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REVIEW ARTICLE

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mTOR Signaling pathway as a master regulator of memory CD8$^+$ T-cells, Th17, and NK cells development and their functional properties

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Abstract

The mammalian target of rapamycin (mTOR) is a member of the evolutionary phosphatidylinositol kinase-related kinases (PIKKs). mTOR plays a pivotal role in the regulation of diverse aspects of cellular physiology such as body metabolism, cell growth, protein synthesis, cell size, autophagy, and cell differentiation. Immunologically, mTOR has a fundamental part in controlling and shaping diverse functions of innate and adaptive immune cells, in particular, T-cell subsets differentiation, survival, and metabolic reprogramming to ultimately regulate the fate of diverse immune cell types. Researchers report that rapamycin, a selective mTOR inhibitor, and immunosuppressive agent, has surprising immunostimulatory effects on inducing both quantitative and qualitative aspects of virus-specific memory CD8$^+$ T-cells differentiation and homeostasis in a T-cell-intrinsic manner. The mTOR signaling pathway also plays a critical role in dictating the outcome of regulatory T cells (Treg), T helper 17 (Th17) cells, and natural killer (NK) cells proliferation and maturation, as well as the effector functions and cytotoxic properties of NK cells. Manipulation of mTOR activity is a critical therapeutic approach for pharmacological agents that seek to inhibit mTOR. This approach should enhance specific memory CD8$^+$ T-cells responses and induce fully functional effector properties of NK cells to provoke their antitumor and antiviral activities.

KEYWORDS
immune responses, memory CD8$^+$ T-cell, mTOR, NK cell, regulatory T-cell, Th17

1 | INTRODUCTION

CD8$^+$ cytotoxic T lymphocytes (CTLs) are a critical part of the host immune defense against intracellular pathogens and for tumor surveillance. These cells directly kill cancer cells or cells infected with viruses by releasing the cytotoxic contents of granules (granzymes [Gzm] and perforin) or indirectly by cytokine secretion of interferon (IFN)-γ and tumor necrosis factor (TNF). During acute viral infections or those caused by intracellular pathogens, antigen-stimulated signaling in conjunction with inflammatory signals, such as Type I IFN and interleukin-12 (IL-12), stimulate naive antigen-specific CD8$^+$ T-cells. These CD8$^+$ T-cells become activated and undergo rapid and efficient clonal expansion into mature, functional pathogen-specific effector CD8$^+$ T-cells (Kaech & Cui, 2012). After elimination of the pathogen, the majority of pathogen-specific effector CD8$^+$ T-cells engage in self-apoptosis. However, approximately 5–10% of the effector CD8$^+$ T-cells remain viable and experience multiple changes in their functional and cellular properties, and different transcriptional program. The remaining CD8$^+$ T-cells subsequently form a stable, highly protective, and long-lived antigen-specific memory CD8$^+$ T-cells pool that provides greater reactivity and protection after rechallenge by the same antigen

(Ahmed & Gray, 1996). Memory $CD8^+$ T-cells are considered to be a pivotal players of protective immunity in the adaptive immune response. Successful induction of potent and effective memory T-cells responses is a major goal of therapeutic vaccinations against chronic infectious disease and poorly immunogenic tumors (Klebanoff, Gattinoni, & Restifo, 2006). Survival, homeostatic proliferation (HP), and an extended life span of self-renewing memory $CD8^+$ T-cells occurs in the absence of the self-major histocompatibility complex (self-MHC; antigen-independent) that is mainly supported through the combination of IL-7 and IL-15 in a cytokine-dependent manner (Geginat, Lanzavecchia, & Sallusto, 2003). The mammalian target of rapamycin (mTOR) is a key signaling serine/threonine-protein kinase.

mTOR complex-1 (mTORC1) has been shown to play a crucial roles cellular physiology include cellular metabolism, proliferation, and differentiation, regulation of cell growth and size, messenger RNA (mRNA) turnover and translation, control of ribosomal biogenesis, cytoskeletal organization, autophagy, mitochondrial metabolism, biogenesis and mitochondrial DNA copy number (Laplante & Sabatini, 2009).

The mTOR protein belongs to the phosphatidylinositol kinase-related kinases (PIKKs) family. mTOR is a 289 kDa protein that contains a highly conserved carboxy-terminal serine/threonine-protein kinase domain (Laplante & Sabatini, 2009). Rapamycin (sirolimus), first isolated in the 1970s from *Streptomyces hygroscopicus*, was found in a soil sample on Easter Island (Rapa Nui). It was originally considered to be a metabolite that had potent antifungal activity. Intracellularly, rapamycin forms a complex with its intracellular receptor, immunophilin 12 kDa FK506-binding protein (FKBP12). This complex blocks the activity of the regulatory associated protein of mTOR (raptor)-bound (mTORC1), but not the rapamycin-insensitive companion of mTOR (rictor)-bound (mTORC2) (Vezina, Kudelski, & Sehgal, 1975). Rapamycin and its derivatives (analogues) termed rapalogs have been shown to have broad growth-inhibitory activity and antiproliferative effects on a wide variety of cells, including T-cell proliferation and cancer cells (Q. Yang & Guan, 2007). They considered to be as antiproliferative therapeutic agent for prevention of allograft rejection, graft-versus-host disease (GVHD), and cancer (Armand et al., 2008; Eisen et al., 2003).

Multiple signals such as growth factors, amino acids, various cytokines, antigen receptors, insulin, toll-like receptor (TLR) ligands, ligated costimulatory molecules, hypoxia, nutrients, energy status, cellular stress, and DNA damage have revealed to control the functions of both mTORC1 and mTORC2 (Thomson, Turnquist, & Raimondi, 2009; Weichhart, Hengstschlager, & Linke, 2015). Recently developed mTOR inhibitors target the mTOR signaling pathway. These inhibitors help us to understand the mechanisms by which mTOR regulates various components of the innate and adaptive immune systems that include T-cell generation, differentiation and development, and memory generation. It has been revealed that mTOR is a crucial regulator of memory $CD8^+$ T-cells, T helper 17 (Th17) cell, and natural killer (NK) Cells differentiation and development (Araki et al., 2009; Rao, Li, Odunsi, & Shrikant, 2010; Weichhart et al., 2015). In vitro and in vivo studies of the main transcriptions involved in $CD8^+$ effector and memory T-cells fate have elucidated an association between mTOR inhibitors and memory $CD8^+$ T-cells generation (Araki, Youngblood, & Ahmed, 2010; Ferrer et al., 2010; Li et al., 2011, 2012; Pearce et al., 2009; Rao, Li, Odunsi, et al., 2010). According to research, mTOR signaling manipulates the main transcriptions involved in $CD8^+$ T-cells development and metabolic reprogramming, ensuring that long-lived memory and terminally differentiated $CD8^+$ effector T-cells are equally produced. Researchers seek to determine the vital role of the mTOR signaling pathway on memory $CD8^+$ T-cells generation and its potential to improve vaccine immunotherapy.

In this review, we intend to discuss recent research on the underlying roles of mTOR in regulatory T-cell (Treg), Th17, NK cell, and memory $CD8^+$ T-cells differentiation.

2 | mTOR SIGNALING PATHWAY

mTOR exists in two-independent cellular multiprotein complexes—mTORC1 and mTORC2, which differ in their regulation and downstream targets (Thomson et al., 2009). mTORC1 contains five components: mTOR, raptor, mammalian lethal with SEC13 protein 8 (mLST8 or GβL), proline-rich Akt substrate of 40 kDa (PRAS40), and DEP-domain containing mTOR interacting protein (DEPTOR; Laplante & Sabatini, 2009).

Rapamycin is an mTORC1-specific inhibitor. When integrated with its intracellular receptor, FKBP12, it forms rapamycin–FKBP12. The rapamycin–FKBP12 complex subsequently binds to the FKBP12–rapamycin-binding (FRB) domain kinase region on mTORC1 and inhibits its kinase activity by disrupting the interaction between Raptor and mTOR (Thomson et al., 2009) (Yang et al., 2011). mTORC1 activity is regulated by a wide range of cellular and environmental signals. It integrates these signals with various biosynthetic processes that include cellular metabolism, cell growth, the cell cycle, and cell proliferation by phosphorylation of large number of critical signaling molecules and transcription factors (4E-BP1; Ma & Blenis, 2009).

The mTORC2 complex comprises six components—several of which are shared with mTORC1. The mTORC2 components are mTOR, mLST8, rictor, mammalian stress-activated protein kinase interacting protein (mSIN1), protein observed with rictor-1 (PROTOR-1), and DEPTOR (Laplante & Sabatini, 2009). Unlike mTORC1, it does not contain RAPTOR and PRAS40. Thus far, there is scant information exists about the precise biological role of mTORC2. Unlike mTORC1, mTORC2 is mostly not sensitive to rapamycin; however, prolonged exposure to rapamycin can downregulate mTORC2 activity (Sarbassov et al., 2006). Rapamycin-mediated inhibition of mTORC2 interferes with newly translated mTOR kinase and blocks the assembly of mTORC2 (Sarbassov et al., 2006). Studies show that Akt phosphorylation by mTORC2 at serine 473 leads to enhanced activation of Akt and increased cell survival (Bayascas & Alessi, 2005).

The tuberous sclerosis complex-1/2–Ras homolog enriched in the brain (TSC1/2–Rheb) pathway controls activation of mTORC1. The TSC1–TSC2 (hamartin–tuberin) heterodimer complex acts as a key upstream negative regulator for mTORC1 by inhibiting Rheb, which is the direct activator of mTORC1 kinase activity (Inoki, Li, Xu, & Guan, 2003). The TSC1–TSC2 heterodimer acts as a GTPase-activating protein (GAP). After inhibitory phosphorylation of TSC2 by Akt, the TSC1/2 complex dissociates and loses its suppression activity, and allows for conversion of GDP-bound Rheb to its GTP-bound state. In the GTP-bound state, it directly interacts with mTORC1 and stimulates its kinase activity (Figure 1; Laplante & Sabatini, 2012). Signaling through diverse stimulators mediates activation of RAS–mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)–protein kinase B (Akt) signaling, and AMP activated protein kinase (AMPK) signaling, which regulates activation of the TSC1–TSC2 complex (Thomson et al., 2009). Thus, a signal by Akt and RAS–MAPK directly activates mTORC1, whereas AMPK indirectly suppresses mTORC1 activation. Activated mTORC1

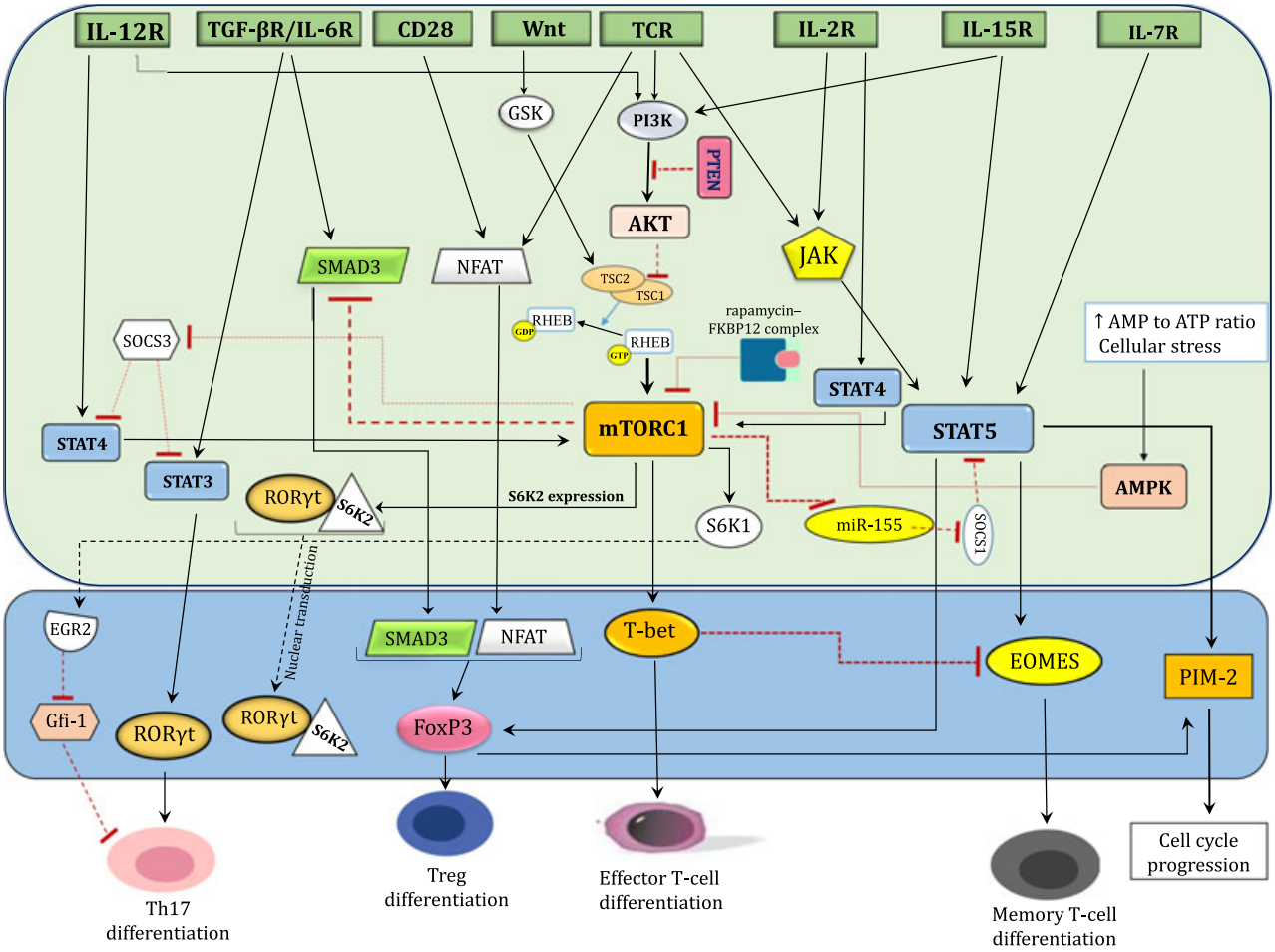

FIGURE 1 Multiple roles of the mTOR signaling pathway in immune cells biology. The different upstream signaling cascade follows diverse stimulators that lead to mTOR activation in immune cells. The TSC1–TSC2 complex acts as key upstream negative regulator for mTORC1 by inhibition of Rheb, which is a direct activator of mTORC1 kinase activity. The TSC1–TSC2 heterodimer acts as a GAP and inhibitory phosphorylation of TSC1 or TSC2. The TSC1/2 complex dissociates and allows the GTP-bound form of Rheb to directly interact with mTORC1 and stimulate its kinase activity. TGF-β dependent activation of SMAD3 is essential in inducing FOXP3. When combined with TCR-induced NFAT, FOXP3 expression is promoted. STAT5 signaling induction and maintenance of FOXP3 expression. miR-155 negatively regulates Treg function by inhibiting the suppressor of SOCS1 expression, a negative regulator of STAT5 signaling. mTOR inhibition drives Tregs to STAT5–pim-2-dependent proliferation. In early-activated $CD8^+$ T-cells, T-bet expression is induced through TCR signaling and IL-12 mediated signals. In addition, IL-12 enhances mTOR activity in $CD8^+$ T-cells via PI3K and the STAT4 pathway. Then, mTOR activity induces sustained T-bet expression. Thus, mTOR activity in synergy with TCR signaling amplifies T-bet expression. Following T-bet expression, the expression of Eomes is induced in a RUNX3-dependent manner and is amplified through the IL-2 signaling pathway; however, it is repressed by IL-12 and mTOR. CD: cluster of differentiation; FOXP3: forkhead box P3; GAP: GTPase-activating protein; GSK3: glycogen synthase kinase 3; GTP: guanosine triphosphate; IL: interluekin; miR-155: microRNA-155; mTOR: mammalian target of rapamycin; mTORC1: mammalian target of rapamycin complex-1; NFAT: nuclear factor of activated T-cells; PI3K: phosphoinositide 3-kinase; pim-2: phosphatidylinositol mannoside-2; RUNX3: runt-related transcription factor 3; SMAD3: mothers against decapentaplegic homolog 3; SOCS1: suppressor of cytokine signaling 1; STAT: signal transducer and activator of transcription; TCR: T-cell receptor; TGF-β: tumor growth factor-β; Th17, T helper 17 cells; Rheb, Ras homolog enriched in brain; TSC: tuberous sclerosis complex; Treg: regulatory T-cell; TSC: tuberous sclerosis complex [Color figure can be viewed at wileyonlinelibrary.com]

promotes protein synthesis by induction of mRNA translation through ribosomal S6 kinase 1 (S6K1) stimulation and inhibition of eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1). The mTOR-dependent phosphorylation of 4E-BP1 is sufficient to drastically disrupt its binding to eIF4E, which results in the release of eIF4E and allows eIF4E to stimulate cap-dependent translation (Laplante & Sabatini, 2009; Weichhart et al., 2015). Activation of glycogen synthase kinase 3 (GSK3) negatively regulates the mTOR pathway by direct phosphorylation of TSC2, which further activates TSC2 GAP activity (Inoki et al., 2006).

3 | REGULATORY ROLE OF mTOR IN THE IMMUNE SYSTEM

3.1 | A review of mTOR signaling in innate immune cells

Recent research focuses on the underlying mechanisms by which inhibition of mTOR affects intracellular signaling pathways in immune cells. The results of these studies show that mTOR plays fundamental roles in the modulation of both innate and adaptive immunity. The mTOR-mediated signaling regulates various aspects of the immune responses such as antigen presentation in dendritic cell (DC) development and polarization, myeloid cells activity, cytokine production, cell migration, activation of effector T-cells, memory generation and maintenance, function and proliferation of Treg cells, and differentiation of T-cell subsets (Thomson et al., 2009; Weichhart et al., 2015). TLR ligands, in addition to growth factors and cytokines such as granulocyte-macrophage colony-stimulating factor (GM–CSF) and FMS-related tyrosine kinase 3 ligands (FLT3L), and IL-4 are among the extracellular and intracellular signals involved in the activation of mTOR signaling in human and mice innate immune cells (Haidinger et al., 2010; Sathaliyawala et al., 2010; Weichhart et al., 2015). PI3K–Akt–mTORC1 pathway is downstream of FLT3L. In its active state, this pathway is critical for DCs development and function, particularly for plasmacytoid DCs (pDCs) and $CD8^+$ DCs (Sathaliyawala et al., 2010). Rapamycin administration can block FLT3L-driven DCs development in vitro. Deletion of phosphatase and tensin homolog (PTEN), a negative regulator of the PI3K–mTOR pathway, has been shown to promote FLT3L-driven DCs development (Sathaliyawala et al., 2010). Pharmacologic inhibition of PI3K, PKB, and mTOR activity reduced pDCs generation from human cord blood-derived $CD34^+$ hematopoietic progenitors (HPCs) as well as their survival and function (van de Laar et al., 2012). Enhanced PKB activity increased the development and activation status, and cytokine production of CD34-derived pDCs in vivo (Van de Laar et al., 2012). The PI3K–Akt–mTOR pathway also controls the homeostasis of Langerhans cells (LCs) in the skin. Raptor, but not rictor-deficient DCs displayed a higher frequency of apoptosis. Diminished expression levels of Langerin, E-cadherin, β-catenin, and C-C chemokine receptor type 7 (CCR7) resulted in an increased tendency to leave the skin, which ultimately promoted migration of DCs toward skin-draining lymph nodes (sLNs). However, decreased expression of CCR7 did not affect the migration of LCs toward sLNs. Rictor/mTORC2 deficiency resulted in a modest reduction of LCs in sLNs (Kellersch & Brocker, 2013). There are also evidences suggesting that PI3K–AKT–mTOR-mediated signaling regulates the molecular mechanisms of macrophage polarization (Weichhart et al., 2015). Rapamycin-mediated inhibition of mTORC1 in human macrophage promoted M1 macrophage polarization and found to shift cytokine/chemokine secretion profile toward M1-like response. In contrast to M1 macrophage, rapamycin treatment-induced macrophage apoptosis in M2 but not in M1 (Mercalli et al., 2013). Myeloid-specific deletion of TSC1 had shown to promote M1 macrophage polarization through activation of Ras GTPase–Raf1–MEK–ERK pathway in an mTOR-independent manner (L. Zhu et al., 2014). These results indicate a crucial role of PI3K–AKT–mTOR in macrophage polarization and survival.

Genetic and pharmacological inhibition of mTORC1 of the mTOR pathway enhanced the production of inflammatory cytokine IL-12, attenuated the expression of anti-inflammatory cytokine IL-10 (Haidinger et al., 2010), decreased IL-2 secretion (Schmitz et al., 2008), and inhibited antiviral cytokine production by DCs via modulating the outcome of IFN secretion (Cao et al., 2008; Fekete et al., 2014). Everolimus is considered as a derivate of rapamycin (Rapamycin analog; Rapalog), and it acts as potential inhibitor of mTORC1. Everolimus treatment increased the frequency of circulating myeloid-derived suppressor cells (MDSCs), monocytes, and DCs in patients with metastatic renal cell carcinoma (mRCC) (Huijts et al., 2017). Taken together, these findings suggested a critical role for mTOR in the control of innate immune cells development and function.

3.2 | mTOR signaling regulates Treg cell development, function, and homeostasis

$CD4^+CD25^+Foxp3^+$ Treg cells appear to control immune responses to self and foreign antigens and play an indispensable role in immune tolerance. Development, function, and survival of Tregs have found to be fundamentally dependent on their constitutive expression of the transcription factor forkhead box P3 (FOXP3; Piccirillo, d'Hennezel, Sgouroudis, & Yurchenko, 2008). Research with conditional mTOR-deficient T-cells has demonstrated that the mTOR signaling pathway was vital for the differentiation of effector $CD4^+$ Th1, Th2, and Th17 T-cells subsets (Delgoffe et al., 2009). Murine T-cells treated with rapamycin-generated high numbers of $CD4^+CD25^{bright}$ T-cells that exhibited suppressive activity. However, rapamycin treatment upregulated FOXP3 expression and enhanced Treg differentiation. Transforming growth factor-β (TGF-β) dependent activation of mothers against decapentaplegic homolog (SMAD) 3 was essential for induction of FOXP3, which, in combination with the T-cell receptor (TCR)-induced nuclear factor of activated T-cells, promoted FOXP3 expression (Fan & Turka, 2018; Tone et al., 2008). Inhibition of SMAD3 through Akt–mTOR pathway-mediated signaling attenuated FOXP3 upregulation and provided a description to observe that mTOR inhibition through rapamycin-induced FOXP3 expression (Song, Wang, Krebs, & Danielpour, 2006).

Direct interaction between mTOR and signal transducer and activator of transcription 5 (STAT5) signaling has been shown to regulate the differentiation of effective T-cells (Teff) and Tregs

(Treg–Teff balance; Shan et al., 2015). STAT5-mediated induction and maintenance of FOXP3 expression play a significant role in Treg development, metabolic status, and homeostasis (Passerini et al., 2008; Shan et al., 2015). According to research, miRNA (miR)-155 negatively regulates Treg function by inhibiting suppressor of cytokine signaling 1 (SOCS1) expression. SOCS1 is a negative regulator of STAT5 signaling. Rapamycin-mediated mTORC1 inhibition upregulated STAT5 signaling via miR-155 upregulation; therefore, it influences proliferation and reciprocal differentiation of Treg and Teff cells (Figure 1; Lu et al., 2009; Yao et al., 2012).

Phosphatidylinositol mannoside-2 (Pim-2) is a serine/threonine-protein kinase that shares overlapping downstream targets, Bad and 4E-BP1, with Akt and mTOR. Pim-2 regulates energy metabolism and cell growth and mediates rapamycin-resistant survival (White, 2003). It has been reported that freshly isolated Treg cells, other than $CD4^+CD25^-$ effector T-cells, exhibit constitutive expression of Pim-2. FOXP3-specific mediated expression of Pim-2 allows for a selective growth advantage in the presence of rapamycin and thereby describes the resistance of Treg cells to rapamycin (Basu, Golovina, Mikheeva, June, & Riley, 2008; Fox, Hammerman, & Thompson, 2005).

Inhibition of mTOR drives Tregs to STAT5–pim-2-dependent proliferation and enhances their proliferation at the late stage of amplification through STAT5–Pim-2–4E-BP1 signaling (Figure 1; Shan et al., 2015). Activation of STAT5–Pim-2–4E-BP1 signaling after mTOR inhibition may explain the incomplete inhibition of Treg proliferation, and explore why rapamycin inhibits effector T-cell growth and delays Treg proliferation.

Recently, researchers who studied distinct levels of mTORC1 activation described a subset of $mTORC1^{hi}$ effector Tregs (eTregs) that had increased glycolytic metabolism and $mTORC1^{lo}$ central Tregs (cTregs). Inhibition of mTOR during T-cell activation favored the long-lived cTreg generation with a memory-like phenotype in mice, which occurred when eTreg generation depended on mTOR function. The *Rptor* deletion was accompanied by decreased expressions of inducible T-cell costimulator (ICOS) and programmed cell death-1 (PD-1) on the eTregs along with decreased suppressive activity (Sun et al., 2018). The authors proposed that strong naïve $CD4^+$ T-cell stimulation contributed to the generation of $mTORC1^{hi}$ eTregs and conversely $mTORC1^{lo}$ cTregs that represented a memory-like phenotype.

Enhanced mTOR signaling was accompanied by improved Treg cell suppressive activity. Inhibition of mTOR activity has also found to mitigate the ability of activated Treg cells to suppress conventional T-cell proliferation and reduces the expression of the Treg-associated molecules, the cytotoxic T-lymphocyte associated antigen (CTLA)−4 and ICOS (Chapman et al., 2018). It had been demonstrated that upregulation of interferon regulatory factor (IRF) 4 and mitochondrial metabolism by mTOR, caused mTOR to induce metabolic reprogramming to glycolytic and nucleotide metabolisms when Tregs were activated (Chapman et al., 2018). According to the results, it was undeniable that mTOR played an essential role in the maintenance of immune homeostasis and suppressive activity of activated Tregs by integration of transcriptional and metabolic programs. TSC1-deficient T-cells have augmented mTORC1 but diminished mTORC2 activity and attenuated Akt-mediated phosphorylation of forkhead box protein O1 (FOXO1)/FOXO3a, which leads to improved stability and function of FOXO1/FOXO3a (Figure 3). Generating high numbers of immunosuppressive Treg cells ex vivo through inhibition of mTOR have been revealed to be a potentially valuable strategy for allotransplanted patients or for the control of autoimmune disorders (Guillot-Delost et al., 2008). mRCC patients treated with mTOR inhibitor everolimus showed a slight increase in highly immunosuppressive $CD25^{hi}$ $FOXP3^+$ Treg cells in their circulation (Beziaud et al., 2016; Huijts et al., 2017).

Thus, mTOR signaling plays a vital role in regulating both the suppressive function of activated Treg cells and immune system homeostasis. mTOR signaling is a potential therapeutic strategy for the manipulation of immune system responses, and a potential therapy for treatment of autoimmunity, transplant rejection, infectious diseases, and cancer.

3.3 | Effector or memory $CD8^+$ T-cells fate is intimately linked to mTOR signaling pathway

3.3.1 | Transcriptional control of memory $CD8^+$ T-cells by mTOR

Researchers have identified multiple transcription factors involved in the regulation of effector and memory $CD8^+$ T-cell development. During acute and chronic infections, antigen-specific $CD8^+$ T-cells undergo several transcriptional changes before they develop into effector and memory cells.

The T-box transcription factor, T-bet, encoded by Tbx21, is a member of the T-box family. The T-box family is a "master regulator" of cell-mediated immunity because it controls the expressions of critical genes that encode numerous effector molecules for NK cells, and $CD4^+$ and $CD8^+$ T-cells (Intlekofer et al., 2005). These phenomena are controlled by complex signaling mechanisms where key molecules T-bet, eomesodermin (Eomes), and B lymphocyte-induced maturation protein 1 (Blimp-1) have important roles (Kaech & Cui, 2012). Eomes is a member of the T-box transcription factor family that expresses in both activated $CD8^+$ T-cells and NK cells (Pearce et al., 2003; Townsend et al., 2004). In early-activated $CD8^+$ T-cells, T-bet expression activates through TCR signaling and IL-12 mediated signals (Joshi et al., 2007; Takemoto, Intlekofer, Northrup, Wherry, & Reiner, 2006). In addition, IL-12 enhances mTOR activity in $CD8^+$ T-cells through PI3K and the STAT4 pathway, after which mTOR activity induces sustained T-bet expression (Rao, Li, Odunsi, et al., 2010). mTOR activity in conjunction with TCR signaling amplifies T-bet expression. Following T-bet expression, runt-related transcription factor 3 (RUNX3) and the IL-2 signaling pathway induce Eomes expression; however, Eomes is repressed by IL-12 and mTOR via inhibition of transcriptional activator FOXO1 (Figures 1,3; Cruz-Guilloty et al., 2009; Pipkin et al., 2010; Rao, Li, Gubbels Bupp, & Shrikant, 2012). There is sustained T-bet expression during viral infections, which induces the development of terminally differentiated $KLRG1^{hi}IL\text{-}7R\alpha^{low}$ effector $CD8^+$ T-cells (Joshi et al., 2007). This indicates an important role for mTOR inhibitors in induction of

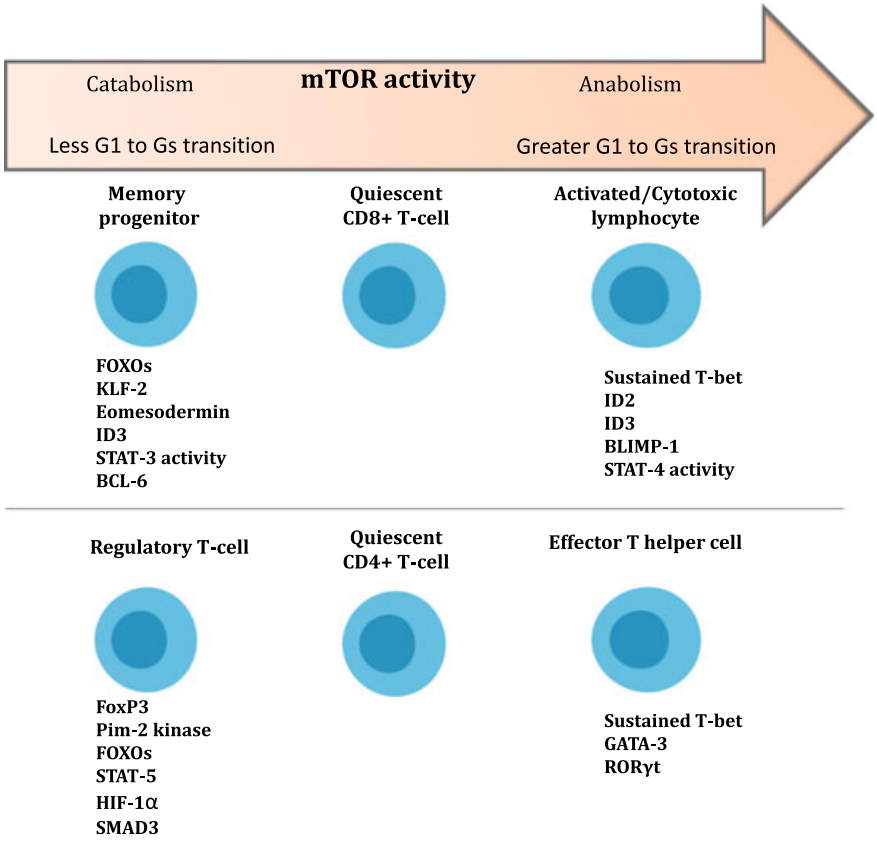

FIGURE 2 Phenotypic changes associated with mTOR activity. The mTOR signaling pathway regulates several transcriptional programs that control immune cells development and homeostasis. The quiescence state in T-cells refers to the active state that is controlled by several factors. Activation and effector generation for both $CD4^+$ and $CD8^+$ T-cells rely on remarkable metabolic reprogramming and results in a shift from catabolism to anabolism. Conversely, the switch between $CD4^+$ and $CD8^+$ T-cells, effector cells, and $CD4^+$ and memory $CD8^+$ T-cells is related to a metabolic switch from anabolic to catabolic pathways. Activation of mTOR supports the increased metabolism in $CD4^+$ and $CD8^+$ effector T-cells. mTOR activation profoundly affects the expression and activity of several transcription factors that determines the fate of effector or memory T-cells and orchestrates cell metabolism. BCL6: B cell lymphoma 6; CD: cluster of differentiation; FOXO: forkhead box protein O; FOXP3: forkhead box P3; HIF-1α: hypoxia-inducible factor-1α; ID: inhibitors of DNA; KFL: Krüppel-like factor; mTOR: mammalian target of rapamycin; Pim-2: phosphatidylinositol mannoside-2; SMAD3: mothers against decapentaplegic homolog 3; STAT: signal transducer and activator of transcription [Color figure can be viewed at wileyonlinelibrary.com]

memory formation. Other crucial transcription factors involved in effector and memory $CD8^+$ T-cells differentiation include inhibitors of DNA binding 2 (ID2), ID3, and Blimp-1 (Figure 2). Effector $CD8^+$ T-cells express both ID2 and ID3, which maintain $CD8^+$ T-cells survival during differentiation from naive to effector cells, and effector to memory cells transition (Ji et al., 2011; C. Y. Yang et al., 2011). ID3 is a key molecule for the development of long-lived memory $CD8^+$ T-cells. Blimp-1 represses ID3 expression as a direct target in effector $CD8^+$ T-cells (Ji et al., 2011; C. Y. Yang et al., 2011). Blimp-1 is required for differentiation into perforin$^+$, GzmB$^+$, and cytolytic effector $CD8^+$ T-cells (Rutishauser et al., 2009) (Figure 2).

3.3.2 | The mTORC1 signaling pathway: A master regulator of memory $CD8^+$ T-cells development and target of mTOR to enhance vaccine immunotherapy

T-cells acquire diverse-positive and -negative signals that control their growth, function, and differentiation during initial exposure to an antigen. However, research has not fully determined the mechanisms by which these signals work together to modulate T-cell memory. $CD8^+$ T-cells provide a fundamental component of protective immunity against cancer. Therefore, induction of an effective $CD8^+$ T-cells response is a main goal for vaccine development against tumors (Herrmann et al., 2010; Klebanoff et al., 2006; Tewari, Sacha, Gao, & Suresh, 2004). The transition between $CD8^+$ effector and memory $CD8^+$ T-cells is related to the metabolic switch from the anabolic to catabolic pathways and by orchestrating relevant and specific transcriptional reprogramming. Several studies have implicated mTOR in the switch between effector and memory cells (Figure 2) (He et al., 2011; Pearce et al., 2009; Rao, Li, & Shrikant, 2010; Rao, Li, Odunsi, et al., 2010). Under physiological conditions the role of mTOR in differentiation of memory $CD8^+$ T-cells appears to be due to AMP-kinase-α1 (AMPK-α1)-mediated sensing of the metabolic stress following glucose deprivation. This, in turn, leads to mTOR inhibition, which favors long-lived central memory $CD8^+$ T-cells development. AMPK-$\alpha1^{null}$ T-cells have shown significant impairment in their capability to develop memory $CD8^+$ T-cells responses following a

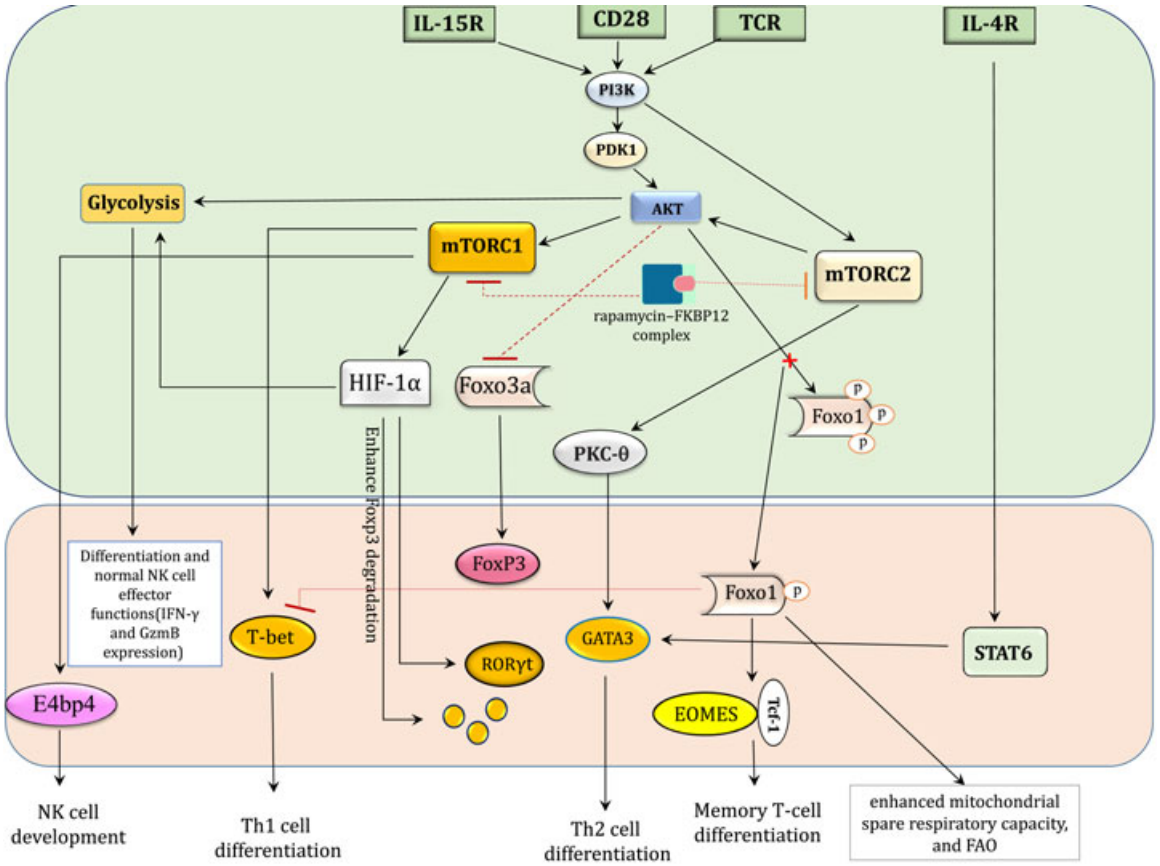

FIGURE 3 Regulation of immune cells development by mTORc1 and mTORc2. The precise functions of mTORC2-mediated metabolic reprogramming in immune cells functions should be explored. Similar to mTORC1, mTORC2 activation is important in regulation of immune cells differentiation. TSC1-deficient T-cells showed augmented mTORC1 but diminished mTORC2 activities and attenuated Akt-mediated phosphorylation of FOXO1/FOXO3a, which leads to improved stability and function of FOXO1/FOXO3a. Tcf-1 activates downstream of the Wnt signaling pathway. Tcf-1 deficient mice lacked the ability to develop memory $CD8^+$ T-cells. Akt-mediated phosphorylation of FOXO1 leads to its cytoplasmic localization, resulting in loss of transcriptional activity. Rictor deficiency leads to reduced activation of Akt, resulting in lower phosphorylation of FOXO1 with subsequent transfer and nuclear accumulation of FOXO1. FOXO1 directly promotes the expression of both Eomes and Tcf-1. In vivo and in vitro studies have shown the PI3K–Akt–mTOR–S6K1/2 pathway positively regulates Th17 differentiation. TGF-β and IL-6 are involved in Th17 differentiation. TGF-β induces Th17 differentiation by induction of critical transcription factor RORγt and inhibits the expression of Gfi1, which negative regulator of Th17 differentiation. mTORC1 pathway promotes Th17 differentiation in a S6K1/2-dependent manner. PI3K–Akt–mTORC1 axis inhibition leads to impaired downregulation of Gfi1 during Th17 differentiation. The PI3K–Akt–mTORC1 axis, by downregulating EGR1 as a negative regulator of Gfi1 expression, promotes Th17 differentiation. Further studies have shown that S6K2, a nuclear counterpart of S6K1, binds to RORγt, and transports RORγt to the nucleus. HIF-1α expression positively regulates Th17 differentiation by directly activating RORγt transcription and synergistic activity with RORγt regulates Th17-related gene expression and inhibits Treg cell generation. Akt: protein kinase B; CD: cluster of differentiation; EGR1: early growth response 1 ; FOXO: forkhead box protein O; Gfi1: growth factor-independent protein 1; HIF-1α: hypoxia-inducible factor-1α; mTORC1: mammalian target of rapamycin complex-1; Rictor: rapamycin-insensitive companion of mTOR; PI3K: phosphoinositide 3-kinase; RORγt: retinoic acid-related orphan receptor gamma t; Tcf-1: transcription factor T-cell factor 1; TGF-β: tumor growth factor-β; Th17: T helper 17 cells; TSC: tuberous sclerosis complex [Color figure can be viewed at wileyonlinelibrary.com]

Listeria monocytogenes infection. AMPK-α1, by monitoring energy stress and subsequent inhibition of mTOR activity in an AMPK-dependent manner, controls $CD8^+$ T-cells memory responses (Rolf et al., 2013). mTOR appeared to be a critical regulator of $CD8^+$ T-cells differentiation, particularly memory T-cells. Rapamycin, as an immunosuppressive drug, has shown to augment the development of memory $CD8^+$ T-cells and promoted a better protective response during vaccination (Araki et al., 2009; D'Souza, Parish, McKay, Kaech, & Shadel, 2011; Ferrer et al., 2010; Rao, Li, Odunsi, et al., 2010).

According to research, virally infected mice treated with rapamycin exhibited surprising immunostimulatory effects on the generation, expansion, and maintenance of viral-specific memory $CD8^+$ T-cells. Mice infected with lymphocytic choriomeningitis virus (LCMV) in the presence of a low dose of rapamycin showed enhanced quantity and quality of virus-specific memory $CD8^+$ T-cells in a T-cell-intrinsic manner. Higher doses of rapamycin inhibited the T-cells response. Researchers observed that rapamycin treatment following vaccination of nonhuman primates with

modified vaccinia virus Ankara (MVA) had enhanced memory T-cell responses. During the expansion phase (Days 0–8 postinfection), rapamycin treatment increased the number of memory precursors that survived and differentiated into long-lived memory T-cells. Interestingly, during transition from effector to memory T-cells from Days 8–30 (contraction phase or memory generation), rapamycin accelerated the transition from effector to memory T-cells and further improved the quality of memory $CD8^+$ T-cells. There was no effect on the number of memory T-cells. Furthermore, the quality and amount of memory $CD8^+$ T-cells improved with rapamycin administration during the expansion and contraction phases (Araki et al., 2009). Thus, in rapamycin-treated mice, there were substantially increased numbers of highly functional memory $CD127^{hi}$, $CD62L^{hi}$, $KLRG1^{low}$, $CD27^{hi}$, and $Bcl2^{hi}CD8^+$ T-cells. The rapamycin-treated mice exhibited strong protective capacity, had increased life-spans, and better recall response in their formed memory $CD8^+$ T-cells. Researchers used a retrovirus based RNA interference (RNAi) system to specifically knockdown mTOR, raptor, S6K1, eIF4E, and FKBP12 expressions in antigen-specific $CD8^+$ T-cells. The results have demonstrated that mTORC1 acted as the key regulator of memory $CD8^+$ T-cell differentiation by direct regulatory action on the mTORC1 pathway in antigen-specific $CD8^+$ T-cells. mTOR, or raptor knockdown cells, remarkably elevated expression levels of memory T-cell markers CD127, CD62L, Bcl-2, and CD27. Significant increases in memory $CD8^+$ T-cell differentiation after knockdown of S6K1 and eIF4E showed that mTOR exerted its effect through these two downstream molecules (Araki et al., 2009). Pearce et al. (2009) reported similar effects of rapamycin on induction of memory $CD8^+$ T-cell differentiation. They observed that mice with a T-cell-specific deletion of TNF receptor-associated Factor 6 (TRAF6) exhibited strong effector responses after immunization, whereas it showed the inability to generate long-lived memory $CD8^+$ T-cells following immunization. TRAF6-deficient $CD8^+$ T-cells had less active AMPK levels and displayed defects in mitochondrial fatty acid oxidation (FAO) after withdrawal of growth Factor IL-2. Administration of metformin, an antidiabetic drug and AMPK activator, rescued FAO in TRAF6-deficient $CD8^+$ T-cells and subsequently induced $CD8^+$ T-cell survival and memory development (Pearce et al., 2009). The activated AMPK-inhibited mTOR activity by direct phosphorylation of two proteins, TSC2 and raptor (Figure 1; Hardie, 2008; Shaw, 2009). Metformin, by indirect inhibition of mTOR, could accelerate memory T-cells development. Treatment with another FAQ-inducing drug, rapamycin, significantly induced memory $CD8^+$ T-cell development in both TRAF6-defective T-cells and wildtype T-cells (Pearce et al., 2009). This study also confirmed the key role of rapamycin treatment in accelerating memory $CD8^+$ T-cell differentiation. Rapamycin also augmented the quantity and quality of the antigen-specific T-cell response following a Listeria infection (Ferrer et al., 2010). Rao, Li, Odunsi, et al. (2010) illustrated the underlying role of mTOR in instructional programming of naive $CD8^+$ T-cells toward the effector and/or memory fate by controlling T-bet and Eomes expressions. IL-12 has been shown to induce mTOR phosphorylation, which is fundamental for sustained T-bet expression during development of effector $CD8^+$ T-cell maturation. The synergistic functions of both PI3K and STAT4 were essential for the effects of IL-12 on mTOR activity. Inhibition of mTOR through rapamycin-induced a switch between T-bet to Eomes expression that resulted in persistent Eomes expression and enhanced memory precursor $CD8^+$ T-cell generation, which contributed to enhanced antitumor efficacy (Figures 1 and 3; Rao, Li, Odunsi, et al., 2010). As shown, changes to the rapamycin treatment regimen and duration could affect the quantity and quality of memory $CD8^+$ responses induced by viral vaccination, which enhanced tumor suppression and increased survival rates in animal models. Notably, mice treated with a short course of rapamycin had enhanced antigen-recall responses in numerous $CD8^+$ T-cells and IFN-γ, in addition to GzmB production. High dose rapamycin caused a greater memory response than the lower dose. An extended duration of high dose rapamycin abrogated memory $CD8^+$ responses due to loss of memory $CD8^+$ T-cells. Extended duration of a low dose of rapamycin was accompanied by a weak antigen-recall response with elevated IFN-γ, but no GzmB expression. Of note, the generation of memory $CD8^+$ T-cell responses following a high dose of rapamycin were independent from IL-15 (Li et al., 2012). Thus, the dose dependency of rapamycin to induce efficient vaccine-induced memory $CD8^+$ T-cell responses in terms of quantity and quality could be a novel therapeutic approach to improve vaccine efficacy and develop new vaccination strategies for cancer or infectious agents. Understanding the exact mechanisms of these phenomena can be important to optimize immunization strategies and cancer immunotherapy.

HP is the proliferation of naive $CD8^+$ T-cells in an antigen-independent manner, including self-antigen-MHC; cytokines such as IL-7 and/or IL-15 play key role in maintenance of normal $CD8^+$ T-cell numbers (Tan et al., 2002). T-bet and Eomes directly target the gene expression of the IL-2 receptor β chain (CD122) which enables IL-15-mediated signaling and maintains memory $CD8^+$ T-cells homeostasis (Intlekofer et al., 2005; Joshi et al., 2007). IL-7, but not IL-15, induced mTOR kinase activation is essential for HP of naïve $CD8^+$ T-cells. Induction of HP through mTOR promotes functional $CD8^+$ T-cell maturation by enhancing T-bet and, subsequently, CD122 expression. IL-7-induced mTOR activity is essential for T-bet expression. T-bet is a key enhancer of CD122 expression. As IL-15 is critical for $CD8^+$ T-cell preservation during HP, it is not surprising that mTOR integrates IL-7 and IL-15 signals to control HP-induced $CD8^+$ T-cell memory. The mTOR signaling blockade can prevent T-bet and CD122 expression, and promote Eomes expression to preserve cell-intrinsic based memory $CD8^+$ T-cells in an IL-15-independent manner (Figures 1 and 3). HP-induced memory $CD8^+$ T-cells that have been generated in both IL-15 dependent or independent manners displayed identical tumor efficacies (Li et al., 2011). These findings showed the important role of HP-induced $CD8^+$ T-cell responses through mTOR kinase in tumor efficacy.

Pharmacological agents can have undesirable immunosuppressive effects due to their broad targets and unwanted side effects. For example, the development of immunosuppressive $FOXP3^+$ Tregs

following inhibition of mTOR with rapamycin (Delgoffe et al., 2009) suppresses the functional activation of DC to become tolerogenic antigen presenting cells (Hackstein et al., 2003; Turnquist et al., 2007). Another insight into the mechanism for mTOR regulation of in vivo memory $CD8^+$ T-cell development could be ascertained from a recent study that used an small interfering RNA (siRNA) targeting raptor conjugated into a specific oligonucleotide aptamer bound to 4-1BB. The result was an enhanced generation of functionally memory $CD8^+$ T-cells. Systemic administration of the 4-1BB aptamer–raptor siRNA to mice was able to decrease mTORC1 activity in $CD8^+$ T-cells and led to the generation of an effective memory response that contributed to prolonged survival, enhanced proliferative capacity, and cytotoxic effector functions. As a result, the researchers observed increased vaccine-induced protective antitumor immunity in tumor-bearing mice. In contrast to 4-1BB aptamer–raptor siRNA conjugates, rapamycin-generated memory $CD8^+$ T-cells impaired cytotoxic effector functions, diminished the alloreactivity of DCs, and could not successfully control tumor growth in rapamycin-treated mice (Berezhnoy, Castro, Levay, Malek, & Gilboa, 2014). The findings indicated that this approach could be a useful strategy for enhancing memory responses and selection of specific targets on activated $CD8^+$ T-cells to further improve its specificity and therapeutic potential.

Aberrant $CD8^+$ T-cell responses and development of memory $CD8^+$ T-cells in ataxia-telangiectasia (A-T) patients and mice that lack the protein kinase A-T mutated (ATM) have a relationship with hyperactivation of Akt and mTORC1 signaling following TCR activation. This defect in memory $CD8^+$ T-cell formation was reversed when $ATM^{-/-}$ mice infected with LCVM received rapamycin, despite the fewer numbers of naïve $CD8^+$ T-cells, which showed an increase in the number of $CD127^{hi}$ $KLRG1^{low}$ memory precursor $CD8^+$ T-cells and $CD62L^{hi}$ T_{CM} cells (D'Souza et al., 2011). Based on evolution, stem cell memory T (T_{SCM}) are located between naïve T-cell and memory T-cells. They are highly proliferative, have the capability for self-renewal and multipotent capacity to differentiate into other T-cell subsets, including T_{CM}, T_{EM}, and effector cells (Farber, Yudanin, & Restifo, 2014; Gattinoni et al., 2011). Activation of WNT–β-catenin signaling has found to play a critical role in induction of T_{SCM} formation (Gattinoni et al., 2009). It has been discovered that inhibition of mTORC1 with rapamycin induced $CD4^+$ and $CD8^+$ T_{SCM} formation independent of Wnt signaling (Scholz et al., 2016). The use of rapamycin for efficient induction of antigen-specific T_{SCM} cells could be a novel, potential T-cell-based immunotherapy for cancer.

Inhibition of mTOR can change the intrinsic properties of T-cells to affect their differentiation into effector or memory cells, and may be an attractive strategy to enhance antitumor responses. The precise molecular mechanisms involved in the development of memory $CD8^+$ T-cells are not fully elucidated. Hence, identification of the exact molecular integration of the mTOR signaling pathway with distinct transcriptional programs associated with memory $CD8^+$ T-cell development may open new horizons for pharmacological agents to enhance T-cell memory responses in humans.

3.3.3 | mTORC2 controls memory $CD8^+$ T-cell differentiation

The physiological role of mTORC2 is not as well defined as mTORC1. In contrast to mTORC1, mTOR in mTORC2 is resistant to rapamycin (Thomson et al., 2009). The precise functions of mTORC2-mediated metabolic reprogramming in immune cells functions have yet to be determined. Rictor-deficient T-cells exhibit reductions in T-bet and GATA-3 expressions, which leads to impaired differentiation into Th1 and Th2 cells (Figure 3; Lee et al., 2010). mTORC2 phosphorylates Akt, which results in inhibition of FOXO3 and FOXO1 (Guertin et al., 2006). mTORC2 directly phosphorylates protein kinase C (PKC), serum and glucocorticoid-regulated kinase 1 (SGK1) to regulate important cellular processes such as reorganization of actin cytoskeleton, in addition to promotion of cell survival and metabolic reprogramming via Akt (Goncharova et al., 2011; Zinzalla, Stracka, Oppliger, & Hall, 2011). The exact upstream activators and downstream signaling mediated by mTORC2 signaling are unknown. Similar to mTORC1, activation of mTORC2 is important for regulation of memory $CD8^+$ T-cell differentiation (Figure 3). Zhang et al. have reported a crucial regulatory role for mTORC2 in memory $CD8^+$ T-cell generation. Rictor-deficient $CD8^+$ T-cells had higher levels of $KLRG1^{low}$ $CD127^{hi}$ memory precursor effector cells (MPECs) and reduced levels of $KLRG1^{hi}$ $CD127^{low}$ short-lived effector cells (SLECs). There was higher BCL-2 expression in Rictor-deficient $CD8^+$ cells compared with wildtype $CD8^+$ cells (Zhang et al., 2016). Rictor-deficient $CD8^+$ cells were rich in IL-2-producing T-cell subsets (Zhang et al., 2016). Of note, IL-2-producing MPECs more efficiently differentiated into long-lived protective memory T-cells that had the capability for self-renewal and potent recall responses (Joshi et al., 2007; Sarkar et al., 2008).

The transcription factor FOXO1 is essential for memory $CD8^+$ T-cell generation as it directly controls Eomes, Krüppel-like Factor 2 or lung Krüppel-like factor (KLF2), and transcription factor T-cell Factor 1 (Tcf-1) expressions (Fabre et al., 2008; Tejera, Kim, Sullivan, Plisch, & Suresh, 2013). Rictor-deficient enhanced memory $CD8^+$ T-cell generation had more potent recall responses after antigen rechallenge. This recall response was related to transcriptional reprogramming, which included upregulation of Eomes and Tcf-1, repression of T-bet, and metabolic reprogramming such as enhanced mitochondrial spare respiratory capacity and FAO in an FOXO1 dependent manner (Figure 3; Zhang et al., 2016). Tcf-1 activation downstream of the Wnt signaling pathway and Tcf-1 deficient mice resulted in impaired development of memory $CD8^+$ T-cells (Jeannet et al., 2010). Akt-mediated phosphorylation of FOXO1 leads to its cytoplasmic localization, resulting in the loss of transcriptional activity. Rictor deficiency leads to reduced activation of Akt, resulting in lower phosphorylation of FOXO1 with subsequent transfer and nuclear accumulation of FOXO1. FOXO1 directly promotes the expressions of Eomes and Tcf-1. Thus, similar to mTORC1, these findings show that the mTORC2–Akt–FOXO1 signaling axis regulates memory $CD8^+$ T-cell generation through specific transcriptional and metabolic reprogramming (Zhang et al., 2016). mRCC patients treated

with everolimus have elevated levels of Eomes^{+}CD8^{+} T-cells (Beziaud et al., 2016).

Based on the current evidence, mTORC2 inhibition can possibly be a useful strategy for immunotherapy approaches to generate effective memory responses against infectious agents and cancer.

3.3.4 | The upstream and downstream mechanisms involved in mTOR-dependent regulation of memory CD8^{+} T-cell generation

There is a lack of information about the exact upstream mechanisms that control mTOR activity or downstream mechanisms regulated through mTOR. It is important to determine how the numerous molecular events that occur upstream and downstream of mTOR regulate and control memory CD8^{+} T-cell development.

Mice with a specific deletion of TSC1, a negative regulator of mTOR signaling, display normal distributions of CD4^{+} and CD8^{+} T-cells, homeostasis, and normal CD62L, CD44, and CD127 expressions. Wildtype and TSC1$^{-/-}$ antigen-experienced CD8^{+} T-cells have shown similar effector responses, while TSC1$^{-/-}$ effector CD8^{+} T-cells exhibited defects in the differentiation of memory T-cell precursors and transition from effector responses into memory T-cells as well as antigen-recall responses. TSC1$^{-/-}$ T-cells enhanced expression of T-bet and Blimp-1, which resulted in excessive mTORC1 activation and metabolic dysregulations that included glycolysis and oxidative phosphorylation after IL-15 stimulation, and affected memory CD8^{+} T-cell differentiation. Enhanced expressions of T-bet and Blimp-1 favored short-lived effector cell (SLRC; CD127^{low}KLRG1^{hi}) differentiation, but there was an inverse association with MPEC (CD127^{hi}KLRG1^{low}; Shrestha et al., 2014). These results indicated that Tsc1 promoted memory CD8^{+} T-cell responses partly by orchestrating transcriptional programs necessary for memory precursor differentiation. Genomics analysis of TSC1$^{-/-}$ antigen-experienced CD8^{+} T-cells have explored the differential gene expression profile that correlated with differentiation of effector and memory CD8^{+} T-cells and included receptors (Il12rb2, IL7r, and Sell), secreting factors (Gzmb), and transcription factors (Tcf7, Bach2, and KLF4), in addition to genes involved in various metabolic pathways (glycolysis and oxidative phosphorylation) in response to IL-15 stimulation. Possibly, TSC1 organizes expression of the immune response and metabolic associated genes in antigen-specific CD8^{+} T-cells and leads to memory CD8^{+} T-cell development (Shrestha et al., 2014). Akt–mTOR activation was negatively regulated by phosphatase and PTEN. At this point, the deletion of PTEN had no significant defects in virus-specific memory CD8^{+} T-cell formation in an LCMV infection (Hand et al., 2010). The combination of anti-CTLA-4 with rapamycin during in vivo CD8^{+} T-cell priming resulted in both increased frequency of long-lived memory CD8^{+} T-cells and enhanced memory responses to tumors and bacterial challenges. This increased effectiveness in mice treated with anti-CTLA-4 and rapamycin during immunization was related to early elevations in expansion and memory precursor differentiation of CD8^{+} T-cells, enhanced FAO, expanded mitochondrial biogenesis, significantly increased respiratory capacity, and effector cytokine production in memory CD8^{+} T-cells (Pedicord, Cross, Montalvo-Ortiz, Miller, & Allison, 2015). The combination of rapamycin plus the OX40 (CD252) agonist showed a similar effect on memory CD8^{+} T-cell formation and protective immunity produced by recombinant adenovirus vaccines (Bassett et al., 2012). The ability of PD-1 to suppress PI3K–Akt–mTOR pathway activation depended on suppression of CD3–CD28-induced signaling (Parry et al., 2005) and subsequently reduced glycolysis, and promoted lipid metabolism and FAO (Patsoukis et al., 2015). On the one hand, negative costimulator PD-1 prevented excessive CD8^{+} T-cell activation and on the other hand, it promoted memory formation (Charlton et al., 2013). Manipulation of the PD-1 pathway and a wide range of signaling molecules downstream and upstream of mTOR, as well as extracellular stimuli and/or inhibitory factors, could enhance the efficacy of certain vaccinations by inducing memory CD8^{+} T-cell generation to induce their antitumor activity and antiviral responses.

3.4 | mTOR signaling pathway enhances Th17 differentiation by multiple mechanisms

mTOR signaling pathway affects all aspects of T-cell subsets—development, differentiation, homeostasis, function, and proliferation (Powell, Pollizzi, Heikamp, & Horton, 2012; Thomson et al., 2009). The mTORC1 pathway has been shown to regulate Th17 differentiation through various mechanisms. The PI3K–Akt–mTOR axis negatively controls FOXP3 expression, and the normal and pathological conditions associated with Treg function (Haxhinasto, Mathis, & Benoist, 2008; Zheng & Rudensky, 2007). In vivo and in vitro studies show that the PI3K–Akt–mTOR–S6K1/2 pathway positively regulates Th17 differentiation. TGF-β and IL-6 are involved in Th17 differentiation. TGF-β induces Th17 differentiation by induction of critical transcription factor retinoic acid-related orphan receptor gamma t (RORγt) (Ivanov et al., 2006; X. O. Yang et al., 2008) and inhibits the expression of growth factor-independent protein 1 (Gfi1), which is a negative regulator of Th17 differentiation (J. Zhu et al., 2009). The mTORC1 pathway promotes Th17 differentiation in a S6K1/2-dependent manner. PI3K–Akt–mTORC1 axis inhibition leads to impaired downregulation of Gfi1 during Th17 differentiation. The PI3K–Akt–mTORC1 axis promotes Th17 differentiation by downregulating early growth response 1, a negative regulator of Gfi1 expression. Studies show that S6K2, a nuclear counterpart of S6K1, binds to RORγt and transports RORγt to the nucleus (Figure 1; Kurebayashi et al., 2012). The ability of mTOR to regulate STAT activation has been previously determined (Zhou et al., 2007). Like mTOR-deficient cells, blockade of the mTOR pathway by the deletion of *Rheb* exhibited decreased tyrosine phosphorylation of STAT3 and STAT4, which resulted in increased SOCS3 expression as a negative regulator of STAT signaling, and inhibited Th17 and Th1 cells differentiation. Surprisingly, the RHEB$^{-/-}$ T-cells failed to generate Th1 and Th17 responses in vitro and in vivo, and did not induce the classical experimental autoimmune encephalomyelitis (EAE). In contrast, Th2 cell development was unimpaired. Alternatively,

Rictor$^{-/-}$ T-cells (mTORC2-deficient T-cells) failed to differentiate into Th2, but retained Th1 and Th17 cells development (Delgoffe et al., 2011; Kurebayashi et al., 2012).

Hypoxia-inducible factor (HIF-1α) has been shown to regulate the balance between Treg and Th17 cells differentiation (Dang et al., 2011). Because Th17 cells relies more on glycolysis than other T-cell subsets, it can be concluded that HIF-1α is important for differentiation of Th17 (Shi et al., 2011). HIF-1α regulates glucose uptake and is one of the crucial elements for the glycolytic response that occurs downstream of mTORC1. Glycolysis is essential for rapid T-cell expansion (Duvel et al., 2010). Furthermore, HIF-1α expression positively regulates Th17 differentiation by direct activation of RORγt transcription. Synergism with RORγt regulates Th17-related gene expression and inhibits Treg cell generation by promoting the active process of FOXP3 depredating (Dang et al., 2011). mTOR signaling is essential to promote HIF-1α induction during Th17 cell differentiation. The HIF-1α dependent glycolytic pathway is an underlying component downstream of mTOR to mediate Th17 and Treg cells differentiation (Shi et al., 2011). Blocking HIF-1α-dependent glycolysis downstream of mTOR signaling results in enhanced Treg development by attenuating proteasome degradation of FOXP3. Concurrently, it could block T17 development by inhibition of RORγt transcription (Battaglia et al., 2006; Shi et al., 2011). Inhibition of mTORC1 by rapamycin attenuated the differentiation of Th17 cells and conversely induced the generation of FOXP3^{+} Treg cells (Kopf, de la Rosa, Howard, & Chen, 2007).

Signaling through IL-1 is critical for induction of Th17 cell differentiation (Chung et al., 2009). Single immunoglobulin IL-1 receptor-related protein (SIGIRR), a negative regulator of the IL-1 receptor and TLR, suppresses Th17 cell differentiation and proliferation through its inhibitory effects on IL-1 signaling. Interestingly, SIGIRR-deficient Th17 cells have been shown to exhibit higher IL-1-induced phosphorylation of JNK and mTOR kinase, and lacked IL-1-induced proliferation in mTOR-deficient Th17 cells (Gulen et al., 2010). These findings explained the ability of IL-1 to enhance Th17 development by stimulating mTORC1 in an IRAK (IL-1 receptor-associated kinase) protein dependent manner and consequently affected IRAK1-mediated disruption of the TSC1–TSC2 complex. In contrast to mTORC1, conditional Rictor-deficient T-cells could normally differentiate into Th1 and Th17 T-cells. mTORC2 dysfunction had no effect on Th17 and FOXP3^{+} Treg cells development (Delgoffe et al., 2011; Lee et al., 2010).

3.5 | The fundamental role of mTOR signaling in the differentiation and function of NK cell

NK cells are group 1 innate lymphoid cells (ILCs). They are a distinct subpopulation of lymphocytes classified as innate immune cells because of their ability to recognize and respond rapidly to target cells in the absence of prior sensitization and in a non-MHC restricted manner. Upon activation, NK cells kill a number of cancer cells, particularly when circulating in the bloodstream (Trinchieri, 1989). Transcription factors Pu.1, Ikaros, E4BP4, ETS1, and ID2 have found to be essential for NK cells to develop from a common lymphoid progenitor (Sun & Lanier, 2011). Reactive NK cells show a hyperactive Akt–mTOR signaling pathway during the steady state. Molecular rheostat of NK cell responsiveness depends on mTOR activity. mTOR pathway regulates the proportional NK cell reactivity in a SHP-1 dependent manner, which is required to maintain an optimal NK cell reactivity. Researchers have demonstrated that upon stimulation through activating NK cell receptors (NKars), the mTOR–Akt pathway augmented calcium flux and lymphocyte function-associated antigen 1 integrin activation as a hallmark of reactive NK cells (Marcais et al., 2017). Rapamycin has been shown to significantly inhibit proliferation of both RNK-16 cells and primary NK cell lines by blocking progression from the G1 to S phases in a dose-dependent manner (Wai, Fujiki, Takeda, Martinez, & Krams, 2008). Rapamycin was not effective on IFN-γ secretion by primary NK cell lines when compared with the control cultures. Rapamycin-treated NK cells showed remarkable defects in their proliferation and modestly inhibited NK cell-mediated killing against YAC-1 targets cells (Wai et al., 2008). Circulating NK cells also decreased in graft recipients treated with rapamycin (Wai et al., 2008). IL-15 plays a key role in the maturation, homeostasis, and peripheral activation of NK cells (Ranson et al., 2003). IL-15 prompts mTOR activity in human and mouse NK cells in a phosphoinositide dependent kinase 1 (PDK1) and Akt dependent manner (Marcais et al., 2014; Nandagopal, Ali, Komal, & Lee, 2014; M. Yang et al., 2015). NK cell exposure to high, but not low concentrations of IL-15, activated mTOR and improved the bioenergetic metabolism. Low doses of IL-15 triggered STAT5 phosphorylation. Thus, mTOR acts as a vital component of the IL-15 signaling pathway; an optimal response of NK cells to IL-15 depends on mTOR. mTOR$^{-/-}$ NK cells have decreased expressions of Eomes and T-bet, which are essential for NK cell maturation, as well as CD122 and IL-15 receptor-2γ (IL-R2γ). NK cell maturation, homeostasis, and activity were also affected by deletions in mTOR. When NK cell differentiation in the bone marrow of mTOR$^{-/-}$ mice was blocked at the CD11b^{lo}CD27^{hi} stage, the peripheral organs exhibited at least a six-fold-decrease in the number of NK cells. NK cell size also decreased in mTOR$^{-/-}$ mic. In addition, NKp46^{+} ILC3 cells were absent in the gut of mice that lacked mTOR, which showed the importance of mTOR for the generation of this population. Rapamycin-treated NK cells in the presence of IL-15, displayed an impaired proliferation capability in the bone marrow. IFN-γ expression resulted in elevated viral burdens in a murine cytomegalovirus infection (Marcais et al., 2014; Nandagopal et al., 2014). Possibly, suppression of NK cell activity likely contributed to the immunosuppressant properties of this drug in clinical studies (Marcais et al., 2014). mTOR$^{-/-}$ NK cells indicated lower expression of transmembrane nutrient receptor CD71 and glucose uptake. This finding suggested a correlation between NK cell metabolism and metabolic checkpoint kinase mTOR activity, and confirmed the crucial role of mTOR as a key part of the IL-15 signaling pathway for metabolic regulation and nutrient uptake during the development and activation of NK cells (Marcais et al., 2014). In accordance

with previous studies, mTOR$^{-/-}$ NK cell survival was not affected (Marcais et al., 2014), which suggested that the prosurvival signals induced by IL-15 activated STAT5 (Eckelhart et al., 2011). Donnelly et al. (2014) reported that mTORC1 signaling in activated NK cell was necessary to induce NK cell effector molecules including IFN-γ and GrzB. Activated mouse NK cells that underwent mTORC1-dependent metabolic reprogramming resulted in enhanced glucose uptake and induced aerobic glycolysis (Donnelly et al., 2014). Inhibition of mTOR signaling by rapamycin derived decreased levels of glycolysis that correlated with inhibited IFN-γ production and GzmB expression in activated NK cells. Therefore, mTORC1 activity, but not mTORC2, was a fundamental key regulator for glycolytic reprogramming of activated NK cells. This metabolic shift was a critical event for normal NK cell effector functions, such as production of IFN-γ and GzmB expression (Figure 3; Donnelly et al., 2014).

Transcription factor E4BP4 (encoded by the Nfil3 gene) has identified as critical for NK cell lineage and promotes NK development. E4bp4 is expressed before Eomes, Id2, and T-bet and by binding directly to the regulatory regions of both Eomes and *Id2* it directly regulates the expression of Eomes and Id2; therefore, it regulates the earliest stages of NK cell development (Male et al., 2014). Yang et al. have shown that PDK1 kinase upstream of mTOR acts as a bridge to connect IL-15 signaling to E4BP4. Pharmacological inhibition, including GSK2334470, Akti-1/2, and Torin1 for suppression of PDK1, Akt, and mTOR, respectively, had less upregulation of E4BP4 following IL-15 stimulation. *PDK1*$^{-/-}$ mice failed to activate mTOR and upregulate E4BP4 expression upon IL-15 stimulation, and showed significant reduction in CD122. As expected, *PDK1*$^{-/-}$ mice had reduced numbers of NK cells in their lymphoid organs. Interestingly, ectopic expression of E4BP4 or bypassed activation of mTOR recovered CD122 expression (M. Yang et al., 2015). These findings showed the importance of the PDK1–mTOR–E4BP4–CD122 axis signaling in early NK cell development. In vitro mouse or human NK treated by TGF-β, a major immunosuppressive cytokine, inhibited IL-15 induced activation of mTOR.

TGF-β, like rapamycin, decreased both metabolic activity and growth of NK cells, the amounts of various NK cell receptors, and the cytotoxic activity of human and mouse NK cells in response to IL-15. mTOR activity induced by IL-15 in NK cells improved with TGF-β blocking antibody. Interestingly, mTOR deletion and constitutive TGF-β signaling in NK cells showed similar effects on NK cell development and maturation, as well as cytotoxic activity. Therefore, mTOR, as an important integrator of cytokine signals in NK cells, has an important role in host defense (Viel et al., 2016). These findings open a new insight for the development of therapeutic approaches that increase mTOR activity in tumor-infiltrating lymphocytes, particularly NK cells, to reinstate their effector activity by counteracting the antiproliferative effects of TGF-β. Evaluation of immunological effects of everolimus treatment in patients with mRCC has shown reduced percentages of immunoregulatory CD56^{bright}CD16-$^{dim/-}$ NK cells. There were no significant changes in the percentage of CD56^{dim}CD16^{+} NK cells (Huijts et al., 2017).

mTOR activity is a vital factor for cell growth and metabolism. It is essential to sustain NK cell proliferation, maturation, and differentiation into their fully functional effector properties. In this regards, mTOR activity may be a critical therapeutic approach due to its enhanced antitumor activity.

4 | CONCLUSION

In this review, we highlighted the critical role of mTOR in regulating immune cell differentiation and function, and memory development. We also discussed the role of mTOR in regulating cellular metabolic function. Despite initial reports on the immunostimulatory effects of mTOR inhibitors on CD8^{+} T-cells, emerging evidence has indicated that different doses of these agents may differently affect CD8^{+} T-cells responses. Subsets of T-cells use different metabolic pathways and these pathways play key roles in regulating the outcome of T-cell differentiation, development, and activation. In this regard, we have emphasized the potential crossroads between mTOR signaling and immune cell biology, especially memory CD8^{+} T-cells development. These results of research studies emphasized the contributions by mTORC1 to metabolic reprogramming of immune cells following physiological or pathological activation of upstream signaling pathways. Promoting the level of understanding of underlying mechanisms that regulate mTOR signaling in immune cells can contribute to the novel strategies that manipulate mTOR activity for treating cancer and other diseases. Collectively, these findings indicate the strength and crucial role of the regulatory properties of mTOR signaling pathway in determining the immune cells fate decisions by integrating transcriptional and metabolic programs.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

Ahmed, R., & Gray, D. (1996). Immunological memory and protective immunity: Understanding their relation. *Science, 272*, 54–60.

Araki, K., Turner, A. P., Shaffer, V. O., Gangappa, S., Keller, S. A., Bachmann, M. F., … Ahmed, R. (2009). mTOR regulates memory CD8 T-cell differentiation. *Nature, 460*, 108–112. https://doi.org/10.1038/nature08155

Araki, K., Youngblood, B., & Ahmed, R. (2010). The role of mTOR in memory CD8 T-cell differentiation. *Immunological Reviews*, *235*, 234–243. https://doi.org/10.1111/j.0105-2896.2010.00898.x

Armand, P., Gannamaneni, S., Kim, H. T., Cutler, C. S., Ho, V. T., Koreth, J., … Antin, J. H. (2008). Improved survival in lymphoma patients receiving sirolimus for graft-versus-host disease prophylaxis after allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning. *Journal of Clinical Oncology*, *26*, 5767–5774. https://doi.org/10.1200/JCO.2008.17.7279

Bassett, J. D., Swift, S. L., VanSeggelen, H., Hammill, J. A., McGray, A. R., Evelegh, C., … Bramson, J. L. (2012). Combined mTOR inhibition and OX40 agonism enhances CD8(+) T cell memory and protective immunity produced by recombinant adenovirus vaccines. *Molecular Therapy*, *20*, 860–869. https://doi.org/10.1038/mt.2011.281

Basu, S., Golovina, T., Mikheeva, T., June, C. H., & Riley, J. L. (2008). Cutting edge: Foxp3-mediated induction of pim 2 allows human T regulatory cells to preferentially expand in rapamycin. *Journal of Immunology*, *180*, 5794–5798.

Battaglia, M., Stabilini, A., Migliavacca, B., Horejs-Hoeck, J., Kaupper, T., & Roncarolo, M. G. (2006). Rapamycin promotes expansion of functional $CD4^+CD25^+FOXP3^+$ regulatory T cells of both healthy subjects and type 1 diabetic patients. *Journal of Immunology*, *177*, 8338–8347.

Bayascas, J. R., & Alessi, D. R. (2005). Regulation of Akt/PKB Ser473 phosphorylation. *Molecular Cell*, *18*, 143–145. https://doi.org/10.1016/j.molcel.2005.03.020

Berezhnoy, A., Castro, I., Levay, A., Malek, T. R., & Gilboa, E. (2014). Aptamer-targeted inhibition of mTOR in T cells enhances antitumor immunity. *Journal of Clinical Investigation*, *124*, 188–197. https://doi.org/10.1172/JCI69856

Beziaud, L., Mansi, L., Ravel, P., Marie-Joseph, E. L., Laheurte, C., Rangan, L., … Adotévi, O. (2016). Rapalogs efficacy relies on the modulation of antitumor T-cell immunity. *Cancer Research*, *76*, 4100–4112. https://doi.org/10.1158/0008-5472.CAN-15-2452

Cao, W., Manicassamy, S., Tang, H., Kasturi, S. P., Pirani, A., Murthy, N., & Pulendran, B. (2008). Toll-like receptor-mediated induction of type I interferon in plasmacytoid dendritic cells requires the rapamycin-sensitive PI(3)K-mTOR-p70S6K pathway. *Nature Immunology*, *9*, 1157–1164. https://doi.org/10.1038/ni.1645

Chapman, N. M., Zeng, H., Nguyen, T. M., Wang, Y., Vogel, P., Dhungana, Y., … Chi, H. (2018). mTOR coordinates transcriptional programs and mitochondrial metabolism of activated Treg subsets to protect tissue homeostasis. *Nature Communications*, *9*, 2095. https://doi.org/10.1038/s41467-018-04392-5

Charlton, J. J., Chatzidakis, I., Tsoukatou, D., Boumpas, D. T., Garinis, G. A., & Mamalaki, C. (2013). Programmed death-1 shapes memory phenotype CD8 T cell subsets in a cell-intrinsic manner. *Journal of Immunology*, *190*, 6104–6114. https://doi.org/10.4049/jimmunol.1201617

Chi, H., Tuberous sclerosis complex 1 (Tsc1) enforces quiescence of naive T cells to promote immune homeostasis and function.

Chung, Y., Chang, S. H., Martinez, G. J., Yang, X. O., Nurieva, R., Kang, H. S., … Dong, C. (2009). Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity*, *30*, 576–587. https://doi.org/10.1016/j.immuni.2009.02.007

Cruz-Guilloty, F., Pipkin, M. E., Djuretic, I. M., Levanon, D., Lotem, J., Lichtenheld, M. G., … Rao, A. (2009). Runx3 and T-box proteins cooperate to establish the transcriptional program of effector CTLs. *Journal of Experimental Medicine*, *206*, 51–59. https://doi.org/10.1084/jem.20081242

D'Souza, A. D., Parish, I. A., McKay, S. E., Kaech, S. M., & Shadel, G. S. (2011). Aberrant CD8+ T-cell responses and memory differentiation upon viral infection of an ataxia-telangiectasia mouse model driven by hyper-activated Akt and mTORC1 signaling. *American Journal of Pathology*, *178*, 2740–2751. https://doi.org/10.1016/j.ajpath.2011.02.022

Dang, E. V., Barbi, J., Yang, H. Y., Jinasena, D., Yu, H., Zheng, Y., … Pan, F. (2011). Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell*, *146*, 772–784. https://doi.org/10.1016/j.cell.2011.07.033

Delgoffe, G. M., Kole, T. P., Zheng, Y., Zarek, P. E., Matthews, K. L., Xiao, B., … Powell, J. D. (2009). The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity*, *30*, 832–844. https://doi.org/10.1016/j.immuni.2009.04.014

Delgoffe, G. M., Pollizzi, K. N., Waickman, A. T., Heikamp, E., Meyers, D. J., Horton, M. R., … Powell, J. D. (2011). The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nature Immunology*, *12*, 295–303. https://doi.org/10.1038/ni.2005

Donnelly, R. P., Loftus, R. M., Keating, S. E., Liou, K. T., Biron, C. A., Gardiner, C. M., & Finlay, D. K. (2014). mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. *Journal of Immunology*, *193*, 4477–4484. https://doi.org/10.4049/jimmunol.1401558

Düvel, K., Yecies, J. L., Menon, S., Raman, P., Lipovsky, A. I., Souza, A. L., … Manning, B. D. (2010). Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Molecular Cell*, *39*, 171–183. https://doi.org/10.1016/j.molcel.2010.06.022

Eckelhart, E., Warsch, W., Zebedin, E., Simma, O., Stoiber, D., Kolbe, T., … Sexl, V. (2011). A novel Ncr1-Cre mouse reveals the essential role of STAT5 for NK-cell survival and development. *Blood*, *117*, 1565–1573. https://doi.org/10.1182/blood-2010-06-291633

Eisen, H. J., Tuzcu, E. M., Dorent, R., Kobashigawa, J., Mancini, D., Valantine-von Kaeppler, H. A., … Bernhardt, P. (2003). Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *New England Journal of Medicine*, *349*, 847–858. https://doi.org/10.1056/NEJMoa022171

Fabre, S., Carrette, F., Chen, J., Lang, V., Semichon, M., Denoyelle, C., … Bismuth, G. (2008). FOXO1 regulates L-selectin and a network of human T cell homing molecules downstream of phosphatidylinositol 3-kinase. *Journal of Immunology*, *181*, 2980–2989.

Fan, M. Y., & Turka, L. A. (2018). Immunometabolism and PI(3)K signaling as a link between IL-2, Foxp3 expression, and suppressor function in regulatory T cells. *Frontiers in Immunology*, *9*, 69. https://doi.org/10.3389/fimmu.2018.00069

Farber, D. L., Yudanin, N. A., & Restifo, N. P. (2014). Human memory T cells: Generation, compartmentalization and homeostasis. *Nature Reviews Immunology*, *14*, 24–35. https://doi.org/10.1038/nri3567

Fekete, T., Pazmandi, K., Szabo, A., Bacsi, A., Koncz, G., & Rajnavölgyi, E. (2014). The antiviral immune response in human conventional dendritic cells is controlled by the mammalian target of rapamycin. *Journal of Leukocyte Biology*, *96*, 579–589. https://doi.org/10.1189/jlb.2A0114-048RR

Ferrer, I. R., Wagener, M. E., Robertson, J. M., Turner, A. P., Araki, K., Ahmed, R., … Ford, M. L. (2010). Cutting edge: Rapamycin augments pathogen-specific but not graft-reactive $CD8^+$ T cell responses. *Journal of Immunology*, *185*, 2004–2008. https://doi.org/10.4049/jimmunol.1001176

Fox, C. J., Hammerman, P. S., & Thompson, C. B. (2005). The Pim kinases control rapamycin-resistant T cell survival and activation. *Journal of Experimental Medicine*, *201*, 259–266. https://doi.org/10.1084/jem.20042020

Gattinoni, L., Lugli, E., Ji, Y., Pos, Z., Paulos, C. M., Quigley, M. F., … Restifo, N. P. (2011). A human memory T cell subset with stem cell-like properties. *Nature Medicine*, *17*, 1290–1297. https://doi.org/10.1038/nm.2446

Gattinoni, L., Zhong, X. S., Palmer, D. C., Ji, Y., Hinrichs, C. S., Yu, Z., … Restifo, N. P. (2009). Wnt signaling arrests effector T cell differentiation and generates $CD8^+$ memory stem cells. *Nature Medicine*, *15*, 808–813. https://doi.org/10.1038/nm.1982

Geginat, J., Lanzavecchia, A., & Sallusto, F. (2003). Proliferation and differentiation potential of human $CD8^+$ memory T-cell subsets in response to antigen or homeostatic cytokines. *Blood*, *101*, 4260–4266. https://doi.org/10.1182/blood-2002-11-3577

Goncharova, E. A., Goncharov, D. A., Li, H., Pimtong, W., Lu, S., Khavin, I., & Krymskaya, V. P. (2011). mTORC2 is required for proliferation and

survival of TSC2-null cells. *Molecular and Cellular Biology*, *31*, 2484–2498. https://doi.org/10.1128/MCB.01061-10

Guertin, D. A., Stevens, D. M., Thoreen, C. C., Burds, A. A., Kalaany, N. Y., Moffat, J., … Sabatini, D. M. (2006). Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Developmental Cell*, *11*, 859–871. https://doi.org/10.1016/j.devcel.2006.10.007

Guillot-Delost, M., Cheraï, M., Hamel, Y., Rosenzwajg, M., Baillou, C., Simonin, G., … Lemoine, F. M. (2008). Clinical-grade preparation of human natural regulatory T-cells encoding the thymidine kinase suicide gene as a safety gene. *Journal of Gene Medicine*, *10*, 834–846. https://doi.org/10.1002/jgm.1220

Gulen, M. F., Kang, Z., Bulek, K., Youzhong, W., Kim, T. W., Chen, Y., … Li, X. (2010). The receptor SIGIRR suppresses Th17 cell proliferation via inhibition of the interleukin-1 receptor pathway and mTOR kinase activation. *Immunity*, *32*, 54–66. https://doi.org/10.1016/j.immuni.2009.12.003

Hackstein, H., Taner, T., Zahorchak, A. F., Morelli, A. E., Logar, A. J., Gessner, A., & Thomson, A. W. (2003). Rapamycin inhibits IL-4-induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. *Blood*, *101*, 4457–4463. https://doi.org/10.1182/blood-2002-11-3370

Haidinger, M., Poglitsch, M., Geyeregger, R., Kasturi, S., Zeyda, M., Zlabinger, G. J., … Weichhart, T. (2010). A versatile role of mammalian target of rapamycin in human dendritic cell function and differentiation. *Journal of Immunology*, *185*, 3919–3931. https://doi.org/10.4049/jimmunol.1000296

Hand, T. W., Cui, W., Jung, Y. W., Sefik, E., Joshi, N. S., Chandele, A., … Kaech, S. M. (2010). Differential effects of STAT5 and PI3K/AKT signaling on effector and memory CD8 T-cell survival. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 16601–16606. https://doi.org/10.1073/pnas.1003457107

Hardie, D. G. (2008). AMPK and Raptor: Matching cell growth to energy supply. *Molecular Cell*, *30*, 263–265. https://doi.org/10.1016/j.molcel.2008.04.012

Haxhinasto, S., Mathis, D., & Benoist, C. (2008). The AKT-mTOR axis regulates de novo differentiation of $CD4^+$ $Foxp3^+$ cells. *Journal of Experimental Medicine*, *205*, 565–574. https://doi.org/10.1084/jem.20071477

He, S., Kato, K., Jiang, J., Wahl, D. R., Mineishi, S., Fisher, E. M., … Zhang, Y. (2011). Characterization of the metabolic phenotype of rapamycin-treated $CD8^+$ T cells with augmented ability to generate long-lasting memory cells. *PLOS One*, *6*, e20107. https://doi.org/10.1371/journal.pone.0020107

Herrmann, A., Kortylewski, M., Kujawski, M., Zhang, C., Reckamp, K., Armstrong, B., … Yu, H. (2010). Targeting Stat3 in the myeloid compartment drastically improves the in vivo antitumor functions of adoptively transferred T cells. *Cancer Research*, *70*, 7455–7464. https://doi.org/10.1158/0008-5472.CAN-10-0736

Huijts, C. M., Santegoets, S. J., de Jong, T. D., Verheul, H. M., de Gruijl, T. D., & van der Vliet, H. J. (2017). Immunological effects of everolimus in patients with metastatic renal cell cancer. *International Journal of Immunopathology and Pharmacology*, *30*, 341–352. https://doi.org/10.1177/0394632017734459

Inoki, K., Li, Y., Xu, T., & Guan, K. L. (2003). Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes and Development*, *17*, 1829–1834. https://doi.org/10.1101/gad.1110003

Inoki, K., Ouyang, H., Zhu, T., Lindvall, C., Wang, Y., Zhang, X., … Guan, K. L. (2006). TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell*, *126*, 955–968. https://doi.org/10.1016/j.cell.2006.06.055

Intlekofer, A. M., Takemoto, N., Wherry, E. J., Longworth, S. A., Northrup, J. T., Palanivel, V. R., … Reiner, S. L. (2005). Effector and memory $CD8^+$ T cell fate coupled by T-bet and eomesodermin. *Nature Immunology*, *6*, 1236–1244. https://doi.org/10.1038/ni1268

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., … Littman, D. R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17^+ T helper cells. *Cell*, *126*, 1121–1133. https://doi.org/10.1016/j.cell.2006.07.035

Jeannet, G., Boudousquie, C., Gardiol, N., Kang, J., Huelsken, J., & Held, W. (2010). Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 9777–9782. https://doi.org/10.1073/pnas.0914127107

Ji, Y., Pos, Z., Rao, M., Klebanoff, C. A., Yu, Z., Sukumar, M., … Gattinoni, L. (2011). Repression of the DNA-binding inhibitor Id3 by Blimp-1 limits the formation of memory $CD8^+$ T cells. *Nature Immunology*, *12*, 1230–1237. https://doi.org/10.1038/ni.2153

Joshi, N. S., Cui, W., Chandele, A., Lee, H. K., Urso, D. R., Hagman, J., … Kaech, S. M. (2007). Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. *Immunity*, *27*, 281–295. https://doi.org/10.1016/j.immuni.2007.07.010

Kaech, S. M., & Cui, W. (2012). Transcriptional control of effector and memory $CD8^+$ T cell differentiation. *Nature Reviews Immunology*, *12*, 749–761. https://doi.org/10.1038/nri3307

Kellersch, B., & Brocker, T. (2013). Langerhans cell homeostasis in mice is dependent on mTORC1 but not mTORC2 function. *Blood*, *121*, 298–307. https://doi.org/10.1182/blood-2012-06-439786

Klebanoff, C. A., Gattinoni, L., & Restifo, N. P. (2006). $CD8^+$ T-cell memory in tumor immunology and immunotherapy. *Immunological Reviews*, *211*, 214–224. https://doi.org/10.1111/j.0105-2896.2006.00391.x

Kopf, H., de la Rosa, G. M., Howard, O. M. Z., & Chen, X. (2007). Rapamycin inhibits differentiation of Th17 cells and promotes generation of $FoxP3^+$ T regulatory cells. *International Immunopharmacology*, *7*, 1819–1824. https://doi.org/10.1016/j.intimp.2007.08.027

Kurebayashi, Y., Nagai, S., Ikejiri, A., Ohtani, M., Ichiyama, K., Baba, Y., … Koyasu, S. (2012). PI3K-Akt-mTORC1-S6K1/2 axis controls Th17 differentiation by regulating Gfi1 expression and nuclear translocation of RORγ. *Cell Reports*, *1*, 360–373.

Laplante, M., & Sabatini, D. M. (2009). mTOR signaling at a glance. *Journal of Cell Science*, *122*, 3589–3594. https://doi.org/10.1242/jcs.051011

Laplante, M., & Sabatini, D. M. (2012). mTOR signaling in growth control and disease. *Cell*, *149*, 274–293. https://doi.org/10.1016/j.cell.2012.03.017

Lee, K., Gudapati, P., Dragovic, S., Spencer, C., Joyce, S., Killeen, N., … Boothby, M. (2010). Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity*, *32*, 743–753. https://doi.org/10.1016/j.immuni.2010.06.002

Li, Q., Rao, R., Vazzana, J., Goedegebuure, P., Odunsi, K., Gillanders, W., & Shrikant, P. A. (2012). Regulating mammalian target of rapamycin to tune vaccination-induced CD8(+) T cell responses for tumor immunity. *Journal of Immunology*, *188*, 3080–3087. https://doi.org/10.4049/jimmunol.1103365

Li, Q., Rao, R. R., Araki, K., Pollizzi, K., Odunsi, K., Powell, J. D., & Shrikant, P. A. (2011). A central role for mTOR kinase in homeostatic proliferation induced CD8+ T cell memory and tumor immunity. *Immunity*, *34*, 541–553. https://doi.org/10.1016/j.immuni.2011.04.006

Lu, L. F., Thai, T. H., Calado, D. P., Chaudhry, A., Kubo, M., Tanaka, K., … Rudensky, A. Y. (2009). Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity*, *30*, 80–91. https://doi.org/10.1016/j.immuni.2008.11.010

Ma, X. M., & Blenis, J. (2009). Molecular mechanisms of mTOR-mediated translational control. *Nature Reviews Molecular Cell Biology*, *10*, 307–318. https://doi.org/10.1038/nrm2672

Male, V., Nisoli, I., Kostrzewski, T., Allan, D. S. J., Carlyle, J. R., Lord, G. M., … Brady, H. J. M. (2014). The transcription factor E4bp4/Nfil3

controls commitment to the NK lineage and directly regulates Eomes and Id2 expression. *Journal of Experimental Medicine*, *211*, 635–642. https://doi.org/10.1084/jem.20132398

Marçais, A., Cherfils-Vicini, J., Viant, C., Degouve, S., Viel, S., Fenis, A., … Walzer, T. (2014). The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. *Nature Immunology*, *15*, 749–757. https://doi.org/10.1038/ni.2936

Marçais, A., Marotel, M., Degouve, S., Koenig, A., Fauteux-Daniel, S., Drouillard, A., … Walzer, T. (2017). High mTOR activity is a hallmark of reactive natural killer cells and amplifies early signaling through activating receptors. *eLife*, *6*, 6. https://doi.org/10.7554/eLife.26423

Mercalli, A., Calavita, I., Dugnani, E., Citro, A., Cantarelli, E., Nano, R., … Piemonti, L. (2013). Rapamycin unbalances the polarization of human macrophages to M1. *Immunology*, *140*, 179–190. https://doi.org/10.1111/imm.12126

Nandagopal, N., Ali, A. K., Komal, A. K., & Lee, S. H. (2014). The critical role of IL-15-PI3K-mTOR pathway in natural killer cell effector functions. *Frontiers in Immunology*, *5*, 187. https://doi.org/10.3389/fimmu.2014.00187

Parry, R. V., Chemnitz, J. M., Frauwirth, K. A., Lanfranco, A. R., Braunstein, I., Kobayashi, S. V., … Riley, J. L. (2005). CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Molecular and Cellular Biology*, *25*, 9543–9553. https://doi.org/10.1128/MCB.25.21.9543-9553.2005

Passerini, L., Allan, S. E., Battaglia, M., Di Nunzio, S., Alstad, A. N., Levings, M. K., … Bacchetta, R. (2008). STAT5-signaling cytokines regulate the expression of FOXP3 in $CD4^+CD25^+$ regulatory T cells and $CD4^+$CD25- effector T cells. *International Immunology*, *20*, 421–431. https://doi.org/10.1093/intimm/dxn002

Patsoukis, N., Bardhan, K., Chatterjee, P., Sari, D., Liu, B., Bell, L. N., … Boussiotis, V. A. (2015). PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nature Communications*, *6*, 6692. https://doi.org/10.1038/ncomms7692

Pearce, E. L., Mullen, A. C., Martins, G. A., Krawczyk, C. M., Hutchins, A. S., Zediak, V. P., & Reiner, S. L. (2003). Control of effector $CD8^+$ T cell function by the transcription factor Eomesodermin. *Science*, *302*, 1041–1043. https://doi.org/10.1126/science.1090148

Pearce, E. L., Walsh, M. C., Cejas, P. J., Harms, G. M., Shen, H., Wang, L. S., … Choi, Y. (2009). Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature*, *460*, 103–107. https://doi.org/10.1038/nature08097

Pedicord, V. A., Cross, J. R., Montalvo-Ortiz, W., Miller, M. L., & Allison, J. P. (2015). Friends not foes: CTLA-4 blockade and mTOR inhibition cooperate during $CD8^+$ T cell priming to promote memory formation and metabolic readiness. *Journal of Immunology*, *194*, 2089–2098. https://doi.org/10.4049/jimmunol.1402390

Piccirillo, C. A., D'hennezel, E., Sgouroudis, E., & Yurchenko, E. (2008). $CD4^+$ $Foxp3^+$ regulatory T cells in the control of autoimmunity: In vivo veritas. *Current Opinion in Immunology*, *20*, 655–662. https://doi.org/10.1016/j.coi.2008.09.006

Pipkin, M. E., Sacks, J. A., Cruz-Guilloty, F., Lichtenheld, M. G., Bevan, M. J., & Rao, A. (2010). Interleukin-2 and inflammation induce distinct transcriptional programs that promote the differentiation of effector cytolytic T cells. *Immunity*, *32*, 79–90. https://doi.org/10.1016/j.immuni.2009.11.012

Powell, J. D., Pollizzi, K. N., Heikamp, E. B., & Horton, M. R. (2012). Regulation of immune responses by mTOR. *Annual Review of Immunology*, *30*, 39–68. https://doi.org/10.1146/annurev-immunol-020711-075024

Ranson, T., Vosshenrich, C. A., Corcuff, E., Richard, O., Muller, W., & Di Santo, J. P. (2003). IL-15 is an essential mediator of peripheral NK-cell homeostasis. *Blood*, *101*, 4887–4893. https://doi.org/10.1182/blood-2002-11-3392

Rao, R. R., Li, Q., Bupp, M. R. G., & Shrikant, P. A. (2012). Transcription factor Foxo1 represses T-bet-mediated effector functions and promotes memory CD8(+) T cell differentiation. *Immunity*, *36*, 374–387. https://doi.org/10.1016/j.immuni.2012.01.015

Rao, R. R., Li, Q., Odunsi, K., & Shrikant, P. A. (2010). The mTOR kinase determines effector versus memory $CD8^+$ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin. *Immunity*, *32*, 67–78. https://doi.org/10.1016/j.immuni.2009.10.010

Rao, R. R., Li, Q., & Shrikant, P. A. (2010). Fine-tuning CD8(+) T cell functional responses: MTOR acts as a rheostat for regulating CD8(+) T cell proliferation, survival and differentiation? *Cell Cycle*, *9*, 2996–3001. https://doi.org/10.4161/cc.9.15.12359

Rolf, J., Zarrouk, M., Finlay, D. K., Foretz, M., Viollet, B., & Cantrell, D. A. (2013). AMPKalpha1: A glucose sensor that controls CD8 T-cell memory. *European Journal of Immunology*, *43*, 889–896. https://doi.org/10.1002/eji.201243008

Rutishauser, R. L., Martins, G. A., Kalachikov, S., Chandele, A., Parish, I. A., Meffre, E., … Kaech, S. M. (2009). Transcriptional repressor Blimp-1 promotes CD8(+) T cell terminal differentiation and represses the acquisition of central memory T cell properties. *Immunity*, *31*, 296–308. https://doi.org/10.1016/j.immuni.2009.05.014

Sarbassov, D. D., Ali, S. M., Sengupta, S., Sheen, J. H., Hsu, P. P., Bagley, A. F., … Sabatini, D. M. (2006). Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Molecular Cell*, *22*, 159–168. https://doi.org/10.1016/j.molcel.2006.03.029

Sarkar, S., Kalia, V., Haining, W. N., Konieczny, B. T., Subramaniam, S., & Ahmed, R. (2008). Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *Journal of Experimental Medicine*, *205*, 625–640. https://doi.org/10.1084/jem.20071641

Sathaliyawala, T., O'Gorman, W. E., Greter, M., Bogunovic, M., Konjufca, V., Hou, Z. E., … Reizis, B. (2010). Mammalian target of rapamycin controls dendritic cell development downstream of Flt3 ligand signaling. *Immunity*, *33*, 597–606. https://doi.org/10.1016/j.immuni.2010.09.012

Schmitz, F., Heit, A., Dreher, S., Eisenächer, K., Mages, J., Haas, T., … Wagner, H. (2008). Mammalian target of rapamycin (mTOR) orchestrates the defense program of innate immune cells. *European Journal of Immunology*, *38*, 2981–2992. https://doi.org/10.1002/eji.200838761

Scholz, G., Jandus, C., Zhang, L., Grandclément, C., Lopez-Mejia, I. C., Soneson, C., … Romero, P. (2016). Modulation of mTOR signalling triggers the formation of stem cell-like memory T cells. *EBioMedicine*, *4*, 50–61. https://doi.org/10.1016/j.ebiom.2016.01.019

Shan, J., Feng, L., Sun, G., Chen, P., Zhou, Y., Xia, M., … Li, Y. (2015). Interplay between mTOR and STAT5 signaling modulates the balance between regulatory and effective T cells. *Immunobiology*, *220*, 510–517. https://doi.org/10.1016/j.imbio.2014.10.020

Shaw, R. J. (2009). LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. *Acta Physiologica*, *196*, 65–80. https://doi.org/10.1111/j.1748-1716.2009.01972.x

Shi, L. Z., Wang, R., Huang, G., Vogel, P., Neale, G., Green, D. R., & Chi, H. (2011). HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *Journal of Experimental Medicine*, *208*, 1367–1376. https://doi.org/10.1084/jem.20110278

Shrestha, S., Yang, K., Wei, J., Karmaus, P. W. F., Neale, G., & Chi, H. (2014). Tsc1 promotes the differentiation of memory $CD8^+$ T cells via orchestrating the transcriptional and metabolic programs. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 14858–14863. https://doi.org/10.1073/pnas.1404264111

Song, K., Wang, H., Krebs, T. L., & Danielpour, D. (2006). Novel roles of Akt and mTOR in suppressing TGF-beta/ALK5-mediated Smad3 activation. *EMBO Journal*, *25*, 58–69. https://doi.org/10.1038/sj.emboj.7600917

Sun, I. H., Oh, M. H., Zhao, L., Patel, C. H., Arwood, M. L., Xu, W., … Powell, J. D. (2018). mTOR complex 1 signaling regulates the generation and function of central and effector Foxp3(+) regulatory T cells. *Journal of Immunology*, *201*, 481–492. https://doi.org/10.4049/jimmunol.1701477

Sun, J. C., & Lanier, L. L. (2011). NK cell development, homeostasis and function: Parallels with CD8(+) T cells. *Nature Reviews Immunology*, *11*, 645–657. https://doi.org/10.1038/nri3044

Takemoto, N., Intlekofer, A. M., Northrup, J. T., Wherry, E. J., & Reiner, S. L. (2006). Cutting edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced $CD8^+$ T cell differentiation. *Journal of Immunology*, *177*, 7515–7519.

Tan, J. T., Ernst, B., Kieper, W. C., LeRoy, E., Sprent, J., & Surh, C. D. (2002). Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype $CD8^+$ cells but are not required for memory phenotype $CD4^+$ cells. *Journal of Experimental Medicine*, *195*, 1523–1532.

Tejera, M. M., Kim, E. H., Sullivan, J. A., Plisch, E. H., & Suresh, M. (2013). FoxO1 controls effector-to-memory transition and maintenance of functional CD8 T cell memory. *Journal of Immunology*, *191*, 187–199. https://doi.org/10.4049/jimmunol.1300331

Tewari, K., Sacha, J., Gao, X., & Suresh, M. (2004). Effect of chronic viral infection on epitope selection, cytokine production, and surface phenotype of CD8 T cells and the role of IFN-gamma receptor in immune regulation. *Journal of Immunology*, *172*, 1491–1500.

Thomson, A. W., Turnquist, H. R., & Raimondi, G. (2009). Immunoregulatory functions of mTOR inhibition. *Nature Reviews Immunology*, *9*, 324–337. https://doi.org/10.1038/nri2546

Tone, Y., Furuuchi, K., Kojima, Y., Tykocinski, M. L., Greene, M. I., & Tone, M. (2008). Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. *Nature Immunology*, *9*, 194–202. https://doi.org/10.1038/ni1549

Townsend, M. J., Weinmann, A. S., Matsuda, J. L., Salomon, R., Farnham, P. J., Biron, C. A., … Glimcher, L. H. (2004). T-bet regulates the terminal maturation and homeostasis of NK and Valpha14i NKT cells. *Immunity*, *20*, 477–494.

Trinchieri, G. (1989). Biology of natural killer cells. *Advances in Immunology*, *47*, 187–376.

Turnquist, H. R., Raimondi, G., Zahorchak, A. F., Fischer, R. T., Wang, Z., & Thomson, A. W. (2007). Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic $CD4^+$ T cells, but enrich for antigen-specific $Foxp3^+$ T regulatory cells and promote organ transplant tolerance. *Journal of Immunology*, *178*, 7018–7031.

van de Laar, L., van den Bosch, A., Boonstra, A., Binda, R. S., Buitenhuis, M., Janssen, H. L. A., … Woltman, A. M. (2012). PI3K-PKB hyperactivation augments human plasmacytoid dendritic cell development and function. *Blood*, *120*, 4982–4991. https://doi.org/10.1182/blood-2012-02-413229

Vézina, C., Kudelski, A., & Sehgal, S. N. (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *Journal of Antibiotics*, *28*, 721–726.

Viel, S., Marçais, A., Guimaraes, F. S. F., Loftus, R., Rabilloud, J., Grau, M., … Walzer, T. (2016). TGF-beta inhibits the activation and functions of NK cells by repressing the mTOR pathway. *Science Signaling*, *9*, ra19–ra19. https://doi.org/10.1126/scisignal.aad1884

Wai, L. E., Fujiki, M., Takeda, S., Martinez, O. M., & Krams, S. M. (2008). Rapamycin, but not cyclosporine or FK506, alters natural killer cell function. *Transplantation*, *85*, 145–149. https://doi.org/10.1097/01.tp.0000296817.28053.7b

Weichhart, T., Hengstschläger, M., & Linke, M. (2015). Regulation of innate immune cell function by mTOR. *Nature Reviews Immunology*, *15*, 599–614. https://doi.org/10.1038/nri3901

White, E. (2003). The pims and outs of survival signaling: Role for the Pim-2 protein kinase in the suppression of apoptosis by cytokines. *Genes and Development*, *17*, 1813–1816. https://doi.org/10.1101/gad.1123103

Yang, C. Y., Best, J. A., Knell, J., Yang, E., Sheridan, A. D., Jesionek, A. K., … Goldrath, A. W. (2011). The transcriptional regulators Id2 and Id3 control the formation of distinct memory $CD8^+$ T cell subsets. *Nature Immunology*, *12*, 1221–1229. https://doi.org/10.1038/ni.2158

Yang, K., Neale, G., Green, D. R., He, W., Chi, H. (2011). The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. *Nature Immunology*, *12*, 888–897. https://doi.org/10.1038/ni.2068

Yang, M., Li, D., Chang, Z., Yang, Z., Tian, Z., & Dong, Z. (2015). PDK1 orchestrates early NK cell development through induction of E4BP4 expression and maintenance of IL-15 responsiveness. *Journal of Experimental Medicine*, *212*, 253–265. https://doi.org/10.1084/jem.20141703

Yang, Q., & Guan, K. L. (2007). Expanding mTOR signaling. *Cell Research*, *17*, 666–681. https://doi.org/10.1038/cr.2007.64

Yang, X. O., Pappu, B. P., Nurieva, R., Akimzhanov, A., Kang, H. S., Chung, Y., … Dong, C. (2008). T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity*, *28*, 29–39. https://doi.org/10.1016/j.immuni.2007.11.016

Yao, R., Ma, Y. L., Liang, W., Li, H. H., Ma, Z. J., Yu, X., & Liao, Y. H. (2012). MicroRNA-155 modulates Treg and Th17 cells differentiation and Th17 cell function by targeting SOCS1. *PLOS One*, *7*, e46082. https://doi.org/10.1371/journal.pone.0046082

Zhang, L., Tschumi, B. O., Lopez-Mejia, I. C., Oberle, S. G., Meyer, M., Samson, G., … Romero, P. (2016). Mammalian target of rapamycin complex 2 controls CD8 T cell memory differentiation in a Foxo1-dependent manner. *Cell Reports*, *14*, 1206–1217. https://doi.org/10.1016/j.celrep.2015.12.095

Zheng, Y., & Rudensky, A. Y. (2007). Foxp3 in control of the regulatory T cell lineage. *Nature Immunology*, *8*, 457–462. https://doi.org/10.1038/ni1455

Zhou, J., Wulfkuhle, J., Zhang, H., Gu, P., Yang, Y., Deng, J., … Zhang, Y. (2007). Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 16158–16163. https://doi.org/10.1073/pnas.0702596104

Zhu, J., Davidson, T. S., Wei, G., Jankovic, D., Cui, K., Schones, D. E., … Paul, W. E. (2009). Down-regulation of Gfi-1 expression by TGF-beta is important for differentiation of Th17 and $CD103^+$ inducible regulatory T cells. *Journal of Experimental Medicine*, *206*, 329–341. https://doi.org/10.1084/jem.20081666

Zhu, L., Yang, T., Li, L., Sun, L., Hou, Y., Hu, X., … Zhao, Y. (2014). TSC1 controls macrophage polarization to prevent inflammatory disease. *Nature Communications*, *5*, 4696. https://doi.org/10.1038/ncomms5696

Zinzalla, V., Stracka, D., Oppliger, W., & Hall, M. N. (2011). Activation of mTORC2 by association with the ribosome. *Cell*, *144*, 757–768. https://doi.org/10.1016/j.cell.2011.02.014

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