. Along with increasing protein translation, mTORC1 activity also upregulates gene expression programs necessary for glucose and lipid metabolism, mitochondrial biogenesis, and inhibition of autophagy. Immunologically, mTORC1 activity leads to the inhibition of SOCS3 and the increased activation of STAT4 and STAT3. This in turn leads to increases in T-bet and ROR γt in response to IL-12 and IL-6, respectively, which promote Th1 and Th17 differentiation. mTORC2 activity leads to the phosphorylation of Akt (at position S473) and SGK1, leading to their activation and in turn resulting in the phosphorylation and sequestration of FOXO proteins in the cytoplasm. This prevents the FOXO proteins from activating the transcription of target genes such as Krüppel-like factor 2 (KLF2), which itself influences the expression of CD62L, CCR7, and S1P1. mTORC2 activity also inhibits SOCS5 expression, thereby enhancing STAT6 phosphorylation in response to IL-4 and subsequent GATA-3 expression and Th2 differentiation. In addition, mTORC2 signaling activates PKCθ, which in turn can also promote Th2 differentiation. Dashed lines indicate that the exact mechanism is unknown, black lines show activating signals, and red lines show inhibitory signals.
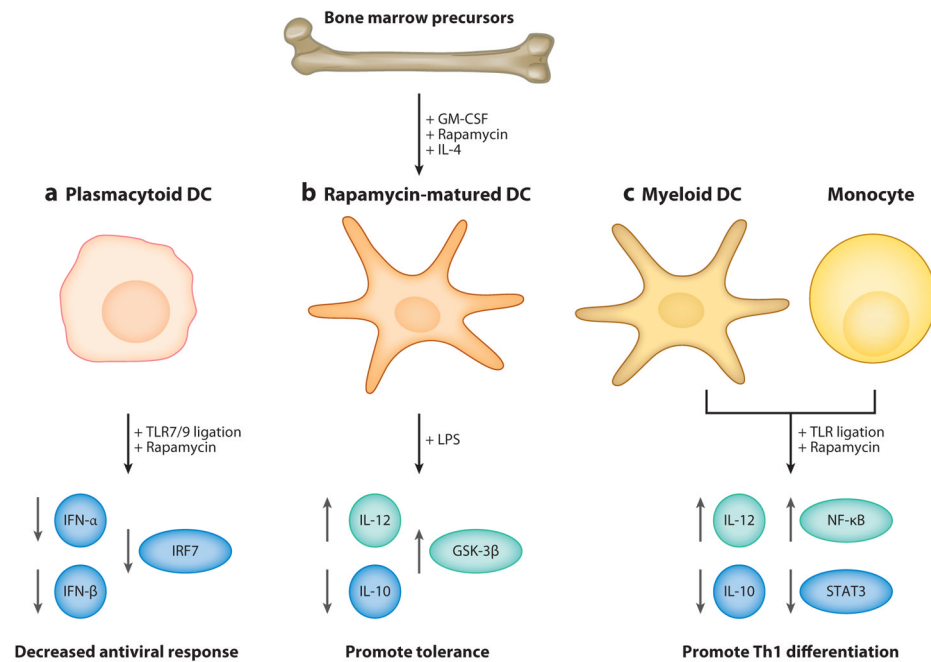
Figure 5.
Pharmacologic inhibitors of mTOR and calcineurin signaling. Rapamycin, cyclosporin A (CsA), and FK506 are all immunosuppressive agents with similar structure yet diverse mechanisms of action. All three agents are activated by binding to *cis-trans* peptidyl-prolyl isomerase proteins called immunophilins. CsA binds to cyclophilin, whereas FK506 and rapamycin bind to FKBP12. Both CsA and FK506 inhibit TCR-induced calcineurin activation and thus inhibit the translocation of NF-AT to the nucleus. In doing so, they block the expression of NF-AT-dependent genes of activation such as IL-2 as well as NF-AT-induced inhibitory genes such as Cbl-b. As such, in addition to being potent immunosuppressive agents, these compounds also block the induction of TCR-induced tolerance. However, rapamycin and similar drugs termed rapalogs, when bound to FKBP12, inhibit the interaction of RAPTOR and mTOR and thus inhibit mTORC1 activation. Prolonged exposure of T cells to rapamycin can also impair mTORC2 activity by an as yet undefined mechanism. In contrast, mTOR kinase inhibitors function as ATP-competitive inhibitors at the mTOR catalytic domain to specifically and potently inhibit both mTORC1 and mTORC2. Wortmannin and LY94002 can block mTOR activity by inhibiting PI3K activity, which is upstream of mTOR. Additionally, molecules that mimic the effects of AMP, such as metformin, can inhibit mTOR activity by activating AMPK, which in turn promotes the ability of TSC2 to inhibit mTORC1. Dashed lines indicate that the exact mechanism is unknown, black lines show activating signals, and red lines show inhibitory signals.

Figure 6.
The role of mTOR in CD4^{+} T cell differentiation. A series of genetic studies has revealed a central role for mTOR in regulating T helper cell differentiation. T cells lacking RHEB, and hence mTORC1 signaling, fail to differentiate into Th1 and Th17 cells under polarizing conditions. This failure to differentiate is associated with decreased STAT activation and decreased expression of lineage-specific transcription factors such as T-bet and RORγt. Similarly, T cells lacking RICTOR and mTORC2 activity demonstrate decreased STAT6 activation, decreased PKC activity, and decreased GATA-3 expression and thus fail to differentiate into Th2 cells under polarizing conditions. T cells lacking mTOR demonstrate increased SMAD3 activation and become Foxp3^{+} Tregs even under normally activating conditions. Black lines indicate the ability of mTOR to activate a pathway, and red lines indicate the ability of mTOR to inhibit a pathway.

Figure 7.
mTOR regulates maturation and function of antigen-presenting cells. Plasmacytoid dendritic cells (DCs) activated by TLR9 and TLR7 agonists in the presence of rapamycin fail to produce interferon-α and -β, indicating an important role for mTOR in promoting type 1 interferon expression (*a*). Activation of monocytes and DCs with LPS in the presence of rapamycin leads to an increase in IL-12 production and a decrease in IL-10 production. Such findings suggest that mTOR inhibits proinflammatory gene expression in these cells (*c*). Alternatively, rapamycin blocks the maturation of bone marrow–derived DCs (*b*). Such cells display decreased MHC and costimulatory molecules and actually promote the induction of anergic and regulatory T cells. Interestingly, in spite of the ability of the rapamycin-matured DCs to promote tolerance, they also produce increased IL-12 upon stimulation with LPS.