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mTOR Regulates T-Cell Differentiation and Activation in Immunity and Autoimmunity

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ABSTRACT: mTOR is an evolutionarily conserved PI3-kinase family member that plays a central role in integrating environmental cues in the form of amino acids, energy, and growth factors. Recently, the kinase mTOR has emerged as an important regulator of the differentiation and function of helper T cells in immune responses and autoimmune diseases. In this review, we summarize the regulatory effects and mechanisms of mTOR complex in the differentiation of $CD4^+$ T and $CD8^+$ T cells, and T-cell homeostasis modulation. This should contribute to understanding the central role for mTOR in regulating immune responses and autoimmune diseases.

KEY WORDS: mTOR; T cell differentiation; immunity; autoimmunity

ABBREVIATIONS: APCs, antigen presenting cells; Akt, AKT8 virus oncogene cellular homologue; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; DCs, dendritic cells; Deptor, DEP-domain-containing; EAE, experimental autoimmune encephalomyelitis; FKBP12, FK506 binding protein 12; mTOR, mammalian target of rapamycin; mLST8, mammalian lethal with Sec13 protein 8; TORC, target of rapamycin complex; Rheb, Ras homologue enriched in brain; Raptor, regulatory-associated companion of mTOR; p70 S6K, p70 S6 kinase; PI-3K, PI3-kinase; STAT3, signal transducer and activator of transcription 3; SLE, systemic lupus erythematosus; TSC, tuberous sclerosis complex; TH cells, T helper cells; TGF-β, transforming growth factor-β; 4E-BP1, eukaryotic initiation factor 4E-binding protein.

I. INTRODUCTION

The mammalian target of the rapamycin (mTOR) signaling pathway integrates both intracellular and extracellular signals and serves as a central regulator of cell metabolism, growth, proliferation, and survival. It belongs to the family of phosphoinositide-3-kinase (PI3K)-related kinases (PIKKs), along with ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR), double-stranded DNA-dependent protein kinase (DNA-PK), and human suppressor of morphogenesis in genitalia-1 (hSMG1).[1,2] All of these proteins have C-terminal protein kinase domains with similarity to the lipid kinase phosphatidylinositol 3-kinase (PI3K), thus giving the family its name. While all members of the family respond to genotoxic stresses, mTOR also responds to many other stresses, including those related to nutrient, energy, and oxygen levels.[3,4]

This response is related to biological evolution status. The appearance of TOR in early eukaryotes enabled these unicellular organisms to sense the availability of nutrients and to promote growth in favorable environmental conditions. With the emergence of multicellularity, TOR acquired additional roles as a central controller of organism growth and homeostasis.[3,5] As such, mTOR is implicated in disease states where growth is deregulated and homeostasis is compromised, namely cancer and metabolic diseases.[3,6] Overstimulation of the mTOR pathway

by excess food consumption may be an essential factor underlying the diabetes epidemic.[7] Finally, recent findings suggest that mTOR signaling controls the adaptive T-cell development and differentiation in immunity, which may be a promising avenue to modulating immune response in physiological or pathological status.[2,8,9] In this review, we summarize the recent research related to the modulation of mTOR on $CD4^+$T and $CD8^+$T cell differentiation and activation in immunity and autoimmunity that has contributed to understanding the precise regulatory mechanisms of mTOR.

II. MTOR COMPLEX SIGNALING PATHWAY

The mTOR protein is a 289-kDa serine threonine protein kinase (Fig. 1). mTOR exists in two distinct multiprotein complexes called complex 1 (mTORC1) and complex 2 (mTORC2).[10,11] mTORC1 has five components: (1) mTOR, which is the catalytic subunit of the complex; (2) regulatory-associated protein of mTOR (Raptor); (3) mammalian lethal with Sec13 protein 8 (mLST8, also known as GbL); (4) proline-rich AKT substrate 40 kDa (PRAS40); and (5) DEP-domain-containing mTOR-interacting protein (Deptor).[12] The exact function of most of the mTOR-interacting proteins in mTORC1 remains unclear.[13] mTORC2 comprises six different proteins: (1) mTOR; (2) rapamycin-insensitive companion of mTOR (Rictor); (3) mammalian stress-activated protein kinase interacting protein (mSIN1); (4) protein observed with Rictor-1 (Protor-1); (5) mLST8; and (6) Deptor .[3,9]

In mTORC1 and mTORC2, which appear to act downstream and upstream of AKT respectively, upstream PI3K activation leads to partial activation of AKT through phosphorylation of threonine 308, an upstream target of mTORC1, and complete activation requires the additional phosphorylation of serine 473, a downstream target of mTORC2.[3] The mTORC1 complex, which is controlled by Rheb (G-protein Rheb, a Ras homologue enriched in the brain), and the TSC (tuberous sclerosis complex) components TSC1 and TSC2 mediate anabolic processes that promote growth by increasing protein synthesis. mTORC1 stimulates translation by directly phosphorylating p70S6 kinase (S6K) and 4E-BP1, which promotes ribosome assembly and translation (Figure 1).[14,15] mTORC1 is sensitive to the bacterial macrolide rapamycin.[16] Rapamycin binds easily to FK506-binding protein of 12 kDa (FKBP12) and interacts with the FKBP12-rapamycin binding domain (FRB) of mTOR, thus inhibiting mTORC1 functions. In contrast to its effect on mTORC1, FKBP12–rapamycin cannot physically interact with or acutely inhibit mTORC2.[14,17] Therefore, mTORC1 and mTORC2 have been respectively characterized as the rapamycin-sensitive and rapamycin-insensitive complexes. But some research also shows that mTORC1 functions are resistant to inhibition by rapamycin, and even in some cases, that chronic rapamycin treatment could inhibit the mTORC2 activity by blocking its assembly.[18] Moreover, mTORC1, which responds to energy, amino acids, growth factors, and oxygen levels (whereas mTORC2 activation is ill-defined), seems to be mediated only by growth factors (Figure 1).[19,20]

Moreover, many positive and negative feedback loops have been described in recent studies. The S6K1-PI3K signaling loop, a very important negative feedback, involves the inhibition of the PI3K pathway by mTORC1. Active S6K inhibits insulin receptor substrate 1 (IRS-1) by phosphorylating it by inducing its degradation and by altering its localization, all of which ultimately dampen PI3K-AKT pathway activation.[21] Tumor suppressor p53 also transactivates negative regulators of mTORC1.[22] Furthermore, p53 could up-regulate the transcription of Sestrins 1 and 2, which bind to the ternary complex TSC1/2–AMP: ATP sensing adenosine monophosphate kinase (AMPK), inducing phosphorylation and activation of TSC2 by AMPK.[23] In addition phosphatase and tensin homolog (PTEN) has been shown to interact with p53, leading to p53 stabilization. Interestingly, protein kinase B (PKB, AKT) is a negative regulator of p53 activity via phosphorylation of an E3 ubiquitin ligase, namely mouse double minute-2 (MDM2), which drives its translocation to the nucleus, where it destabilizes p53.[24,25] These cross regulations of feedback signaling constitute a network coordination of homeostasis after sensing outer stress.

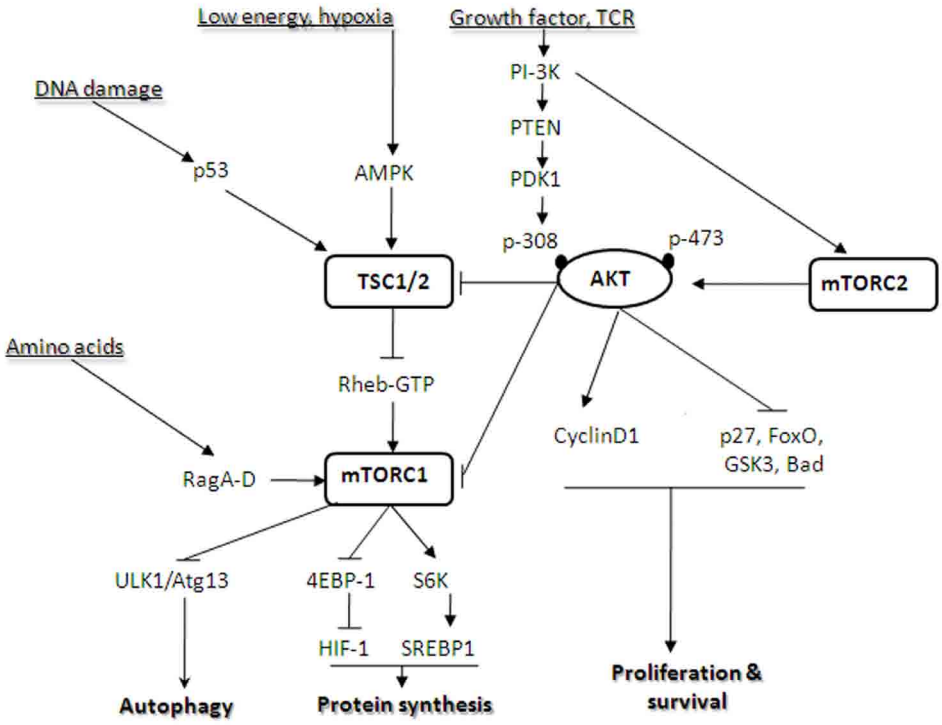

FIGURE 1: The mTOR signaling pathway. mTOR regulates many cellular activities through two distinct complexes: mTORC1 and mTORC2. mTORC1 activity is critically controlled by a small GTPase, Rheb, whose activity is inhibited by a GTPase-activating protein, TSC2, in complex with TSC1. Consequently, inactivation of the TSC1/2 complex via upstream signals emerging from the PI3K/AKT axis and the Erk pathway activate TORC1. Many accessory molecules as well as growth factors signal via the PI3K/AKT and Erk pathways and therefore inevitably activate mTORC1. AKT can further promote TORC1 activity independent of TSC1/2. Low energy or hypoxia leads to the activation of AMPK, which phosphorylates and activates the TSC1/2 complex, resulting in mTORC1 inhibition. Furthermore, mTORC1 activity is dependent on sufficient levels of amino acids, in a process involving recently discovered Rag A–D proteins and the regulator complex.

III. MTOR REGULATES CD4⁺T-CELL DIFFERENTIATION

T-cell immunity is the central modulator in the immune response. mTOR as an important signaling molecule in translating environmental cues into specific types of T-cell responses.[14,26] The essential role for mTOR in sensing the immune microenvironment and dictating immune function and differentiation has begun to emerge.[27,28] Specifically, inhibition of mTOR in TH1 effector cells by rapamycin promotes T-cell tolerance, even in the presence of costimulation.[13,20] This simple study indicates that the mTOR pathway probably related to the induction and maintenance of Foxp3⁺Treg. Moreover, further research results have shown that CD4⁺T cells lacking mTOR fail to differentiate into effector cells under appropriate skewing conditions. Instead, after activation, mTOR-deficient T cells become Foxp3⁺ regulatory cells.[29–31] This inability to differentiate into effector cells in the absence of mTOR is associated with less activation of the transcription factors STAT4 (signal transducers and activators of transcription protein

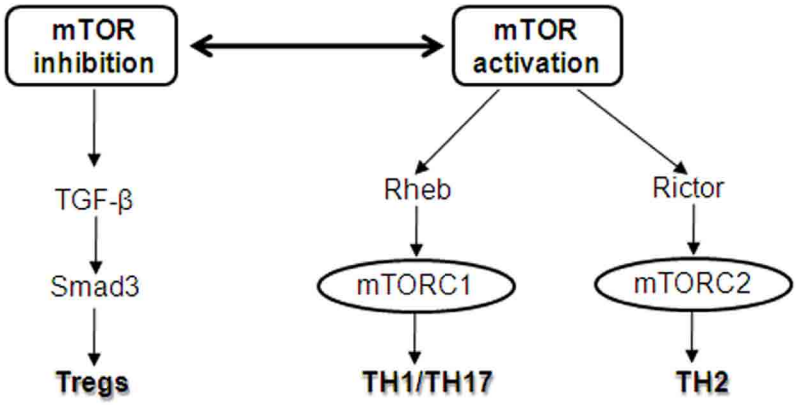

FIGURE 2: mTOR control CD4⁺T cell differentiation. mTORC1 and mTORC2 have different physiological functions. mTORC1 signaling promotes TH1 and TH17 differentiation; mTORC2 signaling promotes TH2 differentiation; and inhibition of mTOR leads to Treg cells via the TGF-β-Smad3 pathway.

4), STAT6, and STAT3 in response to the skewing cytokines interleukin 12 (IL-12), IL-4, and IL-6, respectively.[32,33] These results demonstrate that the mTOR pathway probably controls the balance of regulatory T cells and effector T cells via a series of specific pathways. Recently several key studies have further confirmed and answered this question using a genetically deficient mouse system. These results have shown that mTOR could integrate cytokine signaling and regulate T-cell effector lineage commitment. In Frap1$^{-/-}$ mice (Frap1 is the name of the gene that encodes mTOR protein; mice carrying a floxed Frap1 gene were crossed to mice harboring CD4-Cre to delete the gene in double-positive CD4$^+$CD8$^+$T and CD4 and CD8 single-positive T-cell lymphocytes); results showed that mTOR activation is necessary for TH1, TH2, and TH17 effector T cell differentiation, and even under fully activating conditions, T cells lacking mTOR differentiated into Foxp3$^+$ regulatory T cells.[31] Further, the precise and reciprocal roles for signaling by mTORC1 and mTORC2 were defined in regulation of the differentiation of CD4$^+$ helper T cells.[11]

Other results have shown mTORC1 and mTORC2 to have different physiological functions. mTORC1 signaling promotes TH1 and TH17 differentiation; mTORC2 signaling promotes TH2 differentiation; and inhibition of mTOR leads to Treg cells[34,35] (Figure 2). Small GTPase Rheb has been identified as an activator of mTORC1. Similarly, by selectively knocking out Rheb in T cells, the differentiation of both TH1 and TH17 effector cells required mTORC1 signaling, whereas TH2 differentiation was preserved.[34] Mice with T-cell–specific deletion of Rheb did not mount TH1 or TH17 responses *in vivo* and were resistant to the development of classical experimental autoimmune encephalomyelitis (EAE).[11,35,36]

T cells lacking mTORC1 activity were still able to become TH2 cells. Importantly, Rictor-deficient T cells generated by crossing of mice expressing Cre from the distal promoter of the gene encoding the kinase Lck (dLck-iCre) and mice with loxP-flanked Rictor alleles have been studied.[31,34] mTORC2 activity is significally decreased in T cells; results have shown that naïve T cells were unable to become TH2 cells but maintained their ability to differentiate into TH1 cells and TH17 cells.[11] Thus, all of these results define distinct downstream signaling pathways as the mechanisms by which mTOR regulates the differentiation of helper T cells. Taken together, current studies indicate that mTOR could regulate the dif-

ferent CD4+T cell population differentiation with different signaling complex pathway (Figure 2).

Other than genetic analysis of mTOR in immunity, some specific molecular inhibitor signaling studies have also contributed some interesting experimental evidence. Rapamycin has been shown to promote the generation of Treg cells in the absence of exogenous TGF-β.[13] The ligand for the T-cell inhibitory receptor PD-1 has been shown to promote inducible Treg cells by inhibiting mTOR activation.[37] In addition, local depletion of essential amino acids can promote the generation of Treg cells by inhibiting mTOR activity.[38,39] The simultaneous inhibition of mTORC1 and mTORC2 by an mTOR kinase inhibitor (DMK1) was a potent inducer of Treg cells in the absence of exogenous TGF-β, which indicates that drugs in this class can act as potent agents inducing immunosuppression and tolerance.[37,40]

Furthermore, the TGF-β effects have been also explored in mTOR-mediated Treg differentiation (Figure 2). In T-Frap1$^{-/-}$ mice, Foxp3+Treg differentiation was greater when compared with that of wild-type (WT) mice.[31] Because TGF-β is such a potent skewing agent of antigen-induced Treg cells, one possible explanation for our findings was that cells from T-Frap1$^{-/-}$ mice produce higher amounts of TGF-β. To examine this, splenocytes from WT or T-Frap1$^{-/-}$ mice were cultured in media alone or stimulated with anti-CD3 overnight and examined for TGF-β production by ELISA. For both the WT and T-Frap1$^{-/-}$ cultures, slight increases occurred in TGF-β expression upon activation.[31,41,42] However, the amounts of TGF-β were equivalent, indicating that the development of regulatory cells upon stimulation of the T-Frap1$^{-/-}$ cultures is not the result of increased amounts of TGF-β.

Smad3 plays a critical role in promoting Treg cell differentiation. TGF-β signaling activates Smad3, which, along with TCR-induced NF-AT, contributes to the induction of Foxp3 by promoting acetylation at the Foxp3 enhancer.[10,43,44] To examine Smad3 activation in mTOR-deficient T cells, previously activated WT and T-Frap1$^{-/-}$ T cells were mock-stimulated or stimulated with TGF-β and examined using immunoblotting for Smad3 activation. At baseline, the mTOR-deficient T cells displayed robust phosphorylation of Smad3, and this phosphorylation increased with the addition of TGF-β.[10,43] In contrast, the levels of Smad3 phosphorylation in the WT T cells were very low and they increased modestly upon stimulation with this dose of TGF-β.

Further studies have shown that TGF-β produced in a normally activating inflammatory milieu contributes to the induction of Foxp3+ Treg cells in the absence of mTOR activation.[43,44] This indicates that TGF-β-Smad3 signaling has important effects without mTOR signaling in the differentiation of Foxp3+Treg cells (Figure 2). Meanwhile, some reciprocal modulation also exists in different T-cell subtype differentiations. Our previous studies have shown the differentiation of TH1 cells and anti-inflammatory Treg cells to be reciprocally regulated by S1P1, a receptor for the bioactive lipid sphingosine 1-phosphate (S1P).[10] S1P1 signaled through the kinase mTOR and antagonized the function of TGF-β mainly by attenuating sustained activity of the signal transducer Smad3.[45] Thus, mTOR signaling also can regulate the TGF-β-Smad3 pathway to regulate the different CD4+ T-cell differentiations. Other related studies also provide evidence in agreement with these results.[46,47]

The STAT transcriptional factor is very important in modulating the different subtypes of T-cell lineage differentiations.[19,46,48] mTOR complexes also regulate STAT activation differently. T-Rheb$^{-/-}$ (the regulator of the mTORC1 complex signaling pathway) and T-Rictor$^{-/-}$ (the regulator of the mTORC2 complex signaling pathway) mice were studied in skewing cytokines, and STAT activation was further assessed. Consistent with their inability to become TH1 or TH17 cells, T-Rheb$^{-/-}$ T cells showed less phosphorylation of STAT4 and STAT3 in response to IL-12 and IL-6, respectively. However, T-Rheb$^{-/-}$ T cells demonstrated enhanced STAT6 phosphorylation in response to IL-4 (a TH2-promoting cytokine).[36,49] T-Rictor$^{-/-}$ T cells showed intact IL-12– and IL-6–induced activation of STAT4 and STAT3, whereas IL-4–induced activation of STAT6 was diminished.[50,51]

These findings indicate that the ability of

mTORC1 and mTORC2 to regulate the differentiation of effector T cells differently is due in part to their ability to regulate STAT activation differently. In response to different cytokines, Rheb-deficient T cells showed less IL-23– and IFN-β–induced activation of STAT4 and less IL-10– and IL-21–dependent activation of STAT3.[11,52] Rictor-deficient T cells showed less STAT6 activation in response to IL-13.[53,54] However, for receptors IL-12Rβ1, IL-4R, and IL-6R, expression is comparable in Rheb$^{-/-}$ and Rictor$^{-/-}$ T cells and WT cells.[31] This result indicates that mTOR regulates STAT expression independent of cytokine receptor expressions. STAT activation is partially regulated by the expression of inhibitory SOCS proteins, which can dephosphorylate Janus (Jak) kinase-dependent residues on STAT proteins in different ways.[55,56] In Rheb$^{-/-}$ and Rictor$^{-/-}$ T cells, after activation of T cells for 48, 72, or 96 h, SOCS3 protein remained more abundant in Rheb-deficient T cells, whereas WT and Rictor-deficient cells down-regulated SOCS3. Furthermore, whereas WT and Rheb-deficient cells consistently up-regulated SOCS5 after activation, Rictor-deficient T cells had much more SOCS5 at later time points. In T cells stimulated in TH1 and TH2 differentiation conditions, knockdown of SOCS3 mRNA resulted in more IFN-γ production in Rheb-deficient T cells, whereas knockdown of SOCS5 mRNA in Rictor-deficient T cells resulted in a greater ability to produce IL-4(39, 57, 58). These data demonstrate that SOCS proteins regulate TH1, TH17 and TH2 differentiation in the Rheb- and Rictor-deficient T cells.

IV. MTOR REGULATES CD8$^+$ T-CELL DIFFERENTIATION

Emerging insights into CD8$^+$ T-cell memory generation demonstrates that, in addition to CD4$^+$ T-cell differentiation modulation, mTOR also plays an important role in memory CD8$^+$ T-cell differentiation (32, 59). Naïve CD8$^+$ T-cells are instructed by antigen, costimulatory molecules, and cytokines (IL-2, IL-12, IL-15, etc.) and amino receptor to undergo proliferation, clonal expansion, differentiation, and functional maturation for effector and memory functions.[60,61] The integration of extracellular instructions induces coordinated signaling cascades and transcriptional programs to determine CD8$^+$ T-cell functional fate.

With increasing antigen strength (i.e., avidity, dose, and duration), naïve CD8$^+$ T cells undergo a strength-dependent increase in cell division and effector maturation.[1,62] Also, the extent of antigenic stimulation received by a naïve CD8$^+$ T cell can profoundly influence its functional fate. After an acute viral infection, activated CD8$^+$ T cells clonally expand and differentiate into effector cells that clear virus-infected cells. This expansion phase is followed by a contraction phase, during which 90–95% of the effector T cells die and the surviving 5–10% of the antigen-specific T cells become memory cells.[62,63] Four markers are useful in defining memory CD8$^+$T cells: (1) L-7 receptor α and essential for memory T-cell maintenance (CD127I), (2) lymph node homing receptor and associated with high proliferative capacity (CD62L), (3) killer cell lectin-like receptor subfamily G member 1, inversely correlated with long-lived memory cells (KLRG1), and (4) anti-apoptotic and expressed at high levels in memory T cells (Bcl-2). Thus, the surviving effector cells are considered memory precursor cells and can be distinguished from terminal effector cells by their surface expression of CD127 and KLRG1. CD127 is highly expressed on naïve T cells, but it is uniformly down-regulated on all antigen-specific CD8$^+$T cells after activation. This CD127^{low} T-cell population includes KLRG1^{hi} and KLRG1$^{low/int}$ cells, the latter forming a majority of memory T-cell precursors by re-expressing CD127 subsequently. Administration of rapamycin to mice during both the expansion and contraction phases enhanced not only the magnitude but also the quality of memory CD8$^+$ T cells.[59,63] Knocking down raptor in antigen-specific CD8$^+$ T cells had similar effects to those observed with rapamycin treatment, suggesting that the mTORC1 pathway regulates memory CD8$^+$ T-cell differentiation.[41,59]

Further studies are needed to clarify the effects of mTORC2 (or Rictor) in regulating the memory CD8$^+$ T-cell differentiation. Moreover, mTOR, as a key regulator of cellular metabolism, can facili-

tate transition of effector CD8+T cells to memory. These studies have implicated a novel role for catabolic pathways, such as fatty acid oxidation, in promoting survival of effector CD8+ T cells at the peak of their expansion, and they have identified mTOR as a key regulator of CD8+ T-cell memory generation.[4,32]

Recently, studies have also revealed the molecular mechanisms underpinning the control of effector and memory CD8+ T-cell fate by mTOR. mTOR acts as an integrator of instructions that determines CD8+ T-cell differentiation for effector- versus memory-cell fate by regulating the expression of transcription factors T-bet and eomesodermin (Eomes).[4,31,32,64,65] The transcription factor T-bet (Tbx21) is the master regulator of type I effector-cell differentiation, in which expression is considerably enhanced and sustained in the presence of IL-12.[66] Recent evidence suggests that inflammation-induced T-bet can control effector- and memory-cell fate decisions in CD8+ T cells because increased T-bet expression promotes short-lived effector cells with a KLRG1^{hi} and IL-7R^{lo} phenotypes, whereas low T-bet expression promotes long-lived memory cells.[24,31] Eomes, another T-box–containing transcription factor, whose expression increases from the effector to memory phases of an immune response, has been proposed to promote memory formation.[24] Moreover, IL-12 induces T-bet but inhibits Eomes expression to favor effector- versus memory-cell generation, suggesting the importance of understanding cell-intrinsic factors that regulate T-bet and Eomes expression which may enable desirable CD8+ T-cell functional outcomes. These studies have identified the role of mTOR in instructional programming of naïve CD8+ T cells for effector- and/or memory-cell fate by regulating expression of T-bet and Eomes.[24,67] Inhibition of mTOR activity blocked persistent T-bet expression and promoted memory-precursor generation that showed greater tumor efficacy than type I effector CD8+ T cells.[67]

Furthermore, regulation of transcription factor FoxO1 (a target of AKT) upon mTOR inhibition has been confirmed. mTOR inhibition of antigen-activated CD8+T cells has been shown to increase the expression of Krueppel-like factor 2 (transcription factor KLF-2), which regulates T-cell trafficking by controlling expression of two key molecules (namely S1P1 and CD62L) and the pro-survival cytokine receptor CD127; both are direct gene targets of FoxO1.[68] The activity of FoxO1 is regulated by the Ser/Thr kinase AKT.[69,70] A recent study demonstrated that prolonged mTOR inhibition can regulate both upstream and downstream AKT activity; therefore, it has been suggested that prolonged mTOR inhibition of antigen-activated CD8+ T cells regulates AKT-dependent FoxO1 activity, thus controlling the expression of KLF2 and CD127.[37,71] Thus, these studies suggest a model in which mTOR is a rheostat, which, depending upon the nature and intensity of signals received, regulates the transcriptional balance to control CD8+ T-cell effector function and/or memory generation.

V. CONCLUSION

During the past few years, considerable progress has been made in understanding the lineage relationships between naïve, effector, and memory T cells and in defining the phenotypic and functional changes that underlie T-cell differentiation. But much less is known about the intracellular molecules and pathways that regulate the generation of T-cell differentiation. Recently, mTOR signaling mechanism studies have implied that it plays a great role in integrating different signal and regulating the effector T-cell and memory T-cell differentiation, which should contribute to the understanding of T-cell immunity in physiological and pathological conditions. However, some important questions remain to be answered. For example, how does mTOR regulate the expression of key genes that regulate different T-cell subpopulations of CD4+ and CD8+ T cells? Why does mTOR inhibition produce different effects in CD4+ versus CD8+ T cells? Future studies aimed at answering these questions are likely to identify new ways to improve or modulate CD4+ and CD8+ T-cell responses to regulate immunity and autoimmunity. Moreover, rapamycin has been regarded as an important drug for preventing allograft rejection and

treating autoimmune diseases in clinics, which suggests that modulation of the T-cell differentiation and immunity provides a new approach for enhancing the efficacy of vaccines against autoimmune diseases and cancer.

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