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RESEARCH HIGHLIGHT

Distinct strengths of mTORC1 control T-cell memory via transcriptional FOXO1 and metabolic AMPKα1 pathways in linear cell differentiation and asymmetric cell division models

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$CD8^+$ effector T (T_E) cells play a critical role in immunity against infections. In response to a pathogenic stimulus, antigen-presenting cells (APCs) deliver three signals [via T-cell receptor (TCR), costimulation, and cytokines] to naïve $CD8^+$ T cells, stimulating their entry into a developmental program characterized by T-cell expansion followed by a contraction phase. During the contraction phase, 90–95% of IL-$7R^-CD62L^-KLRG1^+$ T_E cells undergo cell apoptosis, and the remaining 5–10% of T cells differentiate into IL-$7R^+CD62L^+KLRG1^-$ memory T (T_M) cells (Fig. 1A) [1]. In this T_M-cell population, the best characterized subsets are $CD45RA^+CCR7^+$IL-$7R^+CD62L^+KLRG1^-$ stem cell-like T_M (T_{SCM}), $CCR7^+$IL-$7R^+CD62L^+KLRG1^-$ central T_M (T_{CM}), and $CCR7^-$IL-$7R^+CD62L^-KLRG1^-$ effector T_M (T_{EM}) cells (Fig. 1B). A central question in fundamental immunology is the origin of the long-lived T_M cells that confer protection against secondary infection. Two well-known models have been proposed to explain the origin of T_M cells: the "linear cell differentiation" [2] and "asymmetric cell division" models [3].

The "linear cell differentiation (LCD)" or "signal strength" model was originally proposed by Sallusto's group in 2000 and posits that strong and weak strengths of the aforementioned three signals control T-cell differentiation into short-lived T_E and long-lived T_M cells, respectively (Fig. 1B) [2]. Subsequent evidence has accumulated in support of this model, with distinct strengths of TCR or antigen (high and low affinities) and IL-2 (high and low doses) signals favoring T_E and T_M cell differentiation, respectively [4, 5]. Various transcription factors crucial to controlling T-cell phenotypes have been identified, with forkhead box-O-1 (FOXO1), FOXO1-regulated T-cell factor-1 (TCF1), inhibition of DNA-binding protein-3 (Id3) and Eomes favoring T_M cell differentiation and T-bet and Id2 favoring T_E cell differentiation (Fig. 1B) [1]. Adenosine monophosphate-activated protein kinase-α1 (AMPKα1) is a conserved energy sensor that plays central role in controlling cellular metabolism and survival [6]. AMPKα1 stimulates mitochondrial biogenesis and fatty acid oxidation (FAO) to support T_M-cell differentiation by increasing the abundance of Unc-51-like autophagy-activating kinase-1 (ULK1), autophagy-related gene-7 (ATG7), proliferator-activated receptor-γ coactivator-1α (PGC1α) and aquaporin-9 (AQP9). In contrast, mammalian target of rapamycin complex-1 (mTORC1) regulates the expression of hypoxia-inducible factor-1α (HIF-1α) and cMyC, which in turn promote glycolytic metabolism crucial for T_E cell development (Fig. 1B) [6]. However, despite concerted efforts to identify the contributions of key transcription factors and metabolic profiles to T-cell memory, the underlying molecular mechanism(s) controlling distinct T-cell differentiation programs has yet to be discovered.

mTORC1 is an evolutionarily conserved protein complex that senses the strengths of all three signals and plays important role in T-cell proliferation, metabolism, and differentiation [1]. In 2009, Ahmed's group provided the first evidence that rapamycin (Rapa)-mediated inhibition of mTORC1 promotes $CD8^+$ T_M-cell formation [7]. This finding was further supported by evidence that Rapa induces T-cell memory via a FOXO1-dependent transcriptional switch from T-bet to Eomes [8]. However, the molecular mechanism underlying Rapa-promoted T-cell memory is largely unknown.

The proinflammatory cytokine IL-2 and prosurvival IL-7 or IL-15 common γ-chain (γc)-family cytokines induce $CD8^+$ T_E-cell and T_M-cell formation, respectively, by triggering a Janus kinase-3 (JAK3) signal, leading to activation of the PI3K-AKT-mTORC1 pathway [9]. To assess how activation of a single signaling pathway allows the formation of two distinct T-cell lineages, we genetically engineered *IL-7R* gene knockout (KO)/ovalbumin (OVA)-specific TCR transgenic OTI (*IL-7R* KO/OTI) mice and adoptively transferred $CD8^+$ T cells derived from wild-type (WT) OTI or *IL-7R* KO/OTI mice into C57BL/6 mice. Following infection with recombinant *Listeria monocytogenes* rLmOVA, we demonstrated that, unlike T cells derived from WT OTI mice, *IL-7R* KO/OTI mouse-derived T cells downregulated the expression of FOXO1, TCF1, AMPKα1, and ULK1, which is necessary for T_M-cell formation, and exhibited defective cell survival [10]. In addition, we prepared in vitro IL-2- and IL-7-stimulated T (IL-2/T_E and IL-7/T_M) cells derived from OTI mice, which approximated the in vivo T_E- and T_M-cell differentiation programs, for subsequent characterization [10]. Surprisingly, we showed that IL-2 and IL-7 stimulated strong and weak mTORC1 (IL-2/$mTORC1^{Strong}$ and IL-7/$mTORC1^{weak}$) signaling due to persistent expression of cell-surface IL-2R, resulting in IL-2/$mTORC1^{Strong}$ signaling in IL-2/T_E cells, while transient expression of cell-surface

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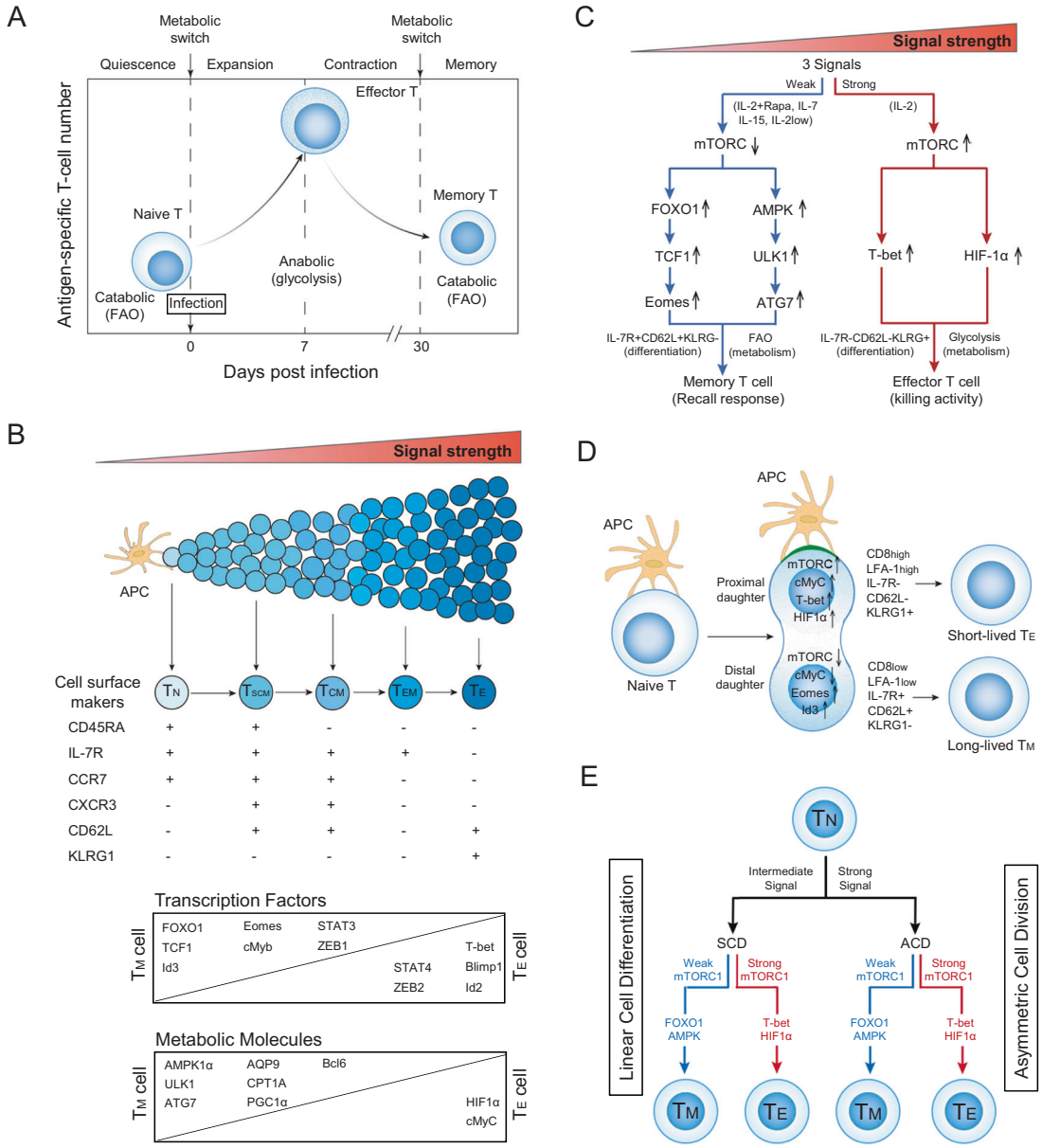

Fig. 1 mTORC1 signaling with distinct strengths controls T-cell memory via the transcriptional FOXO1 and metabolic AMPKα1 pathways in both the linear cell differentiation and asymmetric cell division models. **A** Metabolic changes in $CD8^+$ T cells at various stages of an acute infection. In response to a pathogenic stimulus, naïve $CD8^+$ T cells enter a developmental program characterized initially by T-cell expansion and then by a subsequent contraction phase. During this latter phase, the majority of effector T (T_E) cells undergo apoptosis, while the remaining minority of T cells differentiate into long-lived memory T (T_M) cells. To meet the bioenergetic demand during the expansion phase, naïve T cells switch from mitochondrial respiration to glycolysis. During contraction to the memory phase, the metabolic program reverts to catabolic fatty acid oxidation as T_E cells gradually transition into T_M cells. **B** In the linear cell differentiation model, signals provided at distinct strengths by the antigen-presenting cell (APC) control naïve T-cell differentiation into short-lived T_E and long-lived T_M cells, including stem cell-like T_M (T_{SCM}), central T_M (T_{CM}), and effector T_M (T_{EM}) cells. The phenotypic attributes and expression levels of transcription factors and metabolic molecules controlling these cellular phenotypes are illustrated. **C** Schematic diagram of the distinct strengths of cytokine signaling (strong and weak) that control naïve T-cell differentiation into T_E and T_M cells. A strong cytokine (IL-2, regular dose 100 U/ml) signal stimulates strong mTORC1 ($mTORC1^{Strong}$) signaling, leading to the formation of T_E cells via transcriptional T-bet and metabolic HIF-1α pathways. In contrast, weak cytokine [IL-7, IL-15, low dose (10 U/ml) of IL-2 ($IL\text{-}2^{Low}$) and IL-2 (regular dose) plus rapamycin (IL-2+Rapa)] signals stimulate weak mTORC1 ($mTORC1^{Weak}$) signaling, leading to the formation of T_M cells *via* transcriptional FOXO1-TCF1-Eomes and metabolic AMPK-ULK1-ATG7 pathways. **D** In the asymmetric cell division model, the proximal $CD8^{High}LFA\text{-}1^{High}KLRG1^{+}IL\text{-}7R^{-}CD62L^{-}$ daughter cell displays upregulation of mTORC1, cMyC, T-bet, and HIF-1α and adopts a $CD8^+$ T_E-cell fate during the first cell division. The distal $CD8^{Low}LFA\text{-}1^{Low}KLRG1^{-}IL\text{-}7R^{+}CD62L^{+}$ daughter cell shows up- and downregulation of Id3/Eomes and mTORC1/cMyC, respectively, and is destined to become a T_M cell. The green region at the contact interface between the APC and engaged T cell represents the immunological synapse. **E** Schematic diagram illustrating in both the asymmetric cell division (ACD) model with ACD and linear cell differentiation (LCD) model with symmetric cell division (SCD), how the $mTORC1^{Weak}$ signal promotes T-cell memory via transcriptional FOXO1 and metabolic AMPK pathways and the $mTORC1^{Strong}$ signal induces T_E cell formation via transcriptional T-bet and metabolic HIF-1α pathways

IL-7R resulted in IL-7/mTORC1^{weak} signaling in IL-7/T_M cells [10]. We also demonstrated that the IL-7/mTORC1^{weak} signal upregulated the levels of the phenotypic markers IL-7R and CD62L; increased the expression of the transcription factors FOXO1, TCF1, and Id3 for T_M-cell differentiation and the metabolic molecules AMPKα1, ULK1, ATG7, PGC1α, and AQP9 for stimulating mitochondrial biogenesis and FAO metabolism in IL-7R$^+$CD62L$^+$KLRG1$^-$ IL-7/T_M cells; and promoted long-term T-cell survival and recall responses upon a secondary antigen boost [10]. In contrast, mTORC1^{Strong} signaling in IL-7R$^-$CD62L$^-$KLRG1$^+$ IL-2/T_E cells reduced the collective activity of these pathway-related molecules and instead increased the abundance of the transcription factors T-bet and HIF-1α for T_E-cell differentiation and glycolytic metabolism, respectively [10]. These data collectively indicate that IL-7/mTORC1^{Weak} signaling induces T-cell memory via the transcriptional FOXO1 and metabolic AMPKα1 pathways (Fig. 1C). This conclusion is also supported by the findings that IL-15-stimulated prosurvival IL-15/T_M cells and a low dose of inflammatory IL-2-stimulated IL-2^{Low}/T_M cells exhibited mTORC1^{Weak} signaling, a T_M-cell phenotype and long-term survival after adoptive transfer into C57BL/6 mice (Fig. 1C) [10].

To test molecular pathways crucial for Rapa-promoted T-cell memory, we prepared in vitro IL-2-stimulated OTI T cells in the absence or presence of Rapa to form IL-2(Rapa-)/T (IL-2/T) and IL-2 (Rapa+)/T cells, respectively, for subsequent characterization [11]. We demonstrated that IL-2(Rapa+)/T cells with mTORC^{Weak} signaling upregulated the expression of the transcription factors FOXO1, TCF1, and Eomes and the metabolic regulators AMPKα1, pULK1, and ATG7 to promote mitochondrial biogenesis and FAO and showed long-term survival after adoptive cell transfer into C57BL/6 mice compared to IL-2/T_E cells with mTORC1^{Strong} signaling [11]. These findings indicate that the Rapa-induced mTORC^{Weak} signal promotes T-cell memory via the concerted activity of the transcriptional FOXO1-TCF1-Eomes and metabolic AMPKα1-ULK1-ATG7 networks in IL-2(Rapa+)/T_M cells (Fig. 1C) [11] and further support the above finding that mTORC1^{Weak} signaling induces T-cell memory via the transcriptional FOXO1 and metabolic AMPKα1 pathways [10].

In addition to the "LCD" model, Reiner's group proposed an "asymmetric cell division (ACD)" or "bifurcative differentiation" model in 2007, in which stimulation of a single naïve CD8$^+$ T-cell by an APC gives rise to two descendant daughter cells with distinct fates after the first cell division [3]. A special type of cellular apparatus called the immunological synapse (IS) is formed at the contact interface between the engaged naïve CD8$^+$ T cell and APC. Numerous CD8, cytoskeletal talin, and T-cell/APC molecular conjugates, such as TCR/antigenic peptide/major histocompatibility complex-I (pMHCI), costimulatory CD28/CD80 and adhesive LFA-1/CD54, accumulate at the contact interface to form the IS. The CD8$^+$ T cell then undergoes extensive cytoskeletal remodeling, leading to asymmetric partitioning of cell-surface molecules (CD8, CD62L, IL-7R, LFA-1, and KLRG1) [3, 12] and intracellular cell fate determinants (T-bet, Id3, Eomes, and Bcl6) [13]. Recently, it has been shown that this ACD process is also marked by differential segregation of key molecular components, such as mTORC1, cMyC, and amino acid transporters, into the daughter cells [14, 15]. This asymmetric molecular distribution pattern is maintained during mitosis and leads to the formation of two progenitor daughter cells with distinct fates after the first cell division [3]. As a result, the proximal CD8^{High}LFA-1^{High}KLRG1$^+$IL-7R$^-$CD62L$^-$ daughter cell displays upregulation of mTORC1, cMyC, T-bet, and HIF-1α and adopts a CD8$^+$ T_E-cell fate, while the distal CD8^{Low}LFA-1^{Low}KLRG1$^-$IL-7R$^+$CD62L$^+$ daughter cell shows increased Id3/Eomes expression and decreased mTORC1/cMyC expression and is destined to become a T_M cell (Fig. 1D). In addition, CD8$^+$ T cells exposed to strong signals (high affinity for the antigenic peptide or higher levels of LFA-1 adhesion molecules) have been shown to undergo ACD, whereas T cells activated by below-threshold antigenic stimulation undergo symmetric cell division (SCD), indicating that strong stimuli preferentially lead to ACD, whereas intermediate (less strong) stimuli favor SCD [12]. Asymmetric inheritance of mTORC1 has an impact on lymphocyte metabolic fitness, with the mTORC1^{Weak} distal daughter cell displaying elevated FAO and long-term survival [15].

The above evidence strongly supports the notion that the mTORC1^{Weak} signal in the distal daughter cell promotes T-cell memory in the strong signal-stimulated "ACD" model, while mTORC1^{Weak} signaling is able to promote T-cell memory development during SCD in the intermediate signal-stimulated "LCD" model (Fig. 1E). In both models, however, the mTORC1^{Weak} signal promotes T-cell memory by coordinately regulating the expression of the transcriptional FOXO1 and metabolic AMPKα1 networks, indicating that the distinct strengths of mTORC1 signaling control T-cell memory via transcriptional FOXO1 and metabolic AMPKα1 pathways in the "LCD" and "ACD" models (Fig. 1E).

Understanding the molecular mechanism that governs T-cell memory is of great importance in vaccine or immunotherapy design. The above novel findings not only elucidate the molecular mechanism underlying the origin of T_M cells in the "LCD" and "ACD" models but also have a great impact on the development of efficient immunotherapies and vaccines for cancer and infectious diseases.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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