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CALCIUM REGULATION OF T CELL METABOLISM

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Abstract

T cells are an essential component of the immune system that provide antigen-specific acute and long lasting immune responses to infections and tumors, ascertain the maintenance of immunological tolerance and, on the flipside, mediate autoimmunity in a variety of diseases. The activation of T cells through antigen recognition by the T cell receptor (TCR) results in transient and sustained Ca^{2+} signals that are shaped by the opening of Ca^{2+} channels in the plasma membrane and cellular organelles. The dynamic regulation of intracellular Ca^{2+} concentrations controls a variety of T cell functions on the timescale of seconds to days after signal initiation. Among the more recently identified roles of Ca^{2+} signaling in T cells is the regulation of metabolic pathways that control the function of many T cell subsets. In this review, we discuss how Ca^{2+} regulates several metabolic programs in T cells such as the activation of AMPK and the PI3K-AKT-mTORC1 pathway, aerobic glycolysis, mitochondrial metabolism including tricarboxylic acid (TCA) cycle function and oxidative phosphorylation (OXPHOS), as well as lipid metabolism.

INTRODUCTION

Ca^{2+} plays essential roles in maintaining and controlling biological function as a key regulator of various cell functions including muscle contraction, neurotransmitter release and hormone secretion. The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) is tightly regulated. In non-stimulated cells it is ~50–100 nM, which is $> 10^3$ fold lower than in the extracellular space (~1–2 mM) and the main organellar Ca^{2+} store, the endoplasmic reticulum (ER) (~0.4 mM) [1]. This gradient, together with the negative membrane potential of cells, provides the driving force for Ca^{2+} influx. Ca^{2+} transport across membranes is mediated by a variety of Ca^{2+} channels including voltage- or ligand-gated and store-operated Ca^{2+} channels [2]. The opening of Ca^{2+} channels results in local or global changes in $[\text{Ca}^{2+}]_i$ that function as an important signal transduction mechanism by regulating a wide variety of Ca^{2+} dependent proteins, enzymes and transcription factors. In lymphocytes such as T, B and NK cells,

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which are components of the adaptive immune system, dynamic changes in $[Ca^{2+}]_i$ regulate cell functions on different time scales. Within seconds to minutes, $[Ca^{2+}]_i$ increases following antigen receptor stimulation affect processes like the release of cytotoxic granules by CD8⁺ T cells and NK cells or lymphocyte migration. Within hours after stimulation, Ca^{2+} signals promote the de novo gene expression and production of cytokines, chemokines, cell surface receptors or pro- and anti-apoptotic genes that shape lymphocyte function. At even longer time scales, within days after stimulation, Ca^{2+} signals modulate the expression of genes that determine lymphocyte differentiation with profound impacts on T and B cell fates.

An important aspect of Ca^{2+} signaling in lymphocytes that has come into focus more recently is its role in regulating energy metabolism [3]. Immunometabolism itself has emerged as an important regulator of immune function in the last decade [4,5]. One of the key insights from these studies is that different subsets of macrophages and lymphocytes use distinct metabolic programs at various stages of their life cycle and differentiation, which is thought to serve their specific metabolic demands during the course of an immune response (Figure 1). For example, resting naive T cells have low nutrient consumption, metabolic rates and biosynthesis, which changes dramatically after T cell stimulation. Activated T cells upregulate the expression of glucose and other nutrient transporters, glycolytic enzymes and mitochondrial pathways that support the production of ATP and anabolic metabolites used for the synthesis of lipids, amino acids and nucleotides to enable immune cell growth and proliferation [3,6]. Besides controlling the energetic demands of immune cells, metabolic pathways – through the metabolites they produce – are emerging as important regulators of gene expression through epigenetic modulation of transcription [7,8]. Ca^{2+} was recently found to control several metabolic programs in T cells and other lymphocyte subsets. In this review, we will discuss the role of Ca^{2+} signaling pathways in the regulation of several key metabolic programs in lymphocytes such as (i) phosphoinositide-3-kinase (PI3K)-Akt-mechanistic target of rapamycin (mTOR) signaling, (ii) adenosine monophosphate-activated protein kinase (AMPK) activation, (iii) aerobic glycolysis, (iv) mitochondrial metabolism including tricarboxylic acid (TCA) cycle regulation and oxidative phosphorylation, and (v) lipid metabolism.

Ca^{2+} REGULATES SEVERAL METABOLIC SIGNALING PATHWAYS

Regulation of the PI3K-AKT-mTORC1 pathway by Ca^{2+} .

The stimulation of T cells through the TCR and costimulatory receptors like CD28 results in the activation of the PI3K-AKT-mTORC1 signaling pathway. Activation of mTORC1 plays a vital role in the regulation of many T cell functions including mRNA transcription, protein synthesis, cell growth and proliferation, which collectively control the fate of many T cell subsets [9,10]. In T cells, store-operated Ca^{2+} entry (SOCE) through Ca^{2+} release-activated Ca^{2+} (CRAC) channels was recently shown to be essential for the activation of the PI3K-AKT-mTORC1 pathway (Figure 2) [11]. SOCE is the predominant Ca^{2+} influx pathway in T cells that is induced after TCR stimulation, the production of IP₃ and release of Ca^{2+} from the ER, which triggers the activation of two ER proteins, stromal interaction molecules (STIM) 1 and 2. Activated STIM1 and STIM2 bind to a hexameric ORAI1 protein complex

in the plasma membrane that forms the Ca^{2+} selective CRAC channel [12]. Deletion of *Stim1* and *Stim2* in mouse CD4^+ and CD8^+ T cells abolishes SOCE and inhibits phosphorylation of AKT, mTORC1 and the mTORC1 target S6 kinase following TCR engagement and costimulation through CD28 [11]. A similar defect is observed in T cells of patients with a loss-of-function mutation in the *STIM1* gene that abolishes SOCE [13]. Furthermore, wildtype T cells that are stimulated in the presence of an inhibitor of the Ca^{2+} /calmodulin-dependent phosphatase calcineurin also have reduced phosphorylation of AKT, mTORC1 and S6 kinase [11,14], suggesting that SOCE and calcineurin control the activation of this pathway. A similar role of SOCE in B cell metabolism was recently reported by Freedman and colleagues [15]. B cells lacking *STIM1* and *STIM2* have reduced activation of NFAT and NF- κ B and expression of the NF- κ B subunit c-Rel, resulting in impaired activation of mTORC1 and c-Myc signaling and B cell proliferation. In this study, the Ca^{2+} -regulated checkpoints of B cell survival, proliferation, and mTORC1 activation could be bypassed by costimulating SOCE-deficient B cells through CD40 or TLR9 engagement [15]. This is reminiscent of SOCE-deficient T cells, in which cytokine stimulation through IL-2 and IL-7 receptors, but not CD28, partially restored T cell expansion [11].

The molecular mechanisms underlying the crosstalk between the Ca^{2+} -calcineurin and PI3K-AKT-mTOR pathways in T and B cells are presently not well understood. In Th17 cells, a subset of CD4^+ T cells characterized by the expression of the transcription factor ROR γ t and production of the cytokine IL-17, inhibition or deletion of the Ca^{2+} /calmodulin-dependent kinase 4 (CaMK4) was shown to suppress AKT phosphorylation, IL-17 production and the severity of Th17-mediated autoimmune disease [16]. These effects were, however, indirect as CaMK4 controls the cAMP response element modulator α (CREM- α) and thereby CpG-DNA methylation of the *Il17a* gene [16]. A more direct effect of Ca^{2+} and calmodulin on AKT activation was found in neuroblastoma cells, in which the Ca^{2+} /calmodulin-dependent protein kinase kinase (CaMKK or CaMKK2) activates AKT in a Ca^{2+} -dependent manner [17]. A similar role of CaMKK was found in ovarian cancer cell lines, in which combined inhibition of CaMKK and PI3K resulted in additive effects on AKT phosphorylation [18]. Whether and how PI3K is regulated by Ca^{2+} is not well understood. In HeLa cells, Ca^{2+} -bound calmodulin was found to bind to the class III PI3K (Vps34), rather than canonical class I PI3K used by growth factors and hormones, in response to Ca^{2+} transients evoked by amino acid stimulation [19]. Besides SOCE, Ca^{2+} release from lysosomes through mucolipin transient receptor potential channel 1 (TRPML1) has been reported to regulate mTORC1 activation in non-immune cells by inducing the association of calmodulin with mTOR [20,21]. Whether lysosomal Ca^{2+} release through TRPML1 plays a role in the metabolic regulation of T cells or other immune cells remains to be investigated. Taken together, SOCE and other Ca^{2+} signaling pathways emerge as critical regulators of PI3K-AKT-mTORC1 pathways and thus T cell metabolism.

Ca^{2+} -dependent regulation of AMPK.

AMPK is an important metabolic regulator that detects changes in cellular AMP to ATP ratios and limits ATP consuming metabolic pathways [22,23]. T cells exclusively express one AMPK isoform, AMPK α 1, which is activated by two independent pathways that

synergize to increase AMPK activity [24] (Figure 1B). The first pathway is activated by energetic stress due to a high AMP:ATP ratio, resulting in AMP binding to AMPK, which allows liver kinase B1 (LKB1) to phosphorylate AMPK at Threonine 172 (Thr172) and activate it [25]. The second pathway is dependent on TCR stimulation and increases in $[Ca^{2+}]_i$, which result in the activation of CaMKK2 and phosphorylation of AMPK at Thr172 [24]. The activation of AMPK by Ca^{2+} and CaMKK, unlike that by LKB1, can occur in the absence of energy stress [24]. AMPK plays a vital role in cell metabolism throughout the entire life span of T cells. In resting cells, a high AMP:ATP ratio results in increased AMPK activity and suppression of cell growth. Mechanistically AMPK constrains the metabolic programs associated with T cell growth and proliferation by activating TSC2 (Tuberous Sclerosis Complex 2), an upstream inhibitor of mTORC1 [6,26]. High AMPK and low mTORC1 activity early after TCR stimulation allows T cells to prepare for the engagement of glycolytic metabolism that is the prerequisite for cell growth and proliferation [27]. Whereas AMPK-deficient T cells have normal effector functions under regular nutrient conditions [28], their major defect is a lack of metabolic adaptation to glucose limitation or nutrient replacement, which causes increased apoptosis [29]. Following an initial phase of T cell stimulation, the ATP:AMP ratio increases due to enhanced glycolysis and mitochondrial ATP production resulting in attenuated AMPK activity and release of mTORC1 inhibition, which shifts T cell metabolism to enhanced oxidative phosphorylation (OXPHOS) and glycolysis (Figure 1B). Besides controlling the metabolic shift of naive to effector T cells, AMPK is involved in regulating the metabolic transition of CD8⁺ effector to memory T cells after infection [30,31]. T cell-specific deletion of the tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) was shown to impair AMPK activation and mitochondrial fatty acid oxidation (FAO) [30]. This defect results in compromised development of memory CD8⁺ T cells after infection, but can be overcome by direct activation of AMPK with the anti-diabetic drug metformin. These findings are consistent with another study showing. While these studies [30,31] did not address if the effects of AMPK are dependent on Ca^{2+} , another study showed that the purinergic receptor P2RX7 promotes the transition of CD8⁺ effector T cells to long-lived memory cells by activating AMPK and suppressing mTORC1 [32]. One of the functions of P2RX7 is to mediate Ca^{2+} influx upon binding of extracellular ATP, and Jameson and colleagues [32] suggest that Ca^{2+} influx through P2RX7 may induce both AMPK activation and mitochondrial ATP production to induce and maintain memory CD8⁺ T cells (Figure 1C). In a follow-up study, the same authors demonstrate that P2RX7 controls the development and maintenance of virus-specific, CD103⁺CD69⁺ tissue-resident memory (Trm) CD8⁺ T cells through induction of the TGF- β signaling pathway [33]. Intriguingly, the inhibition of calcineurin function by low-dose FK506 treatment reduced TGF- β -induced CD103 expression in WT effector CD8⁺ T cells *in vitro* to levels observed in *P2rx7*^{-/-} T cells, suggesting that the observed P2RX7-mediated TGF- β sensitivity of memory CD8⁺ T cells is dependent on Ca^{2+} -calcineurin signaling [33]. Whether the P2RX7-dependent expression of TGF- β RII is indeed mediated by Ca^{2+} influx through P2RX7 requires further analysis. A role of SOCE in the regulation of AMPK was described in human SH-SY5Y neuroblastoma cells stimulated through muscarinic acetylcholine receptors (mAChRs) to induce Ca^{2+} influx. Deletion of STIM1 reduced both $[Ca^{2+}]_i$, AMPK activation and glucose uptake, suggesting that SOCE is required for sustained activation of AMPK and neuronal energy metabolism [34]. STIM1 dependent activation of AMPK was

also observed in alveolar epithelial cells in response to hypoxia [35]. In HEK293 cells, STIM2 rather than STIM1 was shown to interact with AMPK and CaMKK2 to regulate Ca^{2+} -induced AMPK activation [36]. Whether STIM proteins and SOCE also regulate AMPK α 1 function in T cells has not been reported. Conversely, however, AMPK α 1-deficient murine T cells were shown to have increased ORAI1 cell surface expression and SOCE [37], suggesting that AMPK and SOCE may reciprocally regulate each other. The consequences of this regulation for T cell-mediated immune responses have not been explored. Taken together, various Ca^{2+} signaling pathways appear to contribute to the regulation of AMPK and T cell metabolism.

Ca^{2+} signaling regulates glycolysis.

An important energy source in activated T cells is glucose. Depriving T cells of glucose or abolishing expression of glucose transporter 1 (GLUT1) impairs TCR-induced proliferation and T cell-mediated immunity [38]. Several signaling pathways and transcription factors have been reported to regulate the metabolic adaptation of activated T cells [39]. This adaptation is required to allow T cells to proliferate, clonally expand and become effector cells. We recently showed that SOCE mediates the activation of calcineurin to control the metabolic reprogramming of quiescent T cells after TCR and CD28 stimulation (Figure 2) [11]. Abolishing SOCE in mouse T cells by deletion of *Stim1* and *Stim2* or inhibition of calcineurin with FK506 impaired TCR-induced proliferation *in vitro* and clonal expansion of virus-specific T cells *in vivo*. SOCE and calcineurin control the expression of the glucose transporters GLUT1 and GLUT3 and the majority of glycolytic enzymes. This critical role of SOCE results from the Ca^{2+} - and calcineurin-dependent expression of transcription factors such as c-Myc, IRF4 and HIF1 α , which are key regulators of glycolysis in activated T cells [11]. Many of the effects of SOCE on glycolytic metabolism in naive T cells are mediated by the calcineurin-dependent activation of NFAT. Deletion of NFATc1 and NFATc2 in T cells strongly impaired glycolytic gene expression, whereas expression of constitutively active NFATc1 in SOCE-deficient T cells partially restored glycolysis and T cell proliferation [11]. These findings are consistent with a study in cytotoxic CD8 $^{+}$ T cells showing that NFATc1 controls metabolic gene expression, the glycolytic switch, and thus T cell function [40]. Although NFATc1 is essential for glycolytic gene expression, NFATc1 binds only a few glycolysis-associated genes (*Glut3* and *Hk2*) directly. Instead, NFATc1 appears to regulate the levels of GLUT1 and glycolytic enzymes indirectly, for instance by controlling the expression of other transcription factors such as IRF4 [11,41], HIF-1 α [11,42], and c-Myc [11,14,40,43]. Besides controlling the glycolytic metabolism of T cells at the transcriptional level, SOCE and calcineurin also regulate the activation of the PI3K-AKT-mTORC1 pathway at the posttranslational level as discussed above. Collectively, these findings suggest that SOCE controls a metabolic checkpoint at which lymphocytes assess adequate nutrient supplies to support their growth and proliferation. They also help explain the lymphocyte proliferation defect and immunodeficiency of SOCE-deficient patients with mutations in *STIM1* and *ORAI1* genes [44] and provide novel insights into the pharmacodynamics of the calcineurin inhibitors tacrolimus (FK506) and cyclosporin A, which are widely used to prevent the rejection of allogeneic organ transplants and for the treatment of T cell-mediated inflammation in eczema and psoriasis. Their potent immunosuppressive effects on T cells have been mostly attributed to the suppression of

proinflammatory cytokine production, but an important new mechanism underlying the effects of both drugs may be their inhibition of T cell metabolism, which is reminiscent of another immunosuppressive drug, rapamycin, which blocks mTORC1 function.

An important role of SOCE in glycolysis was found in Th17 cells, which are critical for providing adaptive immunity to infection with bacterial and fungal pathogens. However, Th17 cells can be pathogenic and mediate inflammation in several autoimmune and inflammatory diseases such as multiple sclerosis (MS), Crohn's disease and psoriasis [45]. A comparative transcriptome analysis of both Th17 cell subsets polarized *in vitro* revealed that SOCE regulates glycolysis only in non-pathogenic Th17, but not pathogenic Th17 cells [13]. Deletion of SOCE in non-pathogenic Th17 cells of *Stim1^{fl/fl}Cd4Cre* mice results in reduced expression of glucose transporters and glycolytic enzymes and, as a consequence, decreases glucose uptake and glycolysis. These effects are mediated by the SOCE dependent regulation of Foxo, HIF-1 α and c-Myc signaling pathways. A dispensable role of SOCE in regulating glycolysis in pathogenic Th17 cells was also observed in another study using mice expressing a hyperactive form of STAT3, which drives the differentiation of proinflammatory Th17 cells. Deletion of *Stim1* in Th17 cells of these mice did not significantly impair mTOR activation and the expression of glycolysis-associated genes, glucose uptake or glycolysis, suggesting that SOCE is not required for the regulation of glycolytic metabolism in pathogenic Th17 cells [46]. Neither does SOCE appear to regulate the expression of glutamine transporters such as ASCT2 (SLC1A5) and LAT1 (SLC7A5), which are required for the induction of EAE by T cells [47,48], in pathogenic Th17 cells [46]. It is intriguing to speculate that the differential regulation of glycolysis by SOCE in these distinct Th17 cell subsets could be exploited for the treatment of Th17 cell-mediated inflammatory diseases. Taken together, Ca²⁺ signaling and especially SOCE appears to have important roles in regulating the metabolism of several T cell subsets.

THE ROLE OF CALCIUM IN MITOCHONDRIAL METABOLISM

From studies in myocytes and other non-immune cell types, Ca²⁺ has long been known to regulate mitochondrial metabolism including the activity of TCA cycle enzymes, electron transport chain (ETC) function and the production of reactive oxygen species (ROS) [49,50]. Conversely, mitochondria are important regulators of intracellular Ca²⁺ homeostasis and signaling by acting as a major organellar Ca²⁺ store [51,52]. Uptake of Ca²⁺ by mitochondria also regulates the function of CRAC channels and SOCE by buffering [Ca²⁺]_i near the ER, thus limiting Ca²⁺ refilling of ER stores, prolonging STIM activation, and opening of CRAC channels. In addition, CRAC channels are subject to negative feedback inhibition called Ca²⁺-dependent inactivation, and lowering of [Ca²⁺]_i near the channel by Ca²⁺ uptake into mitochondria allows for sustained CRAC channel opening and SOCE [51]. This role of mitochondria in regulating [Ca²⁺]_i homeostasis has been reviewed in detail elsewhere [53,54] and will not be discussed here. The conventional function of mitochondria is the generation of ATP from oxidation of glucose, certain amino acids and lipid intermediates. Glucose enters the mitochondrial matrix as pyruvate, which fuels two important metabolic pathways: the TCA cycle and the ETC. In the TCA cycle, pyruvate is oxidized resulting in the generation of the reducing equivalents NADH and FADH₂ that fuel the ETC. The ETC pumps protons across the inner mitochondrial membrane, which create

the mitochondrial membrane potential and driving force for ATP production by the ATP synthase complex. The TCA cycle and ETC are upregulated upon T cell activation and both play critical roles in T cell function [4,5]. The TCA cycle provides intermediates for the synthesis of anabolic metabolites such as amino acids and lipids, whereas the ETC is essential for the production of ATP and also ROS, which were shown to contribute to T cell activation [14,55,56].

Ca²⁺ regulates TCA cycle function.

Upon T cell stimulation, the metabolic flow through the TCA cycle increases to support the production of NADH and FADH₂, ETC function and OXPHOS. In activated T cells, the TCA cycle assumes a predominantly anapleurotic role, producing many biosynthetic intermediates, such as citrate and α -ketoglutarate (AKG) that serve as precursors for fatty acid and amino acid synthesis, and thereby sustains cell growth and proliferation [55]. Ca²⁺ plays essential roles in regulating many aspects of the TCA cycle (Figure 3). Most of these mechanisms have been studied in cardiac and skeletal muscle cells. Ca²⁺ at concentrations of 0.1–2 μ M promotes the activity of three dehydrogenases in the TCA cycle: pyruvate dehydrogenase (PDH), AKG dehydrogenase (KDH), and isocitrate dehydrogenase (IDH) [57]. PDH controls the irreversible entry of pyruvate into the TCA cycle, and its activity is reciprocally regulated by PDH kinase and PDH phosphatase. Increases in mitochondrial Ca²⁺ levels ([Ca²⁺]_m) were shown to stimulate PDH phosphatase, which dephosphorylates the E1 subunit of PDH and activates the PDH complex [58]. Ca²⁺ also promotes the activities of KDH and IDH by increasing their affinity for AKG and isocitrate, respectively [59,60]. Furthermore, Ca²⁺ increases the intrinsic activity of KDH [61]. The net effect of Ca²⁺ is activation of the TCA cycle through the main rate-determining enzymes, leading to increased production of reducing equivalents and biosynthetic precursors. Conversely, mutations of IDH found in various cancer types that result in neomorphic function of IDH and production of the R-enantiomer of 2-hydroxylglutarate (R-2-HG) were found to suppress the anti-tumor immunity of T cells by interfering with Ca²⁺ signaling and NFAT activation [62]. Aside from its interactions with TCA enzymes, Ca²⁺ has additional effects on transporters affiliated with the TCA cycle. Increases in [Ca²⁺] in the mitochondrial intermembrane space stimulate the activity of aspartate-glutamate exchangers [63,64], namely SLC25A12 (Aralar1) and SLC25A13 (Citrin), which contain Ca²⁺ binding EF hands and are essential components of the malate-aspartate shuttle that transports both electrons and glutamate across the inner mitochondrial membrane. The net effect of this Ca²⁺ regulated transport is increased NADH levels in the mitochondrial matrix, which supply electrons to the ETC for ATP production. Taken together, Ca²⁺ promotes TCA activity and NADH levels by interacting with both TCA cycle dehydrogenases and mitochondrial transporters. The effects of these Ca²⁺-regulated metabolic processes in T cells are not well understood. Increases in [Ca²⁺]_i following TCR stimulation, either by activation of SOCE or other Ca²⁺ channels, may result in Ca²⁺ uptake into mitochondria to increase [Ca²⁺]_m, promote TCA cycle activity and thus sustain mitochondrial function. We observed an increased abundance of multiple TCA cycle metabolites in murine CD4⁺ T cells stimulated with anti-CD3/CD28 antibodies. This increase was attenuated by abolishing SOCE or inhibition of calcineurin function [11]. Based on these findings, one can speculate that SOCE enhances TCA activity in T cells through some of the Ca²⁺-dependent mechanisms

discussed above (Figure 4), which warrants further investigation. An alternative explanation however, which will be discussed further below, is that increased TCA cycle function is controlled by SOCE at the level of *de novo* gene expression.

Ca²⁺ controls the activity of the ETC.

The ETC uses high energy electrons provided by NADH and FADH₂ to pump protons into the intermembrane space, creating a proton gradient that is used by the ATP synthase for the generation of ATP (Figure 3). The proton gradient also creates a strongly negative mitochondrial membrane potential (MMP) of approximately -150 mV, which provides the driving force for Ca²⁺ uptake into mitochondria from the cytoplasm. Studies primarily in skeletal and cardiac myocytes have shown that Ca²⁺ promotes ATP production by enhancing the activities of complexes I, II, III and V (F₁F₀ ATP synthase) of the ETC. Increasing [Ca²⁺]_m enhances the conductance of each ETC complex and overall ETC activity [65,66]. Moreover, increases in [Ca²⁺]_m augment F₁F₀ ATP synthase activity, although the exact mechanism remains unclear [67,68]. Studies using radioactive ⁴⁵Ca²⁺ tracing showed that Ca²⁺ can directly bind the F₁ β subunit of ATP synthase [69], which may increase its activity [65]. A recent report demonstrated that Ca²⁺ binding induces conformational changes in F₁F₀ ATP synthase triggering the mitochondrial permeability transition and cell death [70]. Whether these effects of Ca²⁺ binding result in increased ATPase activity, however, was not tested. The Ca²⁺ sensitivity of ATP synthase may also be mediated indirectly via Ca²⁺-responsive phosphorylation events. Besides its effects on ATP synthase and ETC complexes, Ca²⁺ also promotes the function of the malate-aspartate shuttle as discussed earlier [63] and the glycerol-3-phosphate shuttle, which is coupled to the reduction of coenzyme Q, thereby sustaining the transfer of electrons down the ETC [71–73]. T cell stimulation with anti-CD3/CD28 antibodies results in the upregulation of ETC function, OXPHOS and ATP synthase activity, allowing for early energy mobilization to support T cell proliferation and survival [56,74]. Activated T cells have an increased oxygen consumption rate (OCR), which is an indication of OXPHOS [74]. Of note, inhibition of ATP synthase with oligomycin at 24h post-activation, but not 48 or 72h, results in impaired T cell proliferation, suggesting that mitochondrial ATP production is important for early events following T cell activation [74]. SOCE plays an important role in enhancing OXPHOS after T cell stimulation by regulating the expression of many ETC genes as will be discussed further below (Figure 2) [11,13,46]. Other Ca²⁺ channels in T cells that regulate mitochondrial function include P2RX7, which was proposed to promote CD8⁺ memory T cell formation (besides Ca²⁺-dependent activation of AMPK) by increasing Ca²⁺ uptake into mitochondria and OXPHOS to produce ATP [32]. In summary, Ca²⁺ exerts a positive regulatory effect on ETC function and OXPHOS in T cells, thereby promoting the metabolic fitness of T cells.

Ca²⁺ regulates mitochondrial ROS production.

Besides facilitating ATP synthesis, another function of the ETC is the production of mitochondrial ROS (mROS) through the transfer of electrons to O₂ via complexes I, III, and IV, which has important roles in T cell signaling (Figure 2 and 3) [56,75,76]. Intracellular Ca²⁺ signals modulate the production of mROS by several mechanisms. First, the Ca²⁺-dependent activation of TCA cycle enzymes increases the electron flow through the ETC,

resulting in higher electron leakage [77]. Complex I and II, which are sensitive to $[Ca^{2+}]_m$, are major producers of mROS [78]. Furthermore, Ca^{2+} stimulates nitric oxide (NO) synthase and increases NO production, resulting in increased mROS levels [79]. Stimulation of T cells rapidly increases mROS levels [80], which has complex effects on T cell function depending on how much mROS is produced. Moderate ROS levels are required for cytokine production and the metabolic function of T cells as evidenced by diminished T cell proliferation and IL-2 production after treatment of T cells with ROS scavenging antioxidants [14,56,81]. By contrast, excessive mROS levels resulting from impaired glutathione production impairs NFAT activation and IL-2 production [14]. The role of $[Ca^{2+}]$ and ROS in T cell activation has been elucidated by Chandel and colleagues who showed that ETC complexes, especially complex III, are required for mROS production and IL-2 induction [56]. This process is Ca^{2+} -dependent as chelation of Ca^{2+} or inhibition of SOCE resulted in decreased mROS and IL-2 production, which was rescued by increasing intracellular H_2O_2 [56]. More recently, the same lab showed that complex III plays an important role in the suppressive function of T regulatory (Treg) cells, an immunosuppressive T cell subset crucial for the maintenance of immunological tolerance by suppressing autoreactive T cells. Intriguingly, the mechanism how complex III controls Treg function involves the modulation of metabolites regulating DNA demethylases. Deletion of the gene encoding Rieske iron-sulfur protein (RISP), an essential component of complex III, in Treg cells increased DNA methylation as well as the metabolites 2-hydroxyglutarate (2-HG) and succinate that inhibit the ten-eleven translocation (TET) family of DNA demethylases [82].

Although Ca^{2+} influx in T cells is required for mROS production by complex III [56], near complete deletion of SOCE has the opposite effect [46]. Deletion of STIM1 in pathogenic Th17 cells resulted in excessive mROS levels, enhanced DNA damage and cell death. mROS levels were increased in STIM1-deficient Th17 cells despite reduced ETC function and OXPHOS. Two explanations likely account for this seemingly paradoxical finding. One is the abnormal mitochondrial morphology, characterized by loose cristae packing, in STIM1-deficient Th17 cells [46], which likely results in inefficient transfer of electrons through ETC supercomplexes [83,84], resulting in electron leakage and mROS production. The second is the deregulated expression of several antioxidant genes in STIM1-deficient Th17 cells, including Sod2, Gpx1 and Gpx4 as well as the transcription factor Nrf2 that regulates the expression of Sod2 and genes involved in glutathione synthesis [76]. As a consequence of excessively increased mROS levels and DNA damage, STIM1-deficient pathogenic Th17 cells are less viable and fail to induce multiorgan inflammation compared to wildtype Th17 cells [46]. Another reported cause of the death in activated T cells due to increased mROS levels is the upregulation of FasL expression [80]. While TCR and Ca^{2+} signaling induce mROS production, ROS conversely regulates many molecules in the TCR signaling pathway and CRAC channel complex. ROS causes the oxidation of an extracellular cysteine residue in ORAI1 thereby locking the CRAC channel in a closed conformation [85,86]. Besides ORAI1, ROS also modulates the function of TCR signaling molecules upstream of CRAC channel activation [87] and the transcription factors NFAT [56] and NF- κ B [88]. How these complex effects of ROS on SOCE and SOCE-dependent pathways influence T cell immunity remains to be investigated. Some clues come from research on the role of NADPH

oxidase, a major source of ROS in phagocytes, in T cell function. Deletion of the gp91^{phox} (NOX₂) subunit of NADPH oxidase resulted in complete loss of TCR-induced H₂O₂ production, but had no effect on the upregulation of T cell activation markers and proliferation [89]. TCR-induced H₂O₂ production was also abolished in T cells lacking the regulatory gp47^{phox} subunit, but in these cells the activation of Erk and production of the cytokines IFN γ and IL-2 was enhanced [90]. Human T cells express the Ca²⁺-dependent, NOX₂-homologue Duox1. Its deletion results in strongly reduced TCR-induced H₂O₂ production, impaired proximal TCR signaling, Ca²⁺ influx and cytokine production [91]. The complex effects of ROS on T cell function, which depend on the source, kinetics and localization of ROS, have been discussed in detail elsewhere [76]. Taken together, there is compelling evidence that Ca²⁺ signals, in particular SOCE, regulate several aspects of mitochondrial metabolism in T cells, including the activity of the TCA cycle and ETC, and other mitochondrial functions such as mROS production.

Transcriptional regulation of mitochondrial gene expression by Ca²⁺ signals.

Many of the abovementioned effects of Ca²⁺ on mitochondrial metabolism could be explained by direct Ca²⁺ mediated regulation of TCA cycle and ETC function. It is not well understood if baseline [Ca²⁺]_m is sufficient for mitochondrial function or if dynamic changes in [Ca²⁺]_m can further regulate TCA cycle and ETC activity. Mitochondrial Ca²⁺ levels often rise simultaneously with increased [Ca²⁺]_i, especially when mitochondria are situated near local Ca²⁺ domains due to opening of Ca²⁺ channels in the plasma membrane or ER [92]. A plausible cause of the Ca²⁺-dependent enhanced mitochondrial function following TCR stimulation-induced SOCE or ATP-mediated activation of P2RX7 is the uptake of Ca²⁺ from the cytoplasm into mitochondria and subsequent upregulation of TCA cycle and ETC function. Ca²⁺ uptake into mitochondria is mediated by the voltage dependent anion channel (VDAC) in the outer mitochondrial membrane and the mitochondrial Ca²⁺ uniporter (MCU) in the IMM (Figure 3 and 4). The MCU is a multiprotein complex that consists of the pore-forming MCU subunit and several regulatory proteins including MCUb, MICU1, MICU2 and EMRE [93–96]. The MCU is widely considered to be necessary and sufficient for Ca²⁺ uptake and accordingly cells from *Mcu*^{−/−} mice lack mitochondrial Ca²⁺ uptake [96]. Consistent with the role of Ca²⁺ in mitochondrial bioenergetics, pancreatic β cells isolated from *Mcu*^{−/−} mice have decreased glucose-induced ATP production [97]. *In vivo*, however, several independently generated *Mcu*^{−/−} mouse strains showed only few signs of defective mitochondrial function [96] and the reasons for this unexpected finding are a matter of current debate. The role of MCU in mitochondrial Ca²⁺ uptake and bioenergetics in immune cells, specifically in T cells, has not been investigated. Although deletion of either Orai1, STIM1 or IP3R in chicken DT40 B cells impaired mitochondrial Ca²⁺ uptake and function, these effects were due to reduced levels of the MCU, whose expression was shown to be dependent on the Ca²⁺-regulated transcription factor CREB [98]. MCU deletion in T cells might be expected to impair mitochondrial functions such as OXPHOS and mROS production, with potentially the strongest effects on those T cell subsets that rely the most on mitochondrial metabolism such as Treg and memory CD8⁺ T cells [4,5]. Our preliminary analysis of *Mcu*^{f/f} *Cd4Cre* mice with conditional deletion of the MCU in T cells showed normal thymic development of T cells, normal Treg numbers and no spontaneous immune activation. CD4⁺ and CD8⁺ T cell

numbers were largely unaffected in 10-week old mice. Furthermore, T cell differentiation into various T helper subsets *in vitro* appears unaffected. Furthermore, acute infection of *Mcu^{fl/fl} Cd4Cre* mice with lymphocytic choriomeningitis virus (LCMV) did not reveal any gross abnormalities in antiviral T cell responses (M.V. unpublished observations). It is noteworthy that Ca^{2+} efflux from mitochondria is mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger NCLX (*SLC8B1*) in myocytes [99]. A recent study showed that inhibition of NCLX in primary murine B cells inhibited Ca^{2+} influx, migration and proliferation after antigen receptor stimulation, whereas no such effects were observed in murine T cells [100]. While these data do not exclude a potential role of the MCU and NCLX in T cell function, the role of dynamic changes in $[\text{Ca}^{2+}]_m$ in T cell function warrants further careful evaluation.

Recent studies have provided compelling evidence supporting a critical role of SOCE in the transcriptional regulating of many genes encoding mitochondrial proteins, especially those of the ETC, with important implications for mitochondrial function and the metabolism of T cells (Figure 2 and 4). One of the first indications that SOCE regulates ETC gene expression and mitochondrial function came from a study of patients with LOF mutations in *ORAI1* and *STIM1* and SOCE-deficient mice [101]. Human and murine fibroblasts lacking SOCE had reduced expression and function of ETC complexes I and IV and consequently reduced proton pumping, electron transport and O_2 consumption. Furthermore, levels of uncoupler protein (UCP) 2, a proton transporter in the IMM whose opening prevents mitochondrial damage [102] were reduced in SOCE-deficient cells, consistent with the higher MMP and increased numbers of mitochondria undergoing degradation in acidic compartments [101]. Many of the genes encoding mitochondrial proteins, such as CPT1B, ACADVL, UCP2, Cox4i1, and NDUFA1, whose expression was reduced in SOCE-deficient cells, are regulated by PGC-1 α and PPAR α [103]. The levels of both transcriptional regulators were decreased in STIM1/STIM2-deficient cardiomyocytes, providing a potential mechanism how SOCE controls mitochondrial gene expression [101].

In T cells, SOCE is required for the upregulation of mitochondrial metabolism following TCR stimulation [11]. Activation of CD4^+ and CD8^+ T cells lacking STIM1/STIM2 and accordingly SOCE resulted in a failure to increase mitochondrial size and expression of ETC complexes I, II and IV (Figure 2 and 4). As a consequence, mitochondrial respiration and ATP production were severely impaired [11]. Similar defects were observed when calcineurin function was inhibited [11]. These defects were associated with decreased expression of the PGC-1 α homologue PPRC1, whose expression is robustly induced upon stimulation of wildtype T cells. Collectively, these findings suggest that SOCE controls mitochondrial biogenesis and function in T cells at the transcriptional level [11]. SOCE was also found to be essential for mitochondrial gene expression, function, and structural integrity in pathogenic Th17 cells [46]. The expression of nuclear encoded ETC genes was reduced in STIM1-deficient Th17 cells, resulting in strongly impaired mitochondrial respiration and OXPHOS. How SOCE regulates ETC gene expression is not known, but may involve NFAT binding to the promoters of ETC genes such as *Ndufs3*, *Ndufs6*, and *Uqcrc1*, which are downregulated in SOCE-deficient Th17 cells (S.F. unpublished observations) [46].

OXPHOS is required for the function of pathogenic Th17 cells because its inhibition using the ATP synthase blocker oligomycin suppressed IL-17A levels and biased their gene

expression profile toward that of non-pathogenic Th17 cells. Further evidence supporting the importance of OXPHOS for pathogenic Th17 cells comes from a study showing that oligomycin impairs Th17 effector functions and ameliorates *in vivo* colitis induced by trinitrobenzenesulfonic acid (TNBS) [104]. These anti-inflammatory effects are similar to those observed in STAT3-driven pathogenic Th17 cells lacking STIM1, which failed to induce severe inflammation of the lung, skin and intestine [46]. Whereas SOCE regulates OXPHOS in pathogenic Th17 cells, it is dispensable for the regulation of glycolysis as discussed earlier [46]. This finding is confirmed by a transcriptomic comparison of pathogenic and non-pathogenic Th17 cells, which revealed that STIM1 controls mitochondrial gene expression and OXPHOS but not aerobic glycolysis in pathogenic Th17 cells. In non-pathogenic Th17 cells, which are essential for immunity to infection with extracellular bacteria and fungi, STIM1 regulates both metabolic pathways [13]. The Ca^{2+} -permeable channel P2RX7 was recently shown to promote mitochondrial homeostasis and the metabolic fitness of memory CD8^{+} T cells [32]. Deletion of P2RX7 in mice resulted in a dramatic reduction in the numbers of central and tissue-resident memory CD8^{+} T cells after LCMV infection, which was associated with selective suppression of mitochondrial oxygen consumption in memory precursor effector cells, but not short-lived effector cells [32]. Stimulation of CD8^{+} T cells with the ATP analog BzATP resulted in Ca^{2+} influx in wildtype but not *P2rx7*^{-/-} T cells and activation of AMPK, which promotes mitochondrial homeostasis and health [105]. The authors of this study propose that Ca^{2+} influx through P2RX7 promotes Ca^{2+} uptake into mitochondria and ATP production as well as AMPK activation, thereby supporting the maintenance and function of long-lived central and tissue-resident memory CD8^{+} T cells in mice [32]. If these effects of P2RX7 are indeed related to its ability to conduct Ca^{2+} ions remains to be fully investigated. Collectively, these studies demonstrate that Ca^{2+} signaling is an important regulatory pathway that controls mitochondrial gene expression and functions including OXPHOS.

CALCIUM REGULATION OF LIPID METABOLISM

Fatty acids (FA) are a major source of energy in many cells including lymphocytes. FA can be taken up by cells through transport proteins in the plasma membrane or produced by cells in the process of FA synthesis (FAS). FA are stored in the form of triglycerides within cells, from where they can be mobilized and used for energy production by fatty acid oxidation (FAO). Both FAS and FAO are essential pathways in the metabolic regulation of T cell differentiation, proliferation, survival and effector functions.

FAO within mitochondria is used by cells for the conversion of FA into energy. FA are broken down in a process called β -oxidation to generate Acetyl-CoA, which is used to generate ATP by OXPHOS. Several enzymes are critical for FAO, including acyl-CoA dehydrogenase very long chain (ACADVL), an enzyme that catalyzes the first step of β -oxidation, and carnitine palmitoyltransferase 1 (CPT1) that transports long-chain FA into mitochondria [106]. In the immune system, FAO plays important roles in long-lived and anti-inflammatory cells [5]. In T cells, several indirect lines of evidence have supported the idea that the development and maintenance of memory CD8^{+} T cells and Treg cells is dependent on FAO. Stimulation of memory CD8^{+} T cells with IL-15 promotes upregulation of CPT1A and their capacity for FAO [107]. Treg cells also have elevated expression of

CPT1A allowing for increased rates of FAO [108] and treatment of CD4⁺ T cells with the CPT1 inhibitor etomoxir resulted in impaired differentiation into Treg cells *in vitro* [109]. Furthermore, intra-tumor Treg cells were shown to have increased expression of CD36, which binds long-chain fatty acids and facilitates their transport into cells, and CD36-deficient Treg cells had reduced mitochondrial respiration [110]. However, the concept of FAO as an essential pathway for the formation of memory CD8⁺ T cells and Treg cells has recently been called into question by a study using mice with conditional deletion of *Cpt1a* in T cells and thus abolished uptake of long-chain FA into mitochondria, which found no defect in the formation of memory CD8⁺ T cells and Treg cells [111]. These findings suggest that etomoxir, used to establish a role of FAO, may have additional targets besides CPT1 and that FAO fueled by the CPT1-dependent uptake of long chain FAs is not required for the development of memory CD8⁺ T cells and Treg cells. It is possible, however, that short chain FAs, whose uptake into mitochondria is independent of CPT1, fuel FAO in memory CD8⁺ T cells and Treg cells. SOCE is required for FAO because mitochondrial oxygen consumption as a readout for FAO was impaired in ORAI1 or STIM1-deficient fibroblasts cultured in oleic acid; this defect in FAO was more pronounced when FAO was induced by cell starvation. Potential explanations for the attenuated FAO are reduced expression of CPT1 and ACADVL in SOCE deficient fibroblasts and the impaired mobilization of FA from triglycerides stored in lipid droplets because of impaired lipolysis [101]. While these data indicate that SOCE is essential to upregulate FAO in non-immune cells, the role of SOCE in the regulation of FAO in T cells has not been investigated. Intriguingly, the numbers of thymus-derived Foxp3⁺ Treg cells are reduced in mice with conditional deletion of STIM1/STIM2 or ORAI1/ORAI2 and patients with LOF mutations in *ORAI1* or *STIM1* that abolish SOCE [112–117] as is the differentiation of STIM1-deficient mouse CD4⁺ T cells into peripheral Treg cells *in vitro* [118]. Furthermore, mice with conditional deletion of STIM1 and STIM2 in all T cells had impaired maintenance of memory CD8⁺ T cells [119]. Whether these defects in Treg development and memory T cell maintenance are related to dysregulation of FAO in the absence of SOCE remains to be further investigated. Additional evidence for a role of Ca²⁺ signaling in memory CD8⁺ T cells comes from studies in *P2rx7*^{-/-} mice, which not only had impaired AMPK activation (which is known to induce FAO) and reduced OXPHOS, but also impaired development and maintenance of memory CD8⁺ T cells, thus potentially linking P2RX7, Ca²⁺ and FAO to memory CD8⁺ T cell formation [32].

FAS allows cells to generate FA from metabolites produced by glycolysis, the pentose phosphate pathway or the TCA cycle. The induction of FAS depends on mTORC1, which controls the function of the transcriptional regulator sterol regulatory element binding protein (SREBP). SREBP in turn regulates the expression of several key enzymes in the FAS pathway such as FA synthase and acetyl CoA carboxylase (ACC1) [5]. In T cells, FAS appears to be involved in CD8⁺ T cell survival as T cell specific deletion of ACC1 in mice results in impaired persistence and homeostatic proliferation of CD8⁺ T cells as well as a severe defect in antigen-specific CD8⁺ T cells expansion after *Listeria monocytogenes* infection [120]. Genetic deletion or pharmacological inhibition of ACC1 in T cells resulted in impaired the differentiation of Th17 cells by altering the transcriptional and metabolic signature of ACC1-deficient Th17 cells [121]. Th17 cells are very dependent on Ca²⁺

signals as even moderate reduction of SOCE in mice with T cell specific deletion of ORAI1, STIM1, STIM2 or the CRAC channel regulator CRACR2A inhibited the expression of Th17 cytokines including IL-17A and attenuated the severity of EAE, IBD and other Th17-mediated diseases in mice [46,122–125]. Whether impaired Th17 cell function (or T cell function in general) in the absence of SOCE is related to defects in FAS is not known. A possible link between SOCE and FAS could be the Ca^{2+} dependent activation of the AKT-mTORC1 pathway [11] that promotes FAS. Furthermore, glycolysis is dependent on SOCE, resulting in reduced production of the TCA cycle intermediate citrate in STIM1/STIM2 deficient T cells [11], which is the substrate for the generation of acetyl-CoA and FAS. In memory CD8^+ T cells, Ca^{2+} has been suggested to negatively regulate FAS by inducing AMPK. P2RX7-deficient memory CD8^+ T cells (and therefore potentially impaired Ca^{2+} influx) had diminished phosphorylation of ACC1, an AMPK target [32]. Whether and how SOCE and other Ca^{2+} channels regulate lipid metabolism in T cells is not well understood and requires further investigation.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Intracellular Ca^{2+} levels regulate several metabolic pathways in T cells including glycolysis and OXPHOS, whereas the effects of Ca^{2+} on other pathways such as the TCA cycle and lipid metabolism in lymphocytes are not well understood. We are thus just beginning to understand how Ca^{2+} regulates immunometabolism and immune function. The role of Ca^{2+} in regulating immunometabolism is complex because it can regulate metabolic signaling pathways that promote opposing outcomes. This is exemplified by the Ca^{2+} -dependent activation of AMPK, which inhibits energy-intensive protein biosynthesis and instead activates pathways that restore energy levels in the cell such as OXPHOS and FAO. But Ca^{2+} also activates mTOR signaling, which promotes the biosynthetic pathways of glycolysis, glutamine metabolism, and FAS thereby preparing lymphocytes for rapid growth and proliferation. How these pleiotropic effects of Ca^{2+} on opposing metabolic pathways are balanced is not well understood. It is likely that the spatio-temporal control of Ca^{2+} signals plays a role in this regulation, for instance through localized Ca^{2+} signals near the plasma membrane or organellar membranes, where ion channels mediating Ca^{2+} fluxes are located. Moreover, mTORC1 was shown to localize to the surface of lysosomes in non-immune cells in response to nutrients where it is activated by the GTPase Rheb [126] and, as discussed earlier, Ca^{2+} release from lysosomes through ectopically expressed TRPML1 can mediate mTORC1 activation [20]. Whether similar processes are functional in T cells remains to be studied. Another potentially important link between localized Ca^{2+} signals and metabolic function in lymphocytes is the contact between the ER and mitochondria in dedicated structural domains known as mitochondria-associated membranes (MAMs) which allow for the direct transfer of Ca^{2+} from the ER to mitochondria. Ca^{2+} transfer in MAMs occurs through IP3Rs in the ER membrane and juxtaposed VDAC channels in the OMM, resulting in mitochondrial Ca^{2+} uptake through the MCU in the IMM [127]. Constitutive IP3R-mediated Ca^{2+} release from the ER and uptake into mitochondria was shown to be required for providing sufficient reducing equivalents to support OXPHOS in chicken DT40 B cells [128]. When this Ca^{2+} transfer is impaired in the absence of IP3Rs, DT40 cells undergo prosurvival macroautophagy. Dysregulation of ER-mitochondrial contacts in many cell types

and tissues has been implicated in a variety of metabolic diseases [127], but how MAMs regulate Ca^{2+} handling in lymphocytes and affect immune metabolism and immune responses has not been studied.

Ca^{2+} regulates several aspects of mitochondrial metabolism including dehydrogenases of the TCA cycle, several complexes of the ETC, ATP synthase, and various IMM shuttle systems. The direct effects of Ca^{2+} on mitochondrial proteins have been studied in isolated mitochondria and non-immune cells, and although it can be speculated that these pathways are similarly regulated by Ca^{2+} in lymphocytes, this has not been studied directly. If they indeed are, we do not know if the regulation of the TCA cycle and the ETC function requires dynamic changes in $[\text{Ca}^{2+}]_m$ when T cells become activated and differentiate into various T cell subsets such as effector and memory cells. Recent research from our lab has shown that SOCE through CRAC channels plays an important role in the transcriptional regulation of glycolytic enzymes, glucose transporters as well as ETC genes in T cells. Some of the effects of SOCE on T cell metabolism could in addition be due to mitochondrial Ca^{2+} uptake and increases in $[\text{Ca}^{2+}]_m$ resulting in the direct regulation of TCA cycle and ETC activity. Another area of Ca^{2+} -dependent regulation of immunometabolism that is not well understood is lipid metabolism. Our lab showed that SOCE controls several aspects of lipid metabolism in non-immune cells including FAO, expression of PGC-1 α /PPAR α , two neutral lipases and lipolysis, as well as the suppression of lipophagy [101]. When it comes to lymphocytes, however, we can only conjecture the relationship between Ca^{2+} and the regulation of lipid metabolism. As noted, Ca^{2+} regulates both AMPK and mTOR activation, which subsequently have various effects on proteins of the FAO and FAS pathways such as ACC2, ACC1, HSL, and SREBP. Furthermore, at the transcriptional level, Ca^{2+} may regulate genes critical for lipid metabolism such as CPT1 and ACADVL. Understanding the role of different Ca^{2+} signaling mechanisms in lipid metabolism in lymphocytes and immune responses certainly warrants further study. Another intriguing question is how Ca^{2+} affects the metabolism of different T cell subsets and T cells throughout their life cycle. The field of immunometabolism has clearly revealed differences in metabolic dependencies among different T cell subsets. For instance, Th1 and Th17 cells rely on mTORC1 function, Th2 cells on mTORC2, Treg cells on AMPK, and memory CD8 $^{+}$ T cells on AMPK and FAO [108]. Additionally, T cells “exhausted” from repeated stimulation in the context of chronic infection or tumors exhibit reduced glycolysis and OXPHOS [129]. It will be important to better understand how Ca^{2+} signals differentially affect the metabolic states of these different T cell subsets and thereby shape immune responses.

From a pathophysiological and translational perspective, it will be important to study how Ca^{2+} signals are involved in the etiology of common metabolic diseases like type 1 and type 2 diabetes, metabolic syndrome or obesity. As discussed in this review SOCE regulates glycolysis through activation of the mTORC1 pathway and expression of glycolytic enzymes [11], and lipid metabolism through activation of lipolysis and FAO [101]. Conversely, glucose and lipids were shown to regulate the activity of CRAC channels and SOCE. The glycolytic metabolite phosphoenolpyruvate (PEP) regulates $[\text{Ca}^{2+}]_i$ in CD8 $^{+}$ T cells by inhibiting the function of the sarco-endoplasmic reticulum Ca^{2+} -ATPase (SERCA), which pumps Ca^{2+} from the cytosol into the ER, resulting in increased cytosolic Ca^{2+} and NFAT signaling [130]. Tumor-specific T cells could be metabolically reprogrammed by

increasing PEP production through overexpression of PEP carboxykinase 1 (PCK1), which increased T cell effector functions [130]. Long-term increases in glucose levels, as found in diabetic patients, may also affect SOCE. A recent study showed that incubation of murine endothelial cells with methylglyoxal, a by-product of glucose metabolism whose accumulation has been linked to the development of diabetic complications, increased angiotensin II-evoked SOCE likely through the formation of glycation end products [131]. Similarly, lipids were reported to modulate CRAC channel function. CD36, which mediates the transport of long-chain FA, is required for the induction of SOCE following depletion of ER stores in CHO cells [132] or stimulation of fibroblasts with oleic acid [101] suggesting that FA uptake into cells promotes CRAC channel function. By contrast, steatosis in liver cells was shown to inhibit SOCE. This effect was dependent on protein kinase C (PKC) function and may involve phosphorylation of ORAI1 [133], which was reported to suppress ORAI1 activity and SOCE [134]. Steatosis-mediated inhibition of SOCE may function as a positive feedback loop and cause further lipid accumulation. The mechanisms by which glucose and lipids regulate SOCE and whether they also affect T cell function is not understood. These questions certainly deserve further investigation, because the altered metabolic environments in patients with diabetes and obesity characterized by hyperglycemia and hyperlipidemia were shown to alter T cell function and differentiation, which in turn may affect disease progression [135,136]. Understanding whether and how SOCE and other Ca^{2+} signaling pathways are involved in T cell dysfunction in metabolic disorders may provide new opportunities for therapeutic intervention.

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Figure 1. Ca^{2+} regulates metabolic pathways at different stages of the T cell life cycle.

(A) In naïve T cells, OXPHOS and FAO sustain basal cellular metabolism. (B) T cell stimulation through the TCR and CD28 results in SOCE and Ca^{2+} signals, which result in activation of AMPK and initial inhibition of mTORC1 and enhanced OXPHOS. SOCE results in increased mitochondrial respiration and OXPHOS through upregulation of mitochondrial gene expression, especially components of the ETC, resulting in enhanced ATP production, which suppresses AMPK and increases mTORC1 function. In parallel, SOCE mediates the activation of calcineurin and NFAT as well as the PI3K-AKT-mTORC1 pathway, which promote the expression of the transcription factors c-Myc, IRF4 and HIF1a, glycolytic enzymes and the glucose transporters GLUT1 and 3. Aerobic glycolysis allows stimulated T cells to engage the biosynthetic pathways required for cell growth and proliferation. (C) The transition of effector T cells to long-term memory T cells is accompanied by a metabolic switch to OXPHOS and FAO as the main energy source to maintain memory T cells. Ca^{2+} influx through the purinergic receptor P2RX7 (and potentially other Ca^{2+} channels) activates AMPK and suppresses mTORC1, thereby promoting mitochondrial function. *Abbreviations:* AMPK, adenosine monophosphate-activated protein kinase; APC, antigen presenting cell; CaMKK, calmodulin kinase kinase; FA, fatty acid; FAO, fatty acid oxidation; GLUT, glucose transporter; LKB-1, liver kinase B1; MHC, major histocompatibility complex; mTORC, mammalian target of rapamycin; NFAT, nuclear factor of activated T cells; OXPHOS, oxidative phosphorylation; SOCE, store-operated Ca^{2+} entry; TCR, T cell receptor.

Figure 2. Regulation of T cell metabolism by SOCE and Ca²⁺ signaling.

The two main Ca²⁺ channels regulating T cell metabolism are the store-operated CRAC channel and the purinergic P2RX7 channel. (1) CRAC channels formed by ORAI family proteins in the plasma membrane mediate store-operated Ca²⁺ entry (SOCE) in response to TCR stimulation. TCR signaling results in production of IP₃, release of Ca²⁺ from the ER through IP₃ receptor channels (not shown) and activation of STIM1 and STIM2 proteins in the ER membrane, which subsequently bind to and open ORAI channels. SOCE activates many Ca²⁺ dependent enzymes including calcineurin, CaMK and adenylyl cyclases as well as transcription factors such as NFAT and CREB. SOCE has emerged as an important regulator of several metabolic pathways: (a) Activation of calcineurin and NFAT downstream of TCR-induced SOCE regulates, both directly and indirectly through NFAT regulated transcription factors such as IRF4 and HIF-1α, the expression of glucose transporters and glycolytic enzymes, which allows CD4⁺ and CD8⁺ T cells to grow, enter the cell cycle and proliferate. (b) SOCE activates the PI3-AKT-mTORC1 pathway at the posttranslational level further promoting glycolytic metabolism. (c) SOCE enhances the expression of nuclear encoded mitochondrial genes that are components of the ETC, resulting in increased OXPHOS and ATP production. Impaired expression of ETC genes and redox regulation in SOCE-deficient T cells results in high mROS levels and the death of pTh17 cells. (d) In non-immune cells, SOCE regulates the expression of two transcriptional regulators of lipid metabolism, PPARα and PGC1α, and the activation of the transcription factor CREB via the production of cAMP, which control several aspects of lipid metabolism such as expression of neutral lipases and lipolysis, fatty acid oxidation and lipophagy (not shown). Note that activation of ORAI1 is inhibited by FFAs. (2) Activation of the P2RX7 channel by extracellular ATP regulates activation of AMPK and OXPHOS in memory CD8⁺

T cells, presumably by mediating Ca^{2+} influx. “?” indicates pathways whose role in lymphocyte metabolism remain to be elucidated. For additional details see the main text.

Abbreviations: I-IV, ETC complexes I-IV; V, ETC complex V (ATP synthase); AKT, AKT serine/threonine kinase; ATP, adenosine triphosphate; CaMK, Ca^{2+} /calmodulin-dependent protein kinase; cAMP, cyclic adenosine monophosphate; CPT1, carnitine palmitoyltransferase I; CRAC, Ca^{2+} release-activated Ca^{2+} channel; CREB, cAMP response element-binding protein; ER, endoplasmic reticulum; ETC, electron transport chain; FAO, fatty acid oxidation; FFA, free fatty acids; GLUT, glucose transporter; HIF1 α , hypoxia-inducible factor 1 α ; HK2, hexokinase 2; HSL, hormone-sensitive lipase; IP3, inositol 1,4,5-trisphosphate; IRF4, interferon regulatory factor 4; MCU, mitochondrial Ca^{2+} uniporter; mTORC1, mammalian target of rapamycin complex 1; NFAT, nuclear factor of activated T cells; OXPHOS, oxidative phosphorylation; PEP, phosphoenolpyruvate; PGC1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PPAR α , peroxisome proliferator-activated receptor; pTh17, pathogenic Th17 cells; SOCE, store-operated calcium entry; STIM, stromal interaction molecule 1; TCA, tricarboxylic acid; TCR, T cell receptor; Th17, T helper 17 cells.

Figure 3. Effects of Ca^{2+} on mitochondrial metabolism.

Ca^{2+} in the mitochondrial matrix regulates the function of the dehydrogenases IDH, KDH and PDH enzymes in the TCA cycle as well as the malate-aspartate and the glycerol-3-phosphate shuttles, thereby affecting the production of reducing equivalents NADH and FADH_2 and substrates for FAS and other biosynthetic pathways. Ca^{2+} also regulates the function of ETC complexes I, II and III as well as ATP synthase, thereby controlling the production of ATP and mROS. Ca^{2+} enters the mitochondrial matrix through VDAC channels in the OMM and the MCU in the IMM. Ca^{2+} efflux from mitochondria is mediated by the NCLX exchanger. * denotes molecules shown to be regulated by Ca^{2+} in lymphocytes. ? indicates the fact that the role of the MCU in immune cells is unknown. For further details, see text. **Abbreviations:** I-IV, ETC complexes I-IV; α -KG, α -ketoglutarate; ATP, adenosine triphosphate; CRAC, calcium release-activated channels; ETC, electron transport chain; IDH, isocitrate dehydrogenase; IMM, inner mitochondrial membrane; KDH, α -ketoglutarate dehydrogenase; MCU, mitochondrial calcium uniporter; mROS, mitochondrial reactive oxygen species; mtDNA, mitochondrial DNA; NCLX, Na/Li/Ca exchanger; NFAT, nuclear factor of activated T cells; NF- κ B, nuclear factor kappa B; mt, mitochondrial; OMM, outer mitochondrial membrane; PDH, pyruvate dehydrogenase; STIM, stromal interaction molecule 1; TCA, tricarboxylic acid; TCR, T cell receptor; VDAC, voltage-dependent anion channel.

Figure 4. Regulation of mitochondrial function by SOCE.

TCR stimulation activates STIM1 and STIM2 which induce Ca²⁺ influx (SOCE) via ORAI1 and its homologues ORAI2 and 3 that encode the CRAC channel. SOCE results in the activation of Ca²⁺ dependent enzymes and transcription factors such as NFAT, Erk1/2, and NF-κB that regulate the expression mitochondrial proteins, especially those of the ETC, which are encoded by nuclear DNA. Moreover, Ca²⁺ entering cells by SOCE is taken up by mitochondria through VDAC and MCU in the OMM and IMM respectively, where it contributes to dynamic changes in [Ca²⁺]_m and potentially the regulation of Ca²⁺ dependent components of the TCA cycle and ETC. ? indicates that fact that the role of Ca²⁺ in the regulation of genes encoded by mtDNA in lymphocytes is unknown. For further details, see Figure 3 and text. *Abbreviations:* CRAC, Ca²⁺ release-activated Ca²⁺ channel; Erk, extracellular signal-regulated kinase; ETC, electron transport chain; MCU, mitochondrial calcium uniporter; mROS, mitochondrial reactive oxygen species; mtDNA, mitochondrial DNA; NCLX, Na/Li/Ca exchanger; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κappa B; PKC, protein kinase; STIM, stromal interaction molecule; TCA, tricarboxylic acid; TCR, T cell receptor.