T CELL MEMORY

mTORC2 forms iron-clad defense to guard memory

Long-term persistence of memory CD4⁺ T cells is supported by mTORC2-dependent protection from a distinctive form of regulated cell death known as ferroptosis.

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CD4⁺ T helper cells orchestrate adaptive immune responses to invading pathogens, with antigen-specific memory T cells being crucial for mediating long-term protection from reinfection. Compared with memory CD8⁺ T cells, the molecular mechanisms that underlie the preservation of memory CD4⁺ T cells are less well understood. In this issue of *Nature Immunology*, Wang et al.[1] uncover a role for mechanistic (or mammalian) target of rapamycin (mTOR) complex 2 (mTORC2) in sustaining the memory CD4⁺ T cell pool by promoting an antioxidant program to reduce the levels of mitochondrial reactive oxygen species (ROS). Notably, memory CD4⁺ T cells with impaired mTORC2 signaling are prone to cell death via ferroptosis, a unique type of regulated cell death that is dependent on iron and characterized by excessive ROS-dependent lipid peroxidation[2]. These findings hold immediate clinical implications for the improvement of long-term immunity.

During an immune response, T cells traverse through different activation stages that ultimately lead to the generation of short-lived effector or long-lived memory T cells. The differentiation into these stages is shaped by dynamic metabolic states that are regulated by mTOR and other signaling pathways[3]. Signaling via mTOR complex 1 (mTORC1) is an essential driver for the activation and differentiation of T cells[4]. By comparison, mTORC2 signaling roles in T cells are more nuanced with respect to T cell subset and tissue location. For example, mTORC2 is dispensable for the differentiation of T helper 1 (T_H1) cells and early effector function, but is required to establish T_H1 memory. Moreover, mTORC2 is essential for the differentiation and function of T follicular helper (T_{FH}) cells[1,5,6]. Interestingly, mTORC2 and downstream Akt signaling promote early upregulation of glycolysis specifically in memory T cells to support their effector function upon reactivation[7]. The current study demonstrates a positive role for mTORC2 signaling in sustaining CD4⁺ T cell memory. The molecular mechanisms behind such intriguing cell type- and cell state-specific regulation warrant further investigation.

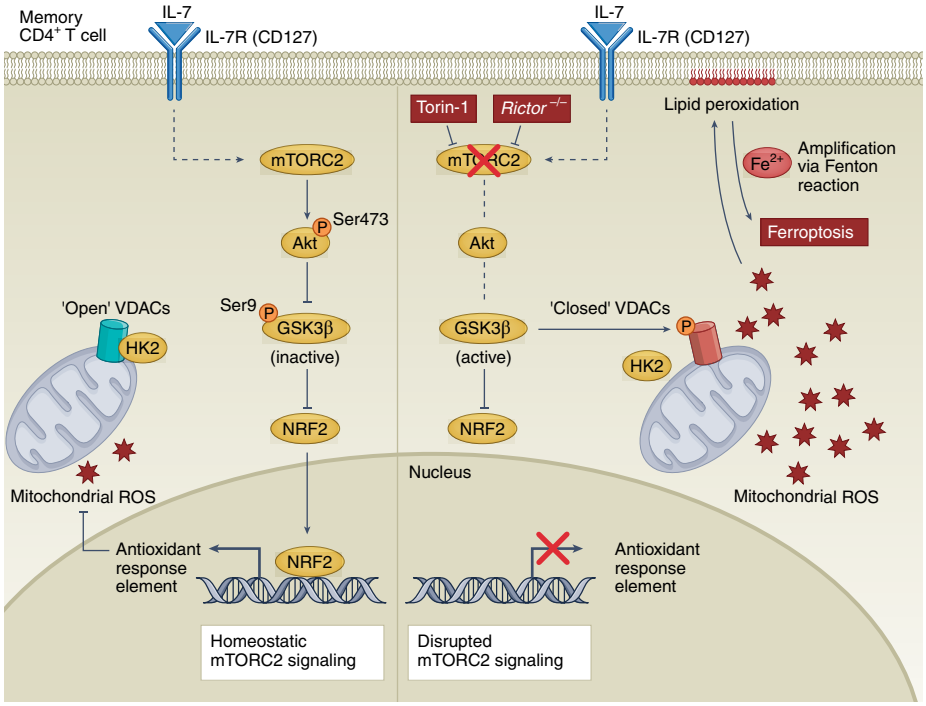

Fig. 1 | The mTORC2–Akt–GSK3β signaling axis sustains CD4⁺ T cell memory by preventing ferroptotic cell death. Left, virus-specific memory CD4⁺ T cells have increased IL-7–IL-7R and mTORC2 signaling to enable proper antioxidant activity and homeostatic levels of mitochondria-derived ROS. Mechanistically, upstream mTORC2–Akt signaling promotes phosphorylation and inactivation of GSK3β. Right, inhibition of mTORC2 signaling in memory CD4⁺ T cells, such as by deletion of *Rictor* or treatment with Torin-1, leads to upregulated GSK3β activity, including the disruption of homeostatic mitochondrial VDAC–HK2 interactions and inhibition of NRF2-dependent antioxidant programs. Consequently, accumulated mitochondrial ROS reacts with membrane lipids, forming lipid peroxides that are further propagated in an iron-dependent manner via the Fenton reaction. Aberrant lipid peroxidation ultimately causes ferroptotic cell death and the collapse of the virus-specific memory CD4⁺ T cell pool.

The authors use a mouse model of acute lymphocytic choriomeningitis virus (LCMV) infection to study virus-specific CD4⁺ T cells in the memory phase[1]. They observe increased mTORC2 signaling in memory CD4⁺ T cells compared with naive CD4⁺ T cells, as evidenced by increased levels of p-Akt(Ser473), a canonical substrate for mTORC2. Furthermore, ablation of mTORC2 signaling via genetic deletion of *Rictor* or by treatment with the small-molecule inhibitor Torin-1 in established memory CD4⁺ T cells impaired

cellular persistence, primarily owing to cell death that was not driven by apoptosis, necrosis or pyroptosis. Instead, ablation of mTORC2 led to an accumulation of mitochondria-derived ROS and peroxidized lipids. These alterations are hallmarks of ferroptosis[2], an iron-dependent regulated cell death process that is caused by uncontrolled mitochondria-derived ROS and the resultant accumulation of membrane lipid peroxides. Mechanistically, mTORC2 signaling inhibits ferroptosis by orchestrating mitochondrial activity (Fig. 1). Specifically, mitochondrial voltage-dependent anion channels (VDACs) have a regulatory role in ferroptosis by maintaining proper membrane permeability and ROS homeostasis. The 'open' state of VDACs is stabilized by binding to hexokinase 2 (HK2), a process that requires the mTORC2–Akt-dependent inhibitory phosphorylation of GSK3β(Ser9)[8]. Consistent with this notion, the authors showed that there was a reduction of HK2-associated VDACs in *Rictor*-deficient memory CD4$^+$ T cells. Furthermore, increased levels of ROS were recently shown to impair HK2 stability and downstream enzymatic antioxidant function in effector T cells[9], which suggests that a perturbance to this regulatory axis could quickly promote uncontrolled ROS accumulation and pro-ferroptosis conditions. Thus, VDACs probably adopt a 'closed' state after mTORC2 inhibition, which leads to dysregulated mitochondrial function and ROS production that further impair HK2 activity and promote ferroptosis.

The current study also demonstrates that increased GSK3β activity that occurs as a consequence of mTORC2 inhibition diminishes NRF2 nuclear localization and transcriptional function[1]. NRF2 is the master transcriptional regulator that promotes expression of glutathione peroxidase 4 (GPX4) and components of the system x_c^- cystine and glutamine antiporters. These antiporters increase intracellular concentrations of cystine that are important for the synthesis of glutathione (GSH), the essential cofactor for GPX4. Ferroptotic cells typically have diminished activity of these elements, making them vulnerable to iron-mediated propagation of lipid peroxides via the Fenton reaction. Consistent with decreased NRF2 activity, memory CD4$^+$ T cells with ablated mTORC2 signaling display decreased system x_c^- gene expression and intracellular cystine concentrations. Notably, the authors find these effects to be specific to memory, but not effector, CD4$^+$ T cells. A previous study found that T cell-specific deletion of *Gpx4* renders both CD4$^+$ and CD8$^+$ T cells to be sensitive to ferroptotic cell death after primary activation[10]. Furthermore, induced deletion of *Gpx4* during the memory phase did not affect antigen-specific CD8$^+$ T cell responses during a secondary challenge, although its effects on CD4$^+$ T cell memory were not reported[10]. Given the differential requirements for mTORC2 among T cell subsets, it is possible that mTORC2-independent signals may support anti-ferroptosis programs (for example, by regulating GPX4 expression or function) in those CD4$^+$ and CD8$^+$ T cell subsets marked by relatively low mTORC2 activity. Finally, the authors show that mTORC2 inhibition-induced ferroptosis can be reversed by ectopic expression of an inactive mutant form of GSK3β in memory CD4$^+$ T cells[1]. Together, these data suggest that ferroptotic cell death in memory CD4$^+$ T cells is prevented via continuous signaling through the mTORC2–Akt–GSK3β axis.

One important question remaining is how upstream signals regulate this process. Cytokines including interleukin 2 (IL-2), IL-7 and IL-21 activate mTOR signaling pathways in CD4$^+$ T cells. In particular, IL-7 promotes the maintenance of memory CD4$^+$ T cells through engagement of IL-7R (with specific subunit CD127), and the current study demonstrates increased CD127 expression in memory CD4$^+$ T cells, which mediates IL-7–IL-7R signaling for increased levels of p-Akt(Ser473) in these cells. Metabolic pathways or nutrients may also contribute to mTORC2 activation in memory CD4$^+$ T cells, as phospholipid metabolism was recently shown to sustain mTORC2 signaling in T_{FH} cells by maintaining the expression of the chemokine receptor CXCR5 on the cell surface[11]. Whether differential regulation of surface receptor expression in CD4$^+$ T cell subsets is mechanistically involved in the regulation of ferroptosis requires further evaluation.

This study[1] has important implications for understanding how to bolster immune memory to improve protection to infections, including SARS-CoV2. The findings suggest that mTORC2 modulation could improve T_{FH} cell responses that promote protective humoral immune responses, thus combating the possibility of waning protective humoral immunity to COVID-19 or other vaccines. Many clinical trials are currently tracking longitudinal immune responses in humans after vaccination, and the results of this current study suggest that mTORC2 signaling activity in memory CD4$^+$ T cells may be a positive predictor of an individual's ability to sustain protective memory responses. Moreover, it would be of interest to examine whether alterations in mTORC2 activity over time correlate with the frequency of memory CD4$^+$ T cells. Augmentation of mTORC2 signaling could improve memory CD4$^+$ T cell retention and support robust reactivation potential. However, one notable caveat is that mTORC2 signaling antagonizes the formation of memory CD8$^+$ T cells by decreasing FOXO1-dependent expression of the memory-promoting transcription factor TCF-1[12]. Thus, the timing of modulating mTORC2 activity (that is, differentiation of memory versus established memory cells) would be crucial to ensure that memory CD8$^+$ T cell differentiation is not hampered.

In addition, these findings suggest that ferroptosis could be wielded as a therapeutic weapon against pathogenic memory CD4$^+$ T cells that are associated with autoimmunity or allergic disease. Future studies are required to address whether mTORC2-dependent protection from ferroptosis observed in acute viral infection is translatable within these immunopathological conditions. Furthermore, could aberrant mTORC2 itself be responsible for chronically sustaining these harmful cell populations? The apparent cellular specificity of mTORC2 makes it an attractive signaling hub to target. In summary, this study highlights a new function of the mTORC2–Akt–GSK3β signaling axis to prevent ferroptosis and enable the persistence of long-term CD4$^+$ T cell memory. ❐

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Competing interests

H.C. a consultant for Kumquat Biosciences.