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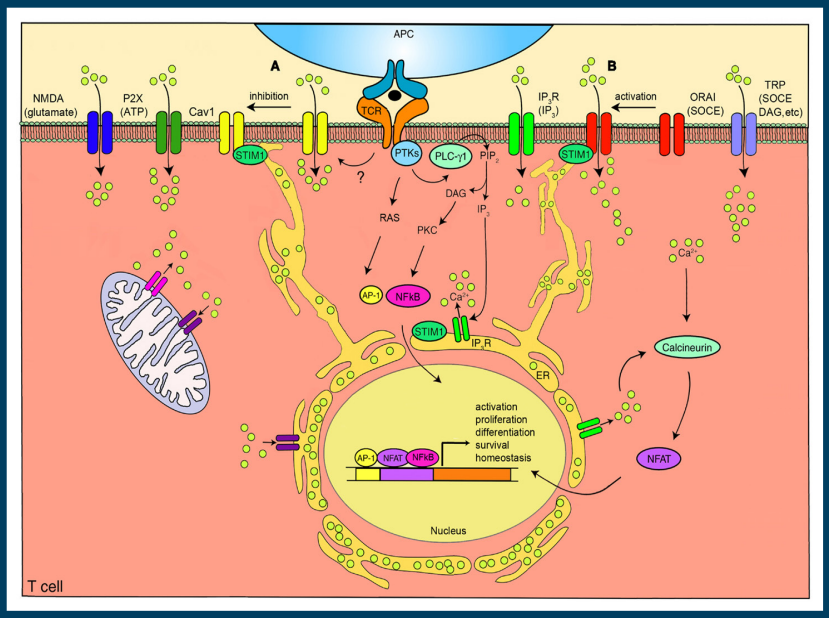

THE REGULATION OF CALCIUM HOMEOSTASIS IN T LYMPHOCYTES

Topic Editor
Gergely Toldi

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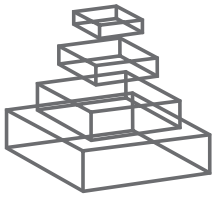

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THE REGULATION OF CALCIUM HOMEOSTASIS IN T LYMPHOCYTES

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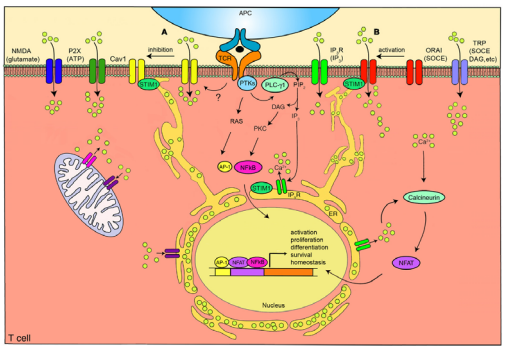

The calcium channels in T cells. T cell receptor (TCR) engagement by a peptide-MHC on an antigen presenting cell (APC), induces protein tyrosine kinases (PTKs) to activate phospholipase C-γ1 (PLC-γ1), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) from plasma membrane phospholipids to generate diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3). Elevated levels of IP3 in the cytosol leads to the release of Ca2+ from IP3R Ca2+ channels located in the ER. Ca2+ depletion from the ER induces Ca2+ influx from the extracellular space through the plasma membrane channel, ORAI1. Several auxiliary channels also operate during TCR-mediated Ca2+ signalling. These include plasma membrane IP3R activated by the ligand IP3, transient receptor potential (TRP) channels that can be operated by DAG and SOCE, adenosine triphosphate (ATP) responsive purinergic P2 (P2X) receptors, glutamate mediated N-methyl-D-aspartate activated (NMDA) channels and voltage-dependent Ca2+ channels (CaV) that may be regulated through TCR signalling events. The mitochondria also control cytoplasmic Ca2+ levels. Increase in intracellular Ca2+ results in activation of calmodulin- calcineurin pathway that induces NFAT nuclear translocation and transcription of target genes to direct T cell homeostasis, activation, proliferation, differentiation and survival. Within this complex network of Ca2+ signalling, a model of the reciprocal regulation of CaV1 and ORAI1 in T cells has been proposed. (A) Low-level TCR signalling through interactions with self-antigens (ie. self-peptides/self MHC molecules) may result in CaV1 (particularly CaV1.4) activation and Ca2+ influx from outside the cell. This allows for filling of intracellular stores and initiation of a signalling cascade to activate a prosurvival program within the naïve T cell. STIM1 is not activated in this scenario and, consequently, ORAI1 remains closed. (B) Strong TCR signalling through engagement by a foreign peptide-MHC induces the downstream signalling events that result in ER Ca2+ store depletion and STIM1 accumulation in puncta in regions of the ER near the plasma membrane allowing interactions with Ca2+ channels. ORAI1 enhances STIM1 recruitment to the vicinity of CaV1 channels. Here, STIM1 can activate ORAI1 while inhibiting CaV1. Yellow circles = Ca2+

Omilusik KD, Nohara LL, Stanwood S and Jefferies WA (2013) Weft, warp, and weave: the intricate tapestry of calcium channels regulating T lymphocyte function. *Front. Immunol.* 4:164. doi: 10.3389/fimmu.2013.00164

The process of T lymphocyte activation has always been a focus of interest in immunology. It determines the course of normal immune function and plays a substantial role in the development of immunological disorders. Calcium is an important messenger through which the kinetics of lymphocyte activation is regulated, and plays an essential role in T lymphocyte homeostasis, proliferation, differentiation, and apoptosis. This essential signaling pathway, involving both intracellular calcium release and calcium entry from the extracellular space has gained increasing attention over the recent decades.

Dynamic alterations of cytoplasmic calcium levels are coordinated by finely tuned cellular mechanisms responsible for the elevation and decrease of calcium. Over the recent years, we have gained insight into the molecular basis of the machinery that is responsible for the storage and release of intracellular calcium as well as for the uptake of extracellular calcium. Several techniques evolved that enable accurate real-time monitoring of the kinetics and regulation of calcium homeostasis and lymphocyte activation, offering valuable information on T lymphocyte function following activation. Mathematical models have also been developed that help a better understanding of calcium movement and fuel more focused research to reach a deeper understanding of key players and aspects of calcium homeostasis.

The relationship between calcium signals and disorders of the immune system is also receiving growing attention. Influencing the actual distribution and availability of cytoplasmic free calcium ions via modulation of the above mechanisms might provide novel therapeutic strategies for the treatment of immune-mediated disease. The complex roles and expression of calcium channel families in different subpopulations of T cells will contribute to the possibility of selective inhibition of a particular T cell subset at a given stage of differentiation, leading to more specific immunosuppressive strategies compared with current immunomodulatory agents.

In this Research Topic, we aim to approach the regulation of calcium homeostasis in T lymphocytes and the role of calcium in the function of T cells under normal and pathologic conditions from several different aspects. We aim to gather current knowledge on calcium handling of T lymphocytes in order to enhance future research and the elaboration of potential therapeutic strategies for the benefit of patients with immune-mediated disorders.

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The regulation of calcium homeostasis in T lymphocytes

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Keywords: calcium, T lymphocytes, lymphocyte activation, CRAC channel, CaV1 channels, autophagy, adenine nucleotides, mathematical modeling

Calcium is an important messenger in every cell type. Its intracellular level is regulated by finely tuned machinery responsible for calcium uptake, release, and intracellular storage. T cells are no exception in this regard. Pathways of calcium homeostasis participate in a number of cellular processes that determine short and long-term function of T lymphocytes. Over the recent year, an increasing number of calcium channels and transporters have been described that play a key role in balancing cytoplasmic calcium levels in T cells. Therapeutic strategies are now evolving based on the modulation of T-lymphocyte calcium homeostasis in order to combat immune-mediated disorders.

Submissions in this Research Topic include reviews on the ion channels that regulate calcium influx from the extracellular space in T cells (1, 2), either by conducting calcium ions or by modulating the membrane potential that provides the driving force for calcium influx (3, 4). The best characterized calcium channel in T cells is the calcium release-activated calcium (CRAC) channel, which is composed of ORAI and stromal interaction molecule (STIM) proteins. Several other channels may also mediate calcium influx directly in T cells including members of the transient receptor potential (TRP) family, P2X receptors, and voltage-gated calcium (Cav) channels. While the role of CRAC channels to T-cell function is well described by findings in ORAI1 and STIM1-deficient patients and mice (5, 6), the contributions of TRP, Cav1, and P2X receptor channels remain to be more clearly defined. These channels could contribute to calcium influx in specific T-cell subsets at distinct stages of T-cell development or following stimuli other than T-cell receptor (TCR) engagement.

Special attention is given to the emerging roles of Cav1 channels and their integration with other channels to generate a specific calcium signature in T lymphocytes (7, 8). The relationships between STIM, ORAI, and Cav1 will be discussed. For instance, STIM was shown to be a negative regulator of Cav1 signaling (9). Furthermore, the involvement of Cav1 channels as players in human disease will also be explored. In a recent study, a mixture of Cav1.2 and Cav1.3 specific antisense oligodeoxynucleotides strongly impaired the TCR-dependent increase of cytoplasmic calcium level and cytokine production in Th2 cells without any effect on Th1 cells, thus protecting mice against the development of asthma (10). These findings suggest that these channels may represent an interesting new approach in the treatment of allergic diseases.

The role of autophagy, a pathway for intracellular degradation in calcium homeostasis in T cells will also be reviewed (11). Autophagy regulates calcium signaling by developmentally maintaining the homeostasis of the ER (12).

Second messengers derived from the adenine dinucleotides, nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide phosphate (NADP) have also been implicated in T-cell calcium signaling (13). Nicotinic acid adenine dinucleotide phosphate (NAADP) acts as a very early second messenger upon TCR/CD3 engagement, while cyclic ADP-ribose (cADPR) is mainly involved in sustained partial depletion of the endoplasmic reticulum by stimulating calcium release via ryanodine receptors. Finally, adenosine diphosphoribose (ADPR), a breakdown product of both NAD and cADPR activates the TRPM2 cation channel, thereby facilitating calcium (and sodium) entry into T cells. Receptor-mediated formation, metabolism, and mode of action of these novel second messengers in T lymphocytes will be reviewed. Their involvement in immune regulation also makes these pathways suitable targets for therapeutic intervention. The NAADP antagonist BZ194 has recently been shown to ameliorate the clinical course of transfer experimental autoimmune encephalitis, an animal model of multiple sclerosis (14).

While the crosstalk between calcium signaling and metabolic regulation in T cells is relatively poorly understood, one of the contributions highlights where such interactions occur (15). Calcium is known not only to mediate T-cell activation but it also modulates the unique metabolic changes that occur in distinct T-cell subsets and developmental stages. The crosstalk between mitochondrial metabolism, reactive oxygen species (ROS) generation, and CRAC channel activity will be highlighted.

In the course of lymphocyte activation, potassium channels maintain the driving force for sustained calcium influx from the extracellular milieu as they grant the efflux of potassium from the cytoplasm, thus conserving an electrochemical potential gradient between the intra- and extracellular spaces. There are two major types of potassium channels in T cells: the voltage-gated Kv1.3 and the calcium-activated IKCa1 channels. The relation between the calcium currents through CRAC channels and the efflux of potassium makes the proliferation and activation of lymphocytes sensitive to pharmacological modulation of Kv1.3 and IKCa1 channels (16), and provides an opportunity for targeted intervention that will also be discussed (17). Specific inhibition of these channels results in a diminished calcium influx in lymphocytes and a lower level of lymphocyte activation.

Finally, a quantitative mathematical model of T-lymphocyte calcium dynamics will be introduced that has been developed

in order to establish a tool which helps to disentangle cause-effect relationships between ion fluxes and observed calcium time courses (18).

ACKNOWLEDGMENTS

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REFERENCES

1. Feske S. Ca2+ influx in T cells: how many Ca2+ channels? *Front Immunol* (2013) **4**:99. doi:10.3389/fimmu.2013.00099
2. Omilusik KD, Nohara LL, Stanwood S, Jefferies WA. Weft, warp, and weave: the intricate tapestry of calcium channels regulating T lymphocyte function. *Front Immunol* (2013) **4**:164. doi:10.3389/fimmu.2013.00164
3. Cahalan MD, Chandy KG. The functional network of ion channels in T lymphocytes. *Immunol Rev* (2009) **231**:59–87. doi:10.1111/j.1600-065X.2009.00816.x
4. Feske S, Skolnik EY, Prakriya M. Ion channels and transporters in lymphocyte function and immunity. *Nat Rev Immunol* (2012) **12**:532–47. doi:10.1038/nri3233
5. Feske S. CRAC channelopathies. *Pflugers Arch* (2010) **460**:417–35. doi:10.1007/s00424-009-0777-5
6. Fuchs S, Rensing-Ehl A, Speckmann C, Bengsch B, Schmitt-Graeff A, Bondzio I, et al. Antiviral and regulatory T cell immunity in a patient with stromal interaction molecule 1 deficiency. *J Immunol* (2012) **188**:1523–33. doi:10.4049/jimmunol.1102507
7. Badou A, Jha MK, Matza D, Flavell RA. Emerging roles of L-type voltage-gated and other calcium channels in T lymphocytes. *Front Immunol* (2013) **4**:243. doi:10.3389/fimmu.2013.00243
8. Pelletier L, Savignac M. Ca2+ signaling in T-cell subsets with a focus on the role of Cav1 channels: possible implications in therapeutics. *Front Immunol* (2013) **4**:150. doi:10.3389/fimmu.2013.00150
9. Park CY, Shcheglovitov A, Dolmetsch R. The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science* (2010) **330**:101–5. doi:10.1126/science.1191027
10. Djata Cabral M, Paulet PE, Robert B, Gomes ML, Renoud M, Savignac C. Knocking-down Cav1 calcium channels implicated in Th2-cell activation prevents experimental asthma. *Am J Respir Crit Care Med* (2010) **181**:1310–7. doi:10.1164/rccm.200907-1166OC
11. Jia W, He M-X, McLeod IX, He Y-W. Autophagy, a novel pathway to regulate calcium mobilization in T lymphocytes. *Front Immunol* (2013) **4**:179. doi:10.3389/fimmu.2013.00179
12. Jia W, Pua HH, Li QJ, He YW. Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. *J Immunol* (2011) **186**:1564–74. doi:10.4049/jimmunol.1001822
13. Ernst IMA, Fliegert R, Guse AH. Adenine dinucleotide second messengers and T-lymphocyte calcium signaling. *Front Immunol* (2013) **4**:259. doi:10.3389/fimmu.2013.00259
14. Cordiglieri C, Odoardi F, Zhang B, Nebel M, Kawakami N, Klinkert WE, et al. Nicotinic acid adenine dinucleotide phosphate-mediated calcium signalling in effector T cells regulates autoimmunity of the central nervous system. *Brain* (2010) **133**:1930–43. doi:10.1093/brain/awq135
15. Fracchia KM, Pai CY, Walsh CM. Modulation of T cell metabolism and function through calcium signaling. *Front Immunol* (2013) **4**:324. doi:10.3389/fimmu.2013.00324
16. Beeton C, Wulff H, Standifer NE, Azam P, Mullen KM, Pennington MW, et al. Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. *Proc Natl Acad Sci U S A* (2006) **103**:17414–9. doi:10.1073/pnas.0605136103
17. Orbán C, Biró E, Grozdics E, Bajnok A, Toldi G. Modulation of T lymphocyte calcium influx patterns via the inhibition of Kv1.3 and IKCa1 potassium channels in autoimmune disorders. *Front Immunol* (2013) **4**:234. doi:10.3389/fimmu.2013.00234
18. Schmeitz C, Hernandez-Vargas EA, Fliegert R, Guse AH, Meyer-Hermann M. A mathematical model of T lymphocyte calcium dynamics derived from single transmembrane protein properties. *Front Immunol* (2013) **4**:277. doi:10.3389/fimmu.2013.00277

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Weft, warp, and weave: the intricate tapestry of calcium channels regulating T lymphocyte function

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Calcium (Ca^{2+}) is a universal second messenger important for T lymphocyte homeostasis, activation, proliferation, differentiation, and apoptosis. The events surrounding Ca^{2+} mobilization in lymphocytes are tightly regulated and involve the coordination of diverse ion channels, membrane receptors, and signaling molecules. A mechanism termed store-operated Ca^{2+} entry (SOCE), causes depletion of endoplasmic reticulum (ER) Ca^{2+} stores following T cell receptor (TCR) engagement and triggers a sustained influx of extracellular Ca^{2+} through Ca^{2+} release-activated Ca^{2+} (CRAC) channels in the plasma membrane. The ER Ca^{2+} sensing molecule, stromal interaction molecule 1 (STIM1), and a pore-forming plasma membrane protein, ORAI1, have been identified as important mediators of SOCE. Here, we review the role of several additional families of Ca^{2+} channels expressed on the plasma membrane of T cells that likely contribute to Ca^{2+} influx following TCR engagement, particularly highlighting an important role for voltage-dependent Ca^{2+} channels (Ca_V) in T lymphocyte biology.

Keywords: calcium, T cell, calcium channels, L-type calcium channels, T cell signaling

In the body's steady-state, a pool of T lymphocytes that express a diverse T cell receptor (TCR) repertoire is maintained in the periphery. In the event of an infection, T lymphocytes, through their TCR, recognize the infectious antigen and are activated and subsequently induced to proliferate and differentiate into effector cells capable of clearing the pathogen. Key components of the signaling events mediating T lymphocyte development, differentiation, homeostasis, effector function, and cell death are the universal second messenger calcium (Ca^{2+}) and the Ca^{2+} channels that regulate the intracellular Ca^{2+} levels (Smith-Garvin et al., 2009).

The activation of a T cell occurs when its TCR recognizes cognate antigen presented on major histocompatibility complex (MHC) by an antigen processing cell. In primary immune responses, this is the function of dendritic cell (DC). DCs take up soluble and particulate antigen as well as cellular debris by phagocytosis, endocytosis, or macropinocytosis and degrade them in endolysosomal compartments where liberated foreign antigens, usually peptides, are subsequently loaded onto MHC-I or MHC-II molecules that cycle to the plasma membrane. Here, the MHC/foreign antigen complex is recognized by a cognate TCR expressed on a specific T lymphocyte (Vyas et al., 2008). A series of signaling events ensue following ligation of the TCR. Ca^{2+} is critical to the TCR signaling processes. TCR engagement triggers an increase in intracellular Ca^{2+} levels resulting from the activation of phospholipase Cγ1 (PLCγ1) and the associated hydrolysis of phosphatidylinositol-3,4-bisphosphate (PIP_2) into inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to IP_3 receptors (IP_3R) in the endoplasmic reticulum (ER) causing release of ER Ca^{2+} stores into the cytoplasm. During the event of store-operated Ca^{2+} entry (SOCE), depletion of ER Ca^{2+} stores triggers a sustained influx of extracellular Ca^{2+} through Ca^{2+} release-activated Ca^{2+} (CRAC) channels in the plasma membrane (Hogan et al., 2010).

The sustained increase in intracellular Ca^{2+} results in the activation of signaling molecules and transcription factors that induce expression of genes required for T cell activation, proliferation, differentiation, and effector function. In T cells, Ca^{2+} can activate a variety of targets including the serine/threonine phosphatase calcineurin and its transcription factor target nuclear factor of activated T cells (NFAT), Ca^{2+}-calmodulin-dependent kinase (CaMK) and its target cyclic AMP-responsive element-binding protein (CREB), myocyte enhancer factor 2 (MEF2) targeted by both calcineurin and CaMK, and nuclear factor kappa B (NFκB) (Oh-Hora, 2009). The best studied downstream effect of Ca^{2+} is the calcineurin-NFAT pathway. Increased Ca^{2+} levels promote the binding of Ca^{2+} to calmodulin inducing a conformational change that allows calmodulin to bind and activate calcineurin. Calcineurin dephosphorylates serines in the amino-terminus of NFAT exposing a nuclear localization signal. This results in the transport of NFAT into the nucleus, where NFAT can interact with other transcription factors, integrating signaling pathways, and inducing gene expression patterns dependent on the context of the TCR signaling (Hogan et al., 2003; Macian,

2005; Smith-Garvin et al., 2009). Ca^{2+} has also been proposed to regulate the Ras/mitogen-activated protein kinase (MAPK) pathway in T cells. RasGRP that activates Ras not only has a DAG binding domain but also has a pair of EF-hand motifs that can directly bind Ca^{2+} (Cullen and Lockyer, 2002). Through this interaction, activation and membrane localization of Ras guanyl nucleotide-releasing protein (RasGRP) is influenced. Upon weak TCR stimulation, RasGRP localizes to the Golgi membrane whereas strong TCR signaling results in recruitment to the plasma membrane. The site of activation may play a role in what extracellular-signal-regulated kinase (ERK) can target downstream thereby contributing to differential signaling dependent on the stimulus (Teixeiro and Daniels, 2010).

There are several families of channels expressed on the plasma membrane of T lymphocytes (Kotturi et al., 2006) that may play important roles in Ca^{2+} entry (**Figure 1**). Recently, through genome wide high-throughput RNA interference screens and analysis of patients with severe combined immunodeficiency

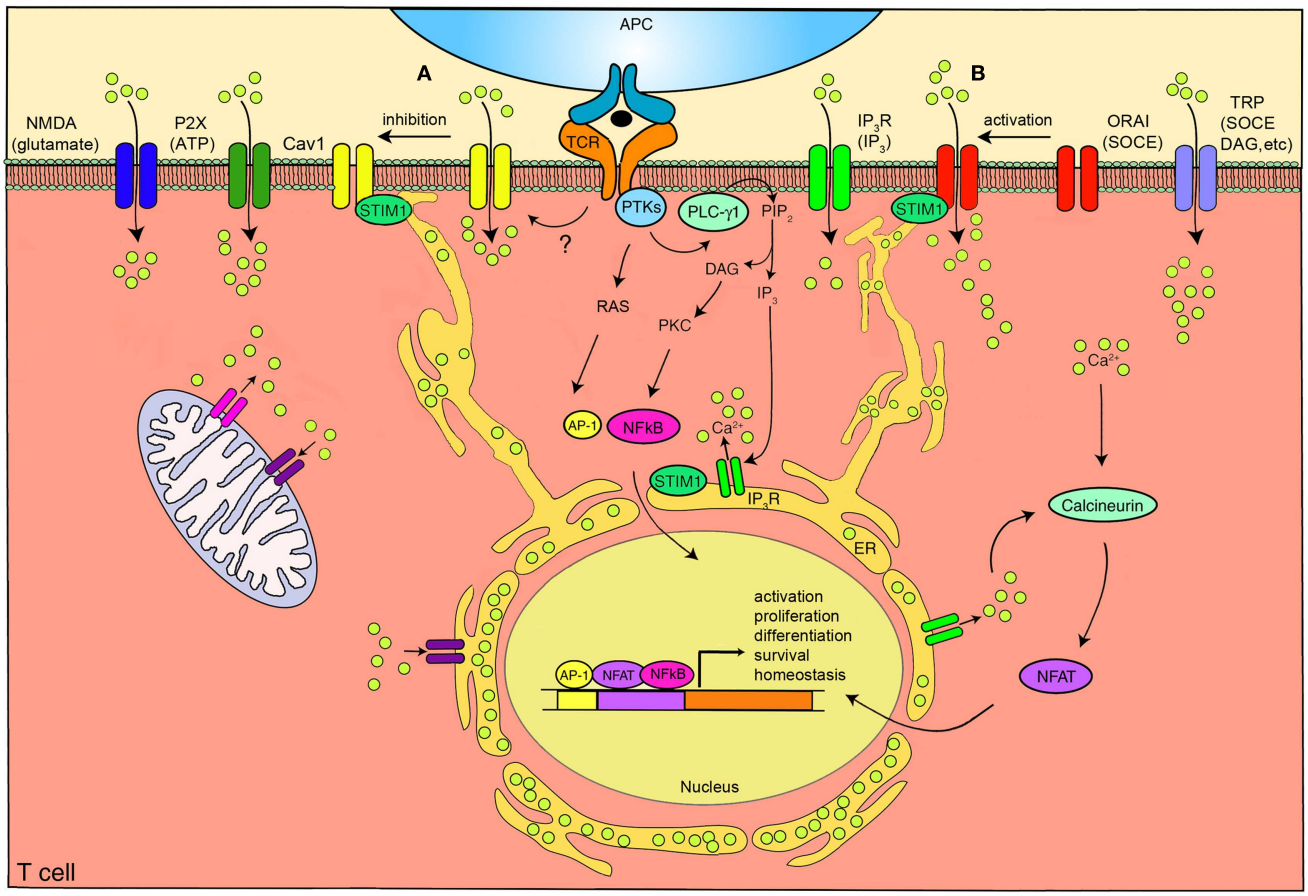

FIGURE 1 | The calcium channels in T cells. T cell receptor (TCR) engagement by a peptide-MHC on an antigen presenting cell (APC), induces protein tyrosine kinases (PTKs) to activate phospholipase C-γ1 (PLC-γ1), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) from plasma membrane phospholipids to generate diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). Elevated levels of IP_3 in the cytosol leads to the release of Ca^{2+} from IP_3R Ca^{2+} channels located in the endoplasmic reticulum (ER). Ca^{2+} depletion from the ER induces Ca^{2+} influx from the extracellular space through the plasma membrane channel, ORAI1. Several auxiliary channels also operate during TCR-mediated Ca^{2+} signaling. These include plasma membrane IP_3R activated by the ligand IP_3, transient receptor potential (TRP) channels that can be operated by DAG and store-operated Ca^{2+} entry (SOCE), adenosine triphosphate (ATP) responsive purinergic P2 (P2X) receptors, glutamate mediated *N*-methyl-D-aspartate activated (NMDA) channels, and voltage-dependent Ca^{2+} channels (Ca_V1) that may be regulated through TCR signaling events. The mitochondria also control cytoplasmic Ca^{2+} levels. Increase in intracellular Ca^{2+} results in activation of calmodulin-calcineurin pathway that induces nuclear factor of activated T cells (NFAT) nuclear translocation and transcription of target genes to direct T cell homeostasis, activation, proliferation, differentiation, and survival. Within this complex network of Ca^{2+} signaling, a model of the reciprocal regulation of Ca_V1 and ORAI1 in T cells has been proposed. **(A)** Low-level TCR signaling through interactions with self-antigens (i.e., self-peptides/self-MHC molecules) may result in Ca_V1 (particularly $Ca_V1.4$) activation and Ca^{2+} influx from outside the cell. This allows for filling of intracellular stores and initiation of a signaling cascade to activate a pro-survival program within the naïve T cell. Stromal interaction molecule 1 (STIM1) is not activated in this scenario and, consequently, ORAI1 remains closed. **(B)** Strong TCR signaling through engagement by a foreign peptide-MHC induces the downstream signaling events that result in ER Ca^{2+} store depletion and STIM1 accumulation in puncta in regions of the ER near the plasma membrane allowing interactions with Ca^{2+} channels. ORAI1 enhances STIM1 recruitment to the vicinity of Ca_V1 channels. Here, STIM1 can activate ORAI1 while inhibiting Ca_V1. PKC, protein kinase C. AP-1, activating protein-1. NFκB, nuclear factor kappa B. Yellow circles, Ca^{2+}.

(SCID), a pore-forming plasma membrane protein, ORAI1 (Feske et al., 2006; Vig et al., 2006; Zhang et al., 2006), and an ER Ca^{2+} sensing molecule, stromal interaction molecule 1 (STIM1) (Liou et al., 2005; Roos et al., 2005), have been identified as the classically defined CRAC channel. Transient receptor potential (TRP) channels have also been the focus of much attention and have been reported to be activated by store depletion in T cells. IP_3 receptors (IP_3R), similar to the ER-associated Ca^{2+} channels, have been shown to be expressed at the plasma membrane of T cells. In addition, T cell expressed adenosine triphosphate (ATP) responsive purinergic P2 (P2X) receptors and glutamate mediated *N*-methyl-D-aspartate (NMDA) activated receptors have shown significant Ca^{2+} permeability. Finally, voltage-dependent Ca^{2+} channels (Ca_V), the focus of this review, have been identified to play a crucial function in T cells (Omilusik et al., 2011).

ORAI AND STIM

The discovery of the pore-forming plasma membrane proteins, ORAI1 and homologs ORAI2 and ORAI3, and the ER Ca^{2+} sensors, STIM1 and STIM2, has led to the development of a well-established paradigm of their coordinated action (Hogan et al., 2010; Feske et al., 2012; Srikanth and Gwack, 2012). TCR engagement triggers the generation of IP_3 and the subsequent activation of IP_3Rs that mediate the release of Ca^{2+} from the ER. The ER transmembrane protein, STIM1, can sense the depletion of Ca^{2+} stores. STIM1 exists as a monomer when Ca^{2+} is present, and its conformation is stabilized through an interaction between its luminal EF-hand domain and sterile α-motif (SAM). When ER Ca^{2+} stores are depleted, the EF-SAM domain interaction in STIM1 becomes unstable resulting in the oligomerization of STIM1 molecules (Park et al., 2009; Stathopulos et al., 2009). STIM1 oligomers accumulate in puncta in regions of ER 10–25 nm beneath the plasma membrane (Liou et al., 2005, 2007; Wu et al., 2006a). Here, ORAI1 at the plasma membrane can interact with STIM1 (Luik et al., 2006; Xu et al., 2006). ORAI1 has been suggested to exist as a dimer in the plasma membrane and upon STIM1 interaction forms tetramers that can function to import Ca^{2+} (Penna et al., 2008).

Analyses of ORAI1 and STIM1 deficiency in human patients, that initially led to the identification of ORAI (Feske et al., 2006), as well as in mouse models, have validated their physiological role in T cell activation. In humans, loss of functional ORAI1 or STIM1 results in SCID (Partiseti et al., 1994; Le Deist et al., 1995; Feske et al., 2001, 2005, 2006; Picard et al., 2009). While lymphocyte numbers are normal in these patients, impaired SOCE leaves T cells with diminished ability to proliferate and produce cytokines upon activation. Analogous phenotypes are observed in animal models. In *ORAI1*$^{-/-}$ and *STIM1*$^{-/-}$ mice, thymic development of conventional TCRαβ T cells appears normal. However, impaired selection of agonist-selected T cells, T regulatory cells (T_{reg}), invariant natural killer T cells and TCRαβ$^+$ CD8αα$^+$ intestinal intraepithelial lymphocytes, owing to a defect in IL-2 or IL-15 signaling has been noted in STIM1- and STIM2-deficient mice (Oh-Hora et al., 2013). ORAI1-deficiency causes a moderate reduction in SOCE and subsequent cytokine production in T cells (Gwack et al., 2008; Vig et al., 2008). STIM1-deficient T cells have no CRAC channel function or SOCE, no subsequent activation of NFAT transcription factor and, as a result, have impaired cytokine secretion (Oh-Hora et al., 2008). This impacts T cell responses and, consequently, confers protection from experimental autoimmune encephalomyelitis (EAE) due to poor generation of Th_1/Th_{17} responses (Schuhmann et al., 2010).

Interestingly, STIM-deficiency is also associated with lymphoproliferative and autoimmune diseases. In SCID patients, this is seen as lymphadenopathy (enlarged lymph nodes) and hepatosplenomegaly (enlarged liver and spleen) as well as autoimmune hemolytic anemia and thrombocytopenia resulting from immune responses directed against the red blood cells and platelets, respectively (Picard et al., 2009). It has been suggested that this autoimmunity observed in STIM1-deficient patients is a consequence of the reduced T_{reg} cell numbers found in the periphery (Feske, 2009; Picard et al., 2009). Similarly, mice lacking both STIM1 and STIM2 experienced autoimmune and lymphomyeloproliferative syndromes again due to a severe reduction in T_{reg} number in the thymus and secondary lymphoid organs and impaired T_{reg} suppressive function (Oh-Hora et al., 2008). This T_{reg} deficiency is presumably a result of poor Ca^{2+}/NFAT-dependent induction of Foxp3 expression (Wu et al., 2006b; Oh-Hora et al., 2008; Tone et al., 2008). Together, these studies highlight the importance of ORAI1/STIM1 in T cell activation and immune tolerance.

T cells also express family members ORAI2 and ORAI3 that exhibit similar structure to ORAI1. ORAI2 and ORAI3 form Ca^{2+}-permeable ion pores; however, these channels differ in their pharmacology, ion selectivity, activation kinetics, and inactivation properties in comparison to ORAI1 (Lis et al., 2007). Overexpression of ORAI2 or ORAI3 with STIM1 can result in Ca^{2+} currents similar but not identical to the CRAC current (DeHaven et al., 2007; Lis et al., 2007). However, ORAI2's contribution to Ca^{2+} signaling in differentiated T cells is questionable as overexpression of ORAI2 in ORAI1$^{-/-}$ T cells does not restore SOCE (Gwack et al., 2008). ORAI2 expression is high in naïve T cells and is down regulated upon activation; therefore, ORAI2 may have a major role in development or peripheral homeostasis (Gwack et al., 2008; Vig et al., 2008). ORAI3 has been shown to form pentamers with ORAI1 to make up the arachidonate-regulated Ca^{2+}-selective (ARC) channels (Mignen et al., 2009). These channels are activated by arachidonic acid rather than store-depletion and require plasma membrane localized STIM1 for their regulation (Mignen et al., 2007). Their role in T cells has yet to be determined.

STIM2 with 42% sequence similarity to STIM1 is also found in T cells. While STIM1 has relatively high and constant expression and can be found to some extent in the plasma membrane as well as the ER, STIM2 is expressed at low levels in naïve T cells but is upregulated upon TCR activation and is exclusively localized to the ER (Williams et al., 2001; Soboloff et al., 2006). Like STIM1, STIM2 functions as an ER Ca^{2+} sensor and is able to mediate SOCE in lymphocytes. Nevertheless, STIM2 does not seem to serve a redundant purpose as its overexpression only partially rescues Ca^{2+} influx deficiency in STIM1$^{-/-}$ T cells (Brandman et al., 2007; Oh-Hora et al., 2008). Upon Ca^{2+} store depletion, STIM2 also oligomerizes and localizes to puncta at ER-plasma membrane contacts; however, STIM2 detects smaller decreases in

ER Ca^{2+} concentration and forms multimers with slower kinetics than STIM1 with some STIM2 already activated in resting cells with replete Ca^{2+} stores (Soboloff et al., 2006; Brandman et al., 2007). This fits with the established role for STIM2 in regulating basal Ca^{2+} influx and stabilizing cytosolic and ER Ca^{2+} levels in resting cells (Brandman et al., 2007). It also explains the fact that STIM2-deficiency has minimal effect on the initial Ca^{2+} entry but impairs the ability of T cells to maintain nuclear translocation of NFAT and cytokine production (Oh-Hora et al., 2008). Where STIM1 readily senses ER Ca^{2+} store depletion and can initiate SOCE, STIM2 remains active in higher Ca^{2+} levels when stores are refilling and can sustain the response (Oh-Hora, 2009).

Although the details of the ORAI-STIM pathway have been the subject of a large amount of recent work, this scheme does not account for the involvement of other currents mediated by additional plasma membrane Ca^{2+} channels that have been shown to be expressed and function in T cells (Kotturi et al., 2003; Kotturi and Jefferies, 2005; Omilusik et al., 2011), nor does it allow for differential patterns in Ca^{2+} response between T cell subsets (Fanger et al., 2000; Weber et al., 2008; Robert et al., 2011). Immunologists are only beginning to acknowledge, accept, and integrate these channels into the pantheon of functions mediated by T cells. Therefore, incorporating multiple Ca^{2+} channels into a comprehensive model is essential for the complete understanding of Ca^{2+} signaling in T cells.

IMPORTANT ADDITIONAL Ca^{2+} CHANNELS IN T LYMPHOCYTES

IP_3 RECEPTORS

The IP_3Rs, similar to those found in the ER, have been suggested to function as Ca^{2+} channels at the plasma membrane (Khan et al., 1992; Kotturi et al., 2006). IP_3 dissipates rapidly after TCR engagement; therefore, IP_3 induced activation of plasma membrane receptors would only contribute to short-term Ca^{2+} signaling (Kotturi et al., 2006). Alternatively, it was suggested that IP_3Rs in the ER, known to bind IP_3 to deplete ER Ca^{2+} stores, change conformation upon ER store depletion, and signal to surface IP_3Rs to open (Berridge, 1993). IP_3Rs have been identified on the cell surface of cultured T cells (Khan et al., 1992; Tanimura et al., 2000). However, IP_3-induced Ca^{2+} currents across the plasma membrane could not be detected (Zweifach and Lewis, 1993). As an alternate function based on the numerous protein binding sites present in the modulatory domain of the channel, IP_3Rs have been proposed to operate at the plasma membrane as scaffolds (Patterson et al., 2004). Further work is required to clearly fit the IP_3R into the Ca^{2+} signaling network in T cells.

TRANSIENT RECEPTOR POTENTIAL CHANNELS

The first TRP family member was discovered in *Drosophila* and was found to have a role in visual transduction (Montell and Rubin, 1989). Subsequently, 28 mammalian TRP channel proteins have been identified. These are grouped into six subfamilies based on amino acid sequence similarities: the classical TRPs (TRPCs) that are most similar to *Drosophila* TRP; the vanilloid receptor TRPs (TRPVs); the melastatin TRPs (TRPMs); the mucolipins (TRPMLs); the polycystins (TRPPs); and ankyrin transmembrane protein 1 (TRPA1) (Clapham et al., 2003; Montell and Rubin, 1989). The six transmembrane domain TRP channels form pores that are permeable to cations including Ca^{2+} (Owsianik et al., 2006). Various TRP channel family members have been shown to be expressed in cultured or primary T cells (Schwarz et al., 2007; Oh-Hora, 2009; Wenning et al., 2011).

Before the discovery of ORAI1 and STIM1, TRP channels were investigated as candidates for the CRAC channel. The TRPV6 channel is highly permeable to Ca^{2+} and has been shown to be activated by store-depletion (Cui et al., 2002). In addition, when a dominant-negative pore-region mutant of TRPV6 was expressed in Jurkat T cells, the CRAC current was diminished (Cui et al., 2002). However, in subsequent studies, the CRAC channel inhibitor, BTP2, had no effect on TRPV6 channel activity (Zitt et al., 2004; He et al., 2005; Schwarz et al., 2006) and the role of TRPV6 as a CRAC channel could not be confirmed (Voets et al., 2001; Bodding et al., 2002). TRPC3 channels were also under consideration as CRAC channels following the discovery that Jurkat T cell lines with mutated TRPC3 channels had reduced Ca^{2+} influx following TCR stimulation. This impairment could be overcome by overexpression of a wild-type TRPC3 (Fanger et al., 1995; Philipp et al., 2003). Furthermore, siRNA knockdown of TRPC3 expression in human T cells resulted in reduced proliferation following TCR stimulation (Wenning et al., 2011). However, while TRPC3 has been shown to be activated in response to store-depletion (Vazquez et al., 2001), the major stimulus gating TRPC3 seems to be DAG (Hofmann et al., 1999).

Although not store-operated, the TRPM2 channel in T cells has also been examined. TRMP2 is a non-selective Ca^{2+} channel that is activated by the intracellular secondary messengers ADP-ribose (ADPR), nicotinamide adenine dinucleotide (NAD^+), hydrogen peroxide (H_2O_2), and cyclic ADPR (Perraud et al., 2001; Hara et al., 2002; Massullo et al., 2006). It has been proposed that activation of T cells can increase endogenous ADPR levels in T cells which results in Ca^{2+} entry through TRPM2 and subsequent induction of cell death demonstrating that TRPM2 can contribute to some degree to Ca^{2+} signaling in T cells (Gasser et al., 2006). Recently, the TRPM2 channels have been implicated in T cell effector function. $CD4^+$ T cells from TRPM2-deficient mice were shown to have reduced ability to proliferate and secrete cytokines following TCR activation. Furthermore, TRPM2-deficient mice had decreased inflammation and demyelinating spinal cord lesions in an EAE model (Melzer et al., 2012). Although important to T cell function, the current role of TRP receptors in Ca^{2+} signaling is still under investigation.

ATP-RESPONSIVE PURINERGIC P2 RECEPTORS (P2X)

The P2X receptors are ATP-gated ion channels that permit the influx of extracellular cations including Ca^{2+} ions (reviewed in Junger, 2011). Four family members in particular, P2X1, P2X2, P2X4, and P2X7, have been associated with T cells and may serve to amplify the TCR signal to ensure antigen recognition and T cell activation through an autocrine feedback mechanism (Bours et al., 2006; Yip et al., 2009; Woehrle et al., 2010; Junger, 2011). Upon TCR engagement, ATP is released through Pannexin 1 hemichannels that localize to the immunological synapse where they release ATP that acts on the P2X channels to promote Ca^{2+} influx and

enhance signaling (Filippini et al., 1990; Schenk et al., 2008; Yip et al., 2009). In particular, P2X1, 4, and 7 have been shown to contribute to the increase in intracellular Ca^{2+}, NFAT activation, proliferation, and IL-2 production in murine and human T cells following stimulation (Baricordi et al., 1996; Schenk et al., 2008; Yip et al., 2009; Woehrle et al., 2010). Initial analysis of P2X7 receptor-deficient mice revealed no major defects in T cell development (Solle et al., 2001). However, additional studies did identify a deficiency in T_{reg} stability and function as well as Th_{17} differentiation (Schenk et al., 2011). Also, T cells from C57Bl/6 mice with a natural mutation in the P2X7 gene that reduces ATP sensitivity have been shown to produce reduced amounts of IL-2 following stimulation compared to Balb/c mice with a fully functional receptor further delineating a role for P2X receptors in T cell function (Adriouch et al., 2002; Yip et al., 2009). Likewise, in two models of T cell-dependent inflammation, treatment with a P2XR antagonist impeded the development of colitogenic T cells in inflammatory bowel disease and induced unresponsiveness in anti-islet TCR transgenic T cells in diabetes (Schenk et al., 2008). Therefore, it is clear that P2X channels are playing an important role in T cell Ca^{2+} signaling; however, the specific mechanistic details of how they fit into shaping the T cell Ca^{2+} environment need further exploration.

N-METHYL-D-ASPARTATE ACTIVATED RECEPTORS

The NMDA receptors are a class of ligand-gated glutamate ionotropic receptors found in the central nervous system that play a crucial role in neuronal function. These receptors are heterotetramers composed of two subunits, NR1 and NR2, that form an ion channel which is highly permeable to K^+, Na^+, and Ca^{2+} (Boldyrev et al., 2012). Ca^{2+} entry through the receptors into the cell occurs when the NMDA receptors are activated by binding to their ligands, glutamate and glycine. In neurons, this allows for long-lasting memory formation (Boldyrev et al., 2012). Interestingly, NMDA receptors have been shown to be expressed on rodent and human T cells and contribute to the increase in intracellular Ca^{2+} level following T cell activation (Lombardi et al., 2001; Boldyrev et al., 2004; Miglio et al., 2005, 2007; Mashkina et al., 2007, 2010). Zainullina et al. (2011) further demonstrated that activation of T cells with thapsigargin, an inhibitor of a Ca^{2+}-ATPase of the ER that induces Ca^{2+} store depletion and activation of plasma membrane Ca^{2+} channels, in the presence of an NMDA receptor antagonist did not affect the movement of Ca^{2+} from intracellular stores. However, it reduced the influx of Ca^{2+} from the extracellular space suggesting that NMDA receptors participate in SOCE, at least to some degree. In this scenario, the NMDA receptors may be mainly contributing to Ras/Rac-dependent signaling in T cells following TCR engagement (Zainullina et al., 2011). Analogous to neuronal synapses, a recent study of thymocytes showed that upon TCR stimulation, NMDA receptors localize to the immunological synapse (Affaticati et al., 2011). Here, DCs rapidly release glutamate that activates the NMDA receptors on the T cells contributing to the increase in intracellular Ca^{2+} concentration. It is suggested that glutamate signaling through these receptors may participate in negative selection in the thymus by inducing apoptosis in thymocytes while it may influence proliferation in peripheral T cells (Affaticati et al., 2011). Further studies are required to determine the role glutamate plays in shaping the Ca^{2+} signal in T cells.

VOLTAGE-DEPENDENT Ca^{2+} CHANNELS

Ca_V channels function typically in excitable cells such as nerve, muscle, and endocrine cells where they open in response to membrane depolarization to allow Ca^{2+} entry (Buraei and Yang, 2010). The Ca_V channels were initially classified based on the voltage required for activation into the subgroups high-voltage activated (HVA) and low-voltage activated (LVA) channels. Further analysis of the Ca_V channels allowed for additional classification of the channels into groups with distinct biophysical and pharmacological properties: T (tiny/transient)-, N (neuronal)-, P/Q (Purkinje)-, R (toxin-resistant)-, L (long-lasting)-type channels (Lacinova, 2005; Buraei and Yang, 2010).

The Ca_V channels are heteromultimeric protein complexes composed of five subunits: α_1, α_2, β, δ, and γ. The α_2 and δ subunits are linked together through disulfide bonds to form a single unit referred to as $\alpha_2\delta$. The α_1 subunit of the channel is the pore-forming component responsible for the channel's unique properties while the $\alpha_2\delta$, β, and γ subunits regulate the structure and activity of α_1 (Buraei and Yang, 2010). The α_1 subunit consists of four homologous repeated motifs (I–IV) each composed of six transmembrane segments (S1–S6) with a re-entrant pore-forming loop (P-loop) between S5 and S6. The P-loop contains four highly conserved negatively charged amino acids responsible for selecting and conducting Ca^{2+} while the S6 segments form the inner pore (Buraei and Yang, 2010). The S4 segments are positively charged and constitute the voltage sensor. The pore opens and closes through voltage-mediated movement of this sensor (Lacinova, 2005).

Ten mammalian α_1 subunits are divided into three subfamilies based on similarities in amino acid sequence. The Ca_V1 family contains L-type channels; the Ca_V2 family consists of N-, P/Q-, and R-type channels; and the Ca_V3 family are T-type channels (Buraei and Yang, 2010). Initially, "voltage-operable" current seemingly activated by TCR engagement or store depletion with electrophysiological properties different than the CRAC current in the plasma membrane of Jurkat T cells was identified (Densmore et al., 1992, 1996). Subsequently, numerous pharmacological and genetic studies have demonstrated the existence of Ca_V1 or L-type channels in T cells (**Table 1**). The Ca_V1 channels exist as four isoforms: $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$, and $Ca_V1.4$. In excitable cells, L-type Ca^{2+} channels require high-voltage activation and have slow current decay kinetics. They have a unique sensitivity to 1,4-dihydropyridines (DHPs), a wide drug class that can either activate (for example: Bay K 8644) or inhibit (for example: nifedipine) the activity of the channel (Lacinova, 2005).

Early studies suggesting that L-type Ca^{2+} channels contributed to T cell Ca^{2+} signaling relied on pharmaceutical analysis (Grafton and Thwaite, 2001; Kotturi et al., 2003; Gomes et al., 2004). These include *in vitro* experiments where the DHP antagonist nifedipine was shown to block proliferation of human T cells or peripheral blood mononuclear cells or impair increase in intracellular Ca^{2+} following stimulation with mitogens (Birx et al., 1984; Gelfand et al., 1986; Dupuis et al., 1993). This effect of nifedipine seemed to be dose-dependent when T cells were stimulated

Table 1 | Ca_V1 Ca^{2+} channel expression in T cells.

Subtype	Distribution	Analysis	Reference
$Ca_V1.1$	Mouse CTLs	Protein	Matza et al. (2009)
	Mouse effector $CD8^+$ T cells	mRNA (PCR); protein	Jha et al. (2009)
	Mouse $CD4^+$ T cells	mRNA (PCR); protein	Badou et al. (2006), Matza et al. (2008)
$Ca_V1.2$	Human peripheral blood T cells; human Jurkat, MOLT-4, CEM T cell lines	mRNA (partial sequence); protein (truncated/full)	Stokes et al. (2004)
	Mouse CTLs	Protein	Matza et al. (2009)
	Mouse $CD8^+$ T cells	mRNA (PCR)	Jha et al. (2009)
	Mouse $CD4^+$ T cells	mRNA (PCR); protein	Badou et al. (2006), Matza et al. (2008)
	Mouse $CD4^+$ Th2 cells	mRNA (sequence); protein	Cabral et al. (2010)
	Mouse BDC2.5 $CD4^+$ T cells	mRNA (PCR)	Lee et al. (2008)
$Ca_V1.3$	Human Jurkat T cell line	mRNA (partial sequence); protein (truncated)	Stokes et al. (2004)
		mRNA (PCR)	Colucci et al. (2009)
	Mouse $CD8^+$ T cells	mRNA (PCR)	Jha et al. (2009)
	Mouse $CD4^+$ Th2 cells	mRNA (sequence); protein	Cabral et al. (2010)
$Ca_V1.4$	Human Jurkat T cell line; human spleen; human peripheral blood $CD4^+/CD8^+$ T cells	mRNA (sequence); protein	Kotturi et al. (2003), Kotturi and Jefferies (2005)
	Human spleen and thymus; rat spleen and thymus	mRNA (PCR); protein	McRory et al. (2004)
	Mouse T cells	mRNA (PCR); protein (truncated)	Omilusik et al. (2011)
	Mouse naïve $CD8^+$ T cells	mRNA (PCR); protein	Jha et al. (2009)
	Mouse $CD4^+$ T cells	mRNA (PCR)	Badou et al. (2006), Colucci et al. (2009)

in the presence of the immunosuppressive agent cyclosporine A (Marx et al., 1990; Padberg et al., 1990). In a resultant study performed by Kotturi et al. (2003), treatment of Jurkat T cells and human peripheral blood T cells with the DHP agonist Bay K 8644 was shown to increase intracellular Ca^{2+} levels and induce ERK 1/2 phosphorylation, while treatment with the DHP antagonist nifedipine blocked Ca^{2+} influx, ERK 1/2 phosphorylation, NFAT activation, IL-2 production, and T cell proliferation. At micromolar concentrations, DHPs can also affect the function of K^+ channels and therefore conclusions drawn from these pharmaceutical studies (Grafton and Thwaite, 2001; Kotturi et al., 2003, 2006; Gomes et al., 2004) regarding contribution of Ca_V1 to T cell function have been criticized (Wulff et al., 2003, 2004). However, inhibitory effects have been noted when DHP antagonists were used at concentrations well below those influencing K^+ channels (Sadighi Akha et al., 1996; Kotturi et al., 2003) as well as with the more specific Ca_V1 blocker, calciseptine, that also obstructs Ca^{2+} influx in T cells (de Weille et al., 1991; Matza and Flavell, 2009).

Subsequent genetic studies have confirmed the expression of L-type Ca^{2+} channels in T cells and have gone on to compare their structure to those found in excitable cells. $Ca_V1.4$ was the first Ca_V1 channel identified in T cells (Kotturi et al., 2003; Kotturi and Jefferies, 2005; Omilusik et al., 2011). The $Ca_V1.4$ α_1 subunit is encoded by the *CACNA1F* gene originally cloned from human retina (Fisher et al., 1997) where $Ca_V1.4$ mediates Ca^{2+} entry into the photoreceptors promoting tonic neurotransmitter release (Strom et al., 1998). Kotturi et al. identified the $Ca_V1.4\alpha_1$ subunit mRNA and protein in Jurkat T cells as well as in human peripheral blood T cells (Kotturi et al., 2003; Kotturi and Jefferies, 2005). This human lymphocyte form of Cav1.4 was shown to undergo alternative splicing, resulting in a protein smaller in size compared to a retinoblastoma version (Kotturi and Jefferies, 2005). Sequence analysis revealed that the $Ca_V1.4$ expressed in human T cells exists as two novel splice variants (termed $Ca_V1.4a$ and $Ca_V1.4b$) distinct from the retina (Kotturi and Jefferies, 2005). $Ca_V1.4a$ lacks exons 31, 32, 33, 34, and 37 resulting in deletions of transmembrane segments S3, S4, S5, and half of S6 in motif IV. As a result, the voltage sensor domain and part of the DHP binding site and EF-hand Ca^{2+} binding motif are deleted from the channel. While the removal of the voltage sensor may alter the voltage-gated activation of this channel, partial deletion of the DHP binding site may decrease the sensitivity of T cell-specific $Ca_V1.4$ channels. This explained why large doses of DHP antagonists are required to completely block Ca^{2+} influx through Ca_V channels in T cells (Dupuis et al., 1993). Remarkably, the splice event caused a frameshift that changed the carboxy-terminus to a sequence that resembles (40% identity) the $Ca_V1.1$ channel found in skeletal muscle (Kotturi and Jefferies, 2005). The second splice variant, $Ca_V1.4b$, lacks exons 32 and 36 causing a deletion of the extracellular loop between S3 and S4 in motif IV. $Ca_V1.4b$ also has an early stop codon that prematurely truncates the channel. The voltage sensing motif is not spliced out; however, it has been proposed that removal of the extracellular loop may alter the voltage sensing function of this channel (Kotturi and Jefferies, 2005). Upon membrane depolarization, the S4 voltage sensor domain moves and this splicing event may leave the domain in a conformation that prevents S4 movement (Bezanilla, 2002; Jurkat-Rott and Lehmann-Horn, 2004). Since their discovery in T cells (Kotturi and Jefferies, 2005), alternative splice variants of other Ca_V channels have been found. Analogous structural changes have been

subsequently noted for $Ca_V1.1$ (Matza and Flavell, 2009) with one isoform similarly lacking the extracellular loop between S3 and S4 in motif IV that translated to shifted voltage sensitivity in muscle cells (Tuluc et al., 2009). These structural changes likely explain the insensitivity of T cell Ca_V1 channels to be activated by cell depolarization and instead, gating in T cells may be through alternate mechanisms such as ER store-depletion or TCR signaling. Supporting this hypothesis, Jha et al. (2009) recently found $Ca_V1.4$ to be localized to lipid rafts in the plasma membrane of murine T cells. $Ca_V1.4$ was found to be associated with components of the T cell signaling complex. Given its location, Ca_V1 channel activity could be regulated in T cells by downstream TCR signaling events.

Recent *in vivo* studies have directly addressed the controversy regarding the importance of voltage-dependent Ca^{2+} channels in T cell function. Mice with targeted deletions in the regulatory β subunits that mediate Ca_V channel assembly, plasma membrane targeting, and activation have been described (Badou et al., 2006; Buraei and Yang, 2010). The β3 and β4 family members are expressed in naïve $CD4^+$ T cells and upregulated in activated T cells. Upon TCR cross-linking, $CD4^+$ T cells from β3 or β4-deficient mice showed impaired Ca^{2+} influx, NFAT nuclear translocation, and cytokine secretion (Badou et al., 2006). Cav1.1 expression was found to be reduced in the β4-deficient T cells providing a possible role for Ca_V1 in lymphocyte function (Badou et al., 2006). The same group later examined $CD8^+$ T cell populations in a β3-deficient mouse (Jha et al., 2009). $\beta3^{-/-}$ mice have reduced numbers of $CD8^+$ T cells possibly due to increased spontaneous apoptosis induced by higher expression of Fas. Upon activation, these $CD8^+$ T cells have decreased Ca^{2+} entry, proliferation, and NFAT nuclear translocation. β3 was found to associate with $Ca_V1.4$ and several TCR signaling proteins suggesting its role in TCR gated Ca^{2+} signaling (Jha et al., 2009). Similarly, when the AHNAK1 protein, a large scaffold protein required for $Ca_V1.1$ surface expression, was disrupted, T cells had reduced Ca^{2+} influx and NFAT activation that equated to poor effector function (Matza et al., 2008, 2009). Recently, Cabral et al. (2010) began to address differential Ca^{2+} signaling in T cell subsets. This study demonstrated that $Ca_V1.2$ and $Ca_V1.3$ channels were expressed in Th2 but not Th1 differentiated effector T cells. Knockdown of $Ca_V1.2$ and/or $Ca_V1.3$ expression in Th2 cells with antisense oligodeoxynucleotides resulted in reduced Ca^{2+} influx following TCR stimulation and impaired cytokine secretion (Cabral et al., 2010). In addition, Th2 cells with disrupted Ca_V1 expression were impaired in their ability to induce asthma in an adoptive transfer model (Cabral et al., 2010). Further studies defining the Cav1 channel subtype or splice variant essential to various stages of development and activation of the T cell subsets will likely provide an explanation for differences in Ca^{2+} responses.

Omilusik et al. (2011) used a murine model deficient for $Ca_V1.4$ (Mansergh et al., 2005), one of the pore-forming subunits of a Ca_V channel, to unequivocally establish a T cell-intrinsic role for Ca_V1s in the activation, survival, and maintenance of naïve $CD4^+$ and $CD8^+$ T cells *in vivo*. $Ca_V1.4$ was shown to be essential for TCR-induced regulation of cytosolic free Ca^{2+} and downstream TCR signaling, impacting activation of the Ras/ERK and NFAT pathways, IL-7 receptor expression and IL-7 responsiveness. The loss of $Ca_V1.4$ and subsequently naïve peripheral T cells resulted in deficient immune responses when challenged with the model bacteria, *L. monocytogenes*. Instead of being activated by Ca^{2+} store release as in the case of ORAI1, it appears that $Ca_V1.4$ may operate to create intracellular Ca^{2+} stores in the ER. Low-level TCR signaling through interactions with self-antigens (i.e., self-peptides/self-MHC molecules) may result in $Ca_V1.4$-mediated Ca^{2+} influx from outside the cell, allowing the filling of intracellular stores and the initiation of a pro-survival program. This recent data supports the concept that in the absence of $Ca_V1.4$, there is a reduction in the influx of extracellular Ca^{2+} coupled to self/MHC-TCR interaction, resulting in low cytoplasmic Ca^{2+} levels and depleted Ca^{2+} ER stores (Omilusik et al., 2011). Therefore, when $Ca_V1.4$-deficient T cells are stimulated through the TCR, there is a defective Ca^{2+} release from the ER as a result of lower levels of stored Ca^{2+}, decreased subsequent SOCE, and diminished inward Ca^{2+} flux through CRAC channels leading to weakened Ca^{2+}-dependent signaling. Overall, the absence of tonic survival signals provided by $Ca_V1.4$ results in failure of naïve T cells to thrive and perpetuates a state of immunological activation and exhaustion (Omilusik et al., 2011). Studies on other immune cells support this contention. For example, $Ca_V1.2$ expressed in mast cells has been reported to protect against antigen-induced cell death by maintaining mitochondria integrity and inhibiting the mitochondrial cell death pathway (Suzuki et al., 2009). Using pharmacological agents and siRNA specific knockdown, Suzuki et al. (2009) demonstrated that $Ca_V1.2$ channels protect mast cells from undergoing apoptosis following FcεRI activation as discerned by assessing mitochondrial membrane potential, cytochrome *c* release, and caspase-3/7 activation. Furthermore, though it remains unclear, it appears that Ca^{2+} influx through $Ca_V1.2$ at the plasma membrane may be important for maintenance of the mitochondrial Ca^{2+} concentration, thereby providing the cell with pro-survival signals (Suzuki et al., 2009). In conclusion, it is of importance to note that knockouts of the components of Ca_V1 channels in T cells have, by large, more severe phenotypes than those of other categories of Ca^{2+} channels in T cells and, certainly, this argues strongly that Ca_V1 channels play a significant role in regulating and orchestrating T cell biology.

It is interesting to consider and likely profoundly important for integrating the multiple functions of T cells with other homeostatic processes, that Ca_V1 coexist in excitable and non-excitable cells with other Ca^{2+} channels and the interplay between the channels all likely contribute to the highly regulated Ca^{2+} signaling system. Ca_V1 channels have been shown to interact with the ER/sarcoplasmic reticulum (SR) ryanodine receptors (RyRs) in excitable cells (Lanner et al., 2010). In skeletal muscle, $Ca_V1.1$ channels are activated by membrane depolarization and through a physical interaction with RyR1 stimulate the release of Ca^{2+} from the SR. Similarly, in cardiac muscle, $Ca_V1.2$ is triggered to mediate entry of extracellular Ca^{2+} which in turn activates RyR2 channels to release intracellular Ca^{2+} stores (Lanner et al., 2010). Both mechanisms have also been observed in neurons (Chavis et al., 1996; Mouton et al., 2001). Although T cells express RyRs (Hosoi et al., 2001) and these receptors have been shown to contribute to Ca^{2+} signaling following TCR activation (Hohenegger et al., 1999;

Schwarzmann et al., 2002; Conrad et al., 2004), further studies are needed to demonstrate a Ca_V1–RyR interaction.

An interplay between voltage-gated sodium channels (VGSC) and Ca_V1 has also been suggested to shape the T cell Ca^{2+} signal. In excitable cells such as muscle and neurons, membrane depolarization by VGSC leads to an increase in cytosolic Ca^{2+} through the activation of Ca_V channels (Dravid et al., 2004; Fekete et al., 2009; Catterall, 2010). A recent study in T cells has determined an essential role for a VGSC in positive selection (Lo et al., 2012). Pharmacological inhibition and shRNA-mediated knockdown was used to demonstrate that the VGSC composed of a pore-forming SCN5a and a regulatory SCN5b subunit is necessary for Ca^{2+} influx during positive selection of $CD4^+$ T cells. It is proposed that this SCN5a-SCN5b channel is expressed in double positive T cells in order to convert a weak positive selection signal into a sustained Ca^{2+} flux necessary for positive selection to take place. However, once in the periphery, T cells no longer express the channel to eliminate the possibility of autoimmunity (Lo et al., 2012). ORAI1 and STIM1 do not seem to contribute to thymic development of conventional TCRαβ T cells (Oh-Hora et al., 2013); therefore, it is an interesting idea that VGSC activation by kinases downstream of the TCR (Rook et al., 2012) can induce Ca^{2+} signaling by Ca_V1 in developing T cells. Further studies are required to formally demonstrate a functional link between Ca_V1 and VGSC channels in lymphocytes.

Recently, an interesting reciprocal relationship between $Ca_V1.2$ and ORAI1 has been described (Park et al., 2010; Wang et al., 2010). After Ca^{2+} store depletion in the ER, STIM1 oligomers form at ER-plasma membrane junctions allowing the STIM1 CRAC-activating domain (CAD) to interact with the C-terminus of ORAI1 and $Ca_V1.2$ channels. ORAI1 channels are activated by STIM1 and subsequently open causing sustained Ca^{2+} influx from the extracellular space. Conversely, STIM1 inhibits Ca^{2+} influx through $Ca_V1.2$ and promotes its internalization, further shutting down the activity of the channel (Park et al., 2010; Wang et al., 2010). It is interesting to speculate that strong TCR signaling through engagement by a foreign peptide-MHC may trigger this activation of ORAI1 and inhibition of Ca_V1 channels (**Figure 1**). However, low-level TCR signaling through interactions with self-antigens (i.e., self-peptides/self-MHC molecules) may not induce STIM1 to localize to the plasma membrane thereby activating Ca_V and co-ordinately inhibiting ORAI1. This results in Ca_V1-mediated Ca^{2+} influx from outside the cell, filling of depleted intracellular stores, and induction of a signaling cascade to activate a pro-survival program within the naïve T cell. The activation and inhibition of Ca_V1 channels through STIM1 or other TCR-mediated events is an intriguing concept and will likely be the focus of many new studies.

Although Ca_V1 function is vital for T cell Ca^{2+} signaling, their specific functions have yet to be fully explored. Further work is required to clarify the role played by each Ca_V1 channel family member as well as the other Ca^{2+} channels in shaping the Ca^{2+} signal. Altogether, these studies do provide a new framework for understanding the regulation of lymphocyte biology through the function of several Ca^{2+} channels, particularly the L-type Ca^{2+} channels, in the storage of intracellular Ca^{2+} and operative Ca^{2+} regulation during antigen receptor-mediated signal transduction.

Overall, the translational aspects of the current research in the field of Ca^{2+} channel biology have direct implications in designing new modalities for modifying T cell responses using drugs that are known to control Ca^{2+} channels activities, such as the plethora of drugs that already exist for modifying Ca_V1 channels. Agents that target the Ca_V1 splice variants expressed in lymphocytes and inhibit the activity of the channel may serve as more specific immunosuppressants than the current options. Relevant applications for these agents may include therapy for autoimmune diseases, reduction of transplant rejection risk, and treatment of other disorders requiring suppression or in the case of existing immunodeficiency, activation of the immune system.

REFERENCES

Adriouch, S., Dox, C., Welge, V., Seman, M., Koch-Nolte, F., and Haag, F. (2002). Cutting edge: a natural P451L mutation in the cytoplasmic domain impairs the function of the mouse P2X7 receptor. *J. Immunol.* 169, 4108–4112.

Affaticati, P., Mignen, O., Jambou, F., Potier, M. C., Klingel-Schmitt, I., Degrouard, J., et al. (2011). Sustained calcium signalling and caspase-3 activation involve NMDA receptors in thymocytes in contact with dendritic cells. *Cell Death Differ.* 18, 99–108. doi:10.1038/cdd.2010.79

Badou, A., Jha, M. K., Matza, D., Mehal, W. Z., Freichel, M., Flockerzi, V., et al. (2006). Critical role for the beta regulatory subunits of Cav channels in T lymphocyte function. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15529–15534. doi:10.1073/pnas.0607262103

Baricordi, O. R., Ferrari, D., Melchiorri, L., Chiozzi, P., Hanau, S., Chiari, E., et al. (1996). An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. *Blood* 87, 682–690.

Berridge, M. J. (1993). Inositol trisphosphate and calcium signalling. *Nature* 361, 315–325. doi:10.1038/361315a0

Bezanilla, F. (2002). Voltage sensor movements. *J. Gen. Physiol.* 120, 465–473. doi:10.1085/jgp.20028660

Birx, D. L., Berger, M., and Fleisher, T. A. (1984). The interference of T cell activation by calcium channel blocking agents. *J. Immunol.* 133, 2904–2909.

Bodding, M., Wissenbach, U., and Flockerzi, V. (2002). The recombinant human TRPV6 channel functions as Ca2+ sensor in human embryonic kidney and rat basophilic leukemia cells. *J. Biol. Chem.* 277, 36656–36664. doi:10.1074/jbc.M202822200

Boldyrev, A. A., Bryushkova, E. A., and Vladychenskaya, E. A. (2012). NMDA receptors in immune competent cells. *Biochemistry Mosc.* 77, 128–134. doi:10.1134/S0006297912020022

Boldyrev, A. A., Kazey, V. I., Leinsoo, T. A., Mashkina, A. P., Tyulina, O. V., Johnson, P., et al. (2004). Rodent lymphocytes express functionally active glutamate receptors. *Biochem. Biophys. Res. Commun.* 324, 133–139. doi:10.1016/j.bbrc.2004.09.019

Bours, M. J., Swennen, E. L., Di Virgilio, F., Cronstein, B. N., and Dagnelie, P. C. (2006). Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol. Ther.* 112, 358–404.

Brandman, O., Liou, J., Park, W. S., and Meyer, T. (2007). STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca2+ levels. *Cell* 131, 1327–1339. doi:10.1016/j.cell.2007.11.039

Buraei, Z., and Yang, J. (2010). The ss subunit of voltage-gated Ca2+ channels. *Physiol. Rev.* 90, 1461–1506. doi:10.1152/physrev.00057.2009

Cabral, M. D., Paulet, P. E., Robert, V., Gomes, B., Renoud, M. L., Savignac, M., et al. (2010). Knocking down Cav1 calcium channels implicated in Th2 cell activation prevents experimental asthma. *Am. J. Respir. Crit. Care Med.* 181, 1310–1317. doi:10.1164/rccm.200907-1166OC

Catterall, W. A. (2010). Signaling complexes of voltage-gated sodium and calcium channels. *Neurosci. Lett.* 486, 107–116. doi:10.1016/j.neulet.2010.08.085

Chavis, P., Fagni, L., Lansman, J. B., and Bockaert, J. (1996). Functional coupling between ryanodine receptors and L-type calcium channels in neurons. *Nature* 382, 719–722. doi:10.1038/382719a0

Clapham, D. E., Montell, C., Schultz, G., and Julius, D. (2003). International Union of Pharmacology. XLIII. Compendium of voltage-gated ion channels: transient receptor potential channels. *Pharmacol. Rev.* 55, 591–596. doi:10.1124/pr.55.4.6

Colucci, A., Giunti, R., Senesi, S., Bygrave, F. L., Benedetti, A., and Gamberucci, A. (2009). Effect of nifedipine on capacitive calcium entry in Jurkat T lymphocytes. *Arch. Biochem. Biophys.* 481, 80–85. doi:10.1016/j.abb.2008.10.002

Conrad, D. M., Hanniman, E. A., Watson, C. L., Mader, J. S., and Hoskin, D. W. (2004). Ryanodine receptor signaling is required for anti-CD3-induced T cell proliferation, interleukin-2 synthesis, and interleukin-2 receptor signaling. *J. Cell. Biochem.* 92, 387–399. doi:10.1002/jcb.20064

Cui, J., Bian, J. S., Kagan, A., and McDonald, T. V. (2002). CaT1 contributes to the stores-operated calcium current in Jurkat T-lymphocytes. *J. Biol. Chem.* 277, 47175–47183. doi:10.1074/jbc.M205870200

Cullen, P. J., and Lockyer, P. J. (2002). Integration of calcium and Ras signalling. *Nat. Rev. Mol. Cell Biol.* 3, 339–348. doi:10.1038/nrm808

de Weille, J. R., Schweitz, H., Maes, P., Tartar, A., and Lazdunski, M. (1991). Calciseptine, a peptide isolated from black mamba venom, is a specific blocker of the L-type calcium channel. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2437–2440. doi:10.1073/pnas.88.6.2437

DeHaven, W. I., Smyth, J. T., Boyles, R. R., and Putney, J. W. Jr. (2007). Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J. Biol. Chem.* 282, 17548–17556. doi:10.1074/jbc.M611374200

Densmore, J. J., Haverstick, D. M., Szabo, G., and Gray, L. S. (1996). A voltage-operable current is involved in Ca2+ entry in human lymphocytes whereas ICRAC has no apparent role. *Am. J. Physiol.* 271, C1494–C1503.

Densmore, J. J., Szabo, G., and Gray, L. S. (1992). A voltage-gated calcium channel is linked to the antigen receptor in Jurkat T lymphocytes. *FEBS Lett.* 312, 161–164. doi:10.1016/0014-5793(92)80926-8

Dravid, S. M., Baden, D. G., and Murray, T. F. (2004). Brevetoxin activation of voltage-gated sodium channels regulates Ca dynamics and ERK1/2 phosphorylation in murine neocortical neurons. *J. Neurochem.* 89, 739–749. doi:10.1111/j.1471-4159.2004.02407.x

Dupuis, G., Aoudjit, F., Ricard, I., and Payet, M. D. (1993). Effects of modulators of cytosolic Ca2+ on phytohemagglutin-dependent Ca2+ response and interleukin-2 production in Jurkat cells. *J. Leukoc. Biol.* 53, 66–72.

Fanger, C. M., Hoth, M., Crabtree, G. R., and Lewis, R. S. (1995). Characterization of T cell mutants with defects in capacitative calcium entry: genetic evidence for the physiological roles of CRAC channels. *J. Cell Biol.* 131, 655–667. doi:10.1083/jcb.131.3.655

Fanger, C. M., Neben, A. L., and Cahalan, M. D. (2000). Differential Ca2+ influx, KCa channel activity, and Ca2+ clearance distinguish Th1 and Th2 lymphocytes. *J. Immunol.* 164, 1153–1160.

Fekete, A., Franklin, L., Ikemoto, T., Rozsa, B., Lendvai, B., Sylvester Vizi, E., et al. (2009). Mechanism of the persistent sodium current activator veratridine-evoked Ca elevation: implication for epilepsy. *J. Neurochem.* 111, 745–756. doi:10.1111/j.1471-4159.2009.06368.x

Feske, S. (2009). ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca2+ entry in the immune system and beyond. *Immunol. Rev.* 231, 189–209. doi:10.1111/j.1600-065X.2009.00818.x

Feske, S., Giltnane, J., Dolmetsch, R., Staudt, L. M., and Rao, A. (2001). Gene regulation mediated by calcium signals in T lymphocytes. *Nat. Immunol.* 2, 316–324. doi:10.1038/86318

Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S. H., Tanasa, B., et al. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441, 179–185. doi:10.1038/nature04702

Feske, S., Prakriya, M., Rao, A., and Lewis, R. S. (2005). A severe defect in CRAC Ca2+ channel activation and altered K+ channel gating in T cells from immunodeficient patients. *J. Exp. Med.* 202, 651–662. doi:10.1084/jem.20050687

Feske, S., Skolnik, E. Y., and Prakriya, M. (2012). Ion channels and transporters in lymphocyte function and immunity. *Nat. Rev. Immunol.* 12, 532–547. doi:10.1038/nri3233

Filippini, A., Taffs, R. E., and Sitkovsky, M. V. (1990). Extracellular ATP in T-lymphocyte activation: possible role in effector functions. *Proc. Natl. Acad. Sci. U.S.A.* 87, 8267–8271. doi:10.1073/pnas.87.21.8267

Fisher, S. E., Ciccodicola, A., Tanaka, K., Curci, A., Desicato, S., D'urso, M., et al. (1997). Sequence-based exon prediction around the synaptophysin locus reveals a gene-rich area containing novel genes in human proximal Xp. *Genomics* 45, 340–347. doi:10.1006/geno.1997.4941

Gasser, A., Glassmeier, G., Fliegert, R., Langhorst, M. F., Meinke, S., Hein, D., et al. (2006). Activation of T cell calcium influx by the second messenger ADP-ribose. *J. Biol. Chem.* 281, 2489–2496. doi:10.1074/jbc.M506525200

Gelfand, E. W., Cheung, R. K., Grinstein, S., and Mills, G. B. (1986). Characterization of the role for calcium influx in mitogen-induced triggering of human T cells. Identification of calcium-dependent and calcium-independent signals. *Eur. J. Immunol.* 16, 907–912. doi:10.1002/eji.1830160806

Gomes, B., Savignac, M., Moreau, M., Leclerc, C., Lory, P., Guery, J. C., et al. (2004). Lymphocyte calcium signaling involves dihydropyridine-sensitive L-type calcium channels: facts and controversies. *Crit. Rev. Immunol.* 24, 425–447. doi:10.1615/CritRevImmunol.v24.i6.30

Grafton, G., and Thwaite, L. (2001). Calcium channels in lymphocytes. *Immunology* 104, 119–126. doi:10.1046/j.0019-2805.2001.01321.x

Gwack, Y., Srikanth, S., Oh-Hora, M., Hogan, P. G., Lamperti, E. D., Yamashita, M., et al. (2008). Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol. Cell. Biol.* 28, 5209–5222. doi:10.1128/MCB.00360-08

Hara, Y., Wakamori, M., Ishii, M., Maeno, E., Nishida, M., Yoshida, T., et al. (2002). LTRPC2 Ca2+-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol. Cell* 9, 163–173. doi:10.1016/S1097-2765(01)00438-5

He, L. P., Hewavitharana, T., Soboloff, J., Spassova, M. A., and Gill, D. L. (2005). A functional link between store-operated and TRPC channels revealed by the 3,5-bis-trifluoromethyl-pyrazole derivative, BTP2. *J. Biol. Chem.* 280, 10997–11006. doi:10.1074/jbc.M411797200

Hofmann, T., Obukhov, A. G., Schaefer, M., Harteneck, C., Gudermann, T., and Schultz, G. (1999). Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* 397, 259–263. doi:10.1038/16711

Hogan, P. G., Chen, L., Nardone, J., and Rao, A. (2003). Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev.* 17, 2205–2232.

Hogan, P. G., Lewis, R. S., and Rao, A. (2010). Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu. Rev. Immunol.* 28, 491–533. doi:10.1146/annurev.immunol.021908.132550

Hohenegger, M., Berg, I., Weigl, L., Mayr, G. W., Potter, B. V., and Guse, A. H. (1999). Pharmacological activation of the ryanodine receptor in Jurkat T-lymphocytes. *Br. J. Pharmacol.* 128, 1235–1240. doi:10.1038/sj.bjp.0702935

Hosoi, E., Nishizaki, C., Gallagher, K. L., Wyre, H. W., Matsuo, Y., and Sei, Y. (2001). Expression of the ryanodine receptor isoforms in immune cells. *J. Immunol.* 167, 4887–4894.

Jha, M. K., Badou, A., Meissner, M., McRory, J. E., Freichel, M., Flockerzi, V., et al. (2009). Defective survival of naive CD8+ T lymphocytes in the absence of the beta3 regulatory subunit of voltage-gated calcium channels. *Nat. Immunol.* 10, 1275–1282. doi:10.1038/ni.1793

Junger, W. G. (2011). Immune cell regulation by autocrine purinergic signalling. *Nat. Rev. Immunol.* 11, 201–212. doi:10.1038/nri2938

Jurkat-Rott, K., and Lehmann-Horn, F. (2004). The impact of splice isoforms on voltage-gated calcium channel alpha1 subunits. *J. Physiol. (Lond.)* 554, 609–619. doi:10.1113/jphysiol.2003.052712

Khan, A. A., Steiner, J. P., Klein, M. G., Schneider, M. F., and Snyder, S. H. (1992). IP3 receptor: localization to plasma membrane of T cells and cocapping with the T cell receptor. *Science* 257, 815–818. doi:10.1126/science.1323146

Kotturi, M. F., Carlow, D. A., Lee, J. C., Ziltener, H. J., and Jefferies, W. A. (2003). Identification and functional characterization of voltage-dependent calcium channels in T lymphocytes. *J. Biol. Chem.* 278, 46949–46960. doi:10.1074/jbc.M309268200

Kotturi, M. F., Hunt, S. V., and Jefferies, W. A. (2006). Roles of CRAC and Cav-like channels in T cells: more than one gatekeeper? *Trends Pharmacol. Sci.* 27, 360–367. doi:10.1016/j.tips.2006.05.007

Kotturi, M. F., and Jefferies, W. A. (2005). Molecular characterization of L-type calcium

channel splice variants expressed in human T lymphocytes. *Mol. Immunol.* 42, 1461–1474. doi:10.1016/j.molimm.2005.01.014

Lacinova, L. (2005). Voltage-dependent calcium channels. *Gen. Physiol. Biophys.* 24(Suppl. 1), 1–78.

Lanner, J. T., Georgiou, D. K., Joshi, A. D., and Hamilton, S. L. (2010). Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harb. Perspect. Biol.* 2, a003996. doi:10.1101/cshperspect.a003996

Le Deist, F., Hivroz, C., Partiseti, M., Thomas, C., Buc, H. A., Oleastro, M., et al. (1995). A primary T-cell immunodeficiency associated with defective transmembrane calcium influx. *Blood* 85, 1053–1062.

Lee, L. F., Lih, C. J., Huang, C. J., Cao, T., Cohen, S. N., and McDevitt, H. O. (2008). Genomic expression profiling of TNF-alpha-treated BDC2.5 diabetogenic CD4+ T cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10107–10112. doi:10.1073/pnas.0803336105

Liou, J., Fivaz, M., Inoue, T., and Meyer, T. (2007). Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca2+ store depletion. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9301–9306. doi:10.1073/pnas.0702866104

Liou, J., Kim, M. L., Heo, W. D., Jones, J. T., Myers, J. W., Ferrell, J. E. Jr., et al. (2005). STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr. Biol.* 15, 1235–1241. doi:10.1016/j.cub.2005.05.055

Lis, A., Peinelt, C., Beck, A., Parvez, S., Monteilh-Zoller, M., Fleig, A., et al. (2007). CRACM1, CRACM2, and CRACM3 are store-operated Ca2+ channels with distinct functional properties. *Curr. Biol.* 17, 794–800. doi:10.1016/j.cub.2007.03.065

Lo, W. L., Donermeyer, D. L., and Allen, P. M. (2012). A voltage-gated sodium channel is essential for the positive selection of CD4(+) T cells. *Nat. Immunol.* 13, 880–887. doi:10.1038/ni.2379

Lombardi, G., Dianzani, C., Miglio, G., Canonico, P. L., and Fantozzi, R. (2001). Characterization of ionotropic glutamate receptors in human lymphocytes. *Br. J. Pharmacol.* 133, 936–944. doi:10.1038/sj.bjp.0704134

Luik, R. M., Wu, M. M., Buchanan, J., and Lewis, R. S. (2006). The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J. Cell Biol.* 174, 815–825. doi:10.1083/jcb.200604015

Macian, F. (2005). NFAT proteins: key regulators of T-cell development and function. *Nat. Rev. Immunol.* 5, 472–484. doi:10.1038/nri1632

Mansergh, F., Orton, N. C., Vessey, J. P., Lalonde, M. R., Stell, W. K., Tremblay, F., et al. (2005). Mutation of the calcium channel gene Cacna1f disrupts calcium signaling, synaptic transmission and cellular organization in mouse retina. *Hum. Mol. Genet.* 14, 3035–3046. doi:10.1093/hmg/ddi336

Marx, M., Weber, M., Merkel, F., Meyer Zum Buschenfelde, K. H., and Kohler, H. (1990). Additive effects of calcium antagonists on cyclosporin A-induced inhibition of T-cell proliferation. *Nephrol. Dial. Transplant.* 5, 1038–1044. doi:10.1093/ndt/5.12.1038

Mashkina, A. P., Cizkova, D., Vanicky, I., and Boldyrev, A. A. (2010). NMDA receptors are expressed in lymphocytes activated both in vitro and in vivo. *Cell. Mol. Neurobiol.* 30, 901–907. doi:10.1007/s10571-010-9519-7

Mashkina, A. P., Tyulina, O. V., Solovyova, T. I., Kovalenko, E. I., Kanevski, L. M., Johnson, P., et al. (2007). The excitotoxic effect of NMDA on human lymphocyte immune function. *Neurochem. Int.* 51, 356–360. doi:10.1016/j.neuint.2007.04.009

Massullo, P., Sumoza-Toledo, A., Bhagat, H., and Partida-Sanchez, S. (2006). TRPM channels, calcium and redox sensors during innate immune responses. *Semin. Cell Dev. Biol.* 17, 654–666. doi:10.1016/j.semcdb.2006.11.006

Matza, D., Badou, A., Jha, M. K., Willinger, T., Antov, A., Sanjabi, S., et al. (2009). Requirement for AHNAK1-mediated calcium signaling during T lymphocyte cytolysis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9785–9790. doi:10.1073/pnas.0902844106

Matza, D., Badou, A., Kobayashi, K. S., Goldsmith-Pestana, K., Masuda, Y., Komuro, A., et al. (2008). A scaffold protein, AHNAK1, is required for calcium signaling during T cell activation. *Immunity* 28, 64–74. doi:10.1016/j.immuni.2007.11.020

Matza, D., and Flavell, R. A. (2009). Roles of Ca(v) channels and AHNAK1 in T cells: the beauty and the beast. *Immunol. Rev.* 231, 257–264. doi:10.1111/j.1600-065X.2009.00805.x

McRory, J. E., Hamid, J., Doering, C. J., Garcia, E., Parker, R., Hamming, K., et al. (2004). The CACNA1F gene encodes an L-type calcium channel with unique biophysical properties and tissue distribution. *J. Neurosci.* 24, 1707–1718. doi:10.1523/JNEUROSCI.4846-03.2004

Melzer, N., Hicking, G., Gobel, K., and Wiendl, H. (2012). TRPM2 cation channels modulate T cell effector functions and contribute to autoimmune CNS inflammation. *PLoS ONE* 7:e47617. doi:10.1371/journal.pone.0047617

Miglio, G., Dianzani, C., Fallarini, S., Fantozzi, R., and Lombardi, G. (2007). Stimulation of N-methyl-D-aspartate receptors modulates Jurkat T cell growth and adhesion to fibronectin. *Biochem. Biophys. Res. Commun.* 361, 404–409. doi:10.1016/j.bbrc.2007.07.015

Miglio, G., Varsaldi, F., and Lombardi, G. (2005). Human T lymphocytes express N-methyl-D-aspartate receptors functionally active in controlling T cell activation. *Biochem. Biophys. Res. Commun.* 338, 1875–1883. doi:10.1016/j.bbrc.2005.10.164

Mignen, O., Thompson, J. L., and Shuttleworth, T. J. (2007). STIM1 regulates Ca2+ entry via arachidonate-regulated Ca2+-selective (ARC) channels without store depletion or translocation to the plasma membrane. *J. Physiol. (Lond.)* 579, 703–715. doi:10.1113/jphysiol.2006.122432

Mignen, O., Thompson, J. L., and Shuttleworth, T. J. (2009). The molecular architecture of the arachidonate-regulated Ca2+-selective ARC channel is a pentameric assembly of Orai1 and Orai3 subunits. *J. Physiol. (Lond.)* 587, 4181–4197. doi:10.1113/jphysiol.2009.174193

Montell, C., and Rubin, G. M. (1989). Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. *Neuron* 2, 1313–1323. doi:10.1016/0896-6273(89)90069-X

Mouton, J., Marty, I., Villaz, M., Feltz, A., and Maulet, Y. (2001). Molecular interaction of dihydropyridine receptors with type-1 ryanodine receptors in rat brain. *Biochem. J.* 354, 597–603. doi:10.1042/0264-6021:3540597

Oh-Hora, M. (2009). Calcium signaling in the development and function of T-lineage cells. *Immunol. Rev.* 231, 210–224. doi:10.1111/j.1600-065X.2009.00819.x

Oh-Hora, M., Komatsu, N., Pishyareh, M., Feske, S., Hori, S., Taniguchi, M., et al. (2013). Agonist-selected T cell development requires strong T cell receptor signaling and store-operated calcium entry. *Immunity* 38, 881–895. doi:10.1016/j.immuni.2013.02.008

Oh-Hora, M., Yamashita, M., Hogan, P. G., Sharma, S., Lamperti, E., Chung, W., et al. (2008). Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat. Immunol.* 9, 432–443. doi:10.1038/ni1574

Omilusik, K., Priatel, J. J., Chen, X., Wang, Y. T., Xu, H., Choi, K. B., et al. (2011). The CaV1.4 calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. *Immunity* 35, 349–360. doi:10.1016/j.immuni.2011.07.011

Owsianik, G., Talavera, K., Voets, T., and Nilius, B. (2006). Permeation and selectivity of TRP channels. *Annu. Rev. Physiol.* 68, 685–717. doi:10.1146/annurev.physiol.68.040204.101406

Padberg, W. M., Bodewig, C., Schafer, H., Muhrer, K. H., and Schwemmle, K. (1990). Synergistic immunosuppressive effect of low-dose cyclosporine A and the calcium antagonist nifedipine, mediated by the generation of suppressor cells. *Transplant. Proc.* 22, 2337.

Park, C. Y., Hoover, P. J., Mullins, F. M., Bachhawat, P., Covington, E. D., Raunser, S., et al. (2009). STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 136, 876–890. doi:10.1016/j.cell.2009.02.014

Park, C. Y., Shcheglovitov, A., and Dolmetsch, R. (2010). The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science* 330, 101–105. doi:10.1126/science.1191027

Partiseti, M., Le Deist, F., Hivroz, C., Fischer, A., Korn, H., and Choquet, D. (1994). The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodeficiency. *J. Biol. Chem.* 269, 32327–32335.

Patterson, R. L., Boehning, D., and Snyder, S. H. (2004). Inositol 1,4,5-trisphosphate receptors as signal integrators. *Annu. Rev. Biochem.* 73, 437–465. doi:10.1146/annurev.biochem.73.071403.161303

Penna, A., Demuro, A., Yeromin, A. V., Zhang, S. L., Safrina, O., Parker, I., et al. (2008). The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature* 456, 116–120. doi:10.1038/nature07338

Perraud, A. L., Fleig, A., Dunn, C. A., Bagley, L. A., Launay, P., Schmitz, C., et al. (2001). ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature* 411, 595–599. doi:10.1038/35079100

Philipp, S., Strauss, B., Hirnet, D., Wissenbach, U., Mery, L., Flockerzi, V., et al. (2003). TRPC3 mediates T-cell receptor-dependent calcium entry in human T-lymphocytes. *J. Biol. Chem.* 278, 26629–26638. doi:10.1074/jbc.M304044200

Picard, C., McCarl, C. A., Papolos, A., Khalil, S., Luthy, K., Hivroz, C., et al. (2009). STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N. Engl. J. Med.* 360, 1971–1980. doi:10.1056/NEJMoa0900082

Robert, V., Triffaux, E., Savignac, M., and Pelletier, L. (2011). Calcium signalling in T-lymphocytes. *Biochimie* 93, 2087–2094. doi:10.1016/j.biochi.2011.06.016

Rook, M. B., Evers, M. M., Vos, M. A., and Bierhuizen, M. F. (2012). Biology of cardiac sodium channel Nav1.5 expression. *Cardiovasc. Res.* 93, 12–23. doi:10.1093/cvr/cvr252

Roos, J., Digregorio, P. J., Yeromin, A. V., Ohlsen, K., Lioudyno, M., Zhang, S., et al. (2005). STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J. Cell Biol.* 169, 435–445. doi:10.1083/jcb.200502019

Sadighi Akha, A. A., Willmott, N. J., Brickley, K., Dolphin, A. C., Galione, A., and Hunt, S. V. (1996). Anti-Ig-induced calcium influx in rat B lymphocytes mediated by cGMP through a dihydropyridine-sensitive channel. *J. Biol. Chem.* 271, 7297–7300. doi:10.1074/jbc.271.13.7297

Schenk, U., Frascoli, M., Proietti, M., Geffers, R., Traggiai, E., Buer, J., et al. (2011). ATP inhibits the generation and function of regulatory T cells through the activation of purinergic P2X receptors. *Sci. Signal.* 4, ra12. doi:10.1126/scisignal.2001270

Schenk, U., Westendorf, A. M., Radaelli, E., Casati, A., Ferro, M., Fumagalli, M., et al. (2008). Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels. *Sci. Signal.* 1, ra6. doi:10.1126/scisignal.1160583

Schuhmann, M. K., Stegner, D., Berna-Erro, A., Bittner, S., Braun, A., Kleinschnitz, C., et al. (2010). Stromal interaction molecules 1 and 2 are key regulators of autoreactive T cell activation in murine autoimmune central nervous system inflammation. *J. Immunol.* 184, 1536–1542. doi:10.4049/jimmunol.0902161

Schwarz, E. C., Wissenbach, U., Niemeyer, B. A., Strauss, B., Philipp, S. E., Flockerzi, V., et al. (2006). TRPV6 potentiates calcium-dependent cell proliferation. *Cell Calcium* 39, 163–173. doi:10.1016/j.ceca.2005.10.006

Schwarz, E. C., Wolfs, M. J., Tonner, S., Wenning, A. S., Quintana, A., Griesemer, D., et al. (2007). TRP channels in lymphocytes. *Handb. Exp. Pharmacol.* 179, 445–456. doi:10.1007/978-3-540-34891-7_26

Schwarzmann, N., Kunerth, S., Weber, K., Mayr, G. W., and Guse, A. H. (2002). Knock-down of the type 3 ryanodine receptor impairs sustained Ca2+ signaling via the T cell receptor/CD3 complex. *J. Biol. Chem.* 277, 50636–50642. doi:10.1074/jbc.M209061200

Smith-Garvin, J. E., Koretzky, G. A., and Jordan, M. S. (2009). T cell activation. *Annu. Rev. Immunol.* 27, 591–619. doi:10.1146/annurev.immunol.021908.132706

Soboloff, J., Spassova, M. A., Hewavitharana, T., He, L. P., Xu, W., Johnstone, L. S., et al. (2006). STIM2 is an inhibitor of STIM1-mediated store-operated Ca2+ Entry. *Curr. Biol.* 16, 1465–1470. doi:10.1016/j.cub.2006.05.051

Solle, M., Labasi, J., Perregaux, D. G., Stam, E., Petrushova, N., Koller, B. H., et al. (2001). Altered cytokine production in mice lacking P2X(7) receptors. *J. Biol. Chem.* 276, 125–132. doi:10.1074/jbc.M006781200

Srikanth, S., and Gwack, Y. (2012). Orai1, STIM1, and their associating partners. *J. Physiol. (Lond.)* 590, 4169–4177. doi:10.1113/jphysiol.2012.231522

Stathopulos, P. B., Zheng, L., and Ikura, M. (2009). Stromal interaction molecule (STIM) 1 and STIM2 calcium sensing regions exhibit distinct unfolding and oligomerization kinetics. *J. Biol. Chem.* 284, 728–732. doi:10.1074/jbc.C800178200

Stokes, L., Gordon, J., and Grafton, G. (2004). Non-voltage-gated L-type Ca2+ channels in human T cells: pharmacology and molecular characterization of the major alpha pore-forming and auxiliary beta-subunits. *J. Biol. Chem.* 279, 19566–19573. doi:10.1074/jbc.M401481200

Strom, T. M., Nyakatura, G., Apfelstedt-Sylla, E., Hellebrand, H., Lorenz, B., Weber, B. H., et al. (1998). An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nat. Genet.* 19, 260–263. doi:10.1038/940

Suzuki, Y., Yoshimaru, T., Inoue, T., and Ra, C. (2009). Ca(v)1.2 L-type Ca2+ channel protects mast cells against activation-induced cell death by preventing mitochondrial integrity disruption. *Mol. Immunol.* 46, 2370–2380. doi:10.1016/j.molimm.2009.03.017

Tanimura, A., Tojyo, Y., and Turner, R. J. (2000). Evidence that type I, II, and III inositol 1,4,5-trisphosphate receptors can occur as integral plasma membrane proteins. *J. Biol. Chem.* 275, 27488–27493.

Teixeiro, E., and Daniels, M. A. (2010). ERK and cell death: ERK location and T cell selection. *FEBS J.* 277, 30–38. doi:10.1111/j.1742-4658.2009.07368.x

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

Tuluc, P., Molenda, N., Schlick, B., Obermair, G. J., Flucher, B. E., and Jurkat-Rott, K. (2009). A CaV1.1 Ca2+ channel splice variant with high conductance and voltage-sensitivity alters EC coupling in developing skeletal muscle. *Biophys. J.* 96, 35–44. doi:10.1016/j.bpj.2008.09.027

Vazquez, G., Lievremont, J. P., St, J. B. G., and Putney, J. W. Jr. (2001). Human Trp3 forms both inositol trisphosphate receptor-dependent and receptor-independent store-operated cation channels in DT40 avian B lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 98, 11777–11782. doi:10.1073/pnas.201238198

Vig, M., Dehaven, W. I., Bird, G. S., Billingsley, J. M., Wang, H., Rao, P. E., et al. (2008). Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat. Immunol.* 9, 89–96. doi:10.1038/nrg2314

Vig, M., Peinelt, C., Beck, A., Koomoa, D. L., Rabah, D., Koblan-Huberson, M., et al. (2006). CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science* 312, 1220–1223. doi:10.1126/science.1127883

Voets, T., Prenen, J., Fleig, A., Vennekens, R., Watanabe, H., Hoenderop, J. G., et al. (2001). CaT1 and the calcium release-activated calcium channel manifest distinct pore properties. *J. Biol. Chem.* 276, 47767–47770.

Vyas, J. M., Van Der Veen, A. G., and Ploegh, H. L. (2008). The known unknowns of antigen processing and presentation. *Nat. Rev. Immunol.* 8, 607–618. doi:10.1038/nri2368

Wang, Y., Deng, X., Mancarella, S., Hendron, E., Eguchi, S., Soboloff, J., et al. (2010). The calcium store sensor, STIM1, reciprocally controls Orai and CaV1.2 channels. *Science* 330, 105–109. doi:10.1126/science.1191086

Weber, K. S., Miller, M. J., and Allen, P. M. (2008). Th17 cells exhibit a distinct calcium profile from Th1 and Th2 cells and have Th1-like motility and NF-AT nuclear localization. *J. Immunol.* 180, 1442–1450.

Wenning, A. S., Neblung, K., Strauss, B., Wolfs, M. J., Sappok, A., Hoth, M., et al. (2011). TRP expression pattern and the functional importance of TRPC3 in primary human T-cells. *Biochim. Biophys. Acta* 1813, 412–423. doi:10.1016/j.bbamcr.2010.12.022

Williams, R. T., Manji, S. S., Parker, N. J., Hancock, M. S., Van Stekelenburg, L., Eid, J. P., et al. (2001). Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem. J.* 357, 673–685. doi:10.1042/0264-6021:3570673

Woehrle, T., Yip, L., Elkhal, A., Sumi, Y., Chen, Y., Yao, Y., et al. (2010). Pannexin-1 hemichannel-mediated ATP release together with P2X1 and P2X4 receptors regulate T-cell activation at the immune synapse. *Blood* 116, 3475–3484. doi:10.1182/blood-2010-04-277707

Wu, M. M., Buchanan, J., Luik, R. M., and Lewis, R. S. (2006a). Ca2+ store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J. Cell Biol.* 174, 803–813. doi:10.1083/jcb.200604014

Wu, Y., Borde, M., Heissmeyer, V., Feuerer, M., Lapan, A. D., Stroud, J. C., et al. (2006b). FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* 126, 375–387. doi:10.1016/j.cell.2006.05.042

Wulff, H., Beeton, C., and Chandy, K. G. (2003). Potassium channels as therapeutic targets for autoimmune disorders. *Curr. Opin. Drug Discov. Devel.* 6, 640–647.

Wulff, H., Knaus, H. G., Pennington, M., and Chandy, K. G. (2004). K+ channel expression during B cell differentiation: implications for

immunomodulation and autoimmunity. *J. Immunol.* 173, 776–786.
Xu, P., Lu, J., Li, Z., Yu, X., Chen, L., and Xu, T. (2006). Aggregation of STIM1 underneath the plasma membrane induces clustering of Orai1. *Biochem. Biophys. Res. Commun.* 350, 969–976. doi:10.1016/j.bbrc.2006.09.134
Yip, L., Woehrle, T., Corriden, R., Hirsh, M., Chen, Y., Inoue, Y., et al. (2009). Autocrine regulation of T-cell activation by ATP release and P2X7 receptors. *FASEB J.* 23, 1685–1693. doi:10.1096/fj.08-126458
Zainullina, L. F., Yamidanov, R. S., Vakhitov, V. A., and Vakhitova, Y. V. (2011). NMDA receptors as a possible component of store-operated Ca(2)(+) entry in human T-lymphocytes. *Biochemistry Mosc.* 76, 1220–1226. doi:10.1134/S0006297911110034
Zhang, S. L., Yeromin, A. V., Zhang, X. H., Yu, Y., Safrina, O., Penna, A., et al. (2006). Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9357–9362. doi:10.1073/pnas.0603161103
Zitt, C., Strauss, B., Schwarz, E. C., Spaeth, N., Rast, G., Hatzelmann, A., et al. (2004). Potent inhibition of Ca2+ release-activated Ca2+ channels and T-lymphocyte activation by the pyrazole derivative BTP2. *J. Biol. Chem.* 279, 12427–12437. doi:10.1074/jbc.M309297200
Zweifach, A., and Lewis, R. S. (1993). Mitogen-regulated Ca2+ current of T lymphocytes is activated by depletion of intracellular Ca2+ stores. *Proc. Natl. Acad. Sci. U.S.A.* 90, 6295–6299. doi:10.1073/pnas.90.13.6295

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Ca^{2+} influx in T cells: how many Ca^{2+} channels?

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Ca^{2+} signals are critical for T cell function. A number of ion channels regulate Ca^{2+} influx from the extracellular space in T cells, either by conducting Ca^{2+} ions or by modulating the membrane potential that provides the driving force for Ca^{2+} influx (Cahalan and Chandy, 2009; Feske et al., 2012). The best characterized Ca^{2+} channel in T cells is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel, which mediates store-operated Ca^{2+} entry (SOCE) in response to T cell receptor (TCR) activation and is composed of ORAI and stromal interaction molecules (STIM) family proteins. Several other channels may also mediate Ca^{2+} influx directly in T cells including members of the transient receptor potential (TRP) family, P2X receptors, and voltage-gated Ca^{2+} (Ca_v) channels. Compared to CRAC channels, however, their contribution to TCR-induced Ca^{2+} influx and immunity is less well defined.

Ca^{2+} release-activated Ca^{2+} channels were first identified in T cells (and mast cells) over 20 years ago (Lewis and Cahalan, 1989; Hoth and Penner, 1992; Zweifach and Lewis, 1993). They mediate Ca^{2+} influx and have well defined electrophysiological properties (Parekh and Penner, 1997; Prakriya and Lewis, 2003). The long elusive molecular identity of the CRAC channel was solved with the discovery of ORAI1 by genome-wide RNAi screens and positional cloning in patients lacking CRAC channel function (Feske et al., 2006; Vig et al., 2006b; Zhang et al., 2006). ORAI1 and its two homologs, ORAI2 and ORAI3, are integral membrane proteins (**Figure 1**). Mutagenesis and structural analyses have showed that ORAI1 forms the pore of the CRAC channel through which Ca^{2+} ions enter the cell (Prakriya et al., 2006; Vig et al., 2006a; Yeromin et al., 2006; Hou et al., 2012; McNally et al., 2012). CRAC channels open after TCR-induced production of inositol 1,4,5-trisphosphate (InsP3) and release of Ca^{2+} from ER stores. Reduced Ca^{2+} levels in the ER trigger the activation of STIM 1 and 2 located in the ER membrane. After translocation to ER-plasma membrane junctions, STIM proteins bind to ORAI1 and open the CRAC channel pore, resulting in sustained Ca^{2+} influx. The molecular regulation of CRAC channel function has been described in detail elsewhere (Shaw et al., 2012).

The essential role of CRAC channels for T cell function and adaptive immunity is best illustrated by patients with loss-of-function or null mutations in *ORAI1* or *STIM1* genes, whose T cells lack CRAC channel function and SOCE (Partiseti et al., 1994; Le Deist et al., 1995; Feske et al., 1996; McCarl et al., 2009; Picard et al., 2009; Feske, 2011; Fuchs et al., 2012). CRAC channel-deficient T cells proliferate poorly *in vitro* and have a profound defect in the production of cytokines such as IFNγ, TNFα, IL-2, and IL-17. Similar defects are found in $CD4^+$ and $CD8^+$ T cells from *Stim1*$^{-/-}$, *Orai1*$^{-/-}$, and *Orai1*^{R91W} knock-in mice (Gwack et al., 2008; Beyersdorf et al., 2009; McCarl et al., 2010). SOCE-deficient T cells were found to be more resistant to apoptotic cell death and showed migration defects *in vitro* and *in vivo* (Ma et al., 2010; Kim et al., 2011; Greenberg et al., 2013) (and Stefan Feske unpublished data). Interestingly, SOCE is dispensable for the development and selection of conventional $TCR\alpha\beta^+$ $CD4^+$ and $CD8^+$ T cells in SOCE-deficient patients and mice. However, their T cell function is severely compromised *in vivo*, apparent in absent delayed type hypersensitivity (DTH) responses to recall antigens in patients and mice (Le Deist et al., 1995; Feske et al., 1996; McCarl et al., 2010) and impaired skin allograft rejection in *Orai1*^{R93W} knock-in mice (McCarl et al., 2010). Most importantly, impaired T cell function in ORAI1 and STIM1-deficient patients results in recurrent and chronic infections with a wide spectrum of viral, bacterial and fungal pathogens (Partiseti et al., 1994; Le Deist et al., 1995; Feske et al., 1996; McCarl et al., 2009; Picard et al., 2009; Byun et al., 2010; Feske, 2010; Fuchs et al., 2012).

Besides immunity to infection, CRAC channels in T cells regulate immunological tolerance and inflammation. $CD4^+$ T cells from mice lacking ORAI1 or STIM1 function showed strongly impaired expression of proinflammatory cytokines such as IFN-γ and IL-17 (Ma et al., 2010; McCarl et al., 2010). Importantly, these mice were resistant to T cell-mediated intestinal and CNS inflammation in animal models of colitis and multiple sclerosis. Complete absence of CRAC channel function in mice with T cell-specific deletion of *Stim1* and *Stim2* genes, in addition, results in impaired development and function of $Foxp3^+$ regulatory T (Treg) cells (Oh-Hora et al., 2008). As a result, STIM1/2-deficient mice over time develop severe myelo-lymphoproliferative disease with lymphadenopathy, splenomegaly, and pulmonary inflammation (Oh-Hora et al., 2008). Intriguingly, these mice show an exocrine gland autoimmune disease resembling Sjogren's syndrome in humans (Cheng et al., 2012). Reduced numbers of Treg cells are also found in ORAI1- and STIM1-deficient patients (Picard et al., 2009) (and unpublished data), most of which suffer from autoimmune thrombocytopenia and hemolytic anemia due to autoantibodies against erythrocytes and platelets (Feske, 2011). The complete lack of SOCE in STIM1/2-deficient mice not only impaired the development of Treg cells but also that of natural killer T (NKT) cells and $TCR\alpha\beta^+$ $CD8\alpha\alpha^+$ intraepithelial lymphocytes (IEL) in the gut (Oh-Hora et al., 2013). These findings indicate that low to moderate SOCE is sufficient for the postselection maturation of agonist-selected T cells (Treg

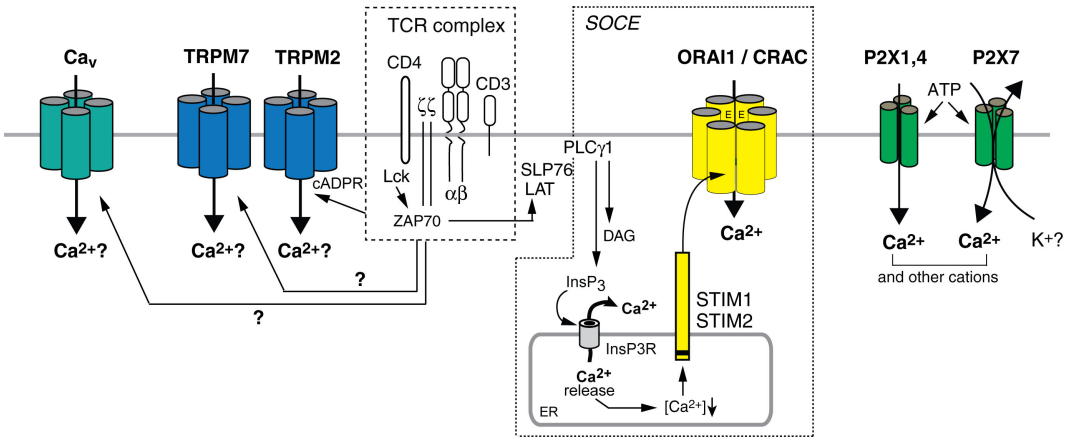

FIGURE 1 | Ca^{2+} influx pathways in T cells. Stimulation of T cells through the TCR complex results in Ca^{2+} influx, which is involved in the regulation of many T cell functions. CRAC channels mediate store-operated Ca^{2+} entry (SOCE) following activation of PLCγ1 and production of $InsP_3$. $InsP_3$ binds to and opens Ca^{2+} permeable $InsP_3$ receptors ($InsP_3R$) in the ER, resulting in the release of Ca^{2+} from ER stores (Lewis, 2001; Feske, 2007). Ca^{2+} release from the ER causes the activation of STIM 1 and 2, which oligomerize and translocate to ER-plasma membrane junctions. STIM1 and STIM2 bind to ORAI1, the pore-forming subunit of the CRAC channel, thereby mediating its opening and sustained Ca^{2+} influx. The subunit composition of the CRAC channel awaits further studies; both tetrameric and hexameric assemblies of ORAI1 subunits were proposed (Ji et al., 2008; Mignen et al., 2008; Penna et al., 2008; Maruyama et al., 2009; Hou et al., 2012). TRPM2 is a Ca^{2+} permeable cation channel that can be activated by cADPR and NAADP in human T cells (Beck et al., 2006). Increased cADPR levels after TCR stimulation (Guse et al., 1999) activate SOCE by releasing Ca^{2+} from the ER through RyR channels and potentially activate TRPM2 channels directly. TRPM7 is a non-selective cation channel implicated in Mg^{2+} homeostasis in T cells; whether its ability to conduct Ca^{2+} contributes to T cell function and how it is activated by TCR stimulation is not understood. The L-type Ca_v channels $Ca_v1.2$, $Ca_v1.3$, and $Ca_v1.4$, which mediate depolarization-dependent Ca^{2+} influx in excitable cells including neurons may contribute to Ca^{2+} influx in T cells but their activation mechanism is unknown and their current properties are not well defined. P2X receptors are non-selective Ca^{2+} channels activated by extracellular ATP. Several homologs, P2X1, P2X4, and P2X7, were reported to mediate Ca^{2+} influx in T cells *in vitro*.

cells, NKT cells, IEL), whereas strong SOCE is required for the proinflammatory function of Th1 and Th17 cells.

Transient receptor potential channels belong to a large family of ion channels, which conduct monovalent and divalent cations including Ca^{2+} (Nilius and Owsianik, 2011). Before the discovery of ORAI1 as the CRAC channel, several TRPC channels were proposed to mediate Ca^{2+} influx in T cells. However, a significant role of TRPC channels in Ca^{2+} influx and T cell mediated immune function has not been established. By contrast, TRPM7 is essential for T cell development as mice with T cell-specific deletion of *Trpm7* had a severe block in T cell development at the $CD4^-CD8^-$ double negative stage (Jin et al., 2008). This is the most profound effect of any ion channel on lymphocyte development demonstrated so far. TRPM7 is Mg^{2+} permeable and widely considered to regulate cellular Mg^{2+} homeostasis. However, T cells from *Trpm7*$^{-/-}$ mice had normal Mg^{2+} influx and total Mg^{2+} levels, raising the question whether impaired T cell development is caused by dysregulated Mg^{2+} homeostasis or rather by impaired influx of other cations including Ca^{2+} which TRPM7 is able to conduct as well. Another TRP channel, TRPM2 is a non-selective, Ca^{2+} permeable cation channel and in human T cells, TRPM2 can be activated by a variety of intracellular agonists including adenosine diphosphate ribose (ADPR), cyclic ADPR (cADPR), and Nicotinic acid adenine dinucleotide phosphate (NAADP) (Beck et al., 2006). TCR stimulation was reported to result in increased intracellular cADPR levels and Ca^{2+} release from the ER through ryanodine receptors (RyR) (Guse et al., 1999), thereby initiating SOCE; alternatively, elevated cADPR levels could directly activate TRPM2 channels. However, the physiological function of TRPM2 channels in T cells is unknown. It is intriguing to speculate that TRPM2 may be involved in inflammatory T cell responses similar to their role in CXCL2 chemokine expression and NADPH oxidase function in monocytes (Yamamoto et al., 2008) and phagocytes (Di et al., 2011).

Voltage-gated Ca^{2+} (Ca_v) channels are highly Ca^{2+} selective channels that play an important role in Ca^{2+} influx and the function of electrically excitable cells such as neurons following cell depolarization (Tsien et al., 1987). In T cells, several members of the L-type family of Ca_v channels (Ca_v1) were reported to be expressed but their contribution to Ca^{2+} influx has remained controversial (Hogan et al., 2010). Recent studies showed that genetic deletion of $Ca_v1.4$ in mouse T cells and knockdown of Cav1.2 and Cav1.3 in human T cells attenuates TCR-induced Ca^{2+} influx (Cabral et al., 2010; Omilusik et al., 2011). Similarly, mutation of the regulatory β3 and β4 subunits of Ca_v1 channels in mice results in reduced Ca^{2+} influx and impaired IL-4, IFNγ, and TNFα production in $CD4^+$ and $CD8^+$ T cells following TCR stimulation (Badou et al., 2006; Jha et al., 2009). $CD8^+$ T cells lacking functional β3 regulatory subunits or Cav1.4 channels were more susceptible to apoptosis (Jha et al., 2009; Omilusik et al., 2011). Cav1.4-deficient mice also showed reduced cytotoxic function of $CD8^+$ T cells *in vitro* and impaired $CD8^+$ T cell responses to infection with *Listeria monocytogenes in vivo* (Omilusik et al., 2011). Despite these intriguing findings, the pathways by which TCR signaling activates Ca_v1 channels are unknown. In contrast to excitable cells, depolarization of T cells fails to open Ca_v1 channels and mediate Ca^{2+} influx. It has been speculated that Ca_v1 channels in T cells are activated by an alternative,

voltage-independent mechanism, but the nature of this mechanism remains to be elucidated. In addition, native Ca_V1 channel-like Ca^{2+} currents have so far been reported only once despite efforts by numerous labs and appear to be restricted to naïve $CD4^+$ and $CD8^+$ T cells (Omilusik et al., 2011). Intriguingly, these currents were abolished in T cells from *Cav1.4*$^{-/-}$ mice. Future studies will need to investigate the electrophysiological properties of Ca_V1 channels in T cells, clarify the molecular mechanisms that regulate their activation, and investigate the role of Ca_V1 channels in adaptive immunity.

P2X receptors are not, unlike the Ca^{2+} channels discussed above, activated by TCR stimulation but by extracellular ATP. Three P2X receptors, P2X1, P2X4 and P2X7 (Yip et al., 2009; Woehrle et al., 2010), were reported to mediate Ca^{2+} influx in T cells. The opening of P2X7 causes Ca^{2+} influx and activation of enzymes such as calcineurin, resulting in T cell proliferation (Baricordi et al., 1996) and IL-2 production (Adinolfi et al., 2005; Woehrle et al., 2010). Inhibition of P2X1, P2X4, and P2X7 function by RNAi or chemical antagonists attenuated Ca^{2+} influx and IL-2 production in Jurkat cells and human $CD4^+$ T cells *in vitro* (Yip et al., 2009; Woehrle et al., 2010). A Ca^{2+} dependent role for individual P2X receptors in T cell immunity *in vivo*, however, is not well established. Using *P2X7*$^{-/-}$ mice, one study found that P2X7 is proinflammatory in T cells by promoting the differentiation and function of Th17 cells and inhibiting the stability of Treg cells (Schenk et al., 2011). The role of P2X7 in T cell-mediated autoimmunity, however, is ambiguous as both increased (Chen and Brosnan, 2006) and decreased (Sharp et al., 2008) CNS inflammation were observed in *P2X7*$^{-/-}$ mice when analyzed in animal models of multiple sclerosis. In addition, it is unclear whether the main function of P2X7 receptors in T cells is to mediate Ca^{2+} influx. At the high ATP concentrations (~1 mM) used to measure Ca^{2+} influx in most studies, P2X7 channels form a large pore (Junger, 2011) that is permeable to a variety of anorganic and organic cations (Chused et al., 1996; Adriouch et al., 2002). Similarly high ATP concentrations are used to activate inflammasomes in innate immune cells, in which P2X7 channels are thought to mediate K^+ efflux and thereby production of IL-1β (Ferrari et al., 2006; Tschopp and Schroder, 2010). Future studies need to evaluate if P2X7 receptors modulate T cell function through Ca^{2+} influx or other mechanisms. P2X1 and P2X4 conduct Ca^{2+} more selectively and open at lower (micromolar) ATP concentrations (Junger, 2011). However, P2X1 and P2X4-deficient mice have no reported immunological phenotype (Mulryan et al., 2000; Yamamoto et al., 2006) and their role in T cell immunity *in vivo* remains poorly understood.

Ca^{2+} signals have long been recognized as essential for T cell function and several channels may contribute to Ca^{2+} influx in T cells. Whereas the role of CRAC channels to T cell function and adaptive immunity is well documented by findings in ORAI1 and STIM1-deficient patients and mice, the contributions of TRP, Ca_V1, and P2X receptor channels remain to be more clearly defined. These channels could contribute to Ca^{2+} influx in specific T cell subsets, at distinct stages of T cell development or following stimuli other than TCR engagement. A better understanding of the contributions of different Ca^{2+} influx pathways in T cells will be essential to define potential drug targets for the modulation of T cell function in a variety of diseases caused by aberrant T cell function.

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REFERENCES

Adinolfi, E., Callegari, M. G., Ferrari, D., Bolognesi, C., Minelli, M., Wieckowski, M. R., et al. (2005). Basal activation of the P2×7 ATP receptor elevates mitochondrial calcium and potential, increases cellular ATP levels, and promotes serum-independent growth. *Mol. Biol. Cell* 16, 3260–3272.

Adriouch, S., Dox, C., Welge, V., Seman, M., Koch-Nolte, F., and Haag, F. (2002). Cutting edge: a natural P451L mutation in the cytoplasmic domain impairs the function of the mouse P2×7 receptor. *J. Immunol.* 169, 4108–4112.

Badou, A., Jha, M. K., Matza, D., Mehal, W. Z., Freichel, M., Flockerzi, V., et al. (2006). Critical role for the beta regulatory subunits of Cav channels in T lymphocyte function. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15529–15534.

Baricordi, O. R., Ferrari, D., Melchiorri, L., Chiozzi, P., Hanau, S., Chiari, E., et al. (1996). An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. *Blood* 87, 682–690.

Beck, A., Kolisek, M., Bagley, L. A., Fleig, A., and Penner, R. (2006). Nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose regulate TRPM2 channels in T lymphocytes. *FASEB J.* 20, 962–964.

Beyersdorf, N., Braun, A., Vogtle, T., Varga-Szabo, D., Galdos, R. R., Kissler, S., et al. (2009). STIM1-independent T cell development and effector function in vivo. *J. Immunol.* 182, 3390–3397.

Byun, M., Abhyankar, A., Lelarge, V., Plancoulaine, S., Palanduz, A., Telhan, L., et al. (2010). Whole-exome sequencing-based discovery of STIM1 deficiency in a child with fatal classic Kaposi sarcoma. *J. Exp. Med.* 207, 2307–2312.

Cabral, M. D., Paulet, P. E., Robert, V., Gomes, B., Renoud, M. L., Savignac, M., et al. (2010). Knocking down Cav1 calcium channels implicated in Th2 cell activation prevents experimental asthma. *Am. J. Respir. Crit. Care Med.* 181, 1310–1317.

Cahalan, M. D., and Chandy, K. G. (2009). The functional network of ion channels in T lymphocytes. *Immunol. Rev.* 231, 59–87.

Chen, L., and Brosnan, C. F. (2006). Exacerbation of experimental autoimmune encephalomyelitis in P2×7R-/- mice: evidence for loss of apoptotic activity in lymphocytes. *J. Immunol.* 176, 3115–3126.

Cheng, K. T., Alevizos, I., Liu, X., Swaim, W. D., Yin, H., Feske, S., et al. (2012). STIM1 and STIM2 protein deficiency in T lymphocytes underlies development of the exocrine gland autoimmune disease, Sjogren's syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 109, 14544–14549.

Chused, T. M., Apasov, S., and Sitkovsky, M. (1996). Murine T lymphocytes modulate activity of an ATP-activated P2Z-type purinoceptor during differentiation. *J. Immunol.* 157, 1371–1380.

Di, A., Gao, X. P., Qian, F., Kawamura, T., Han, J., Hecquet, C., et al. (2011). The redox-sensitive cation channel TRPM2 modulates phagocyte ROS production and inflammation. *Nat. Immunol.* 13, 29–34.

Ferrari, D., Pizzirani, C., Adinolfi, E., Lemoli, R. M., Curti, A., Idzko, M., et al. (2006). The P2×7 receptor: a key player in IL-1 processing and release. *J. Immunol.* 176, 3877–3883.

Feske, S. (2007). Calcium signalling in lymphocyte activation and disease. *Nat. Rev. Immunol.* 7, 690–702.

Feske, S. (2010). CRAC channelopathies. *Pflugers Arch.* 460, 417–435.

Feske, S. (2011). Immunodeficiency due to defects in store-operated calcium entry. *Ann. N. Y. Acad. Sci.* 1238, 74–90.

Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S. H., Tanasa, B., et al. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441, 179–185.

Feske, S., Muller, J. M., Graf, D., Kroczek, R. A., Drager, R., Niemeyer, C., et al. (1996). Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male siblings. *Eur. J. Immunol.* 26, 2119–2126.

Feske, S., Skolnik, E. Y., and Prakriya, M. (2012). Ion channels and transporters in lymphocyte function and immunity. *Nat. Rev. Immunol.* 12, 532–547.

Fuchs, S., Rensing-Ehl, A., Speckmann, C., Bengsch, B., Schmitt-Graeff, A., Bondzio, I., et al. (2012). Antiviral and regulatory T cell immunity in a patient with stromal interaction molecule 1 deficiency. *J. Immunol.* 188, 1523–1533.

Greenberg, M. L., Yu, Y., Leverrier, S., Zhang, S. L., Parker, I., and Cahalan, M. D. (2013). Orai1 function is essential for T cell homing to lymph nodes. *J. Immunol.* 190, 3197–3206.

Guse, A. H., Da Silva, C. P., Berg, I., Skapenko, A. L., Weber, K., Heyer, P., et al. (1999). Regulation of calcium signalling in T lymphocytes by the second messenger cyclic ADP-ribose. *Nature* 398, 70–73.

Gwack, Y., Srikanth, S., Oh-Hora, M., Hogan, P. G., Lamperti, E. D., Yamashita, M., et al. (2008). Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol. Cell. Biol.* 28, 5209–5222.

Hogan, P. G., Lewis, R. S., and Rao, A. (2010). Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu. Rev. Immunol.* 28, 491–533.

Hoth, M., and Penner, R. (1992). Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 355, 353–356.

Hou, X., Pedi, L., Diver, M. M., and Long, S. B. (2012). Crystal structure of the calcium release-activated calcium channel Orai. *Science* 338, 1308–1313.

Jha, M. K., Badou, A., Meissner, M., McRory, J. E., Freichel, M., Flockerzi, V., et al. (2009). Defective survival of naive CD8+ T lymphocytes in the absence of the beta3 regulatory subunit of voltage-gated calcium channels. *Nat. Immunol.* 10, 1275–1282.

Ji, W., Xu, P., Li, Z., Lu, J., Liu, L., Zhan, Y., et al. (2008). Functional stoichiometry of the unitary calcium-release-activated calcium channel. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13668–13673.

Jin, J., Desai, B. N., Navarro, B., Donovan, A., Andrews, N. C., and Clapham, D. E. (2008). Deletion of Trpm7 disrupts embryonic development and thymopoiesis without altering Mg2+ homeostasis. *Science* 322, 756–760.

Junger, W. G. (2011). Immune cell regulation by autocrine purinergic signalling. *Nat. Rev. Immunol.* 11, 201–212.

Kim, K. D., Srikanth, S., Yee, M. K., Mock, D. C., Lawson, G. W., and Gwack, Y. (2011). ORAI1 deficiency impairs activated T cell death and enhances T cell survival. *J. Immunol.* 187, 3620–3630.

Le Deist, F., Hivroz, C., Partiseti, M., Thomas, C., Buc, H. A., Oleastro, M., et al. (1995). A primary T-cell immunodeficiency associated with defective transmembrane calcium influx. *Blood* 85, 1053–1062.

Lewis, R. S. (2001). Calcium signaling mechanisms in T lymphocytes. *Annu. Rev. Immunol.* 19, 497–521.

Lewis, R. S., and Cahalan, M. D. (1989). Mitogen-induced oscillations of cytosolic Ca2+ and transmembrane Ca2+ current in human leukemic T cells. *Cell Regul.* 1, 99–112.

Ma, J., McCarl, C. A., Khalil, S., Luthy, K., and Feske, S. (2010). T-cell-specific deletion of STIM1 and STIM2 protects mice from EAE by impairing the effector functions of Th1 and Th17 cells. *Eur. J. Immunol.* 2010, 9.

Maruyama, Y., Ogura, T., Mio, K., Kato, K., Kaneko, T., Kiyonaka, S., et al. (2009). Tetrameric Orai1 is a teardrop-shaped molecule with a long, tapered cytoplasmic domain. *J. Biol. Chem.* 284, 13676–13685.

McCarl, C. A., Khalil, S., Ma, J., Oh-Hora, M., Yamashita, M., Roether, J., et al. (2010). Store-operated Ca2+ entry through ORAI1 is critical for T cell-mediated autoimmunity and allograft rejection. *J. Immunol.* 185, 5845–5858.

McCarl, C. A., Picard, C., Khalil, S., Kawasaki, T., Rother, J., Papolos, A., et al. (2009). ORAI1 deficiency and lack of store-operated Ca2+ entry cause immunodeficiency, myopathy, and ectodermal dysplasia. *J. Allergy Clin. Immunol.* 124, e1317.

McNally, B. A., Somasundaram, A., Yamashita, M., and Prakriya, M. (2012). Gated regulation of CRAC channel ion selectivity by STIM1. *Nature* 482, 241–245.

Mignen, O., Thompson, J. L., and Shuttleworth, T. J. (2008). Orai1 subunit stoichiometry of the mammalian CRAC channel pore. *J. Physiol. (Lond.)* 586, 419–425.

Mulryan, K., Gitterman, D. P., Lewis, C. J., Vial, C., Leckie, B. J., Cobb, A. L., et al. (2000). Reduced vas deferens contraction and male infertility in mice lacking P2×1 receptors. *Nature* 403, 86–89.

Nilius, B., and Owsianik, G. (2011). The transient receptor potential family of ion channels. *Genome Biol.* 12, 218.

Oh-Hora, M., Komatsu, N., Pishyareh, M., Feske, S., Hori, S., Taniguchi, M., et al. (2013). Agonist-selected T cell development requires strong T cell receptor signaling and store-operated calcium entry. *Immunity* 38, 1–15.

Oh-Hora, M., Yamashita, M., Hogan, P. G., Sharma, S., Lamperti, E., Chung, W., et al. (2008). Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat. Immunol.* 9, 432–443.

Omilusik, K., Priatel, J. J., Chen, X., Wang, Y. T., Xu, H., Choi, K. B., et al. (2011). The Ca(v)1.4 calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. *Immunity* 35, 349–360.

Parekh, A. B., and Penner, R. (1997). Store depletion and calcium influx. *Physiol. Rev.* 77, 901–930.

Partiseti, M., Le Deist, F., Hivroz, C., Fischer, A., Korn, H., and Choquet, D. (1994). The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodeficiency. *J. Biol. Chem.* 269, 32327–32335.

Penna, A., Demuro, A., Yeromin, A. V., Zhang, S. L., Safrina, O., Parker, I., et al. (2008). The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature* 456, 116–120.

Picard, C., McCarl, C. A., Papolos, A., Khalil, S., Luthy, K., Hivroz, C., et al. (2009). STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N. Engl. J. Med.* 360, 1971–1980.

Prakriya, M., Feske, S., Gwack, Y., Srikanth, S., Rao, A., and Hogan, P. G. (2006). Orai1 is an essential pore subunit of the CRAC channel. *Nature* 443, 230–233.

Prakriya, M., and Lewis, R. S. (2003). CRAC channels: activation, permeation, and the search for a molecular identity. *Cell Calcium* 33, 311–321.

Schenk, U., Frascoli, M., Proietti, M., Geffers, R., Traggiai, E., Buer, J., et al. (2011). ATP inhibits the generation and function of regulatory T cells through the activation of purinergic P2X receptors. *Sci. Signal.* 4, ra12.

Sharp, A. J., Polak, P. E., Simonini, V., Lin, S. X., Richardson, J. C., Bongarzone, E. R., et al. (2008). P2×7 deficiency suppresses development of experimental autoimmune encephalomyelitis. *J. Neuroinflammation* 5, 33.

Shaw, P. J., Qu, B., Hoth, M., and Feske, S. (2012). Molecular regulation of CRAC channels and their role in lymphocyte function. *Cell. Mol. Life Sci.* PMID: 23052215. [Epub ahead of print].

Tschopp, J., and Schroder, K. (2010). NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nat. Rev. Immunol.* 10, 210–215.

Tsien, R. W., Hess, P., McCleskey, E. W., and Rosenberg, R. L. (1987). Calcium channels: mechanisms of selectivity, permeation, and block. *Annu. Rev. Biophys. Biophys. Chem.* 16, 265–290.

Vig, M., Beck, A., Billingsley, J. M., Lis, A., Parvez, S., Peinelt, C., et al. (2006a). CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr. Biol.* 16, 2073–2079.

Vig, M., Peinelt, C., Beck, A., Koomoa, D. L., Rabah, D., Koblan-Huberson, M., et al. (2006b). CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science* 312, 1220–1223.

Woehrle, T., Yip, L., Elkhal, A., Sumi, Y., Chen, Y., Yao, Y., et al. (2010). Pannexin-1 hemichannel-mediated ATP release together with P2×1 and P2×4 receptors regulate T-cell activation at the immune synapse. *Blood* 116, 3475–3484.

Yamamoto, K., Sokabe, T., Matsumoto, T., Yoshimura, K., Shibata, M., Ohura, N., et al. (2006). Impaired flow-dependent control of vascular tone and remodeling in P2×4-deficient mice. *Nat. Med.* 12, 133–137.

Yamamoto, S., Shimizu, S., Kiyonaka, S., Takahashi, N., Wajima, T., Hara, Y., et al. (2008). TRPM2-mediated Ca2+ influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nat. Med.* 14, 738–747.

Yeromin, A. V., Zhang, S. L., Jiang, W., Yu, Y., Safrina, O., and Cahalan, M. D. (2006). Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 443, 226–229.

Yip, L., Woehrle, T., Corriden, R., Hirsh, M., Chen, Y., Inoue, Y., et al. (2009). Autocrine regulation of T-cell activation by ATP release and P2×7 receptors. *FASEB J.* 23, 1685–1693.

Zhang, S. L., Yeromin, A. V., Zhang, X. H., Yu, Y., Safrina, O., Penna, A., et al. (2006). Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9357–9362.

Zweifach, A., and Lewis, R. S. (1993). Mitogen-regulated Ca2+ current of T lymphocytes is activated by depletion of intracellular Ca2+ stores. *Proc. Natl. Acad. Sci. U.S.A.* 90, 6295–6299.

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Emerging roles of L-type voltage-gated and other calcium channels in T lymphocytes

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In T lymphocytes, calcium ion controls a variety of biological processes including development, survival, proliferation, and effector functions. These distinct and specific roles are regulated by different calcium signals, which are generated by various plasma membrane calcium channels. The repertoire of calcium-conducting proteins in T lymphocytes includes store-operated CRAC channels, transient receptor potential channels, P2X channels, and L-type voltage-gated calcium (Ca_v1) channels. In this paper, we will focus mainly on the role of the Ca_v1 channels found expressed by T lymphocytes, where these channels appear to operate in a T cell receptor stimulation-dependent and voltage sensor independent manner. We will review their expression profile at various differentiation stages of CD4 and CD8 T lymphocytes. Then, we will present crucial genetic evidence in favor of a role of these Ca_v1 channels and related regulatory proteins in both CD4 and CD8 T cell functions such as proliferation, survival, cytokine production, and cytolysis. Finally, we will provide evidence and speculate on how these voltage-gated channels might function in the T lymphocyte, a non-excitable cell.

Keywords: Ca_v1 channels, calcium channels, CD4 T cells, CD8 T cells, CRAC channel

INTRODUCTION

T cells require Ca^{2+} for their development and function (1–4). A canonical pathway for Ca^{2+} entry into T cells has been described thus far. Accordingly, ligation of T Cell Receptor (TCR) leads to activation of phosphoinositide-specific phospholipase C (PLC)γ. PLCγ breaks down phosphatidylinositol-4,5-bisphosphate to generate inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 activates the release of Ca^{2+} into the cytoplasm by binding to IP_3 receptors (IP_3R) located on the surface of internal Ca^{2+} stores, such as the endoplasmic reticulum (ER). Store-operated calcium (SOC) channels in the plasma membrane are then activated by the store depletion (5–7). A requirement for sustained signaling arises largely from the need to recruit and retain Nuclear Factor of Activated T cells (NFAT), a key transcriptional regulator of the IL-2 gene and other cytokine genes, in the nucleus (8).

There are several families of plasma membrane channels expressed in T cells. The most studied channels in lymphocytes are known as "calcium release-activated calcium" (CRAC) channels (5, 9, 10). A breakthrough in their characterization occurred after the identification of stromal interaction molecule (STIM), which is an ER-resident Ca^{2+} sensor, and ORAI/CRACM (CRAC modulator), which is their pore-forming subunit (11–14). The transient receptor potential (TRP) channels have also been detected in T cells and reported to be functionally involved in Ca^{2+} entry possibly after store depletion (15–17). Finally, evidence for the expression of P2X receptor channels on the plasma membrane and for their contribution to Ca^{2+} entry in lymphocytes was also shown (18–20).

EXPRESSION OF Ca_v CHANNELS IN T CELLS

Ca_v channels are heteromultimers that are composed of a pore-forming α1 subunit, β regulatory subunit, and α2, γ, and δ subunits (21). The topology of the α1 pore subunit is predicted to have four repeated motifs (I–IV), each of which is hexahelical and contains a loop between the S5 and S6 transmembrane segments that forms the channel pore. The S4 transmembrane segments in each motif contain conserved positively charged amino acids that are voltage sensors and that move outwards upon membrane depolarization, thereby opening the channel (22).

Several studies, including our own, have shown that $CD4^+$ and $CD8^+$ T cells express high levels of the Ca_v1 pore-forming subunit subfamily ($Ca_v1.1$–1.4 or α1S, α1C, α1D, and α1F, respectively), but not Ca_v2 (α1A, α1B, α1E) or Ca_v3 (α1G, α1H, α1I) subfamilies (see **Table 1**); moreover they express these molecules at levels comparable with those in excitable cells (23–32).

We showed that the $Ca_v1.1$ pore subunit is expressed in naïve $CD4^+$ T cells and its expression is upregulated during primary stimulation of these cells (27, 29, 35). In $CD8^+$ T cells, this subunit is only expressed in effector cells, late after primary stimulation and during secondary stimulation (31, 33).

The $Ca_v1.2$ pore subunit is apparently not detected in naïve $CD4^+$ or $CD8^+$ T cells. In the CD4 compartment, effector Th2 $CD4^+$ cells selectively express this subunit and it is not expressed in effector $CD8^+$ T cells. In $CD8^+$ T cells, its protein expression seems to be upregulated briefly during primary stimulation (usually peaks at day 3 or 4 after *in vitro* stimulation of $CD8^+$ T cells using anti-CD3 and anti-CD28 coated plates) (33).

Table 1 | Role of distinct Ca^{2+}-permeable channels in T lymphocyte development and functions.

Channel	Role in T lymphocytes			Evidence	Reference
	Development	Naive	Differentiated		
$Ca_v1.1$	ND	Expression was detected in naïve CD4+ T cells and a role in TCR-mediated Ca2+ influx	Expression was detected in effector CD8+ T cells. Contribution in TCR-mediated Ca^{2+} entry and CTL effector functions	β4 and AHNAK1-deficient T cells express low levels of the $Ca_v1.1$ protein	[(27) #3; (29) #15; (33) #14]
$Ca_v1.2$ $Ca_v1.3$	ND	No apparent expression	Involvement in TCR-mediated calcium influx in Th2 cells and in Th2 effector functions *in vitro* and *in vivo*	dihydropyridines antagonists and knockdown with Ca_v1 antisense oligodeoxynucleotides	[(34) #6; (60) #195; (27) #3; (36) #280]
$Ca_v1.4$	Involvement in thymic development	Requirement for TCR-induced calcium influx in naïve T cells Essential for survival and naive T cell maintenance	Requirement for CD4+ and CD8+ T cell immune responses	$Ca_vβ3$ KO mice and $Ca_v1.4$ KO mice	[(31) #13; (32) #283]
ORAI1	No apparent effect in ORAI1-deficient mice	No apparent effect in ORAI1-deficient mice	Involvement in TCR-mediated Ca^{2+} influx and effector functions (in T cells from SCID patients) and contribution to TCR-mediated Ca^{2+} influx and effector functions (in ORAI1-deficient T cells from mice)	T cell lines from SCID patients and primary murine T cells from ORAI1 KO mice	[(13) #211; (14) #212; (74) #249]
TRPC3	ND	ND	Contribution to TCR-dependent calcium influx suggested.	T cell lines and primary human T cells/overexpression and siRNA	[(16) #244; (82) #257]
TRPM2	No apparent effect in TRPM2-deficient mice	Reduced TCR-mediated proliferation	Contribution to production of pro-inflammatory cytokines after stimulation via TCR	TRPM2 KO mice	[(89) #266]
TRPM7	defect in T cell development in the thymus	ND	ND	TRPM7 KO mice	[(91) #268]
P2X7, P2X1, and P2X4 receptor channels	No apparent effect in P2X7 deficient mice and ND for P2X1 and P2X4	ND	Critical for TCR-dependent, ATP-mediated Ca^{2+} influx and downstream signaling events accompanying T cell activation	P2X7 receptor KO mice and siRNA for P2X7, P2X1, and P2X4 receptor channels. Jurkat cells and human peripheral CD4+ T cells were used	[(18) #270; (19) #276; (20) #271]

In this table, we consider major Ca^{2+} permeable channels, which may contribute either directly or indirectly to TCR-mediated Ca^{2+} influx, development, initial activation of naïve T cells and effector functions in differentiated T cells. Our goal in distinguishing roles of different channels at different differentiation stages is to emphasize areas where more research efforts are needed in order to understand the contribution of these Ca^{2+} channels in T lymphocyte development and functions. Cav, voltage-gated Ca^{2+} channel; TRP, transient receptor potential; P2XR, P2X receptors; TCR, T cell receptor; Th, T helper; ND, not determined.

The $Ca_v1.3$ pore subunit is expressed in effector Th2, but not in naïve, $CD4^+$ T cells (36). Its mRNA was detected in naïve and effector $CD8^+$ T cells but no information is yet available regarding the protein expression profile in these cells. Finally, the $Ca_v1.4$ pore subunit is expressed in naïve $CD4^+$ and $CD8^+$ T cells (31, 32). Apparently T cells express all the rest of the Ca_v complex subunits, including the regulatory β subunits, γ subunits, as well as α2, and δ subunits. It is therefore likely that these cells express a fully functional Ca_v channel, possibly similar to the ones found in excitable cells (25, 27). Other studies have also shown that these channels are widely expressed in various other immune cell types, such as Dendritic cells (DC), B-lymphocytes, and monocytes (37–39).

In addition to the expression of a full Ca_v complex, other similarities exist between excitable and non-excitable cells in relation to the Ca_v pathway. In striated muscle, Ca_v channels, expressed on the plasma membrane, are physically linked to Ryanodine receptors (RyR), expressed in the Sarcoplasmic Reticulum (SR). During a process called excitation-contraction coupling (E-C coupling), depolarization of the t-tubule membrane (i.e., excitation) induces extracellular Ca^{2+} flow through Ca_v channels (which are gated by the function of their voltage sensor) that lead to activation of RyR channel in the SR membrane. The activation of RyR channels leads to massive Ca^{2+} release from the SR, which in turn initiates contraction (40). Therefore, unlike T cells, muscle cells first obtain Ca^{2+} from the extracellular space that initiates the entire process of Ca^{2+} release from intracellular stores.

It seems that T cells also express all the components necessary for such a mechanism described above, i.e., RyRs and Ca_v channels. Primary T cells express RyR2, and they upregulate its expression after treatment with stromal cell-derived factor 1 (SDF-1), macrophage-inflammatory protein-1 α (MIP1α), or TGF-β. Other hemopoietic cells also express RyRs (41, 42). RyRs, expressed in T cells, can be activated pharmacologically to mobilize Ca^{2+} from intracellular stores independently from IP_3R (43). On the other hand, pharmacological blocking of RyRs in T cells results in reduced proliferation and IL-2 production (44). Knockdown of RyR3, the RyR that is expressed mainly by Jurkat T cells (primary T cells express RyR2 mostly), resulted in a significant reduction in Ca^{2+} entry in response to TCR cross-linking using anti-CD3 (45).

Finally, a recent study has suggested that, similar to excitable cells, store-operated Ca^{2+} entry via TCR stimulation precedes Ca^{2+} release from intracellular stores via IP_3R and RyRs (46). Further studies are required to determine if Ca_v channels are associated with RyRs in T cells and what are their roles in T cell activation.

ROLE OF β REGULATORY SUBUNITS AND Ca_v1 CHANNELS IN T CELL ACTIVATION AND FUNCTION

Numerous lines of evidence demonstrating the expression of Ca_v channels have indicated roles of these channels in T cell biology (see **Table 1**). A potential role for Ca_v channels in T cells became evident in mice with *lethargic* mutation, which arose spontaneously in the inbred mouse strain BALB/cGn in 1962. Homozygotes are recognizable at 2 weeks of age by ataxia, seizures, and lethargic behavior (47, 48). In 1997, Burgess et al. demonstrated that the ataxia and seizures in the lethargic mouse arise from a mutation of the β4 subunit gene (49). Neither full-length nor truncated β4 protein is expressed in the mutant mice (49). Interestingly, these mice experience an immunological disorder, including a defect in their cell-mediated immune response (50). β regulatory subunits, β1–β4, are crucial for normal Ca_v channel function (51), since they are required for the expression of functional channels at the plasma membrane (52), and modulate their biophysical properties by interacting with pore-forming α subunit (51). The mechanism of immune disorder described in these lethargic β4 mutant mice was unknown but of great interest since it implicitly supported the hypothesis that components of Ca_v1 channels are expressed in immune cells and play a crucial role in the activation and function of immune cells.

We and others demonstrated that human and mouse T cells express regulatory β3 and β4 subunits (23–25, 27, 31, 33, 53). In 2006, we provided genetic evidence, for the first time, that $CD4^+$ T cells deficient in either β3 and β4 are impaired in Ca^{2+} response, NFAT activation, and cytokine production (27). Interestingly, in the β4-deficient T cells, we have also detected a notable and specific suppression of the $Ca_v1.1$ pore-forming α1 subunit protein. On the other hand, no significant effect was observed in the expression of the $Ca_v1.2$ channel protein. This observation suggests that the deficiency observed in the β4-deficient mice might be due to the lack of expression of the $Ca_v1.1$ channel (27). However, the exact mechanism of the requirement of multiple β regulatory subunits in effector T cell stage is still unknown.

In $CD8^+$ T cells, we found that β3 is highly expressed in naïve and activated $CD8^+$ T cells and β3 deficiency leads to enhanced apoptosis of naïve T cells and decrease in homeostatic survival of these cells (31). We found that the impaired Ca^{2+} influx in β3-deficient $CD8^+$ T cells was associated with a lack of $Ca_v1.4$ protein expression (31). The functional defect in both β4- and β3-deficient T cells reflected the contribution of these subunits to Ca_v1 channel-dependent calcium response in T lymphocytes (27, 31).

Consistent with our findings (31), Omilusik et al. analyzed $Ca_v1.4$-deficient mice and reported that $CD4^+$ and $CD8^+$ T cells from $Ca_v1.4$-deficient mice had impaired homeostatic maintenance (32). β3 or $Ca_v1.4$-deficient T cells also had increased rates of cell death (31, 32). Naive $CD4^+$ and $CD8^+$ T cells were shown to be dependent on $Ca_v1.4$ function for SOCE, TCR-induced rises in cytosolic Ca^{2+} and downstream TCR signal transduction. The generation of antigen-specific T cell responses was altered in the absence of β3 or $Ca_v1.4$ (31, 32) since these mice failed to mount an effective T cell response to antigen challenge, and this was associated with reduced effector function of $CD8^+$ T cells (32).

Unexpectedly, we found that β3 and $Ca_v1.4$ were associated with a T cell signaling complex in primary T cells that was not dependent on TCR stimulation, which suggested that a preformed complex of these proteins exists in naive T cells (31). Furthermore, we identified a fraction of $Ca_v1.4$ as a lipid raft-resident Ca^{2+} channel protein (31). The reported interaction of $Ca_v1.4$ with filamins in spleen cells (54) combined with our finding of its association with Lck and Vav highlight a Ca_v channel-dependent molecular architecture of a signaling complex in specialized microdomains of T cells. These observations further gain importance given the widely accepted model that the specificity, reliability, and accurate execution of signaling processes depend on

tightly regulated spatiotemporal Ca^{2+} signals restricted to precise microdomains that contain Ca^{2+}-permeable channels and their modulators (55, 56).

Similar to β3 deficiency, analyses of thymocytes lacking a functional $Ca_v1.4$ channel revealed unperturbed or subtle changes in T cell compartment (31, 32). In thymus, the expression of various maturation and activation markers such as TCRβ, CD44, CD69, and CD62L were similar on $Ca_v1.4^{-/-}$ and WT double positive (DP) and $TCR\beta^+$ SP subpopulations (32). $Ca_v1.4$-deficient SP thymocytes exhibited very moderate decreases in TCR- or thapsigargin-induced rises in cytosolic-free Ca^{2+} relative to WT. In contrast to thymocytes, $Ca_v1.4^{-/-}$ peripheral naive, and memory T cells were significantly impaired in TCR- or thapsigargin-induced rises in cytosolic-free Ca^{2+} compared to WT peripheral naive and memory T cells (32). This indicates the great complexity involved in Ca^{2+} regulation, dynamically changing with T cell differentiation, and suggests that differential responses are important for functional outcomes upon TCR engagement. These two independent studies indicated that $Ca_v1.4/\beta3$ complex-mediated influx of Ca^{2+} from outside the cell probably induces a signaling cascade as well as contributes to tonic filling of intracellular Ca^{2+} stores critical for TCR survival signaling (31, 32).

DIFFERENTIAL REGULATION OF T CELL SURVIVAL BY Ca_v VS. CRAC CHANNELS

While $\beta3^{-/-}$ or $Ca_v1.4^{-/-}$ naïve T cells die spontaneously (31, 32), it was surprising to find an enhanced T cell survival and proliferation in the absence of ORAI1/CRACM1 (57). $CD4^+$ T cells from *Orai1*$^{-/-}$ mice showed robust proliferation with repetitive stimulations and strong resistance to stimulation-induced cell death due to reduced mitochondrial Ca^{2+} uptake and altered gene expression of proapoptotic and antiapoptotic molecules. *Orai1*$^{-/-}$ mice showed strong resistance to T cell depletion induced by injection of anti-CD3 Ab. Furthermore, ORAI1-deficient T cells showed enhanced survival after adoptive transfer into immunocompromised hosts. Together, therefore these data suggest a unique requirement of Ca_v1 calcium channels, not ORAI1/CRACM1 channel, in the survival, homeostasis, and proliferation of naïve T cells. While ORAI1/CRACM1 channels are undoubtedly required for the effector/late T cell functions (see **Figure 1**), others and our data also argue for a requirement for Ca_v1 calcium channels in the effector stage of T cells (27, 31–33). Although, it is clear now that both types of calcium channels (Ca_v1 and ORAI1/CRACM1) play critical roles in T cell biology, the present state of knowledge does not rule out a cross talk between Ca_v1 and ORAI1/CRACM1 calcium channels at the effector stage of T cells where all different kinds of calcium channels ($Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$, $Ca_v1.4$, and ORAI1/CRACM1) are co-expressed and deficiencies in these channels show immune defects. Indeed, STIM1 was shown to reciprocally control $Ca_v1.2$ and ORAI1 channels. While STIM1 activates the ORAI1 channel, it blocks $Ca_v1.2$ channel activity (58, 59). When $Ca_v1.2$ was introduced into Jurkat T cell lines expressing reduced levels of STIM1, the authors were able to measure a significant depolarization-induced increase in $[Ca^{2+}]$i compared to WT Jurkat cells (59). This suggests that loss of STIM1 allowed $Ca_v1.2$ activation in these cells. This was further confirmed by using shRNA to suppress STIM1. The regulation of $Ca_v1.2$ by STIM1 occurs through direct interaction since by using co-immunoprecipitation, it was shown that these two proteins co-interact after overexpression but also at their physiological expression level in neuroblastoma cells. Furthermore, it was reported that STIM1 binds to the C terminal region of $Ca_v1.2$ through its CRAC activation domain (CAD) (58, 59). These observations may explain how these two widely expressed channel families, Ca_v1 and ORA1, could function in the same cell type to trigger different signaling pathways, potentially leading to the control of different functions (**Figure 1**).

ROLE OF Ca_v CHANNELS IN T CELL DIFFERENTIATION AND INFLAMMATORY DISORDERS

Savignac et al. demonstrated that expression of Ca_v1 channels was induced during Th2 cell differentiation (60). Agonists and antagonists for Ca_v1 channels modulate the TCR-dependent increase in $[Ca^{2+}]_i$ and IL-4 production by Th2 cells, whereas they failed to alter the Th1 cell responses. The administration of nicardipine, a specific and clinically approved inhibitor for Ca_v1 channels, was found beneficial in three models of Th2-mediated immunopathology but did not prevent experimental autoimmune encephalomyelitis (EAE), an experimental model of Th1-mediated autoimmune disease (60, 61). These studies highlighted that TCR-dependent calcium signaling differs between Th2 and Th1 cells and suggested an important role of Ca_v1 channels in the selective regulation of $[Ca^{2+}]_i$ on stimulation through the TCR in Th2 cells. It is important to note that drugs targeting Ca_v1 channels may be beneficial in the treatment of pathologies associated with Th2 cell-mediated immunopathology.

Further, it is reported that differentiation in Th2 cells but not in Th1 cells was associated with the up-regulation of $Ca_v1.2$ and $Ca_v1.3$ channels both at the mRNA and protein level (36). Depletion of $Ca_v1.2$ and $Ca_v1.3$ expression by antisense oligodeoxynucleotides in T cells reduced TCR-induced Ca^{2+} influx in Th2 cells, attenuated IL-4 production and reduced airway inflammation in a mouse model of allergic asthma (36). Moreover, ovalbumin (OVA)-specific transgenic Th2 cells transfected with Ca_v1-specific antisense (Ca_v1AS) oligodeoxynucleotides were no longer able to induce asthma on adoptive transfer in BALB/c mice given intranasal OVA. The intranasal administration of Ca_v1AS at the time of intranasal challenge with OVA was effective in active experimental asthma, preventing airway inflammation, Th2 cell activation in the lung draining lymph nodes, and airway hyperreactivity (36).

MECHANISM OF Ca_v1 CHANNEL MEDIATED REGULATION OF CA^{2+} SIGNALING IN T LYMPHOCYTES

Others and we demonstrated the presence and significance of Ca_v1 channels in T cells (see **Table 1**) (23, 25, 27, 31, 33, 53). However, it is not known how these Ca_v1 channels open in T cells to conduct calcium. In excitable cell types, Ca_v channels conduct Ca^{2+} upon depolarization (62, 21). The basic question here is whether Ca_v1 channels are activated by TCR stimulation or by depolarization. From a physiological standpoint, T cells should respond only to antigen stimulation through cognate TCR. A voltage-dependent opening in the absence of TCR dependence would lead to a random opening of Ca_v channels

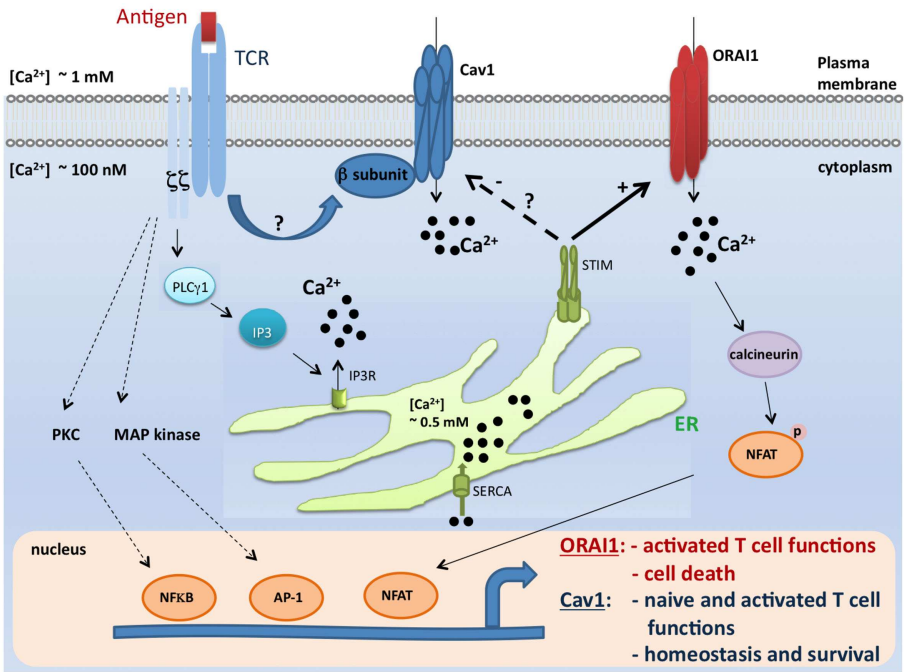

FIGURE 1 | A model for coordinated control of Ca_v1 and ORAI1 channels in T lymphocytes. Antigen encounter by T cells results in the activation of numerous pathways including the Ca^{2+} pathway. Mechanisms of Ca^{2+} influx through two major Ca^{2+}-permeable channels, Ca_v1 and ORAI1, are depicted in this scheme. During the course of biological functions that require activation of the STIM/ORAI pathway (such as effector functions and apoptosis), STIM1 blocks Ca_v1 channel activity and all depending T cell functions. In contrast, this inhibitory effect would be lifted when Ca_v1-dependent T cell functions (such as survival and naïve T cell activation) take place (27, 29, 31–33, 36, 57–59, 77, 78). It is, however, important to point out that the crosstalk described in this model was shown solely for $Ca_v1.2$ channel, and no information is available to date for the relationship between STIM and other Ca_v1 channels. TCR, T cell receptor; Ca_v, voltage-gated Ca^{2+} channels; ER, endoplasmic reticulum; IP3, inositol-1,4,5-trisphosphate; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; STIM1, stromal interaction molecule 1; PLCγ1, phospholipase Cγ1; MAP kinase, Mitogen-activated protein kinase; PKC, protein kinase C; NFkB, nuclear factor kB; AP-1, activator protein-1; and NFAT, nuclear factor of activated T cells.

and subsequent activation of T cells, which could lead to immune activation in the absence of antigen. Unlike excitable cells, T cells migrate and roam the body through variable extracellular environments and tissues with various ion concentrations. It is therefore conceivable that Ca_v channels expressed by T cells have developed a more specific control of their opening than mere voltage sensing. Notably, $Ca_v1.4$, as well as $Ca_v1.3$, has been found to have low activation thresholds that do not require strong depolarization for their activation (63). Earlier surprising findings showed that $Ca_v1.3$ channels can be activated at voltages of approximately -60 mV under physiological calcium concentrations (64).

Since Ca_v1 channels are expressed in T lymphocytes before and after TCR stimulation (23, 25, 27, 31, 33, 53), we tested the susceptibility of T cell Ca_v channels to depolarization induced by KCl. Artificial depolarization of $CD4^+$ T cells, which have been differentiated under Th1 (IL-12 plus anti-IL-4), Th2 (IL-4 plus anti-IFNγ), or Th0 (no cytokine) conditions, with KCl did not lead to calcium influx (27). KCl was used at 40 mM, a dose that induces a significant depolarization of T cells (27). However, under the same conditions and as expected, KCl triggered a transient calcium response in the C2C12 skeletal muscle excitable cell line as previously reported (65). In addition, all four groups of cells, Th0, Th1, Th2, and C2C12, were able to mount a calcium response after stimulation with the calcium ionophore, ionomycin (27). In agreement with our findings, other studies also have shown that treatment of T cells with KCl does not lead to calcium entry (25, 66) and in fact KCl addition seems to inhibit proliferation and IL-2 production (67). These observations demonstrate that, unlike in excitable cells, depolarization of T cells does not induce Ca_v channel opening.

OTHER CA^{2+}-PERMEABLE CHANNELS EXPRESSED BY T LYMPHOCYTES

The encounter of peptide-antigen presenting cell (APC) by naïve T cells induces a quick increase of intracellular calcium concentration in T lymphocytes (4). This calcium increase could be sustained for hours at levels higher than basal standards in order to mediate appropriate T lymphocyte functions such as activation, proliferation, expression of various activation-associated genes such as cytokines and chemokines (4, 7, 68). During their maturation stages, naïve T lymphocyte differentiate into distinct T cell subpopulations (such as Th1, Th2, Th17, and Treg), all of which require calcium signal. In light of these multitude and specific functions governed by T cells, it is logical to discover the expression, by these cell types, of various plasma membrane calcium channels, or even different levels of expression of the same channel at different stages of differentiation. In this section, we review the role of three major

families of Ca^{2+} permeable channels expressed by T lymphocytes, SOC channels, TRP channels, and P2X receptor channels.

STORE-OPERATED CALCIUM CHANNELS

One well studied mechanism of calcium entry into T cells is the store-operated Ca^{2+} (SOC) entry process. This mechanism was suggested by Putney (69). In this study, the authors presented evidence showing that the Ca^{2+} released from ER stores could "directly" induce Ca^{2+} influx through plasma membrane calcium channels in cells that are non-excitable (69). Numerous and independent electrophysiological studies showed that T cells indeed express channels that can be opened in response to store depletion by distinct stimuli (4, 5, 70). These channels have been designated CRAC channels in T cells, and have been extensively characterized at the electrophysiological level (4, 71) and are distinguished by a high selectivity for Ca^{2+} and a low conductance (4, 71). However, the molecular identity of the channels and their related regulatory proteins had remained unknown. In the year 2005, it has first been proposed, using RNA interference (RNAi)-based screen, that STIM 1, a conserved protein, is required for SOC influx both in *Drosophila* S2 cells and in Jurkat T cells (72). In a second study, by generating a point mutation in the STIM1 Ca^{2+} binding domain, it has been proposed that STIM1 operates as a Ca^{2+} store sensor, which functions by connecting Ca^{2+} store depletion to Ca^{2+} influx (11). In 2006, by using genome-wide approaches designed to identify regulators of store-operated Ca^{2+} entry, three separate groups have proposed a protein containing four transmembrane segments, ORAI1 (also named CRACM1) as the putative CRAC channel, or an essential component or related regulatory protein of the CRAC channel (13, 73, 74). It was still not clear, at this stage, whether ORAI1 forms the CRAC channel itself. Using site-directed mutagenesis, three additional studies have shown that it is indeed ORAI1 itself that forms the Ca^{2+} selectivity filter of the CRAC channel complex, providing strong evidence that ORAI1 is the pore-forming subunit of the CRAC channel (14, 75, 76). In addition, the protein ORAI1 was proposed as the prototypical CRAC channel, especially after the discovery that human patients presenting with a SCID disease lack functional CRAC channels and SOCE in T cells (13). However, ORAI1 deficiency in mice resulted surprisingly in no defect in T cell development in the thymus, no defect in T cell proliferation and only a partial inhibition of IL-2 and IFNγ production (77). In contrast, these mice exhibited a major defect in mast cell effector functions (77). It was also reported, in a second independent study, that T cell development is normal in ORAI1$^{-/-}$ mice and that ORAI1-deficient naive CD4+ T cells and CD8+ T cells show no significant decrease of SOC influx after stimulation by thapsigargin or by anti-CD3 mAb (78). Consistently, ORAI1-deficient naive T cells exhibited normal proliferation upon stimulation with anti-CD3 and anti-CD28 mAbs (78). However, ORAI1 seemed to be of more importance to differentiated CD4+ and CD8+ T cells. Indeed, the impairment in Ca^{2+} influx in the absence of ORAI1 was most apparent in Th1 cells, followed by CTLs and then Th2 cells (78). Furthermore, when cytokine production was assessed, only a partial inhibition was observed in differentiated CD4+ and CD8+ T cells from ORAI1$^{-/-}$ mice (78). Interestingly, when cyclosporine A was applied, cytokine production was completely abolished (78), indicating that other calcium/calcineurin-dependent, ORAI1-independent pathways are involved in this cytokine production in differentiated CD4 and CD8 T cells. The lack of a major contribution of ORAI1 is likely not due to a compensatory process by ORAI2 or ORAI3. In fact, while reconstitution with ORAI1 restored SOC influx in differentiated ORAI1-deficient T cells, reconstitution with ORAI2 protein showed no effect, and reconstitution with ORAI3 exhibited only a small SOC influx upon stimulation with thapsigargin but not anti-CD3 mAb (78). These observations suggest that ORAI1 is dispensable for T cell development and for initial intracellular calcium increases detected in naïve T cells upon the initial antigen encounter (see **Table 1**; **Figure 1**). However, ORAI1 is likely to contribute at least partially to CD4 and CD8 effector functions (see **Table 1**). This discovery is perhaps not completely surprising as the discovery phase of research on CRAC channels derived from studies in cell lines such as Jurkat, which are more similar, to some extent, to differentiated effector T cells rather than naive primary T cells. This observation also suggests that Ca^{2+} channels other than CRAC proteins are likely involved in T cell functions.

TRANSIENT RECEPTOR POTENTIAL CHANNELS

Before the discovery of ORAI1 as the main channel responsible for SOC influx in T cells, members of the TRP family were considered as key candidates for T cell calcium channels. In human cells, TRP superfamily of channels can be classified into 7 subfamilies (TRPC, TRPV, TRPM, TRPA, TRPN, TRPP, and TRPML) with a total of 27 cation channels (79). These channels, which share six transmembrane domains, form ion-conducting proteins that are mostly non-selective and permeable to several cations, including Ca^{2+} and Na^{+} (80). TRP channels can be activated via diverse mechanisms. In fact, some TRP channels could respond to stimuli ranging from heat to natural product compounds, pro-inflammatory agents, and exocytosis (79). TRPC, TRPM, and TRPV seem to be the major subfamilies expressed by murine (81) and human T cells (82) (see **Table 1**). In 2003, Hoth and colleagues, by analyzing mutant T cell lines exhibiting defects in Ca^{2+} entry and Ca^{2+}-dependent gene expression (83), suggested an alteration of TRPC3 gene in these mutant cell lines relative to wild type cells. When the wild type TRPC3 gene was reintroduced in mutant cell lines through transient transfection, it was able to restore TCR-mediated Ca^{2+} influx. It was then concluded that TRPC3 channel contributes to TCR-induced Ca^{2+} entry into T cells, and is therefore critical for Ca^{2+}-dependent activation of T cells (16). In this study, the authors used cell lines and overexpression approaches, and therefore, the conclusions needed to be confirmed in a more physiological system. A few years later, using murine immune cells the expression profile of diverse subsets of TRPC, TRPV, and TRPM was reported (81). Similarly, consistent mRNA expression of TRPC1, TRPC3, TRPV1, TRPM2, and TRPM7 was detected in primary human CD4+ T cells purified from healthy donors. TRPC3 and TRPM2 transcripts were upregulated after stimulation via TCR; and knockdown of TRPC3 channel by siRNA showed that this channel may contribute to Ca^{2+}-dependent proliferation of primary T cells (82). Another study pointed out a significant role of TRPC5 channel in the mechanism of effector T cell suppression by Treg cells (84). Interaction of these two cell types was described

as involving cross-linking of GM1 ganglioside (expressed by effector T cells) by galectin-1 (expressed by Treg cells); and the TRPC5 channel was shown to be involved in this regulatory process. In this paper, the authors described the up-regulation of TRPC5 channel transcript, but not TRPC4, in effector murine CD4, and CD8 T cells relative to naïve T cells (84). They also showed that knockdown of TRPC5 channel in effector T cells by short hairpin RNA inhibited both contact-dependent inhibition of effector T cell proliferation and galectin-1-induced Ca^{2+} influx (84). TRPM2 forms non-selective Ca^{2+}-permeable cation channel. This channel is expressed in the brain but also in immune cells (85–87) and it can be opened by the intracellular messenger, adenosine 5′-diphosphoribose (ADPR) (85–87). One of the first reports on the role of TRPM2 (formerly LTRPC2) channel in calcium influx in immune cells demonstrated that the TRPM2 channel mediates Ca^{2+} influx into monocytes (86). This report showed that ADPR and nicotinamide adenine dinucleotide (NAD) can directly stimulate TRPM2 channel activity to mediate Ca^{2+} entry (86). A key question is whether these second messengers, NAD and/or ADPR, are involved in this process upon receptor stimulation. Guse and colleagues showed that indeed intracellular ADPR concentrations are increased upon stimulation of Jurkat T cells by ConA; and that this messenger mediates Ca^{2+} influx through TRPM2 channels (87). In this study, the authors also showed that inhibition of ADPR formation or knockdown of TRPM2 both inhibited this stimulation-dependent TRPM2-mediated Ca^{2+} influx (87). By modifying intracellular NAD concentration and using siRNA knockdown, another recent study similarly emphasized the role of NAD and ADPR in mitogen-induced Ca^{2+} rise in human T lymphocytes through the involvement of TRPM2 channels (88). Subsequently, using TRPM2-deficient mice, it was shown that this channel contributes to T lymphocyte proliferation and production of pro-inflammatory cytokines after stimulation via TCR (89). When evaluated *in vivo*, $TRPM2^{-/-}$ mice displayed amelioration in EAE development. The authors attributed this improved EAE phenotype to reduced T cell effector functions and proposed TRPM2 channel as a potential therapeutic target (89).

Initial evidence for a role of the TRPM7 channel in immune cells emanated from its disruption in DT-40 B cell lines (90). TRPM7 deficient cells exhibited a defect in proliferation and required elevated extracellular Mg^{2+} for their survival (90). In a related interesting study, Clapham and colleagues used lck-Cre mice, since $TRPM7^{-/-}$ mice died prenatally, to selectively delete TRPM7 in T cells. Surprisingly, $TRPM7flox^{-/-}$ Lck-Cre mice displayed a notable defect in T cell development in the thymus. The authors detected a block in transition from the double negative (CD4 − CD8−) to DP (CD4 + CD8+) stage in TRPM7 deficient thymocytes. As a result, both the number and the percentage of T cells in the periphery are reduced. Interestingly, TRPM7 deficient thymocytes did not show any significant defect in Mg^{2+} uptake. And using inductively coupled plasma mass spectrometry, the authors showed that total Mg^{2+} concentration in wild type and deficient T cells is similar suggesting that TRPM7 is dispensable for cellular Mg^{2+} homeostasis in T cells (91). TRPM7 is a channel protein permeable to Ca^{2+} and Mg^{2+} but also contains a regulatory serine-threonine kinase domain in the same structure (92). A role for the kinase domain is likely to be also excluded. In fact, a recent study showed that the defect of TRPM7 deficient T cells in Fas-mediated apoptosis depends on its activity as a channel rather than a kinase (92). Therefore, after the involvement of Mg^{2+} and the kinase domain in this process have been excluded, the question arises as to whether the effects of the TRPM7 channel on T cell development are related to Ca^{2+}.

In addition to TRPC and TRPM channels, other TRP channels such as TRPV1 and TRPV2 appear to show an interesting and consistent expression profile in primary human T cells (82), however, their role in T cell function is still elusive.

P2X RECEPTOR CHANNELS

P2 receptors are broadly distributed in many cell types. Two distinct subfamilies have been described, the G-protein-coupled seven-transmembrane P2Y receptors and the ligand-gated P2X receptors (P2XR) (18). There are seven mammalian P2X receptor members (P2X1–7). These proteins form non-selective cation channels that are gated by extracellular ATP to allow influx of cations including Ca^{2+}, and Na^{2+}. In T lymphocytes, three distinct P2X members have been suggested to contribute to calcium entry in human T cells, P2X1, P2X4, and P2X7 (20) (see **Table 1**). One of the first reports to suggest a potential expression of ATP-gated receptor channels on T cells was published in 1996. In this article, it was shown that extracellular ATP (ATPe) was able to induce intracellular Ca^{2+} concentration increases in PBLs and purified human T cells (93). ATPe exhibited also a synergistic effect with PHA and anti-CD3 mAb on PBL proliferation. It was suggested, in this study, that the ATP-mediated Ca^{2+} influx and the ATP contribution in proliferation were both dependent on P2X and/or P2Z receptors since these effects were blocked using oxidized ATP (oATP), a covalent blocker of these two channels (93). In other reports, it was shown that ATPe was able to induce thymocyte apoptosis (94); and that the biochemical and morphological changes induced by ATPe and leading to apoptosis, are preceded by a rapid intracellular calcium increase (94). It was then documented that P2X7 receptor is critical for apoptosis of BALB/c thymocytes induced by ATPe (18). In fact, the potent P2X7 receptor agonist, benzoylbenzoyl-ATP, was able to mimic the ATPe effect. Furthermore, two P2X7 receptor antagonists (oATP and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid) inhibited the effect of ATPe. However, notable evidence emanated from the use of thymocytes prepared from $P2X7R^{-/-}$ mice, where ATPe-induced apoptosis was completely abolished (18). Interestingly, ATPe could also induce activation of T cells (19, 95); and it appears that whether cells will undergo apoptosis or activation would depend on the level of expression of the P2X7 receptor and on concentrations of ATPe (19, 95). High concentrations of ATPe induce apoptosis, in contrast, lower ATPe doses closer to those secreted in an autocrine or paracrine manner would induce T cell activation (19). Indeed, Junger and colleagues showed that P2X7 receptors are critical for TCR-mediated Ca^{2+} influx and downstream signaling events accompanying T cell activation. The authors were able to reveal secretion of ATP ($<100\,\mu M$) by Jurkat cells after TCR stimulation. Subsequently, they showed that released ATPe activates P2X7 receptors, in an autocrine manner, contributing to Ca^{2+} influx, which induces T cell activation via the activation of NFAT and IL-2 gene transcription (19). In

addition to P2X7 receptor, the expression and involvement of two other critical members, P2X1 and P2X4, in calcium entry and T cell activation was reported (20). In this study, it was shown that P2X1, P2X4 receptors and pannexin-1 hemichannels translocate to the immunological synapse of activated T cells. Inhibition of pannexin-1, using the gap junction inhibitor carbenoxolone, resulted in the inhibition of TCR-mediated ATP release, Ca^{2+} influx and T cell activation. Similarly, inhibition or silencing of P2X1 and P2X4 receptors suppresses Ca^{2+} entry and subsequent signaling events leading to T cell activation, such as NFAT activation and IL-2 gene induction (20). These reports indicate that P2X1, P2X4, and P2X7 receptors play critical roles in TCR-mediated Ca^{2+} signal amplification upon stimulation of T lymphocytes.

PERSPECTIVES AND CONCLUDING REMARKS

Calcium ion is a critical and universal second messenger, which is involved in T lymphocyte function at various stages including development, survival, activation, differentiation, cytokine production, and cell death. In this review, we presented our views on the crucial role played by L-type Ca_v1 channels in T cells. We also summarized the important discovery of the main elements controlling Ca^{2+} entry through CRAC channels in T cells, STIM, and ORAI. The contribution of other Ca^{2+} entry pathways such as the TRP family of channels and ligand-gated P2X receptors was also taken into consideration. Collectively, data reviewed in this manuscript show that T lymphocytes express a considerable number of Ca^{2+} permeable channels (see **Table 1**), which highly likely communicate together in order to regulate development and distinct functions of T cells. However, many questions still remain to be answered. While there is no doubt for a role of Ca_v1 channel proteins in contributing to Ca^{2+} entry in T cells, it has still not been established that it is the Ca_v1 pore-forming protein that conducts Ca^{2+} after TCR stimulation. Site-directed mutagenesis experiments could answer this important question. We also have presented evidence showing that Ca_v1 channels expressed by T cells are not voltage-sensitive and contribute to Ca^{2+} entry after TCR stimulation (27, 29, 31, 32). How Ca_v1 channels are gated after TCR stimulation is still not clear. Another major point is how different Ca_v1, CRAC, TRP, and P2XR subsets contribute, physiologically, to development of T cells, but especially to their differentiation into various effector T cell subpopulations. As shown with distinct subsets of Ca_v1 family of channels, the repertoire of Ca^{2+} channels operating in T cells changes during various stages of differentiation. A more profound study of the expression level of various channels after TCR stimulation, at various differentiation stages and under physiological conditions, will be of major interest. We believe that it will be of importance, therapeutically, to target a channel that is expressed at a specific stage on a specific T cell subpopulation rather than robust blockage of the entire immune system, which leads to major side effects. We also need to uncover factors that are implicated in physiological regulation of these channels. Ultimately, it will be crucial to understand how all these channels interact with each other to finely regulate T lymphocyte functions.

REFERENCES

1. Cantrell D. T cell antigen receptor signal transduction pathways. *Annu Rev Immunol* (1996) **14**:259–74. doi:10.1146/annurev.immunol.14.1.259
2. Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI. Differential activation of transcription factors induced by Ca2+ response amplitude and duration. *Nature* (1997) **386**:855–8. doi:10.1038/386855a0
3. Esser MT, Haverstick DM, Fuller CL, Gullo CA, Braciale VL. Ca2+ signaling modulates cytolytic T lymphocyte effector functions. *J Exp Med* (1998) **187**:1057–67. doi:10.1084/jem.187.7.1057
4. Lewis RS. Calcium signaling mechanisms in T lymphocytes. *Annu Rev Immunol* (2001) **19**:497. doi:10.1146/annurev.immunol.19.1.497
5. Hogan PG, Rao A. Dissecting ICRAC, a store-operated calcium current. *Trends Biochem Sci* (2007) **32**:235–45. doi:10.1016/j.tibs.2007.03.009
6. Lewis RS. The molecular choreography of a store-operated calcium channel. *Nature* (2007) **446**:284–7. doi:10.1038/nature05637
7. Feske S, Skolnik EY, Prakriya M. Ion channels and transporters in lymphocyte function and immunity. *Nat Rev Immunol* (2012) **12**:532–47. doi:10.1038/nri3233
8. Gallo EM, Cante-Barrett K, Crabtree GR. Lymphocyte calcium signaling from membrane to nucleus. *Nat Immunol* (2006) **7**:25–32. doi:10.1038/ni1295
9. Vig M, Kinet JP. Calcium signaling in immune cells. *Nat Immunol* (2009) **10**:21–7. doi:10.1038/ni.f.220
10. Feske S. Ca(2+) influx in T cells: how many ca(2+) channels? *Front Immunol* (2013) **4**:99. doi:10.3389/fimmu.2013.00099
11. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, et al. STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr Biol* (2005) **15**:1235–41. doi:10.1016/j.cub.2005.05.055
12. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, et al. STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. *Nature* (2005) **437**:902–5. doi:10.1038/nature04147
13. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* (2006) **441**:179–85. doi:10.1038/nature04702
14. Vig M, Beck A, Billingsley JM, Lis A, Parvez S, Peinelt C, et al. CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr Biol* (2006) **16**:2073–9. doi:10.1016/j.cub.2006.08.085
15. Venkatachalam K, Ma HT, Ford DL, Gill DL. Expression of functional receptor-coupled TRPC3 channels in DT40 triple receptor InsP3 knockout cells. *J Biol Chem* (2001) **276**:33980–5. doi:10.1074/jbc.C100321200
16. Philipp S, Strauss B, Hirnet D, Wissenbach U, Mery L, Flockerzi V, et al. TRPC3 mediates T-cell receptor-dependent calcium entry in human T-lymphocytes. *J Biol Chem* (2003) **278**:26629–38. doi:10.1074/jbc.M304044200
17. Putney JW Jr. Capacitative calcium entry: sensing the calcium stores. *J Cell Biol* (2005) **169**:381–2. doi:10.1083/jcb.200503161
18. Le Stunff H, Auger R, Kanellopoulos J, Raymond MN. The Pro-451 to Leu polymorphism within the C-terminal tail of P2X7 receptor impairs cell death but not phospholipase D activation in murine thymocytes. *J Biol Chem* (2004) **279**:16918–26. doi:10.1074/jbc.M313064200
19. Yip L, Woehrle T, Corriden R, Hirsh M, Chen Y, Inoue Y, et al. Autocrine regulation of T-cell activation by ATP release and P2X7 receptors. *FASEB J* (2009) **23**:1685–93. doi:10.1096/fj.08-126458
20. Woehrle T, Yip L, Elkhal A, Sumi Y, Chen Y, Yao Y, et al. Pannexin-1 hemichannel-mediated ATP release together with P2X1 and P2X4 receptors regulate T-cell activation at the immune synapse. *Blood* (2010) **116**:3475–84. doi:10.1182/blood-2010-04-277707
21. Catterall WA. Structure and regulation of voltage-gated Ca2+ channels. *Annu Rev Cell Dev Biol* (2000) **16**:521–55. doi:10.1146/annurev.cellbio.16.1.521
22. Jiang Y, Ruta V, Chen J, Lee A, Mackinnon R. The principle of gating charge movement in a voltage-dependent K+ channel. *Nature* (2003) **423**:42–8. doi:10.1038/nature01580

23. Kotturi MF, Carlow DA, Lee JC, Ziltener HJ, Jefferies WA. Identification and functional characterization of voltage-dependent calcium channels in T lymphocytes. *J Biol Chem* (2003) **278**: 46949–60. doi:10.1074/jbc.M309268200
24. Gomes B, Savignac M, Moreau M, Leclerc C, Lory P, Guery JC, et al. Lymphocyte calcium signaling involves dihydropyridine-sensitive L-type calcium channels: facts and controversies. *Crit Rev Immunol* (2004) **24**:425–47. doi:10.1615/CritRevImmunol.v24.i6.30
25. Stokes L, Gordon J, Grafton G. Non-voltage-gated L-type Ca2+ channels in human T cells: pharmacology and molecular characterization of the major alpha pore-forming and auxiliary beta-subunits. *J Biol Chem* (2004) **279**:19566–73. doi:10.1074/jbc.M401481200
26. Kotturi MF, Jefferies WA. Molecular characterization of L-type calcium channel splice variants expressed in human T lymphocytes. *Mol Immunol* (2005) **42**:1461–74. doi:10.1016/j.molimm.2005.01.014
27. Badou A, Jha MK, Matza D, Mehal WZ, Freichel M, Flockerzi V, et al. Critical role for the beta regulatory subunits of Cav channels in T lymphocyte function. *Proc Natl Acad Sci U S A* (2006) **103**:15529–34. doi:10.1073/pnas.0607262103
28. Kotturi MF, Hunt SV, Jefferies WA. Roles of CRAC and Cav-like channels in T cells: more than one gatekeeper? *Trends Pharmacol Sci* (2006) **27**:360–7. doi:10.1016/j.tips.2006.05.007
29. Matza D, Badou A, Kobayashi KS, Goldsmith-Pestana K, Masuda Y, Komuro A, et al. A scaffold protein, AHNAK1, is required for calcium signaling during T cell activation. *Immunity* (2008) **28**:64–74. doi:10.1016/j.immuni.2007.11.020
30. Colucci A, Giunti R, Senesi S, Bygrave FL, Benedetti A, Gamberucci A. Effect of nifedipine on capacitive calcium entry in Jurkat T lymphocytes. *Arch Biochem Biophys* (2009) **481**:80–5. doi:10.1016/j.abb.2008.10.002
31. Jha MK, Badou A, Meissner M, McRory JE, Freichel M, Flockerzi V, et al. Defective survival of naive CD8+ T lymphocytes in the absence of the beta3 regulatory subunit of voltage-gated calcium channels. *Nat Immunol* (2009) **10**:1275–82. doi:10.1038/ni.1793
32. Omilusik K, Priatel JJ, Chen X, Wang YT, Xu H, Choi KB, et al. The Ca(v)1.4 calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. *Immunity* (2011) **35**:349–60. doi:10.1016/j.immuni.2011.07.011
33. Matza D, Badou A, Jha MK, Willinger T, Antov A, Sanjabi S, et al. Requirement for AHNAK1-mediated calcium signaling during T lymphocyte cytolysis. *Proc Natl Acad Sci U S A* (2009) **106**:9785–90. doi:10.1073/pnas.0902844106
34. Badou A, Savignac M, Moreau M, Leclerc C, Pasquier R, Druet P, et al. $HgCl_2$-induced interleukin-4 gene expression in T cells involves a protein kinase C-dependent calcium influx through L-type calcium channels. *J Biol Chem* (1997) **272**:32411–8. doi:10.1074/jbc.272.51.32411
35. Matza D, Flavell RA. Roles of Ca(v) channels and AHNAK1 in T cells: the beauty and the beast. *Immunol Rev* (2009) **231**:257–64. doi:10.1111/j.1600-065X.2009.00805.x
36. Cabral MD, Paulet PE, Robert V, Gomes B, Renoud ML, Savignac M, et al. Knocking down Cav1 calcium channels implicated in Th2 cell activation prevents experimental asthma. *Am J Respir Crit Care Med* (2010) **181**:1310–7. doi:10.1164/rccm.200907-1166OC
37. Sadighi Akha AA, Willmott NJ, Brickley K, Dolphin AC, Galione A, Hunt SV. Anti-Ig-induced calcium influx in rat B lymphocytes mediated by cGMP through a dihydropyridine-sensitive channel. *J Biol Chem* (1996) **271**:7297–300. doi:10.1074/jbc.271.13.7297
38. Grafton G, Stokes L, Toellner KM, Gordon J. A non-voltage-gated calcium channel with L-type characteristics activated by B cell receptor ligation. *Biochem Pharmacol* (2003) **66**:2001–9. doi:10.1016/j.bcp.2003.07.005
39. Vukcevic M, Spagnoli GC, Iezzi G, Zorzato F, Treves S. Ryanodine receptor activation by Ca v 1.2 is involved in dendritic cell major histocompatibility complex class II surface expression. *J Biol Chem* (2008) **283**:34913–22. doi:10.1074/jbc.M804472200
40. Fill M, Copello JA. Ryanodine receptor calcium release channels. *Physiol Rev* (2002) **82**: 893–922.
41. Hakamata Y, Nishimura S, Nakai J, Nakashima Y, Kita T, Imoto K. Involvement of the brain type of ryanodine receptor in T-cell proliferation. *FEBS Lett* (1994) **352**:206–10. doi:10.1016/0014-5793(94)00955-4
42. Hosoi E, Nishizaki C, Gallagher KL, Wyre HW, Matsuo Y, Sei Y. Expression of the ryanodine receptor isoforms in immune cells. *J Immunol* (2001) **167**:4887–94.
43. Hohenegger M, Berg I, Weigl L, Mayr GW, Potter BV, Guse AH. Pharmacological activation of the ryanodine receptor in Jurkat T-lymphocytes. *Br J Pharmacol* (1999) **128**:1235–40. doi:10.1038/sj.bjp.0702935
44. Conrad DM, Hanniman EA, Watson CL, Mader JS, Hoskin DW. Ryanodine receptor signaling is required for anti-CD3-induced T cell proliferation, interleukin-2 synthesis, and interleukin-2 receptor signaling. *J Cell Biochem* (2004) **92**:387–99. doi:10.1002/jcb.20064
45. Schwarzmann N, Kunerth S, Weber K, Mayr GW, Guse AH. Knock-down of the type 3 ryanodine receptor impairs sustained Ca2+ signaling via the T cell receptor/CD3 complex. *J Biol Chem* (2002) **277**:50636–42. doi:10.1074/jbc.M209061200
46. Dadsetan S, Zakharova L, Molinski TF, Fomina AF. Store-operated Ca2+ influx causes Ca2+ release from the intracellular Ca2+ channels that is required for T cell activation. *J Biol Chem* (2008) **283**:12512–9. doi:10.1074/jbc.M709330200
47. Dickie MM. Lethargic (lh). *Mouse News Lett* (1964) **30**:31.
48. Sidman RL, Green MC, Appel SH. *Catalog of the Neurological Mutants of the Mouse.* Cambridge, MA: Harvard University Press (1965). 34 p.
49. Burgess DL, Jones JM, Meisler MH, Noebels JL. Mutation of the Ca2+ channel beta subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. *Cell* (1997) **88**: 385–92. doi:10.1016/S0092-8674(00)81877-2
50. Morrison DG, Moyer MP, Dung HC, Rogers W, Moyer RC. Tumor growth rate varies with age in lethargic mutant BALB/cGnDu mice. *Dev Comp Immunol* (1984) **8**:435–42. doi:10.1016/0145-305X(84)90050-8
51. Dolphin AC. Beta subunits of voltage-gated calcium channels. *J Bioenerg Biomembr* (2003) **35**:599–620. doi:10.1023/B:JOBB.0000008026.37790.5a
52. Bichet D, Lecomte C, Sabatier JM, Felix R, De Waard M. Reversibility of the Ca(2+) channel alpha(1)-beta subunit interaction. *Biochem Biophys Res Commun* (2000) **277**:729–35. doi:10.1006/bbrc.2000.3750
53. McRory JE, Hamid J, Doering CJ, Garcia E, Parker R, Hamming K, et al. The CACNA1F gene encodes an L-type calcium channel with unique biophysical properties and tissue distribution. *J Neurosci* (2004) **24**:1707–18. doi:10.1523/JNEUROSCI.4846-03.2004
54. Doering CJ, Rehak R, Bonfield S, Peloquin JB, Stell WK, Mema SC, et al. Modified Ca(v)1.4 expression in the Cacna1f(nob2) mouse due to alternative splicing of an ETn inserted in exon 2. *PLoS ONE* (2008) **3**:e2538. doi:10.1371/journal.pone.0002538
55. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* (2003) **4**:517–29. doi:10.1038/nrm1155
56. Pani B, Singh BB. Lipid rafts/caveolae as microdomains of calcium signaling. *Cell Calcium* (2009) **45**:625–33. doi:10.1016/j.ceca.2009.02.009
57. Kim KD, Srikanth S, Yee MK, Mock DC, Lawson GW, Gwack Y. ORAI1 deficiency impairs activated T cell death and enhances T cell survival. *J Immunol* (2011) **187**:3620–30. doi:10.4049/jimmunol.1100847
58. Park CY, Shcheglovitov A, Dolmetsch R. The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science* (2010) **330**:101–5. doi:10.1126/science.1191027
59. Wang Y, Deng X, Mancarella S, Hendron E, Eguchi S, Soboloff J, et al. The calcium store sensor, STIM1, reciprocally controls Orai and CaV1.2 channels. *Science* (2010) **330**:105–9. doi:10.1126/science.1191086
60. Savignac M, Gomes B, Gallard A, Narbonnet S, Moreau M, Leclerc C, et al. Dihydropyridine receptors are selective markers of Th2 cells and can be targeted to prevent Th2-dependent immunopathological disorders. *J Immunol* (2004) **172**:5206–12.
61. Gomes B, Cabral MD, Gallard A, Savignac M, Paulet P, Druet P, et al. Calcium channel blocker prevents T helper type 2 cell-mediated airway inflammation. *Am J Respir Crit Care Med*

(2007) **175**:1117–24. doi:10.1164/rccm.200607-1026OC
62. Tsien RW, Fox AP, Hess P, Mccleskey EW, Nilius B, Nowycky MC, et al. Multiple types of calcium channel in excitable cells. *Soc Gen Physiol Ser* (1987) **41**:167–87.
63. Lipscombe D, Helton TD, Xu W. L-type calcium channels: the low down. *J Neurophysiol* (2004) **92**:2633–41. doi:10.1152/jn.00486.2004
64. Koschak A, Reimer D, Huber I, Grabner M, Glossmann H, Engel J, et al. Alpha 1D (Cav1.3) subunits can form l-type Ca2+ channels activating at negative voltages. *J Biol Chem* (2001) **276**:22100–6. doi:10.1074/jbc.M101469200
65. Rosenberg P, Hawkins A, Stiber J, Shelton JM, Hutcheson K, Bassel-Duby R, et al. TRPC3 channels confer cellular memory of recent neuromuscular activity. *Proc Natl Acad Sci U S A* (2004) **101**:9387–92. doi:10.1073/pnas.0308179101
66. Gelfand EW, Cheung RK, Grinstein S. Mitogen-induced changes in Ca2+ permeability are not mediated by voltage-gated K+ channels. *J Biol Chem* (1986) **261**:11520–3.
67. Freedman BD, Price MA, Deutsch CJ. Evidence for voltage modulation of IL-2 production in mitogen-stimulated human peripheral blood lymphocytes. *J Immunol* (1992) **149**:3784–94.
68. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* (2010) **28**:491–533. doi:10.1146/annurev.immunol.021908.132550
69. Putney JW Jr. A model for receptor-regulated calcium entry. *Cell Calcium* (1986) **7**:1–12. doi:10.1016/0143-4160(86)90026-6
70. Parekh AB, Putney JW Jr. Store-operated calcium channels. *Physiol Rev* (2005) **85**:757–810. doi:10.1152/physrev.00057.2003
71. Zweifach A, Lewis RS. Mitogen-regulated Ca2+ current of T lymphocytes is activated by depletion of intracellular Ca2+ stores. *Proc Natl Acad Sci U S A* (1993) **90**:6295–9. doi:10.1073/pnas.90.13.6295
72. Roos J, Digregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, et al. STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J Cell Biol* (2005) **169**:435–45. doi:10.1083/jcb.200502019
73. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science* (2006) **312**:1220–3. doi:10.1126/science.1127883
74. Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, et al. Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. *Proc Natl Acad Sci U S A* (2006) **103**:9357–62. doi:10.1073/pnas.0603161103
75. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* (2006) **443**:230–3. doi:10.1038/nature05122
76. Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* (2006) **443**:226–9. doi:10.1038/nature05108
77. Vig M, Dehaven WI, Bird GS, Billingsley JM, Wang H, Rao PE, et al. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat Immunol* (2008) **9**:89–96. doi:10.1038/nrg2314
78. Gwack Y, Srikanth S, Oh-Hora M, Hogan PG, Lamperti ED, Yamashita M, et al. Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol Cell Biol* (2008) **28**:5209–22. doi:10.1128/MCB.00360-08
79. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem* (2007) **76**:387–417. doi:10.1146/annurev.biochem.75.103004.142819
80. Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. *Annu Rev Physiol* (2006) **68**:619–47. doi:10.1146/annurev.physiol.68.040204.100431
81. Inada H, Iida T, Tominaga M. Different expression patterns of TRP genes in murine B and T lymphocytes. *Biochem Biophys Res Commun* (2006) **350**:762–7. doi:10.1016/j.bbrc.2006.09.111
82. Wenning AS, Neblung K, Strauss B, Wolfs MJ, Sappok A, Hoth M, et al. TRP expression pattern and the functional importance of TRPC3 in primary human T-cells. *Biochim Biophys Acta* (2011) **1813**:412–23. doi:10.1016/j.bbamcr.2010.12.022
83. Fanger CM, Hoth M, Crabtree GR, Lewis RS. Characterization of T cell mutants with defects in capacitative calcium entry: genetic evidence for the physiological roles of CRAC channels. *J Cell Biol* (1995) **131**:655–67. doi:10.1083/jcb.131.3.655
84. Wang J, Lu ZH, Gabius HJ, Rohowsky-Kochan C, Ledeen RW, Wu G. Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. *J Immunol* (2009) **182**:4036–45. doi:10.4049/jimmunol.0802981
85. Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, et al. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature* (2001) **411**:595–9. doi:10.1038/35079100
86. Sano Y, Inamura K, Miyake A, Mochizuki S, Yokoi H, Matsushime H, et al. Immunocyte Ca2+ influx system mediated by LTRPC2. *Science* (2001) **293**:1327–30. doi:10.1126/science.1062473
87. Gasser A, Glassmeier G, Fliegert R, Langhorst MF, Meinke S, Hein D, et al. Activation of T cell calcium influx by the second messenger ADP-ribose. *J Biol Chem* (2006) **281**:2489–96. doi:10.1074/jbc.M506525200
88. Magnone M, Bauer I, Poggi A, Mannino E, Sturla L, Brini M, et al. NAD+ levels control Ca2+ store replenishment and mitogen-induced increase of cytosolic Ca2+ by Cyclic ADP-ribose-dependent TRPM2 channel gating in human T lymphocytes. *J Biol Chem* (2012) **287**:21067–81. doi:10.1074/jbc.M111.324269
89. Melzer N, Hicking G, Gobel K, Wiendl H. TRPM2 cation channels modulate T cell effector functions and contribute to autoimmune CNS inflammation. *PLoS ONE* (2012) **7**:e47617. doi:10.1371/journal.pone.0047617
90. Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R, et al. Regulation of vertebrate cellular Mg2+ homeostasis by TRPM7. *Cell* (2003) **114**:191–200. doi:10.1016/S0092-8674(03)00556-7
91. Jin J, Desai BN, Navarro B, Donovan A, Andrews NC, Clapham DE. Deletion of Trpm7 disrupts embryonic development and thymopoiesis without altering Mg2+ homeostasis. *Science* (2008) **322**:756–60. doi:10.1126/science.1163493
92. Desai BN, Krapivinsky G, Navarro B, Krapivinsky L, Carter BC, Febvay S, et al. Cleavage of TRPM7 releases the kinase domain from the ion channel and regulates its participation in Fas-induced apoptosis. *Dev Cell* (2012) **22**:1149–62. doi:10.1016/j.devcel.2012.04.006
93. Baricordi OR, Ferrari D, Melchiorri L, Chiozzi P, Hanau S, Chiari E, et al. An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. *Blood* (1996) **87**:682–90.
94. Zheng LM, Zychlinsky A, Liu CC, Ojcius DM, Young JD. Extracellular ATP as a trigger for apoptosis or programmed cell death. *J Cell Biol* (1991) **112**:279–88. doi:10.1083/jcb.112.2.279
95. Aswad F, Dennert G. P2X7 receptor expression levels determine lethal effects of a purine based danger signal in T lymphocytes. *Cell Immunol* (2006) **243**:58–65. doi:10.1016/j.cellimm.2006.12.003

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Ca^{2+} signaling in T-cell subsets with a focus on the role of Ca_v1 channels: possible implications in therapeutics

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The role of voltage-dependent calcium (Ca_v1) channels is prominent in excitable cells while store-operated calcium channels (SOCC) were considered as characteristic of non-excitable cells. Ca_v1 channels are implicated in excitation transcription. Store-operated calcium channels (SOCC) activity is increased during cardiac stress and would contribute to Ca^{2+} influx and expression of genes responsible for cardiac hypertrophy and heart failure (Luo et al., 2012). Several lines of evidence now show the importance of Ca_v1 channels in non-excitable cells including lymphocytes (reviewed in Robert et al., 2011, 2013). Ca_v1 channels are defined by their voltage sensitivity and their sensitivity to drugs as dihydropyridines, phenylalkylamines, benzothiazepines, known to alter T-cell functions. However the drug concentrations needed were higher compared to excitable cells. The absence of cell membrane depolarization upon activation and possible non-specific effects of the drugs questioned the putative role of Ca_v1 channels in T-cells.

Ca_v1 channels are formed by the ion forming pore α1 subunit encoded by four genes conferring some tissue-specific expression pattern in excitable cells. $Ca_v1.1$ is characteristic of skeletal muscle cells. $Ca_v1.2$ is found in neurons, heart, and smooth muscle cells while $Ca_v1.3$ is detected in neuroendocrine cells. $Ca_v1.2$ and $Ca_v1.3$ can be found in the same tissues even if their role is not redundant as shown by the differential phenotypes of $Ca_v1.2$ and $Ca_v1.3$ null mice. $Ca_v1.4$ is the retinal form. Ca_v1 channel isoforms differ by their sensitivity to depolarization and to antagonizing drugs such as dihydropyridines (DHP) as well as by their inactivation properties (Lipscombe et al., 2004). For example, $Ca_v1.4$ channels activate at more negative potentials than $Ca_v1.3$ and $Ca_v1.2$, which highlights the potential involvement of $Ca_v1.4$ in non-excitable cells as mast cells (McRory et al., 2004) and more recently in mouse T-lymphocytes (Omilusik et al., 2011).

CALCIUM IN T-LYMPHOCYTES: PROMINENT ROLE OF THE STIM-ORAI PATHWAY

In T-lymphocytes, Ca^{2+} ions are important for the activation of many enzymes including phospholipase C gamma (PLCγ), classical protein kinases C, for proper protein folding, for the accessibility of key enzymes in T-cell transduction, and as a second messenger (Vig and Kinet, 2009). Variations in the intracellular calcium concentration ($[Ca]_i$) are responsible for modulating the transcription of more than 75% of genes induced or down-regulated by T-cell receptor engagement in T-lymphocytes (Feske et al., 2001). The intracellular $[Ca]_i$ that decides the cellular fate is tightly regulated in both resting and activated conditions. The calcium concentration in the external medium is about 1–2 mM, whereas the $[Ca]_i$ is about 50–100 nM and depends on the calcium channels expressed at both the cell and endoplasmic reticulum (ER) membranes, on exchangers, pumps, … Activation of potassium channels that extrude the potassium from the cell is needed for supporting the electrochemical driving force allowing the calcium influx. In T-lymphocytes, TCR engagement results in a cascade of tyrosine kinase activation, the constitution of a platform transducing the signal with the recruitment of adapters and enzymes such as PLCγ that generates inositol trisphosphate (IP3) and diacylglycerol. IP3 binds to its receptors on the ER membrane leading to the release of ER Ca^{2+} stores, which induces a conformational change of STIM1, an ER Ca^{2+} sensor. STIM1 then localizes near the cell membrane, and activates the SOCC ORAI1 at the cell membrane (Barr et al., 2009; Oh-hora, 2009; Vig and Kinet, 2009; Zhou et al., 2010). The sustained entry of Ca^{2+} into the cell through ORAI channels is responsible for the activation of calcineurin, resulting in the nuclear translocation of the transcription factor NFAT as well as the activation of calmodulin kinase-dependent pathways. The severe immunodeficiency observed in mice or Humans with defective STIM1 (Picard et al., 2009) and ORAI1 testifies the importance of these molecules in T-cell biology (Partiseti et al., 1994; Feske et al., 2006, 2012).

However, this scheme accounts neither for the heterogeneity of calcium responses induced by TCR stimulation depending upon the state of activation and differentiation of T-lymphocytes nor for the possible implication of other calcium channels at the T-cell membrane.

Ca_v1 CHANNELS IN T-CELLS

An increasing line of evidence pleads for the involvement of Ca_v1 channels in T-lymphocyte biology (Kotturi et al., 2003, 2006; Stokes et al., 2004; Kotturi and Jefferies, 2005; Badou et al., 2006; Matza et al., 2008, 2009; Jha et al., 2009). Thus, the analysis of mice with ablation of the auxiliary subunits $Ca_vβ3$ (Jha et al., 2009) and $Ca_vβ4$ (Badou et al., 2006) and more recently of mice deleted for $Ca_v1.4$ (Omilusik et al., 2011) reveals the role of Ca_v1 channels in T-lymphocyte survival and activation. $Ca_v1.4$ was recently described as interacting with Vav and lck src kinase (Jha et al., 2009), which could result in Ca^{2+} entry required for maintaining $[Ca]_i$ and the ER Ca^{2+} stores (**Figure 1A**). As a consequence, $Ca_v1.4$ defective T-cells are more prone to apoptosis and have a reduced homeostatic proliferation capacity. Naïve $Ca_v1.4$ null T-cells also harbor defective calcium

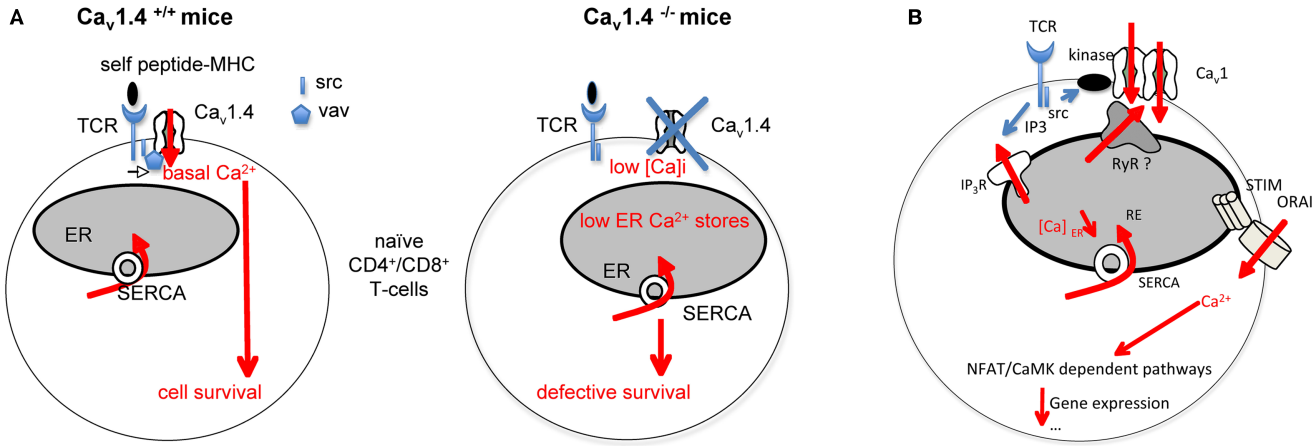

FIGURE 1 | Role of Ca$_v$1 channels in T-cell Ca^{2+} responses and functions. (A) Ca$_v$1.4 is found localized in preformed complexes containing src kinase and Vav. Self peptide-MHC interactions with the TCR, independently of the antigen specificity would induce a survival signal in naïve T-cells. This signal requires some calcium entry depending upon the Ca$_v$1.4 containing complex. Ca$_v$1.4 would be also important for maintaining correct endoplasmic reticulum (ER) Ca^{2+} stores. Ca$_v$1.4 null T-cells exhibit defective calcium homeostasis associated with defective survival. **(B)** The scheme depicts how we assume Ca$_v$1.2 channel regulation in Th2-cells. TCR activation would lead to src and PKC enzyme activation. Possible PKC-Ca$_v$1.2 interactions would induce Ca$_v$1.2 channel opening. Ca$_v$1.2 channels can interact with Ryanodine receptors (RyR) at the membrane of the endoplasmic reticulum (ER). These channels release Ca^{2+} from the ER into the cytosol. The depletion of ER Ca^{2+} stores would allow conformational changes of the Ca^{2+} sensor STIM and the subsequent activation of ORAI channels. IP3R, IP3 receptors; MHC, major histocompatibility complex; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; TCR, T-cell receptor.

influx upon TCR stimulation suggesting the involvement of these channels in TCR-dependent Ca^{2+} signaling (Omilusik et al., 2011). Interestingly, the human Timothy syndrome which is associated to mutation in gene encoding for Ca$_v$1.2 resulting in excessive Ca^{2+} entry is associated in most patients with an immunosuppression suggesting a role for Ca$_v$1.2 channels in immune functions (Liao and Soong, 2010). It will be interesting to determine if and how the Ca$_v$1.2 mutation affects immune cell functions.

Ca$_v$1 CHANNELS IN Th2-CELLS

Depending upon the strength of TCR stimulation, the chronicity of antigenic exposure, the route of antigen administration, and the cytokines present during T-cell differentiation, CD4$^+$ T-cells can differentiate into Th1, Th2, and Th17-cells that produce distinct sets of cytokines and exert different functions. In addition, these subpopulations express lineage specific and common transcription factors. Th1-cells produce gamma interferon (IFN-γ) and are implicated in the eradication of intracellular pathogens, viruses; Th2-cells produce interleukin (IL)-4, IL-5, and IL-13, contribute to the elimination of parasites and Th17, producing IL-17 and IL-22, participate in the elimination of extracellular pathogens as fungi. These subsets may also be pathogenic. Th1 and Th17 can promote autoimmune diseases, whereas Th2-cells can cause allergic diseases. Especially, Th2-cells can induce all the cardinal features of allergic asthma through all the cytokines they produce.

The calcium signature differs between Th1, Th2, and Th17-cells suggesting that components regulating calcium entry may differ between each T-cell subsets. The resting [Ca]i is the lowest in Th1, the highest in Th2, and intermediate in Th17. Conversely the TCR-dependent increase in [Ca]i is the highest in Th1, intermediate in Th17, and less important and sustained in Th2-cells, which could be related to the differential dependence of calcium-regulated transcription factors as NFAT, NFkB, and CREB (Dolmetsch et al., 1997) in the different T-cell subsets. It was suggested that these differences could result from lower equipment in pumps or in potassium channels required for maintaining the electrochemical driving force that supports calcium entry in Th2-cells, compared with the other T-cell subsets (Fanger et al., 2000). Our group identified voltage-dependent calcium Ca$_v$1.2 and Ca$_v$1.3 channels as selectively overexpressed in Th2-cells (Badou et al., 1997; Savignac et al., 2001, 2004; Gomes et al., 2006; Djata Cabral et al., 2010). Knocking down Ca$_v$1.2 and/or Ca$_v$1.3 α1 subunits by transfection with specific antisense oligodeoxynucleotides (Ca$_v$1AS) did not affect the proliferative response of Th2-cells but strongly impaired the TCR-dependent increase in [Ca]$_i$ and Th2 cytokine production without any effect on Th1-cells. We have then injected OVA-specific DO11.10 transgenic Th2-cells transfected or not with Ca$_v$1.2 plus Ca$_v$1.3 AS into BALB/c mice that were given intranasal OVA. Th2 Ca$_v$1AS localized into the lungs and proliferated as well as control Th2-cells. However they were unable to support a sustained inflammation characteristic of asthma. On the contrary, Th1 Ca$_v$1AS were as effective as control Th1-cells in the induction of inflammation. Antisense oligodeoxynucleotides were shown to remain localized into the airways when given by inhalation (Tanaka and Nyce, 2001). A mixture of Ca$_v$1.2 and Ca$_v$1.3 AS given by this route protected mice against the development of asthma (Djata Cabral et al., 2010), suggesting that these channels may represent an interesting new approach in the treatment of allergic diseases. Interestingly TCR stimulation is associated with polarized signaling as shown by an enrichment of Ca^{2+} (Lioudyno et al., 2008) and other ionic channels near the immune synapse, an area where the T-cell membrane contacts the antigen-presenting cell (Cahalan

and Chandy, 2009). It will be important to assess whether Ca_v1 channels traffic at the immune synapse upon TCR activation, in which areas and to identify the partners with which they associate.

Another important feature of the regulation of $[Ca]_i$ will be the understanding of ionic channels as potassium and non-specific cationic channels in Ca_v1 channel opening. Indeed K^+ channels comprising voltage and Ca^{2+}-activated channels maintain the electrochemical gradient of Ca^{2+} required for Ca^{2+} entry and tend to hyperpolarize the cell membrane favoring Ca_v1 channel inactivation. Conversely, TCR-dependent TRPM4 activation induces Na^+ entry described as limiting the Ca^{2+} entry and permitting Ca^{2+} oscillations (Launay et al., 2004). It would be interesting to determine if TRPM4 can favor Ca_v1 channel opening.

REGULATION OF Ca_V1 CHANNELS IN LYMPHOCYTES

All the authors showing the presence of Ca_v1 channels in T-lymphocytes agree that these channels are not voltage-operated in physiological conditions. Therefore, how they are regulated in T-lymphocytes must be explained. Differences in the sequence/structure of Ca_v1 channels in T-cells relative to the canonical forms in excitable cells have been reported and could provide an account for the absence of voltage sensitivity in T-cells (Stokes et al., 2004; Kotturi and Jefferies, 2005). However, the authors must demonstrate that the truncated Ca_v1 channels are true Ca^{2+} channels. The analysis of T-lymphocytes from $Ca_v1.4$ null and sufficient mice reveals the presence of voltage-gated currents in control cells, which were undetectable in null T-cells (Omilusik et al., 2011). Noticeably, the authors used peculiar conditions for their patch clamp experiments and mentioned that normal T-cells were pre-activated before recordings. This suggests that TCR activation could induce or enhance the number of Ca_v1 channels at the cell membrane. The TCR-induced opening of Ca_v1 channels may alternatively be explained by the existence of partners able to drive channel recruitment via two non-exclusive pathways: (i) post-translational modifications regulating $Ca_v1.2$ channel availability at the cell membrane without voltage change and/or (ii) modification of channel trafficking, targeting, recycling, or degradation induced by TCR stimulation. We demonstrate that the sequence of Ca_v1 channels in Th2-lymphocytes is similar to neuronal forms of the channel. However, Ca_v1 channels do not seem to be voltage-operated in Th2-lymphocytes. We have already demonstrated that TCR-induced L-type dependent calcium influx is at least sensitive to Src kinases and the PKC in an IL-4 producing T-cell hybridoma (Savignac et al., 2001). In fact, the application of PP2, an inhibitor of Src kinases or an inhibitor of PKCα on Th2-cells suppresses the Ca_v1 channel-dependent Ca^{2+} influx. In addition, we showed that PKC activator induced an entry of Ca^{2+}, suppressed by an antagonist of Ca_v1 channels (Savignac et al., 2001). These data mean that kinase activation is implicated in Ca_v1 dependent currents (**Figure 1B**). PKCα is a good candidate since $Ca_v1.2$ channels can be constitutively activated at the resting potential of smooth arteriolar cells due to their interaction with PKCα (Navedo et al., 2005; Santana and Navedo, 2010). Ryanodine receptors (RyR) are channels releasing Ca^{2+} from the ER into the cytosol. They are activated directly or not by Ca_v1 channels. It is not known if Ca_v1 channels interact with RyR in T-lymphocytes, inducing ER Ca^{2+} depletion and the activation of the STIM-ORAI pathway (**Figure 1B**).

The pending questions deal with how Ca_v1 channels work in lymphocytes and their integration with other channels to generate a specific calcium signature. The relationships between STIM, ORAI, and Ca_v1 are puzzling. STIM was shown as a negative regulator of Ca_v1 signaling (Park et al., 2010). The possibility of a checkpoint controlling ORAI versus Ca_v1 channel-dependent calcium responses merits to be explored.

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REFERENCES

Badou, A., Jha, M. K., Matza, D., Mehal, W. Z., Freichel, M., Flockerzi, V., et al. (2006). Critical role for the beta regulatory subunits of Cav channels in T lymphocyte function. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15529–15534. doi: 10.1073/pnas.0607262103

Badou, A., Savignac, M., Moreau, M., Leclerc, C., Pasquier, R., Druet, P., et al. (1997). $HgCl_2$-induced IL-4 gene expression in T cells involves protein kinase C-dependent calcium influx through L-type calcium channels. *J. Biol. Chem.* 272, 32411–32418. doi: 10.1074/jbc.272.51.32411

Barr, V. A., Bernot, K. M., Shaffer, M. H., Burkhardt, J. K., and Samelson, L. E. (2009). Formation of STIM and Orai complexes: puncta and distal caps. *Immunol. Rev.* 231, 148–159. doi: 10.1111/j.1600-065X.2009.00812.x

Cahalan, M. D., and Chandy, K. G. (2009). The functional network of ion channels in T lymphocytes. *Immunol. Rev.* 231, 59–87. doi: 10.1111/j.1600-065X.2009.00816.x

Djata Cabral, M., Paulet, P. E., Robert, B., Gomes, M. L., Renoud, M., and Savignac, C. (2010). Knocking-down Cav1 calcium channels implicated in Th2-cell activation prevents experimental asthma. *Am. J. Respir. Crit. Care Med.* 181, 1310–1317. doi: 10.1164/rccm.200907-1166OC

Dolmetsch, R. E., Lewis, R. S., Goodnow, C. C., and Healy, J. I. (1997). Differential activation of transcription factors induced by Ca2+ response amplitude and duration. *Nature* 386, 855–858. doi: 10.1038/386855a0

Fanger, C. M., Neben, A. L., and Cahalan, M. D. (2000). Differential Ca2+ influx, KCa channel activity, and Ca^{2+} clearance distinguish Th1 and Th2 lymphocytes. *J. Immunol.* 164, 1153–1160.

Feske, S., Giltnane, J., Dolmetsch, R., Staudt, L. M., and Rao, A. (2001). Gene regulation mediated by calcium signals in T lymphocytes. *Nat. Immunol.* 2, 316–324. doi: 10.1038/86318

Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S. H., Tanasa, B., et al. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441, 179–185. doi: 10.1038/nature04702

Feske, S., Skolnik, E. Y., and Prakriya, M. (2012). Ion channels and transporters in lymphocyte function and immunity. *Nat. Rev. Immunol.* 12, 532–547. doi: 10.1038/nri3233

Gomes, B., Savignac, M., Cabral, M. D., Paulet, P., Moreau, M., Leclerc, C., et al. (2006). The cGMP/protein kinase G pathway contributes to dihydropyridine-sensitive calcium response and cytokine production in TH2 lymphocytes. *J. Biol. Chem.* 281, 12421–12427. doi: 10.1074/jbc.M510653200

Jha, M. K., Badou, A., Meissner, M., McRory, J. E., Freichel, M., Flockerzi, V., et al. (2009). Defective survival of naive $CD8^+$ T lymphocytes in the absence of the beta3 regulatory subunit of voltage-gated calcium channels. *Nat. Immunol.* 10, 1275–1282. doi: 10.1038/ni.1793

Kotturi, M. F., Carlow, D. A., Lee, J. C., Ziltener, H. J., and Jefferies, W. A. (2003). Identification and functional characterization of voltage-dependent calcium channels in T lymphocytes. *J. Biol. Chem.* 278, 46949–46960. doi: 10.1074/jbc.M309268200

Kotturi, M. F., Hunt, S. V., and Jefferies, W. A. (2006). Roles of CRAC and Ca(V)-like channels in T cells: more than one gatekeeper? *Trends Pharmacol. Sci.* 27, 360–367. doi: 10.1016/j.tips.2006.05.007

Kotturi, M. F., and Jefferies, W. A. (2005). Molecular characterization of L-type calcium channel splice variants expressed in human T lymphocytes. *Mol. Immunol.* 42, 1461–1474. doi: 10.1016/j.molimm.2005.01.014

Launay, P., Cheng, H., Srivatsan, S., Penner, R., Fleig, A., and Kinet, J. P. (2004). TRPM4 regulates calcium oscillations after T cell activation. *Science* 306, 1374–1377. doi: 10.1126/science.1098845

Liao, P., and Soong, T. W. (2010). CaV1.2 channelopathies: from arrhythmias to autism, bipolar disorder, and immunodeficiency. *Pflugers Arch.* 460, 353–359. doi: 10.1007/s00424-009-0753-0

Lioudyno, M. I., Kozak, J. A., Penna, A., Safrina, O., Zhang, S. L., Sen, D., et al. (2008). Orai1 and STIM1 move

to the immunological synapse and are up-regulated during T cell activation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2011–2016. doi: 10.1073/pnas.0706122105

Lipscombe, D., Helton, T. D., and Xu, W. (2004). L-type calcium channels: the low down. *J. Neurophysiol.* 92, 2633–2641. doi: 10.1152/jn.00486.2004

Luo, X., Berdymammet, H., Jiang, N., Wang, Z. V., Tandan, S., Rakalin, A., et al. (2012). STIM1-dependent store-operated Ca2+ entry is required for pathological cardiac hypertrophy. *J. Mol. Cell. Cardiol.* 52, 136–147. doi: 10.1016/j.yjmcc.2011.11.003

Matza, D., Badou, A., Jha, M. K., Willinger, T., Antov, A., Sanjabi, S., et al. (2009). Requirement for AHNAK1-mediated calcium signaling during T lymphocyte cytolysis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9785–9790. doi: 10.1073/pnas.0902844106

Matza, D., Badou, A., Kobayashi, K. S., Goldsmith-Pestana, K., Masuda, Y., Komuro, A., et al. (2008). A scaffold protein, AHNAK1, is required for calcium signaling during T cell activation. *Immunity* 28, 64–74. doi: 10.1016/j.immuni.2007.11.020

McRory, J. E., Hamid, J., Doering, C. J., Garcia, E., Parker, R., Hamming, K., et al. (2004). The CACNA1F gene encodes an L-type calcium channel with unique biophysical properties and tissue distribution. *J. Neurosci.* 24, 1707–1718. doi: 10.1523/JNEUROSCI.4846-03.2004

Navedo, M. F., Amberg, G. C., Votaw, V. S., and Santana, L. F. (2005). Constitutively active L-type Ca2+ channels. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11112–11117. doi: 10.1073/pnas.0500360102

Oh-hora, M. (2009). Calcium signaling in the development and function of T-lineage cells. *Immunol. Rev.* 231, 210–224. doi: 10.1111/j.1600-065X.2009.00819.x

Omilusik, K., Priatel, J. J., Chen, X., Wang, Y. T., Xu, H., Choi, K. B., et al. (2011). The Ca(v)1.4 calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. *Immunity* 35, 349–360. doi: 10.1016/j.immuni.2011.07.011

Park, C. Y., Shcheglovitov, A., and Dolmetsch, R. (2010). The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science* 330, 101–105. doi: 10.1126/science.1191027

Partiseti, M., Le Deist, F., Hivroz, C., Fischer, A., Korn, H., and Choquet, D. (1994). The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodeficiency. *J. Biol. Chem.* 269, 32327–32335.

Picard, C., McCarl, C. A., Papolos, A., Khalil, S., Luthy, K., Hivroz, C., et al. (2009). STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N. Engl. J. Med.* 360, 1971–1980. doi: 10.1056/NEJMoa0900082

Robert, V., Triffaux, E., Savignac, M., and Pelletier, L. (2011). Calcium signalling in T-lymphocytes. *Biochimie* 93, 2087–2094. doi: 10.1016/j.biochi.2011.06.016

Robert, V., Triffaux, E., Savignac, M., and Pelletier, L. (2013). Singularities of calcium signaling in effector T-lymphocytes. *Biochim. Biophys. Acta.* 1833, 1595–1602. doi: 10.1016/j.bbamcr.2012.12.001

Santana, L. F., and Navedo, M. F. (2010). Natural inequalities: why some L-type Ca2+ channels work harder than others. *J. Gen. Physiol.* 136, 143–147. doi: 10.1085/jgp.200910391

Savignac, M., Badou, A., Moreau, M., Leclerc, C., Guery, J. C., Paulet, P., et al. (2001). Protein kinase C-mediated calcium entry dependent upon dihydropyridine sensitive channels: a T cell receptor-coupled signaling pathway involved in IL-4 synthesis. *FASEB J.* 15, 1577–1579.

Savignac, M., Gomes, B., Gallard, A., Narbonnet, S., Moreau, M., Leclerc, C., et al. (2004). Dihydropyridine receptors are selective markers of Th2 cells and can be targeted to prevent Th2-dependent immunopathological disorders. *J. Immunol.* 172, 5206–5212.

Stokes, L., Gordon, J., and Grafton, G. (2004). Non-voltage-gated L-type Ca^{2+} channels in human T cells: pharmacology and molecular characterization of the major alpha pore-forming and auxiliary beta-subunits. *J. Biol. Chem.* 279, 19566–19573. doi: 10.1074/jbc.M401481200

Tanaka, M., and Nyce, J. W. (2001). Respirable antisense oligonucleotides: a new drug class for respiratory disease. *Respir. Res.* 2, 5–9. doi: 10.1186/rr153

Vig, M., and Kinet, J. P. (2009). Calcium signaling in immune cells. *Nat. Immunol.* 10, 21–27. doi: 10.1038/ni.f.220

Zhou, Y., Meraner, P., Kwon, H. T., Machnes, D., Masatsugu, O. H., Zimmer, J., et al. (2010). STIM1 gates the store-operated calcium channel ORAI1 in vitro. *Nat. Struct. Mol. Biol.* 17, 112–116. doi: 10.1038/nsmb.1724

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Autophagy, a novel pathway to regulate calcium mobilization in T lymphocytes

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The T lymphocyte response initiates with the recognition of MHC/peptides on antigen presenting cells by the T cell receptor (TCR). After the TCR engagement, the proximal signaling pathways are activated for downstream cellular events. Among these pathways, the calcium-signaling flux is activated through the depletion of endoplasmic reticulum (ER) calcium stores and plays pivotal roles in T cell proliferation, cell survival, and apoptosis. In studying the roles of macroautophagy (hereafter referred to as autophagy) in T cell function, we found that a pathway for intracellular degradation, autophagy, regulates calcium signaling by developmentally maintaining the homeostasis of the ER. Using mouse genetic models with specific deletion of autophagy-related genes in T lymphocytes, we found that the calcium influx is defective and the calcium efflux is increased in autophagy-deficient T cells. The abnormal calcium flux is related to the expansion of the ER and higher calcium stores in the ER. Because of this, treatment with the ER sarco/ER Ca^{2+}-ATPase pump inhibitor, thapsigargin, rescues the calcium influx defect in autophagy-deficient T cells. Therefore, autophagy regulates calcium mobilization in T lymphocytes through ER homeostasis.

Keywords: autophagy, calcium flux, T lymphocytes, ER homeostasis, ER-phagy

INTRODUCTION

The highly conserved intracellular pathway, autophagy, degrades long-lived proteins, or damaged/extra organelles for quality control purposes to protects cells from death, or to provide energy during stress conditions (1). Using mouse genetic models, in which specific autophagy-related genes (Atgs) are deleted and autophagic pathways are blocked, our lab and other groups have found that autophagy-related molecules are expressed in T lymphocytes and T cell receptor TCR stimulation activates autophagy processing pathway (2–4). Autophagy developmentally regulates the homeostasis of endoplasmic reticulum (ER) and mitochondria (5, 6). ER is expanded when the autophagy pathway is impaired in T lymphocytes (7).

A physiological function of ER in T lymphocytes is the initiation of calcium flux after TCR engagement. The current model for calcium flux downstream of TCR activation is store-operated Ca^{2+} entry (SOCE) and this is mediated by the opening of Ca^{2+} release-activated Ca^{2+} (CRAC) channels on the T cell surface, which is in turn initiated by the depletion of ER calcium stores (8). Molecular mechanistic studies indicate that the ER-resident molecule, stromal interaction molecule 1 (STIM1), senses the calcium concentration of ER stores, redistributes itself and binds a pore subunit of CRAC, ORAI1, to begin the calcium influx into T cells (9–11). Calcium flux and signaling in T lymphocytes are tuned at different levels. We found that the calcium mobilization in T lymphocytes is also regulated by autophagy. Autophagy regulates the volume of the ER in both $CD4^+$ and $CD8^+$ T lymphocytes. Expanded ER leads to increased calcium stores when autophagy is impaired. Depletion of calcium stores is incomplete after TCR stimulation and the redistribution of STIM1 is severely reduced. Finally, calcium influx is much lower in autophagy-deficient T lymphocytes (7). Here we review how autophagy regulates the calcium mobilization in T lymphocytes.

THE CALCIUM-SIGNALING PATHWAY IN T LYMPHOCYTES

After the initial TCR-MHC/peptide contact, activation of the Src-family tyrosine kinase, Lck, leads to the phosphorylation of tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAMs) in CD3 chains of the TCR/CD3 complex. Following the phosphorylation of ITAMs, the Syk family kinase ZAP70 is recruited to the TCR/CD3 complex, phosphorylated, and activated by the tyrosine kinase, Lck. Next, ZAP70 phosphorylates and activates the linker for activation of T cells (LAT) and SLP-76. Then phosphatidylinositol-3-kinases (PI3K) are activated and phosphatidylinositol (3,4,5) triphosphate (PIP3) is produced. Following this, the inducible T cell kinase (Itk) is recruited and interacts with LAT and SLP-76 (12). This sequential cascade spreads, activating several different signaling pathways in the proximal signaling transduction in T lymphocytes. Among these pathways, calcium-signaling starts with the activation of phospholipase Cγ1 (PLCγ1) by Itk. PLCγ1 hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to produce the secondary messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). DAG activates PKC-θ and MAPK/Erk pathways. IP3 binds to the IP3 receptor on the ER membrane to release calcium stores from the ER lumen (marked with open arrow head in **Figure 1**) in order to initiate calcium mobilization and activate further downstream signals in T lymphocytes (13).

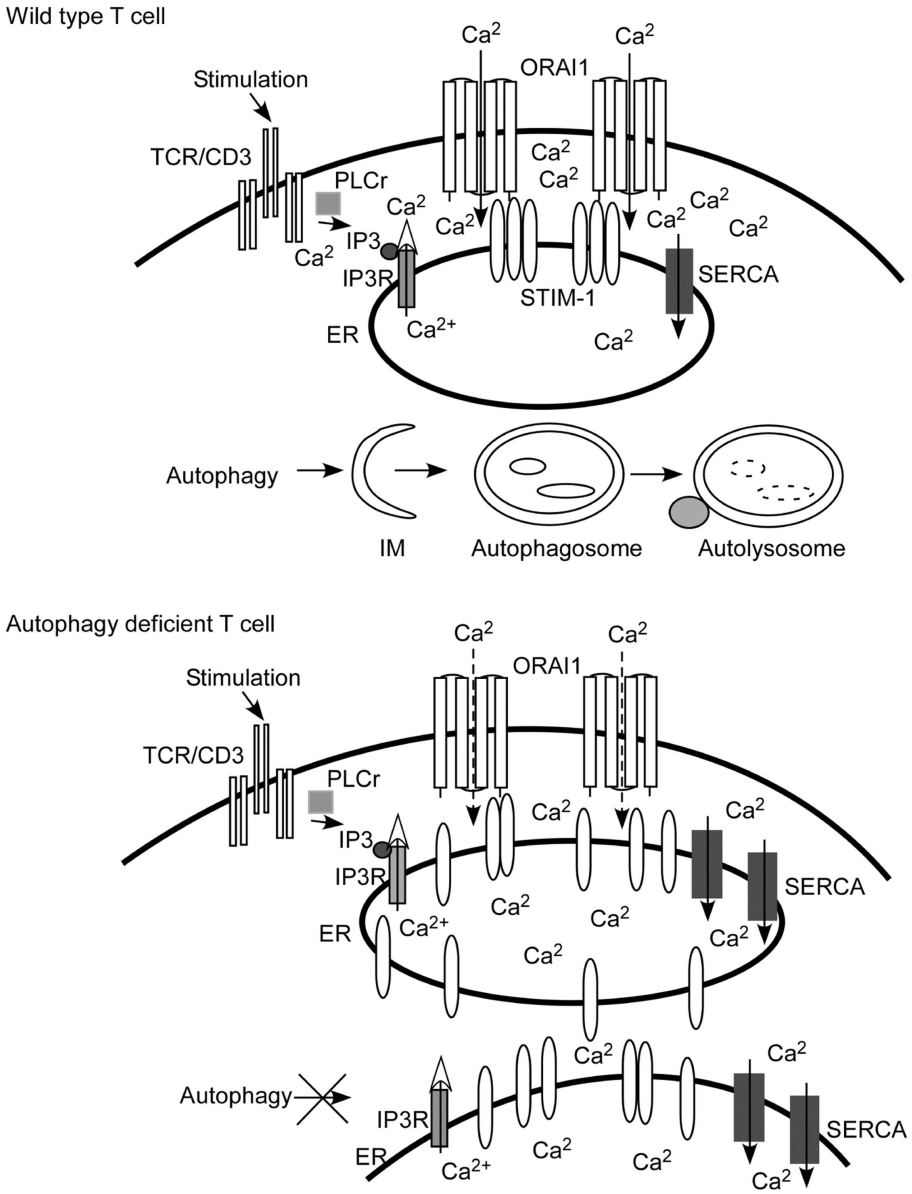

FIGURE 1 | Autophagy regulates calcium mobilization through the control of endoplasmic reticulum (ER) homeostasis. Autophagy is activated when there are damaged, senescent, or extra organelles in order to maintain normal ER contents. In T lymphocytes, inositol 1,4,5-trisphosphate (IP3) is produced after TCR engagement. IP3 binds with the IP3 receptor (IP3R) expressed on ER to initiate the depletion of calcium stores from the ER lumen (marked with an open arrow head). The calcium sensor and ER-resident molecule stromal interaction molecule 1 (STIM1) oligomerizes, and redistributes toward the ER plasma membrane junction after the depletion of calcium stores. Then STIM1 interacts with the pore subunit of Ca^{2+} release-activated Ca^{2+} (CRAC) channels, ORAI1, to open CRAC channels. Extracellular calcium fluxes through CRAC channels into the cytoplasm of T cells (visualized as Ca^{2+} inside of the cells). When autophagy is ablated, as shown in the cartoon figure of autophagy-deficient T cells, the contents of the ER are expanded. Calcium stores are increased since the ER is expanded and more sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPase (SERCA) are expressed. The depletion of calcium stores is incomplete and less STIM1 redistributes to the ER plasma junction. Therefore, less CRAC is opened. The end result is that calcium influx is defective compared to that of wild type T cells. IM, isolation membrane.

The molecular mechanism of the opening of CRAC channels is mediated by the interaction between the ER-resident protein stromal interaction molecule (STIM) 1/2 (14) with CRAC channel components, ORAI protein (ORAI1 and its homologs ORAI2 and ORAI3) (10). ORAI1, ORAI2, and ORAI3 are widely transcribed in different tissues and ORAI1 is the dominant component of the CRAC channel in T lymphocytes (15, 16). Both STIM1 and STIM2 are expressed in T lymphocytes. However, STIM1 is the predominant regulator for SOCE in T cells, while the STIM2 plays a relatively less important role during SOCE (16, 17). STIM senses the concentration of ER calcium stores through an N-terminal EF-hand and a sterile α motif domain (EF-SAM) (18),

forms oligomers, and redistributes itself toward a plasma membrane junction after calcium depletion from ER stores. Then the C-terminus of STIM1 interacts with the CRAC channel components ORAI1 to open the CRAC channels on the T cell surface (19). Consequent extracellular calcium influxes into T cells further activate downstream molecules of TCR signal transduction pathways. The influx of calcium activates the serine/threonine phosphatase calcineurin, which in turn phosphorylates nuclear factor of activated T cells (NFAT). NFAT translocates to nucleus to turn on the transcription of target genes, such as the cytokines IL-2, IL-17A, IL-22, IL-21, and the transcription factor Foxp3 depending on the situation (20–22). This signal transduction in T lymphocytes is finely regulated by different mechanisms.

AUTOPHAGY IN T LYMPHOCYTES

Autophagy is a highly conserved cellular homeostasis and degradation pathway present in all eukaryotic species (23, 24). According to specific characteristics, three different types of autophagy have been described, termed microautophagy, macroautophagy, and chaperone-mediated autophagy (25). Most research focuses on macroautophagy. Macroautophagy degrades long-lived proteins, provides energy during stress conditions, maintains organellar homeostasis, and eliminates various invading intracellular pathogens (26). Panoply of cellular stress conditions, such as growth factor withdrawal, nutrient depletion, or T cell activation can activate the autophagy pathway. Autophagy starts with an overtly crescent membrane structure, called an isolation membrane (IM) in mammalian cells and a phagophore in yeast cells. These membranes are originally derived from Golgi membranes (27), plasma membrane (28), mitochondria (29), or ER (30).

In a manner remarkably homologous to the yeast system, two kinase complexes are essential for the induction of autophagy in mammalian cells. One is the class III PI3K complex and the other is UNC-51-like kinase (ULK) complex. The PI3K complex is composed of the class III PI3K catalytic subunit Vps34, the class III PI3-kinase regulatory subunit p150 (the homolog of Vps15 in yeast), Beclin 1 (the homolog of Vps30/Atg6 in yeast), and Barkor [Beclin 1-associated autophagy-related key regulator, also named KIAA0831 (31), or the Atg14-like molecule (Atg14L) (32), the homolog of Atg14 in yeast] (33). Several Beclin 1 interacted molecules, such as UV-irradiation-resistance-associated gene (UVRAG) (34), vacuole membrane protein 1 (VMP1) (35), activating molecule in Beclin 1-regulated autophagy 1 (Ambra1) (36), Bif-1 (37), and Rubicon (38) are also present in the PI3K complex and regulate autophagy. Vps34 phosphorylates phosphatidylinositol (PI) to produce phosphatidylinositol-3-phosphate (PI3P). The energy sensor, AMP-activated protein kinase (AMPK), phosphorylates T163/S165 of Vps34 to reduce the production of PI3P and therefore inhibits the induction of autophagy. While under conditions of nutrient stress, AMPK phosphorylates S91/S94 of Beclin 1 to activate the autophagic processing pathway. Atg14L distinguishes between nutrient rich or starvation conditions through the inhibition of the phosphorylation of Vps34 induced by AMPK, but promotes the phosphorylation of Beclin 1 caused by AMPK under starvation conditions (39). In T lymphocytes, Vps34 controls the trafficking, recycling, and signaling capacity of the IL-7 receptor (IL-7R), which provides a major survival signal for naïve T cells (40). In another model, Vps34-deficient T cells showed impaired autophagy and abnormal homeostasis of mitochondria (41). The ULK complex includes the mammalian Atg13, FIP200 (Atg17 in yeast) (42), Atg101 (43, 44), and one ULK1 or one ULK2. ULK is the homologous molecule of the serine/threonine kinase Atg1 in yeast. mTOR phosphorylates Atg13, ULK1, and ULK2, and inhibits ULK1 and ULK2 kinase activity to inhibit autophagy induction. Atg13 mediates the interactions between ULK1/2 and FIP200, and is essential for the phosphorylation of FIP200 by ULK (45). Atg101 is required for the stability and phosphorylation of ULK and Atg13 (43, 44).

During the elongation phase, the IM is further expanded and directed by autophagy-related molecules to form a characteristic double membrane structure, termed an autophagosome, to enwrap cytosolic materials. The enveloped components of the autophagosome can be long-lived proteins, organelles, or even invading pathogens (46). Two protein/lipid conjugation systems mediated by Atg molecules regulate autophagosome formation outward from the IM structures. One is the Atg12-conjugation system and the other is the microtubule-associated protein 1 light chain 3 (LC3, Atg8 in the yeast system)-conjugation system. Atg7, an ubiquitin E1-like molecule, is involved in both conjugation systems. Atg10 and Atg3 are ubiquitin E2-like molecules and participate in either the Atg12 or LC3-conjugation system, respectively. Atg12, Atg5, and Atg16L form a large complex (47), the culmination of the Atg12-conjugation system, which further functions as an ubiquitin E3-like molecule to enhance the formation of the lipid form of phosphatidylethanolamine (PE)-LC3 (LC3-II) in the LC3-conjugation system. The lipid form of LC3 (LC3-II) is widely used as a marker for the detection of autophagy induction (48). Finally, at the maturation stage, the autophagosomes fuse with preexisting lysosomes to become mature autolysosomes and lysosomal enzymes degrade the enclosed materials. Macromolecular transporters in the autolysosome then allow for the recycling of degraded materials back to the cytoplasm (49, 50).

The discovery of autophagy related to the adaptive immune system was first reported in the late 1960s. Abnormal granules were observed in human lymphocytes from sarcoidosis patients treated with chloroquine and these granules in the cytosol of lymphocytes were hypothesized as autophagy-related structures (51). In 1984, Seglen first identified that there was autophagosome formation in human primary lymphocytes as well as in leukemic cells (52). In 2004, Gerland reported that several Atgs were expressed in long-term (>14 weeks) cultured human $CD8^+$ T cells. Autophagy was induced in these senescent cells and related to cell death (53). In 2006, Espert found that HIV-1 envelope glycoproteins induced autophagy and accumulation of Beclin 1 in HIV-uninfected $CD4^+$ T cells through CXCR4 to cause cell death (54). Our lab and other groups have thoroughly analyzed the autophagic processing pathways in mouse T lymphocytes using mouse genetic models (2, 3, 55). Many Atgs, such as Atg5, Beclin 1 and LC3, are expressed in thymocytes, most highly during double negative (DN) thymocyte development, but also expressed in mature $CD4^+$ and $CD8^+$ T cell sub-populations. Both $CD4^+$ and $CD8^+$ lymphocytes continue expressing autophagy genes after TCR stimulation and activation. The expression of autophagy machinery was further confirmed by the observation of characteristic double membrane structures

of autophagosomes in T lymphocytes by electron microscopy (EM). Compared to freshly isolated T lymphocytes, the formation of LC3-II was moderately promoted by starvation, but strongly induced by anti-CD3 antibody-mediated TCR stimulation. The detection of LC3-II indicates that autophagic flux occurs in T lymphocytes after T cells are activated by TCR stimulation (3). By using mouse genetic models in which Atg5 (3, 6), Atg7 (5, 55), Atg3 (56), Vps34 (41), or Beclin 1 (57), were specifically deleted in T lymphocytes, it is apparent that autophagy developmentally regulates the homeostasis of organelles such as mitochondria or ER in T lymphocytes (5–7). Through the use of BAC Beclin 1-GFP transgenic mice, Arsov reported that the expression of Beclin 1 was developmentally regulated in both T and B lymphocytes. Beclin 1-GFP is highly expressed in DN thymocytes, down-regulated in double positive (DP) thymocytes and re-expressed in mature thymocytes (4). On top of this, recombination activating gene 1 $(Rag1)^{-/-}$ chimeric mice reconstituted with Beclin $1^{-/-}$ embryonic stem cells (ESCs) indicated that Beclin 1 is involved in the development of early progenitors of thymocytes (58). The functions of autophagy in T lymphocytes have been reviewed in detail (59, 60). Autophagy is essential for the survival of mature T lymphocytes (3, 56). More specifically, autophagy regulates calcium mobilization in T lymphocytes (7).

AUTOPHAGY DEVELOPMENTALLY REGULATES THE HOMEOSTASIS OF ER IN T CELLS

One of the basic physiological functions of autophagy is to remove damaged, senescent, or extra organelles before they become cytotoxic. Contrary to the non-selective bulk degradation of cytosol materials, autophagy selectively reduces organelles to maintain homeostatic volumes. Selective autophagy for the degradation of ER and mitochondria are termed as ER-phagy (or reticulophagy) (61, 62) and mitophagy (63), respectively. ER-phagy can be induced by starvation or the unfolded protein response (UPR). ER-phagy eliminates the expanded ER volume when the UPR is not needed (62). Our data suggests that autophagy maintains the volumes of organelles in certain levels at different stages during T cell development. Analysis of mouse genetic models demonstrates that the deficiency of Atg5, Atg7, Atg3, or Vps34 blocks the autophagy machinery in T lymphocytes. Both the mitochondrial contents and ER volumes are abnormal in autophagy-deficient thymocytes and mature $CD4^+$ and $CD8^+$ T cells (5–7, 41, 56).

During thymocyte development, the contents of ER are dynamic. The thymocytes at DN stage have highest level of the ER volumes and ER content decreases at the DP and single positive (SP) stages. Mature T cells have relatively lower ER contents. Autophagy-deficient thymocytes have similar ER contents in the DN, DP, and SP thymocytes compared to that of wild type thymocytes. However, the ER contents expand in both mature $CD4^+$ and $CD8^+$ autophagy-deficient T cells. Therefore, autophagy maintains ER membrane and content at relative lower levels in mature T cell populations (7). In an inducible-deletion system, the level of ER or mitochondria membranes start increasing at day 10 and significantly increase by day 21 after Atg3 is inducibly deleted and autophagy processing pathway is blocked. Therefore, autophagy regulates the homeostasis of the ER in a temporal manner (56).

Autophagy provides protective roles for cell survival during ER stress (64). Autophagy-deficient T cells constitutively express ER-stress markers, such as disulfide isomerase (PDI), and ER chaperones, such as glucose-regulated protein 78 (Grp78), and Grp94 (7). This suggests that the ER-stress response is activated in autophagy-deficient T cells and ER-stress caused by abnormal homeostasis of ER is one of the reasons why autophagy-deficient T cells show increased susceptibility to apoptosis.

CALCIUM STORES ARE INCREASED IN AUTOPHAGY-DEFICIENT T CELLS

One of the main functions of the ER in T lymphocytes is to regulate calcium mobilization. Upon TCR engagement, the calcium flux starts with the depletion of calcium stores in the ER lumen. The calcium stores are dramatically increased in autophagy impaired T lymphocytes. The higher calcium stores in ER are consistent with the expansion of ER contents in autophagy-deficient mature $CD4^+$ and $CD8^+$ T cells. The calcium stores are maintained by the sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPase (SERCA) pumps expressed on the surface of the ER. Autophagy-deficient T cells express twofold more SERCA pumps than wild type T cells (7). Over the life span of the cell, more calcium is imported by the SERCA pumps in autophagy-deficient T lymphocytes, which leads to increased calcium stores. Higher expressed SERCA pumps also affect the depletion of calcium stores in ER after TCR engagement in autophagy-deficient T cells.

The abnormal and excessive calcium stores in the ER and defective depletion directly affect the oligomerization and redistribution of the calcium sensor STIM1. Although autophagy-deficient T cells express more STIM1, the puncta intensity of STIM1 after TCR stimulation is much lower in autophagy-deficient T cells than that of wild type cells. The autophagy-deficient T cells express a similar level of ORAI1 compared to that of wild type T cells and CRAC channels remain intact in autophagy-deficient T cells (7). Therefore, the insufficient opening of CRAC channels is caused by the higher calcium stores, incomplete depletion, and less oligomerization of STIM1 after TCR activation in autophagy-deficient T cells.

AUTOPHAGY REGULATES THE CALCIUM MOBILIZATION THROUGH THE CONTROL OF ER HOMEOSTASIS IN T CELLS

Mouse genetic models provide novel methods to investigate the physiological functions of autophagy. The specific deletion of Atg7, Atg3, or other Atgs blocks the autophagy processing pathway. The calcium influx in autophagy-deficient T cells is defective upon receipt of TCR signaling. The reason behind the calcium influx defect is due to the higher calcium stores mediated by the expansion of ER organelles in autophagy impaired T lymphocytes. The SERCA pump inhibitor, thapsigargin, inhibits the SERCA pumps from taking up calcium, corrects oligomerization of STIM1, and rescues the defective calcium influx in autophagy-deficient T lymphocytes. A model of the regulation of calcium mobilization in T lymphocytes by autophagy is summarized in **Figure 1**. Individual cell calcium influx analysis indicates that it takes longer for calcium influx in autophagy-deficient T cells to reach the peak of $[Ca^{2+}]_i$, in addition to less total calcium influx after stimulation. The average time for wild type cells to reach the peak of $[Ca^{2+}]_i$ is 56 s, while it takes 76 s for autophagy-deficient T cell to reach a lower peak of $[Ca^{2+}]_i$ (7).

Autophagy also regulates the homeostasis of mitochondria (5, 6). In contrast to the constant trimming of the ER, autophagy decreases the contents of mitochondria from SP compartment of thymocytes to mature $CD4^+$ or $CD8^+$ T cells in a developmentally stage-specific manner. Although there is abnormal expansion of total mitochondrial levels in autophagy-deficient T cells and mitochondria also contributes to the regulation of calcium flux through taking up calcium from the cytosol, the defect of calcium influx in autophagy-deficient T cells is not related to the abnormal expansion of mitochondria. When autophagy-deficient T cells were treated with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) before or after stimulation with thapsigargin, the calcium influx was not different between wild type and autophagy-deficient T cells (our unpublished data).

Although the calcium storage and influx is defective in autophagy-deficient T cells, the IL-2 production is not decreased and actually more IL-2 is produced in autophagy-deficient T cells. Since the calcium influx is not totally abolished in autophagy-deficient T cells, the observed level of calcium proves to be sufficient for turning on the transcription and translation of IL-2 (7). However, Hubbard reported that upon activation $CD4^+$ T cells, IL-2, and IFN-γ production were defective in Atg7-deficient T cells (55). A recent study demonstrates that the autophagy adaptor protein, p62, is important for the ability of Bcl10 to signal to NF-κB, but also for its degradation by autophagy, explaining the enhanced IL-2 production by autophagy-deficient T cells (65).

The regulation of the ER by autophagy is not completely surprising. The ER is one of the purported sites of membrane donor activity for autophagy (66). Additionally, ER stress and the UPR are potent inducers of autophagy (67, 68). The knockdown of inositol trisphosphate receptor (IP3R) expressed on ER membranes or treatment with an IP3R antagonist can induce autophagy (69). Therefore, when autophagy is genetically inhibited for long periods of time, autophagosomes are not formed from the ER-mitochondrial membrane junctions. Since the ER membranes are not trimmed to provide substrates for the elongation of autophagic membranes, they accumulate and express ER-stress markers (7). This process is especially important in cells that make large amounts of secreted proteins, such as plasma cells (70, 71), but also in cells with very little cytoplasmic volume, such as naïve T cells (7). Thus, autophagy is a pro-survival stress response.

AUTOPHAGY-RELATED CALCIUM HOMEOSTASIS IS INVOLVED IN THE PATHOGENESIS OF DISEASES

A mutation in the gene encoding α-synuclein has been shown to be related to the familial forms of Parkinson disease. Cellular α-synuclein maintains the morphology of mitochondria and regulates pools of Ca^{2+} transferred from ER stores to the mitochondria. Homeostatic levels of α-synuclein control the uptake of calcium by mitochondria. Autophagic flux is enhanced when calcium uptake in mitochondria is reduced, due to the inability of mitochondria to buffer Ca^{2+} concentrations (72). Another report indicates that increased intra-axonal calcium levels are followed by the activation of autophagy-mediated axonal degeneration, which often accompanies traumatic nerve injury or neurodegenerative diseases (73). Autophagy induced by calcium signaling is also involved in cell survival during hypoxia-induced stress. In a mouse liver ischemia-reperfusion injury model, the Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) is activated and induces autophagy to protect hepatocytes from oxidative-stress-induced cell death (74).

In cancer cells, autophagy is often associated with enhanced cell survival. In breast cancer cells, nutrient and growth factor withdrawal decreases ATP and activates Ca^{2+}/calmodulin-dependent protein kinase III, the eukaryotic elongation factor-2 kinase (eEF-2 kinase). Finally, autophagy provides protective roles for cancer cells. Knockdown of eEF-2 kinase inhibits autophagy and imparts sensitivity of breast cancer cells to treatments based on the inhibition of growth factors (75). However, the plant indole, diindolylmethane, found in cruciferous vegetables, has antineoplastic activity through the regulation of autophagy to attenuate the growth of cancer cells. Diindolylmethane induces ER stress in ovarian cancer cells and increases cytosolic calcium, which activates AMPK. The activation of AMPK promotes autophagy and inhibits ovarian cancer cell growth (76). Thus autophagy helps cells adapt to ever changing cellular conditions related to stress, metabolism, but acts as a brake on uncontrolled proliferation.

CONCLUSION

Autophagy regulates the homeostasis of ER in a temporal manner. Abnormal expansion of ER increases the calcium stores in the ER lumen. The excessive calcium stores cause the incomplete depletion of resident ER calcium stores and directly affect the oligomerization and redistribution of STIM1 upon TCR activation. Finally, CRAC channels cannot be opened completely and eventually the calcium influx is much lower after T cells are activated. This suggests that autophagy is a novel pathway to regulate the calcium mobilization in T lymphocytes. When published data are taken into consideration, it is apparent that increased cytosolic calcium could inhibit mTOR to induce autophagy in human tumor cell lines and this pathway is mediated by Ca^{2+}/calmodulin-dependent kinase kinase-β (CaMKK-β) and AMPK. Ectopic expression of Bcl-2 in ER decreased the calcium stored in the ER and inhibited the autophagy induced by increased cytosolic calcium (77). The inhibition of the calcium-signaling impacts autophagy. In T cell lines, it has been demonstrated that glucocorticoids promote autophagy through the downregulation of Fyn and inhibition of IP3-mediated calcium signaling (78). It seems that the autophagic pathway and calcium mobilization are reciprocal and delicately intertwined.

REFERENCES

1. Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. *Nat Cell Biol* (2010) **12**:814–22. doi:10.1038/ncb0910-814
2. Li C, Capan E, Zhao Y, Zhao J, Stolz D, Watkins SC, et al. Autophagy is induced in CD4+ T cells and important for the growth factor-withdrawal cell death. *J Immunol* (2006) **177**: 5163–8.
3. Pua HH, Dzhagalov I, Chuck M, Mizushima N, He YW. A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J Exp Med* (2007) **204**:25–31. doi:10.1084/jem.20061303
4. Arsov I, Li X, Matthews G, Coradin J, Hartmann B, Simon AK, et al. BAC-mediated transgenic expression of fluorescent autophagic protein Beclin 1 reveals a role for Beclin 1 in lymphocyte development. *Cell Death Differ* (2008) **15**:1385–95. doi:10.1038/cdd.2008.59

5. Pua HH, Guo J, Komatsu M, He YW. Autophagy is essential for mitochondrial clearance in mature T lymphocytes. *J Immunol* (2009) **182**:4046–55. doi:10.4049/jimmunol.0801143
6. Stephenson LM, Miller BC, Ng A, Eisenberg J, Zhao Z, Cadwell K, et al. Identification of Atg5-dependent transcriptional changes and increases in mitochondrial mass in Atg5-deficient T lymphocytes. *Autophagy* (2009) **5**:625–35. doi:10.4161/auto.5.5.8133
7. Jia W, Pua HH, Li QJ, He YW. Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. *J Immunol* (2011) **186**:1564–74. doi:10.4049/jimmunol.1001822
8. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* (2010) **28**:491–533. doi:10.1146/annurev.immunol.021908.132550
9. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, et al. STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. *Nature* (2005) **437**:902–5. doi:10.1038/nature04147
10. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* (2006) **443**:230–3. doi:10.1038/nature05122
11. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, et al. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* (2009) **136**:876–90. doi:10.1016/j.cell.2009.02.014
12. Andreotti AH, Schwartzberg PL, Joseph RE, Berg LJ. T-cell signaling regulated by the Tec family kinase, Itk. *Cold Spring Harb Perspect Biol* (2010) **2**:a002287. doi:10.1101/cshperspect.a002287
13. Paul S, Schaefer BC. A new look at T cell receptor signaling to nuclear factor-kappaB. *Trends Immunol* (2013) **34**:269–81. doi:10.1016/j.it.2013.02.002
14. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr., et al. STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr Biol* (2005) **15**:1235–41. doi:10.1016/j.cub.2005.05.055
15. Gwack Y, Srikanth S, Feske S, Cruz-Guilloty F, Oh-Hora M, Neems DS, et al. Biochemical and functional characterization of Orai proteins. *J Biol Chem* (2007) **282**:16232–43. doi:10.1074/jbc.M609630200
16. Shaw PJ, Feske S. Regulation of lymphocyte function by ORAI and STIM proteins in infection and autoimmunity. *J Physiol* (2012) **590**:4157–67. doi:10.1113/jphysiol.2012.233221
17. Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, et al. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol* (2008) **9**:432–43. doi:10.1038/ni1574
18. Stathopulos PB, Zheng L, Li GY, Plevin MJ, Ikura M. Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell* (2008) **135**:110–22. doi:10.1016/j.cell.2008.08.006
19. Liou J, Fivaz M, Inoue T, Meyer T. Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca2+ store depletion. *Proc Natl Acad Sci U S A* (2007) **104**:9301–6. doi:10.1073/pnas.0702866104
20. Kim HP, Korn LL, Gamero AM, Leonard WJ. Calcium-dependent activation of interleukin-21 gene expression in T cells. *J Biol Chem* (2005) **280**:25291–7. doi:10.1074/jbc.M501459200
21. Liu XK, Clements JL, Gaffen SL. Signaling through the murine T cell receptor induces IL-17 production in the absence of costimulation, IL-23 or dendritic cells. *Mol Cells* (2005) **20**:339–47.
22. Vaeth M, Schliesser U, Muller G, Reissig S, Satoh K, Tuettenberg A, et al. Dependence on nuclear factor of activated T-cells (NFAT) levels discriminates conventional T cells from Foxp3+ regulatory T cells. *Proc Natl Acad Sci U S A* (2012) **109**:16258–63. doi:10.1073/pnas.1203870109
23. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* (2011) **469**:323–35. doi:10.1038/nature09782
24. Deretic V, Jiang S, Dupont N. Autophagy intersections with conventional and unconventional secretion in tissue development, remodeling and inflammation. *Trends Cell Biol* (2012) **22**:397–406. doi:10.1016/j.tcb.2012.04.008
25. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* (2011) **147**:728–41. doi:10.1016/j.cell.2011.10.026
26. Schmid D, Munz C. Innate and adaptive immunity through autophagy. *Immunity* (2007) **27**:11–21. doi:10.1016/j.immuni.2007.07.004
27. Geng J, Nair U, Yasumura-Yorimitsu K, Klionsky DJ. Post-Golgi Sec proteins are required for autophagy in *Saccharomyces cerevisiae*. *Mol Biol Cell* (2010) **21**:2257–69. doi:10.1091/mbc.E09-11-0969
28. Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC. Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* (2010) **12**:747–57. doi:10.1038/ncb2078
29. Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK, et al. Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* (2010) **141**:656–67. doi:10.1016/j.cell.2010.04.009
30. Chen X, Li M, Chen D, Gao W, Guan JL, Komatsu M, et al. Autophagy induced by calcium phosphate precipitates involves endoplasmic reticulum membranes in autophagosome biogenesis. *PLoS ONE* (2012) **7**:e52347. doi:10.1371/journal.pone.0052347
31. Itakura E, Kishi C, Inoue K, Mizushima N. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell* (2008) **19**:5360–72. doi:10.1091/mbc.E08-01-0080
32. Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat Cell Biol* (2009) **11**:468–76. doi:10.1038/ncb1854
33. Sun Q, Fan W, Chen K, Ding X, Chen S, Zhong Q. Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* (2008) **105**:19211–6. doi:10.1073/pnas.0810452105
34. Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, et al. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* (2006) **8**:688–99. doi:10.1038/ncb1426
35. Molejon MI, Ropolo A, Re AL, Boggio V, Vaccaro MI. The VMP1-Beclin 1 interaction regulates autophagy induction. *Sci Rep* (2013) **3**:1055. doi:10.1038/srep01055
36. Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, et al. Ambra1 regulates autophagy and development of the nervous system. *Nature* (2007) **447**:1121–5.
37. Yamaguchi H, Woods NT, Dorsey JF, Takahashi Y, Gjertsen NR, Yeatman T, et al. SRC directly phosphorylates Bif-1 and prevents its interaction with Bax and the initiation of anoikis. *J Biol Chem* (2008) **283**:19112–8. doi:10.1074/jbc.M709882200
38. Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat Cell Biol* (2009) **11**:385–96. doi:10.1038/ncb1846
39. Kim J, Kim YC, Fang C, Russell RC, Kim JH, Fan W, et al. Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell* (2013) **152**:290–303. doi:10.1016/j.cell.2012.12.016
40. McLeod IX, Zhou X, Li QJ, Wang F, He YW. The class III kinase Vps34 promotes T lymphocyte survival through regulating IL-7Ralpha surface expression. *J Immunol* (2011) **187**:5051–61. doi:10.4049/jimmunol.1100710
41. Willinger T, Flavell RA. Canonical autophagy dependent on the class III phosphoinositide-3 kinase Vps34 is required for naive T-cell homeostasis. *Proc Natl Acad Sci U S A* (2012) **109**:8670–5. doi:10.1073/pnas.1205305109
42. Hara T, Takamura A, Kishi C, Iemura S, Natsume T, Guan JL, et al. FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. *J Cell Biol* (2008) **181**:497–510. doi:10.1083/jcb.200712064
43. Hosokawa N, Sasaki T, Iemura S, Natsume T, Hara T, Mizushima N. Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* (2009) **5**:973–9. doi:10.4161/auto.5.7.9296
44. Mercer CA, Kaliappan A, Dennis PB. A novel, human

Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. *Autophagy* (2009) **5**:649–62. doi:10.4161/auto.5.5.8249
45. Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* (2009) **20**:1992–2003. doi:10.1091/mbc.E08-12-1249
46. Schmid D, Dengjel J, Schoor O, Stevanovic S, Munz C. Autophagy in innate and adaptive immunity against intracellular pathogens. *J Mol Med (Berl)* (2006) **84**:194–202. doi:10.1007/s00109-005-0014-4
47. Mizushima N, Noda T, Ohsumi Y. Apg16p is required for the function of the Apg12p-Apg5p conjugate in the yeast autophagy pathway. *EMBO J* (1999) **18**:3888–96. doi:10.1093/emboj/18.14.3888
48. Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* (2012) **8**:445–544. doi:10.4161/auto.19496
49. Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. *Nat Rev Immunol* (2007) **7**:767–77. doi:10.1038/nri2161
50. Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* (2011) **27**:107–32. doi:10.1146/annurev-cellbio-092910-154005
51. Fedorko M. Effect of chloroquine on morphology of cytoplasmic granules in maturing human leukocytes – an ultrastructural study. *J Clin Invest* (1967) **46**:1932–42. doi:10.1172/JCI105683
52. Seglen PO, Munthe-Kaas AC, Dybedal MA. Amino acid control of protein degradation in normal and leukemic human lymphocytes. *Exp Cell Res* (1984) **155**:121–8. doi:10.1016/0014-4827(84)90773-0
53. Gerland LM, Genestier L, Peyrol S, Michallet MC, Hayette S, Urbanowicz I, et al. Autolysosomes accumulate during in vitro CD8+ T-lymphocyte aging and may participate in induced death sensitization of senescent cells. *Exp Gerontol* (2004) **39**:789–800. doi:10.1016/j.exger.2004.01.013
54. Espert L, Denizot M, Grimaldi M, Robert-Hebmann V, Gay B, Varbanov M, et al. Autophagy and CD4 T lymphocyte destruction by HIV-1. *Med Sci (Paris)* (2006) **22**:677–8. doi:10.1051/medsci/20062289677
55. Hubbard VM, Valdor R, Patel B, Singh R, Cuervo AM, Macian F. Macroautophagy regulates energy metabolism during effector T cell activation. *J Immunol* (2010) **185**:7349–57. doi:10.4049/jimmunol.1000576
56. Jia W, He YW. Temporal regulation of intracellular organelle homeostasis in T lymphocytes by autophagy. *J Immunol* (2011) **186**:5313–22. doi:10.4049/jimmunol.1002404
57. Kovacs JR, Li C, Yang Q, Li G, Garcia IG, Ju S, et al. Autophagy promotes T-cell survival through degradation of proteins of the cell death machinery. *Cell Death Differ* (2012) **19**:144–52. doi:10.1038/cdd.2011.78
58. Arsov I, Adebayo A, Kucerova-Levisohn M, Haye J, Macneil M, Papavasiliou FN, et al. A role for autophagic protein beclin 1 early in lymphocyte development. *J Immunol* (2011) **186**:2201–9. doi:10.4049/jimmunol.1002223
59. He MX, McLeod IX, Jia W, He YW. Macroautophagy in T lymphocyte development and function. *Front Immunol* (2012) **3**:22. doi:10.3389/fimmu.2012.00022
60. McLeod IX, Jia W, He YW. The contribution of autophagy to lymphocyte survival and homeostasis. *Immunol Rev* (2012) **249**:195–204. doi:10.1111/j.1600-065X.2012.01143.x
61. Bernales S, McDonald KL, Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biol* (2006) **4**:e423. doi:10.1371/journal.pbio.0040423
62. Kraft C, Reggiori F, Peter M. Selective types of autophagy in yeast. *Biochim Biophys Acta* (2009) **1793**:1404–12. doi:10.1016/j.bbamcr.2009.02.006
63. Kanki T, Klionsky DJ. The molecular mechanism of mitochondria autophagy in yeast. *Mol Microbiol* (2010) **75**:795–800. doi:10.1111/j.1365-2958.2009.07035.x
64. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, et al. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* (2006) **26**:9220–31. doi:10.1128/MCB.01453-06
65. Paul S, Kashyap AK, Jia W, He YW, Schaefer BC. Selective autophagy of the adaptor protein Bcl10 modulates T cell receptor activation of NF-kappaB. *Immunity* (2012) **36**:947–58. doi:10.1016/j.immuni.2012.04.008
66. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, et al. Autophagosomes form at ER-mitochondria contact sites. *Nature* (2013) **495**:389–93. doi:10.1038/nature11910
67. Kruse KB, Brodsky JL, McCracken AA. Autophagy: an ER protein quality control process. *Autophagy* (2006) **2**:135–7.
68. Yorimitsu T, Nair U, Yang Z, Klionsky DJ. Endoplasmic reticulum stress triggers autophagy. *J Biol Chem* (2006) **281**:30299–304. doi:10.1074/jbc.M607007200
69. Criollo A, Maiuri MC, Tasdemir E, Vitale I, Fiebig AA, Andrews D, et al. Regulation of autophagy by the inositol trisphosphate receptor. *Cell Death Differ* (2007) **14**:1029–39.
70. Pengo N, Cenci S. The role of autophagy in plasma cell ontogenesis. *Autophagy* (2013) **9**:942–4. doi:10.4161/auto.24399
71. Pengo N, Scolari M, Oliva L, Milan E, Mainoldi F, Raimondi A, et al. Plasma cells require autophagy for sustainable immunoglobulin production. *Nat Immunol* (2013) **14**:298–305. doi:10.1038/ni.2524
72. Cali T, Ottolini D, Negro A, Brini M. Alpha-synuclein controls mitochondrial calcium homeostasis by enhancing endoplasmic reticulum-mitochondria interactions. *J Biol Chem* (2012) **287**:17914–29. doi:10.1074/jbc.M111.302794
73. Knoferle J, Koch JC, Ostendorf T, Michel U, Planchamp V, Vutova P, et al. Mechanisms of acute axonal degeneration in the optic nerve in vivo. *Proc Natl Acad Sci U S A* (2010) **107**:6064–9. doi:10.1073/pnas.0909794107
74. Evankovich J, Zhang R, Cardinal JS, Zhang L, Chen J, Huang H, et al. Calcium/calmodulin-dependent protein kinase IV limits organ damage in hepatic ischemia-reperfusion injury through induction of autophagy. *Am J Physiol Gastrointest Liver Physiol* (2012) **303**:G189–98. doi:10.1152/ajpgi.00051.2012
75. Cheng Y, Li H, Ren X, Niu T, Hait WN, Yang J. Cytoprotective effect of the elongation factor-2 kinase-mediated autophagy in breast cancer cells subjected to growth factor inhibition. *PLoS ONE* (2010) **5**:e9715. doi:10.1371/journal.pone.0009715
76. Kandala PK, Srivastava SK. Regulation of macroautophagy in ovarian cancer cells in vitro and in vivo by controlling glucose regulatory protein 78 and AMPK. *Oncotarget* (2012) **3**:435–49.
77. Hoyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. *Mol Cell* (2007) **25**:193–205. doi:10.1016/j.molcel.2006.12.009
78. Harr MW, McColl KS, Zhong F, Molitoris JK, Distelhorst CW. Glucocorticoids downregulate Fyn and inhibit IP(3)-mediated calcium signaling to promote autophagy in T lymphocytes. *Autophagy* (2010) **6**:912–21. doi:10.4161/auto.6.7.13290

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Adenine dinucleotide second messengers and T-lymphocyte calcium signaling

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Calcium signaling is a universal signal transduction mechanism in animal and plant cells. In mammalian T-lymphocytes calcium signaling is essential for activation and re-activation and thus important for a functional immune response. Since many years it has been known that both calcium release from intracellular stores and calcium entry via plasma membrane calcium channels are involved in shaping spatio-temporal calcium signals. Second messengers derived from the adenine dinucleotides NAD and NADP have been implicated in T cell calcium signaling. Nicotinic acid adenine dinucleotide phosphate (NAADP) acts as a very early second messenger upon T cell receptor/CD3 engagement, while cyclic ADP-ribose (cADPR) is mainly involved in sustained partial depletion of the endoplasmic reticulum by stimulating calcium release via ryanodine receptors. Finally, adenosine diphosphoribose (ADPR) a breakdown product of both NAD and cADPR activates a plasma membrane cation channel termed TRPM2 thereby facilitating calcium (and sodium) entry into T cells. Receptor-mediated formation, metabolism, and mode of action of these novel second messengers in T-lymphocytes will be reviewed.

Keywords: calcium signaling, T-lymphocyte, calcium release, nicotinic acid adenine dinucleotide phosphate, cyclic ADP-ribose, adenosine diphosphoribose, TRPM2 cation channels, calcium entry

Adenine derived Ca^{2+} mobilizing second messengers comprise nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic ADP-ribose (cADPR), and adenosine diphosphoribose (ADPR; **Figure 1**). They are all metabolites of nicotinamide adenine dinucleotide (NAD), a dinucleotide well known as coenzyme of oxidoreductases. NAD is converted by the multifunctional ectoenzyme NAD-glycohydrolase/ADP-ribosyl cyclase CD38 to ADPR and cADPR (**Figure 1**). The fact that the active site of CD38 faces the extracellular space while the targets for its products are located inside the cell, also known as topological paradox (1), has recently been investigated in detail. Importantly, in addition to the type II conformation with the active site facing the extracellular space, it was demonstrated that a smaller portion of CD38 is expressed in type III conformation thereby allowing for production of ADPR and cADPR within the cytosol (2). Another interesting feature of CD38 is the fact that it not only can make cADPR and ADPR, but also can synthesize NAADP, at least *in vitro* (**Figure 1**). However, this base-exchange mechanism needs nicotinamide adenine dinucleotide phosphate (NADP) and an excess of nicotinic acid as substrates and it works at acidic pH. Thus, it remains unclear whether this reaction is of physiological significance for second messenger formation in the cytosol. The substrate for the base-exchange reaction, NADP, is produced from NAD by NAD kinase (3). While mature, naïve T cells express only small amounts of CD38, it is upregulated as a consequence of mitogenic stimulation (4, 5). This is for instance seen after infection with HIV in activated antiviral $CD8^+$ T cells (6). CD38 expression in the CD8 compartment is therefore used to monitor antiretroviral therapy (7). Whether CD38 upregulation in activated T cells affects Ca^{2+} signaling compared to naïve, mature T cells is not known, but it is easy to envision the production of Ca^{2+} mobilizing messengers in effector cells being facilitated by upregulation of CD38, allowing for faster Ca^{2+} responses necessary for secretion of cytokines or granzymes and perforin in contrast to activation of calcineurin and NFAT in naïve cells.

Ca^{2+} signaling is one of the essential intracellular signaling pathways involved in T cell activation. It has long been known that both Ca^{2+} release and Ca^{2+} entry contribute to global Ca^{2+} signaling in T cells. In addition to Ca^{2+} release and Ca^{2+} entry evoked by the adenine derived Ca^{2+} mobilizing second messengers introduced above, two "standard" Ca^{2+} signaling systems are involved: (i) Ca^{2+} release by D-*myo*-inositol 1,4,5-trisphosphate [IP_3; (8)] and store-operated or capacitative Ca^{2+} entry (9). Since these systems have been thoroughly investigated and described in detail, they will not be reviewed in this article. However, due to their importance for T cell Ca^{2+} signaling, their roles will be mentioned and/or depicted, as for example in **Figure 2**.

The initial player in our model of T cell Ca^{2+} signaling is NAADP (**Figure 2**) being formed within seconds upon TCR/CD3

Abbreviations: ADPR, adenosine diphosphoribose; cADPR, cyclic ADP-ribose; $[Ca^{2+}]_i$, free cytosolic Ca^{2+} concentration; $[Ca^{2+}]_{lu}$, free endoplasmic reticular luminal Ca^{2+} concentration; CICR, Ca^{2+}-induced Ca^{2+} release; EAE, experimental autoimmune encephalomyelitis; IP_3, D-*myo* inositol 1,4,5-trisphosphate; IP_3R, D-*myo* inositol 1,4,5-trisphosphate receptor(s); NAADP, nicotinic acid adenine dinucleotide phosphate; NAD(P), nicotinamide adenine dinucleotide (phosphate); pADPr, poly-ADP-ribose; PARG, poly-ADP-ribose glycohydrolase; PARP, poly-ADP-ribose polymerase; RyR, ryanodine receptor(s); Stim1, stromal interaction molecule-1; TPC, two-pore channel(s); TRPM2, transient receptor potential channel, subtype melastatin 2.

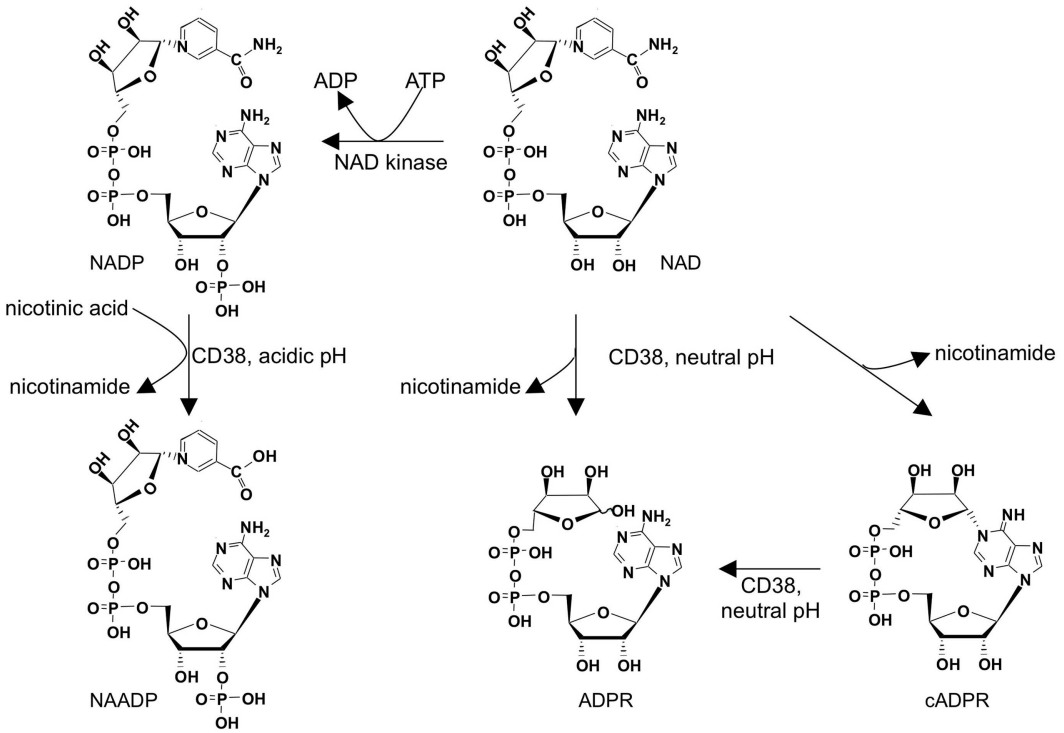

FIGURE 1 | Metabolism of adenine derived second messengers cADPR, NAADP, and ADPR.

ligation (10). However, NAADP is a rather short-lived second messenger, although after a rapid decrease to control levels, a second much smaller rise over several minutes was observed in Jurkat T cells (11). NAADP probably delivers the first local Ca^{2+} signals which then act as co-agonists at IP_3 receptors (IP_3R) and ryanodine receptors (RyR). IP_3 is formed soon after the initial NAADP peak (12) and releases Ca^{2+} via IP_3R (13). Finally, cADPR starts to increase and acts on RyR (14); likely, Ca^{2+} released by NAADP and/or IP_3 facilitates the action of cADPR. Continuous Ca^{2+} release by these consecutively increased second messengers results in continuously decreased luminal Ca^{2+} concentration in the ER ($[Ca^{2+}]_{lu}$). Stromal interaction molecule-1 (Stim1) senses the decreased $[Ca^{2+}]_{lu}$ and activates Ca^{2+} entry via orai/CRAC channels (15–17).

In addition to this Ca^{2+} signaling pathway involved in T cell activation or re-activation, high input signal strength, e.g., obtained by a high concentration of the cross-linking lectin Concanavalin A, activates another different Ca^{2+} entry system operated by ADPR and the transient receptor potential channel, subtype melastatin 2 (TRPM2; **Figure 2**).

Following we will review hallmarks of NAADP, cADPR, and ADPR as second messengers in T cell Ca^{2+} signaling.

NAADP

Upon activation of the TCR/CD3 complex, formation of NAADP rapidly increases within 10–20 s in Jurkat T cells. Following a subsequent decrease within the first minute, a continuously elevated [NAADP] remains for 5–20 min (10). It has been proposed that NAADP may act as an early triggering messenger, mediating initial localized Ca^{2+} events which are subsequently amplified to a global signal, e.g., by recruitment of further channels, other second messengers like cADPR, IP_3, and/or Ca^{2+} induced Ca^{2+} release (CICR). In T-lymphocytes a bell-shaped concentration-response curve following NAADP microinjection is observed. Compared to other second messengers such as IP_3, already low concentrations in the nanomolar range (30–100 nM) induce Ca^{2+} signaling in T-lymphocytes (18).

The mechanism and very early kinetics of receptor-mediated formation of NAADP *in vivo* remains unclear and has been discussed previously [e.g., (19)]. In brief, NAADP is formed *in vitro* by a base-exchange of NADP in presence of nicotinic acid and a pH of 5 [**Figure 1**; (20)]. Further, at pH 5, but also at pH 7.4, 2′-phospho-cADPR may be converted to NAADP (21). Both reactions are catalyzed by the membrane bound, multifunctional enzyme CD38 and require presence of up to millimolar concentrations of nicotinic acid *in vitro* (20, 21). Furthermore, influx of extracellular NAADP may also induce Ca^{2+} signals as shown in a rat basophilic cell line (22). Interestingly, gene silencing of CD38 in Jurkat T-lymphocytes did not result in decreased NAADP levels. Rather, in thymus and spleen of CD38 knock-out mice increased NAADP levels were observed, thus indicating that CD38 may particularly drive degradation of NAADP (23, 24). Accordingly, in T cells to date CD38 may be primarily understood as degrading enzyme while its role in NAADP synthesis *in vivo* remains to be elucidated. In T-lymphocytes and other $CD38^+$ cells, NAADP is degraded to 2′-phospho-ADPR at neutral and acidic pH by CD38,

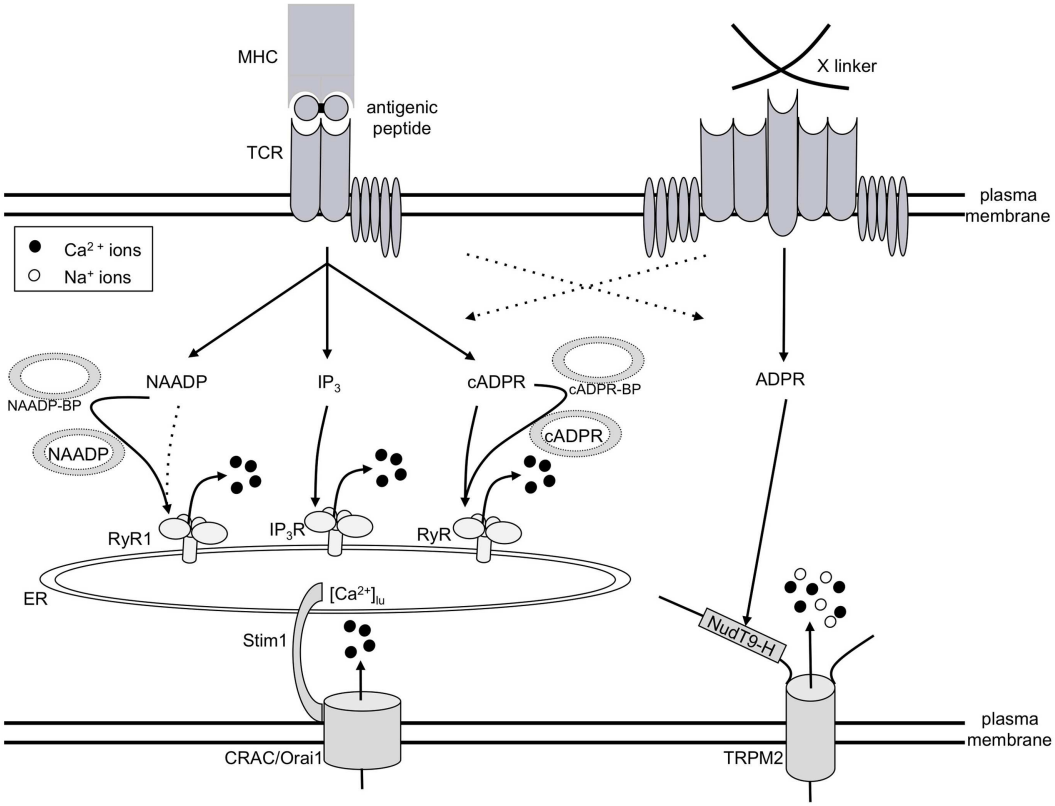

FIGURE 2 | Model of T cell Ca^{2+} signaling. TCR/CD3 ligation by antigenic peptide presented by MHC molecules on antigen presenting cells results in consecutive formation of the second messengers NAADP, IP_3, and cADPR, all of which release Ca^{2+} from the ER. Thus, a continuously decreased intraluminal free Ca^{2+} concentration in the ER ($[Ca^{2+}]_{lu}$) resulting from this constant Ca^{2+} release concomitantly activates CRAC/Orai1 channels in the plasma membrane. The mode of action of both NAADP and cADPR likely involves specific binding proteins for both second messengers (abbreviated here as NAADP-BP or cADPR-BP). A strong stimulus, e.g., cross-linking of receptors by concanavalin A (right side of Figure 2), triggers formation of ADPR and activation of TRPM2, in addition to the mechanisms described on the left side of the figure.

but degradation may also occur non-specifically via nucleotide pyrophosphatases (25).

The targeted receptor(s) and hence target organelle(s) of NAADP are still under debate (19, 26–29). In general, RyR have been implicated as NAADP targets in different cell types, e.g., skeletal muscle cell (30), or pancreatic acinar cells (31, 32). Nonetheless, in the majority of mammalian cells as well as in sea urchin eggs there is evidence that NAADP may primarily target acidic stores and that the RyR located on the ER may rather play a central role in the amplification of the Ca^{2+} signal (33–35).

However focusing particularly on data obtained in T-lymphocytes, NAADP Ca^{2+} signaling strongly depends on RyR activity in the ER (36–40). Following either knock-down or inhibition of RyR by ryanodine in Jurkat T cells, subcellular and global Ca^{2+} signals by NAADP microinjection were inhibited or almost completely abolished (36, 38). In primary effector T-lymphocytes the NAADP antagonist BZ194 inhibits Ca^{2+} signaling, e.g., during formation of the immunological synapse. Furthermore, BZ194 has been shown to selectively inhibit NAADP dependent binding of [^{3}H]ryanodine to RyR1 (39). Interestingly, in primary effector T cells of an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), BZ194 leads to a decrease in cell motility and invasive capacity as well as a decrease in cytokine expression, all of which indicate the central role of NAADP-mediated Ca^{2+} signaling in T cells possibly via RyR (40). In contrast to these results obtained in $CD4^+$ T cells, in cytotoxic T cells NAADP appears to target two-pore channels (TPC) on cytolytic granules (41). In general, overexpression or inhibition of the endolysosomal TPC1 and TPC2 suggest that NAADP initiates Ca^{2+} events via TPC [e.g., (42–44)]. Recently, the N-terminus of TPC1 has been identified as functional region for NAADP-mediated Ca^{2+} signaling (26). In contrast, it was shown that NAADP-mediated Ca^{2+} signaling in TPC1$^{-/-}$/TPC2$^{-/-}$ mice does not differ from wild-type mice (27). Thus, whether NAADP primarily targets TPCs is controversial and particularly the effect of NAADP on TPCs in T-lymphocytes is not yet clear.

Further, in T-lymphocytes and neutrophils TRPM2 is activated by micromolar concentrations of NAADP *per se*, but particularly in synergism with cADPR (45, 46). The effect of cADPR on TRPM2 however, could not be confirmed in HEK293 cells overexpressing TRPM2 and contamination of commercial cADPR

preparations with ADPR have been discussed (47, 48). NAADP has been shown to target the unspecific Ca^{2+} channel TRPML1 in smooth muscle myocytes (49). Whether TRPML1 and TRPM2 are of functional relevance within NAADP – mediated Ca^{2+} signaling in T-lymphocytes, remains to be elucidated.

Despite the questions which organelles are targeted by NAADP and which specific downstream mechanisms may underlie the initiated Ca^{2+} events, also the identity of the NAADP receptor remains unclear. Photoaffinity labeling in mammalian cells using a probe specific for NAADP binding proteins was not altered upon overexpression or knock-out of TPC1 or TPC2, but suggests that a yet not identified 22/23 kDa protein binds NAADP and may hence couple NAADP to its respective Ca^{2+} channels (50, 51), a mechanism recently introduced as unifying hypothesis of NAADP action (29).

CYCLIC ADP-RIBOSE

Cyclic adenosine diphosphoribose (cADPR) was the first Ca^{2+} mobilizing second messenger discovered as derivative of an adenine dinucleotide (52, 53). Though first described in sea urchin egg homogenates, the Ca^{2+} mobilizing activity of cADPR was soon detected in many cells types. In 1995 we published the first report demonstrating specific Ca^{2+} release in human Jurkat T cells (54). Central aspects of the role of cADPR in T cell Ca^{2+} signaling were subsequently published by our laboratory: (i) formation of cADPR upon TCR/CD3 ligation (14, 55), and (ii) mode of action of cADPR by activation of Ca^{2+} release via RyR, as shown by gene silencing of RyR (56). Further, we demonstrated tyrosine phosphorylation of RyR upon TCR/CD3 ligation; in permeabilized T cells enhancement of cADPR evoked Ca^{2+} release by tyrosine kinase p59fyn was observed (57). Importantly, we demonstrated amplification and propagation of pacemaker Ca^{2+} signals by cADPR (58). A connection of cADPR signaling to Ca^{2+} entry was also observed: microinjection of cADPR in the absence of extracellular Ca^{2+} or in the presence of Ca^{2+} channel blockers resulted in much reduced Ca^{2+} signals (59). Finally, using a specific cADPR antagonist it was shown that downstream activation parameters of primary human T cells, such as activation antigen expression or proliferation, were concentration-dependently inhibited (14) suggesting a pivotal role of cADPR in T cell biology.

A detailed structure-activity analysis of cADPR in T cells has been conducted over the past couple of years. The main results from these studies were recently reviewed (60) and are (i) critical dependence of agonist vs. antagonist properties on the substituent at the C-atom 6 of the purine base, (ii) maintenance of biological activity, albeit at a lower level, when both southern and northern ribose were replaced by carbocyclic moieties or simplified ether/alkane bridges, and (iii) the possibility of radical simplification of the purine structure, e.g., the 1,2,3-triazole-4-amide mimic of adenine within cADPR retains biological activity.

ADPR

A relatively new addition to the realm of adenine based Ca^{2+} mobilizing second messengers in T-lymphocytes is ADPR. Presence of ADPR in eukaryotic cells has been known for quite a while (61), but since ADPR is rather dangerous for the cell – its reactive ribose can non-enzymatically form Schiff-bases with amino groups of cellular proteins (62, 63) – it was mostly considered a toxic cellular waste product. This casually explained the presence of efficient mechanisms for the degradation of ADPR in form of cytosolic (64, 65) and mitochondrial ADPR pyrophosphatases (66, 67). These enzymes hydrolyze the pyrophosphate bridge of ADPR yielding AMP and ribose-5′-phosphate that are fed back into metabolism.

Two discoveries suggested that there might be more to ADPR: in 2001 it was reported that TRPM2 (formerly termed TRPC7 or LTRPC2), a Ca^{2+}-permeable cation channel of the melastatin subfamily of TRP channels, can be activated by binding of ADPR to a cytoplasmic domain homologous to the mitochondrial ADPR pyrophosphatase NUDT9 (68, 69). This channel shows expression in a variety of tissues with highest levels being found in brain and cells of the immune system. A year later Bastide et al. showed that ADPR is also able to activate type I RyR isolated from rat skeletal muscle in the presence of micromolar concentrations of Ca^{2+} (70). Since there has been little news on the action of ADPR on RyR, we will focus on the role of ADPR for TRPM2 activation in T-lymphocytes.

Most of the work on ADPR and TRPM2 in T cells so far has been done in Jurkat cells that express TRPM2 on transcript and protein level and respond with a typical TRPM2 current to ADPR infusion (11, 45, 69). Microinjection of ADPR (11) and uncaging of photoactivatable ADPR (71) in these cells results in Ca^{2+} entry-dependent Ca^{2+} signals. By HPLC analysis the cellular ADPR concentration of roughly 40 μmol/L in resting Jurkat cells (72) was shown to nearly double after stimulation with high concentrations of concanavalin A (11).

There are different conceivable ways how this ADPR might be generated. CD38 expressed in Jurkat as well as primary T cells (73) can metabolize β-NAD^+ and cADPR to yield ADPR (74). The topological paradox initially described for cADPR also holds true for ADPR (1). This paradox might be resolved by specific uptake mechanisms for ADPR as have been reported for erythrocytes (75, 76). Another possibility is the presence of CD38 in a type III orientation (2). While the contribution of CD38 to the increase in ADPR after stimulation is still unclear, the basal ADPR seems to be independent of CD38 as the murine T-lymphoma line BW5147 that lacks transcripts for CD38 (77) has even higher basal ADPR levels [73 μmol/L (72)] than Jurkat cells.

Another way that has been discussed for the production of ADPR is the consecutive action of poly-ADPR polymerase (PARP) and poly-ADPR glycohydrolase (PARG) (78). While PARP activity and poly-ADPR levels are quite low in non-stimulated cells, there is a constant turn-over due to the low K_M of PARG [reviewed in (79)] that might contribute to the basal ADPR detected in Jurkat cells. Under DNA damaging conditions like strong oxidative stress the activity of PARP increases to such levels that a large part of cellular β-NAD^+ can be metabolized as has been shown for DT-40 cells (80). Data for a range of cells suggest activation of TRPM2 by oxidative stress results in cell death by apoptosis (78, 81), most likely due to mitochondrial calcium overload and downstream activation of caspases [reviewed in (82)]. While murine $CD4^+$ T cells also die after exposure to hydrogen peroxide, this apparently does not involve TRPM2 (83).

Interestingly there have been reports that in T cells PARP-1 activation can occur after TCR stimulation in a way independent of oxidative stress or DNA damage resulting in poly-ADP ribosylation of NFAT [(84, 85); reviewed in (86)]. It might be speculated that this increased pADPR turn-over will result in increased cellular ADPR and TRPM2 activation hinting to a possible role for ADPR/TRPM2 in TCR signaling. In accordance with this, naïve $CD4^+$ T cells from the wild-type mice upregulated TRPM2 after stimulation with α-CD3/α-CD28-beads and $CD4^+$ T-lymphocytes from $TRPM2^{-/-}$ mice showed not only reduced proliferation, but also reduced production of pro-inflammatory cytokines upon activation (83).

Most work on the role of TRPM2 in the immune response has been done using the $TRPM2^{-/-}$ mouse (87). In a model for ulcerative colitis the inflammation was suppressed, but this was shown to be due to a reduced production of the chemokine CXCL2 in monocytes whereas the infiltration of T cells in the colon was not affected by the knock-out of TRPM2 (87). Recent work has shown that TRPM2 knock-out does not affect airway inflammation either induced by oxidative stressors (88) or as a result of exposure to ovalbumin in a mouse model for acute asthma (89). On the other hand $TRPM2^{-/-}$ noticeably reduced inflammation and spinal cord lesions in EAE induced by a peptide from myelin oligodendrocyte glycoprotein (83). Since the effect of $TRPM2^{-/-}$ on EAE might also involve reduced neuronal cell death or microglia activation, it will be interesting to see whether proliferation and effector functions of T cells from such animals are affected.

Taken together, adenine derived Ca^{2+} mobilizing second messengers play essential roles in T cell Ca^{2+} signaling, both during activation/re-activation or apoptosis. While NAADP is important as rapid Ca^{2+} trigger, particularly in effector T cells, cADPR apparently holds a central role in maintenance of long-lasting Ca^{2+} signaling. Interestingly, also apoptosis induction via TRPM2 involves an adenine derived second messenger, the dinucleotide ADPR. The documented involvement of these adenine derived Ca^{2+} mobilizing second messengers in central aspects of immune regulation make the pathways described in this review suitable targets for therapeutic intervention. In fact, we have recently shown that the NAADP antagonist BZ194 ameliorated the clinical course of transfer EAE, an animal model of multiple sclerosis (40).

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REFERENCES

1. De Flora A, Guida L, Franco L, Zocchi E. The CD38/cyclic ADP-ribose system: a topological paradox. *Int J Biochem Cell Biol* (1997) **29**:1149–66. doi:10.1016/S1357-2725(97)00062-9
2. Zhao YJ, Lam CM, Lee HC. The membrane-bound enzyme CD38 exists in two opposing orientations. *Sci Signal* (2012) **5**:ra67. doi:10.1126/scisignal.2002700
3. Pollak N, Niere M, Ziegler M. NAD kinase levels control the NADPH concentration in human cells. *J Biol Chem* (2007) **282**:33562–71. doi:10.1074/jbc.M704442200
4. Malavasi F, Funaro A, Alessio M, DeMonte LB, Ausiello CM, Dianzani U, et al. CD38: a multilineage cell activation molecule with a split personality. *Int J Clin Lab Res* (1992) **22**:73–80. doi:10.1007/BF02591400
5. Deterre P, Berthelier V, Bauvois B, Dalloul A, Schuber F, Lund F. CD38 in T- and B-cell functions. *Chem Immunol* (2000) **75**:146–68. doi:10.1159/000058767
6. Ho HN, Hultin LE, Mitsuyasu RT, Matud JL, Hausner MA, Bockstoce D, et al. Circulating HIV-specific CD8+ cytotoxic T cells express CD38 and HLA-DR antigens. *J Immunol* (1993) **150**:3070–9.
7. Coetzee LM, Tay SS, Lawrie D, Janossy G, Glencross DK. From research tool to routine test: CD38 monitoring in HIV patients. *Cytometry B Clin Cytom* (2009) **76**:375–84. doi:10.1002/cyto.b.20478
8. Mikoshiba K. IP3 receptor/Ca2+ channel: from discovery to new signaling concepts. *J Neurochem* (2007) **102**:1426–46. doi:10.1111/j.1471-4159.2007.04825.x
9. Putney JW. Capacitative calcium entry: from concept to molecules. *Immunol Rev* (2009) **231**:10–22. doi:10.1111/j.1600-065X.2009.00810.x
10. Gasser A, Bruhn S, Guse AH. Second messenger function of nicotinic acid adenine dinucleotide phosphate revealed by an improved enzymatic cycling assay. *J Biol Chem* (2006) **281**:16906–13. doi:10.1074/jbc.M601347200
11. Gasser A, Glassmeier G, Fliegert R, Langhorst MF, Meinke S, Hein D, et al. Activation of T cell calcium influx by the second messenger ADP-ribose. *J Biol Chem* (2006) **281**:2489–96. doi:10.1074/jbc.M506525200
12. Guse AH, Goldwich A, Weber K, Mayr GW. Non-radioactive, isomer-specific inositol phosphate mass determinations: high-performance liquid chromatography-micro-metal-dye detection strongly improves speed and sensitivity of analyses from cells and micro-enzyme assays. *J Chromatogr B Biomed Appl* (1995) **672**:189–98. doi:10.1016/0378-4347(95)00219-9
13. Guse AH, Roth E, Emmrich F. D-myo-inositol 1,3,4,5-tetrakisphosphate releases Ca2+ from crude microsomes and enriched vesicular plasma membranes, but not from intracellular stores of permeabilized T-lymphocytes and monocytes. *Biochem J* (1992) **288**(Pt 2):489–95.
14. Guse AH, da Silva CP, Berg I, Skapenko AL, Weber K, Heyer P, et al. Regulation of calcium signalling in T lymphocytes by the second messenger cyclic ADP-ribose. *Nature* (1999) **398**:70–3. doi:10.1038/18024
15. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel S-H, Tanasa B, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* (2006) **441**:179–85. doi:10.1038/nature04702
16. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* (2006) **443**:230–3. doi:10.1038/nature05122
17. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science* (2006) **312**:1220–3. doi:10.1126/science.1127883
18. Berg I, Potter BVL, Mayr GW, Guse AH. Nicotinic acid adenine dinucleotide phosphate (Naadp+) is an essential regulator of T-lymphocyte Ca2+-signaling. *J Cell Biol* (2000) **150**:581–8. doi:10.1083/jcb.150.3.581
19. Guse AH, Lee HC. NAADP: a universal Ca2+ trigger. *Sci Signal* (2008) **1**:re10. doi:10.1126/scisignal.144re10
20. Aarhus R, Graeff RM, Dickey DM, Walseth TF, Lee HC. ADP-ribosyl cyclase and CD38 catalyze the synthesis of a calcium-mobilizing metabolite from NADP. *J Biol Chem* (1995) **270**:30327–33. doi:10.1074/jbc.270.51.30327
21. Moreschi I, Bruzzone S, Melone L, De Flora A, Zocchi E. NAADP+ synthesis from cADPRP and nicotinic acid by ADP-ribosyl cyclases. *Biochem Biophys Res Commun* (2006) **345**:573–80. doi:10.1016/j.bbrc.2006.04.096
22. Billington RA, Bellomo EA, Floriddia EM, Erriquez J, Distasi C, Genazzani AA. A transport mechanism for NAADP in a rat basophilic cell line. *FASEB J* (2006) **20**:521–3.
23. Schmid F, Bruhn S, Weber K, Mittrücker H-W, Guse AH. CD38: a NAADP degrading enzyme. *FEBS Lett* (2011) **585**:3544–8. doi:10.1016/j.febslet.2011.10.017

24. Soares S, Thompson M, White T, Isbell A, Yamasaki M, Prakash Y, et al. NAADP as a second messenger: neither CD38 nor base-exchange reaction are necessary for in vivo generation of NAADP in myometrial cells. *Am J Physiol Cell Physiol* (2007) **292**:C227–39. doi:10.1152/ajpcell.00638.2005
25. Graeff R. Acidic residues at the active sites of CD38 and ADP-ribosyl cyclase determine nicotinic acid adenine dinucleotide phosphate (NAADP) synthesis and hydrolysis activities. *J Biol Chem* (2006) **281**:28951–7. doi:10.1074/jbc.M604370200
26. Churamani D, Hooper R, Rahman T, Brailoiu E, Patel S. The N-terminal region of two-pore channel 1 regulates trafficking and activation by NAADP. *Biochem J* (2013) **453**(1):147–51. doi:10.1042/BJ20130474
27. Wang X, Zhang X, Dong X-P, Samie M, Li X, Cheng X, et al. TPC proteins are phosphoinositide- activated sodium-selective ion channels in endosomes and lysosomes. *Cell* (2012) **151**:372–83. doi:10.1016/j.cell.2012.08.036
28. Cang C, Zhou Y, Navarro B, Seo Y-J, Aranda K, Shi L, et al. mTOR regulates lysosomal ATP-sensitive two-pore Na(+) channels to adapt to metabolic state. *Cell* (2013) **152**:778–90. doi:10.1016/j.cell.2013.01.023
29. Guse AH. Linking NAADP to ion channel activity: a unifying hypothesis. *Sci Signal* (2012) **5**:e18. doi:10.1126/scisignal.2002890
30. Hohenegger M, Suko J, Gscheidlinger R, Drobny H, Zidar A. Nicotinic acid-adenine dinucleotide phosphate activates the skeletal muscle ryanodine receptor. *Biochem J* (2002) **367**:423–31. doi:10.1042/BJ20020584
31. Gerasimenko JV, Maruyama Y, Yano K, Dolman NJ, Tepikin AV, Petersen OH, et al. NAADP mobilizes Ca2+ from a thapsigargin-sensitive store in the nuclear envelope by activating ryanodine receptors. *J Cell Biol* (2003) **163**:271–82. doi:10.1083/jcb.200306134
32. Gerasimenko JV, Sherwood M, Tepikin AV, Petersen OH, Gerasimenko OV. NAADP, cADPR and IP3 all release Ca2+ from the endoplasmic reticulum and an acidic store in the secretory granule area. *J Cell Sci* (2006) **119**:226–38. doi:10.1242/jcs.02721
33. Lee HC, Aarhus R. Functional visualization of the separate but interacting calcium stores sensitive to NAADP and cyclic ADP-ribose. *J Cell Sci* (2000) **113**(Pt 24):4413–20.
34. Kinnear NP, Boittin F-X, Thomas JM, Galione A, Evans AM. Lysosome-sarcoplasmic reticulum junctions. A trigger zone for calcium signaling by nicotinic acid adenine dinucleotide phosphate and endothelin-1. *J Biol Chem* (2004) **279**:54319–26. doi:10.1074/jbc.M406132200
35. Cancela JM, Churchill GC, Galione A. Coordination of agonist-induced Ca2+-signalling patterns by NAADP in pancreatic acinar cells. *Nature* (1999) **398**:74–6. doi:10.1038/18032
36. Dammermann W, Guse AH. Functional ryanodine receptor expression is required for NAADP-mediated local Ca2+ signaling in T-lymphocytes. *J Biol Chem* (2005) **280**:21394–9. doi:10.1074/jbc.M413085200
37. Steen M, Kirchberger T, Guse AH. NAADP mobilizes calcium from the endoplasmic reticular Ca(2+) store in T-lymphocytes. *J Biol Chem* (2007) **282**:18864–71. doi:10.1074/jbc.M610925200
38. Langhorst MF, Schwarzmann N, Guse AH. Ca2+ release via ryanodine receptors and Ca2+ entry: major mechanisms in NAADP-mediated Ca2+ signaling in T-lymphocytes. *Cell Signal* (2004) **16**:1283–9. doi:10.1016/j.cellsig.2004.03.013
39. Dammermann W, Zhang B, Nebel M, Cordiglieri C, Odoardi F, Kirchberger T, et al. NAADP-mediated Ca2+ signaling via type 1 ryanodine receptor in T cells revealed by a synthetic NAADP antagonist. *Proc Natl Acad Sci U S A* (2009) **106**:10678–83. doi:10.1073/pnas.0809997106
40. Cordiglieri C, Odoardi F, Zhang B, Nebel M, Kawakami N, Klinkert WEF, et al. Nicotinic acid adenine dinucleotide phosphate-mediated calcium signalling in effector T cells regulates autoimmunity of the central nervous system. *Brain J Neurol* (2010) **133**:1930–43. doi:10.1093/brain/awq135
41. Davis LC, Morgan AJ, Chen JL, Snead CM, Bloor-Young D, Shenderov E, et al. NAADP activates two-pore channels on T cell cytolytic granules to stimulate exocytosis and killing. *Curr Biol* (2012) **22**:2331–7. doi:10.1016/j.cub.2012.10.035
42. Calcraft PJ, Ruas M, Pan Z, Cheng X, Arredouani A, Hao X, et al. NAADP mobilizes calcium from acidic organelles through two-pore channels. *Nature* (2009) **459**:596–600. doi:10.1038/nature08030
43. Brailoiu E, Churamani D, Cai X, Schrlau MG, Brailoiu GC, Gao X, et al. Essential requirement for two-pore channel 1 in NAADP-mediated calcium signaling. *J Cell Biol* (2009) **186**:201–9. doi:10.1083/jcb.200904073
44. Zong X, Schieder M, Cuny H, Fenske S, Gruner C, Rötzer K, et al. The two-pore channel TPCN2 mediates NAADP-dependent Ca(2+)-release from lysosomal stores. *Pflügers Arch* (2009) **458**:891–9. doi:10.1007/s00424-009-0690-y
45. Beck A, Kolisek M, Bagley LA, Fleig A, Penner R. Nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose regulate TRPM2 channels in T lymphocytes. *FASEB J* (2006) **20**:962–4. doi:10.1096/fj.05-5538fje
46. Lange I, Penner R, Fleig A, Beck A. Synergistic regulation of endogenous TRPM2 channels by adenine dinucleotides in primary human neutrophils. *Cell Calcium* (2008) **44**:604–15. doi:10.1016/j.ceca.2008.05.001
47. Kirchberger T, Moreau C, Wagner GK, Fliegert R, Siebrands CC, Nebel M, et al. 8-Bromo-cyclic inosine diphosphoribose: towards a selective cyclic ADP-ribose agonist. *Biochem J* (2009) **422**:139–49. doi:10.1042/BJ20082308
48. Tóth B, Csanády L. Identification of direct and indirect effectors of the transient receptor potential melastatin 2 (TRPM2) cation channel. *J Biol Chem* (2010) **285**:30091–102. doi:10.1074/jbc.M109.066464
49. Zhang F, Jin S, Yi F, Li P-L. TRP-ML1 functions as a lysosomal NAADP-sensitive Ca2+ release channel in coronary arterial myocytes. *J Cell Mol Med* (2009) **13**:3174–85. doi:10.1111/j.1582-4934.2008.00486.x
50. Walseth TF, Lin-Moshier Y, Jain P, Ruas M, Parrington J, Galione A, et al. Photoaffinity labeling of high affinity nicotinic acid adenine dinucleotide phosphate (NAADP)-binding proteins in sea urchin egg. *J Biol Chem* (2012) **287**:2308–15. doi:10.1074/jbc.M111.306563
51. Lin-Moshier Y, Walseth TF, Churamani D, Davidson SM, Slama JT, Hooper R, et al. Photoaffinity labeling of nicotinic acid adenine dinucleotide phosphate (NAADP) targets in mammalian cells. *J Biol Chem* (2012) **287**:2296–307. doi:10.1074/jbc.M111.305813
52. Clapper DL, Walseth TF, Dargie PJ, Lee HC. Pyridine nucleotide metabolites stimulate calcium release from sea urchin egg microsomes desensitized to inositol trisphosphate. *J Biol Chem* (1987) **262**:9561–8.
53. Lee HC, Walseth TF, Bratt GT, Hayes RN, Clapper DL. Structural determination of a cyclic metabolite of NAD+ with intracellular Ca2+-mobilizing activity. *J Biol Chem* (1989) **264**:1608–15.
54. Guse AH, da Silva CP, Emmrich F, Ashamu GA, Potter BV, Mayr GW. Characterization of cyclic adenosine diphosphate-ribose-induced Ca2+ release in T lymphocyte cell lines. *J Immunol* (1995) **1950**(155):3353–9.
55. da Silva CP, Potter BV, Mayr GW, Guse AH. Quantification of intracellular levels of cyclic ADP-ribose by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* (1998) **707**:43–50. doi:10.1016/S0378-4347(97)00622-1
56. Schwarzmann N, Kunerth S, Weber K, Mayr GW, Guse AH. Knock-down of the type 3 ryanodine receptor impairs sustained Ca2+ signaling via the T cell receptor/CD3 complex. *J Biol Chem* (2002) **277**:50636–42. doi:10.1074/jbc.M209061200
57. Guse AH, Tsygankov AY, Weber K, Mayr GW. Transient tyrosine phosphorylation of human ryanodine receptor upon T cell stimulation. *J Biol Chem* (2001) **276**:34722–7. doi:10.1074/jbc.M100715200
58. Kunerth S, Langhorst MF, Schwarzmann N, Gu X, Huang L, Yang Z, et al. Amplification and propagation of pacemaker Ca2+ signals by cyclic ADP-ribose and the type 3 ryanodine receptor in T cells. *J Cell Sci* (2004) **117**:2141–9. doi:10.1242/jcs.01063
59. Guse AH, Berg I, da Silva CP, Potter BV, Mayr GW. Ca2+ entry induced by cyclic ADP-ribose in intact T-lymphocytes. *J Biol Chem* (1997) **272**:8546–50. doi:10.1074/jbc.272.13.8546
60. Guse AH. Structure-activity relationship of cyclic ADP-ribose, an update. *J Chin Pharm Sci* (2013) **22**:127–36. doi:10.5246/jcps.2013.02.017
61. Zocchi E, Guida L, Franco L, Silvestro L, Guerrini M, Benatti U, et al. Free ADP-ribose in human erythrocytes: pathways of intra-erythrocytic conversion and non-enzymic binding to membrane proteins. *Biochem J* (1993) **295**(Pt 1):121–30.
62. Kun E, Chang AC, Sharma ML, Ferro AM, Nitecki D. Covalent modification of proteins by

metabolites of NAD+. *Proc Natl Acad Sci U S A* (1976) **73**:3131–5. doi:10.1073/pnas.73.9.3131
63. Jacobson EL, Cervantes-Laurean D, Jacobson MK. ADP-ribose in glycation and glycoxidation reactions. *Adv Exp Med Biol* (1997) **419**:371–9. doi:10.1007/978-1-4419-8632-0_49
64. Canales J, Pinto RM, Costas MJ, Hernández MT, Miró A, Bernet D, et al. Rat liver nucleoside diphosphosugar or diphosphoalcohol pyrophosphatases different from nucleotide pyrophosphatase or phosphodiesterase I: substrate specificities of Mg(2+)-and/or Mn(2+)-dependent hydrolases acting on ADP-ribose. *Biochim Biophys Acta* (1995) **1246**: 167–77. doi:10.1016/0167-4838(94)00191-I
65. Yang H, Slupska MM, Wei YF, Tai JH, Luther WM, Xia YR, et al. Cloning and characterization of a new member of the Nudix hydrolases from human and mouse. *J Biol Chem* (2000) **275**:8844–53. doi:10.1074/jbc.275.12.8844
66. Bernet D, Pinto RM, Costas MJ, Canales J, Cameselle JC. Rat liver mitochondrial ADP-ribose pyrophosphatase in the matrix space with low Km for free ADP-ribose. *Biochem J* (1994) **299**(Pt 3):679–82.
67. Perraud A-L, Shen B, Dunn CA, Rippe K, Smith MK, Bessman MJ, et al. NUDT9, a member of the Nudix hydrolase family, is an evolutionarily conserved mitochondrial ADP-ribose pyrophosphatase. *J Biol Chem* (2003) **278**:1794–801. doi:10.1074/jbc.M205601200
68. Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, et al. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature* (2001) **411**:595–9. doi:10.1038/35079100
69. Sano Y, Inamura K, Miyake A, Mochizuki S, Yokoi H, Matsushime H, et al. Immunocyte Ca2+ influx system mediated by LTRPC2. *Science* (2001) **293**:1327–30. doi:10.1126/science.1062473
70. Bastide B, Snoeckx K, Mounier Y. ADP-ribose stimulates the calcium release channel RyR1 in skeletal muscle of rat. *Biochem Biophys Res Commun* (2002) **296**: 1267–71. doi:10.1016/S0006-291X(02)02073-9
71. Yu P, Wang Q, Zhang L-H, Lee H-C, Zhang L, Yue J. A cell permeable NPE caged ADP-ribose for studying TRPM2. *PLoS ONE* (2012) **7**:e51028. doi:10.1371/journal.pone.0051028
72. Gasser A, Guse AH. Determination of intracellular concentrations of the TRPM2 agonist ADP-ribose by reversed-phase HPLC. *J Chromatogr B Analyt Technol Biomed Life Sci* (2005) **821**:181–7. doi:10.1016/j.jchromb.2005.05.002
73. Gelman L, Deterre P, Gouy H, Boumsell L, Debré P, Bismuth G. The lymphocyte surface antigen CD38 acts as a nicotinamide adenine dinucleotide glycohydrolase in human T lymphocytes. *Eur J Immunol* (1993) **23**:3361–4. doi:10.1002/eji.1830231245
74. Howard M, Grimaldi JC, Bazan JF, Lund FE, Santos-Argumedo L, Parkhouse RM, et al. Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen CD38. *Science* (1993) **262**:1056–9. doi:10.1126/science.8235624
75. Kim UH, Han MK, Park BH, Kim HR, An NH. Function of NAD glycohydrolase in ADP-ribose uptake from NAD by human erythrocytes. *Biochim Biophys Acta* (1993) **1178**:121–6. doi:10.1016/0167-4889(93)90001-6
76. Albeniz I, Demir O, Nurten R, Bermek E. NAD glycohydrolase activities and ADP-ribose uptake in erythrocytes from normal subjects and cancer patients. *Biosci Rep* (2004) **24**:41–53. doi:10.1023/B:BIRE.0000037755.42767.a4
77. Ferrero E, Saccucci F, Malavasi F. The making of a leukocyte receptor: origin, genes and regulation of human CD38 and related molecules. *Chem Immunol* (2000) **75**:1–19. doi:10.1159/000058763
78. Fonfria E, Marshall ICB, Benham CD, Boyfield I, Brown JD, Hill K, et al. TRPM2 channel opening in response to oxidative stress is dependent on activation of poly(ADP-ribose) polymerase. *Br J Pharmacol* (2004) **143**:186–92. doi:10.1038/sj.bjp.0705914
79. Bonicalzi M-E, Haince J-F, Droit A, Poirier GG. Regulation of poly(ADP-ribose) metabolism by poly(ADP-ribose) glycohydrolase: where and when? *Cell Mol Life Sci* (2005) **62**:739–50. doi:10.1007/s00018-004-4505-1
80. Buelow B, Song Y, Scharenberg AM. The Poly(ADP-ribose) polymerase PARP-1 is required for oxidative stress-induced TRPM2 activation in lymphocytes. *J Biol Chem* (2008) **283**:24571–83. doi:10.1074/jbc.M802673200
81. Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, et al. LTRPC2 Ca2+-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol Cell* (2002) **9**:163–73. doi:10.1016/S1097-2765(01)00438-5
82. Miller BA. The role of TRP channels in oxidative stress-induced cell death. *J Membr Biol* (2006) **209**: 31–41. doi:10.1007/s00232-005-0839-3
83. Melzer N, Hicking G, Göbel K, Wiendl H. TRPM2 cation channels modulate T cell effector functions and contribute to autoimmune CNS inflammation. *PLoS ONE* (2012) **7**:e47617. doi:10.1371/journal.pone.0047617
84. Olabisi OA, Soto-Nieves N, Nieves E, Yang TTC, Yang X, Yu RYL, et al. Regulation of transcription factor NFAT by ADP-ribosylation. *Mol Cell Biol* (2008) **28**: 2860–71. doi:10.1128/MCB.01746-07
85. Valdor R, Schreiber V, Saenz L, Martínez T, Muñoz-Suano A, Dominguez-Villar M, et al. Regulation of NFAT by poly(ADP-ribose) polymerase activity in T cells. *Mol Immunol* (2008) **45**: 1863–71. doi:10.1016/j.molimm.2007.10.044
86. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* (2012) **13**:907–15. doi:10.1038/ni.2386
87. Yamamoto S, Shimizu S, Kiyonaka S, Takahashi N, Wajima T, Hara Y, et al. TRPM2-mediated Ca2+influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nat Med* (2008) **14**:738–47. doi:10.1038/nm1758
88. Hardaker L, Bahra P, de Billy BC, Freeman M, Kupfer N, Wyss D, et al. The ion channel transient receptor potential melastatin-2 does not play a role in inflammatory mouse models of chronic obstructive pulmonary diseases. *Respir Res* (2012) **13**:30. doi:10.1186/1465-9921-13-30
89. Sumoza-Toledo A, Fleig A, Penner R. TRPM2 channels are not required for acute airway inflammation in OVA-induced severe allergic asthma in mice. *J Inflamm (Lond)* (2013) **10**:19. doi:10.1186/1476-9255-10-19

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Modulation of T cell metabolism and function through calcium signaling

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As a vital second messenger in the activation of lymphocytes, the divalent cation Ca^{2+} plays numerous roles in adaptive immune responses. Importantly, Ca^{2+} signaling is essential for T cell activation, tolerance of self-antigens, and homeostasis. Supporting the essential role of Ca^{2+} signaling in T cell biology, the Ca^{2+} regulated protein phosphatase calcineurin is a key target of pharmacologic inhibition for preventing allograft rejection and for autoimmune therapy. Recent studies have highlighted the unique role of Stim1 and Orai1/2 proteins in the regulation of store-operated/calcium release activated calcium (CRAC) channels in the context of T cells. While Ca^{2+} is known to modulate T cell activation via effects on calcineurin and its target, nuclear factor of activated T cells (NFAT), this second messenger also regulates other pathways, including protein kinase C, calmodulin kinases, and cytoskeletal proteins. Ca^{2+} also modulates the unique metabolic changes that occur during in distinct T cell stages and subsets. Herein, we discuss the means by which Ca^{2+} mobilization modulates cellular metabolism following T cell receptor ligation. Further, we highlight the crosstalk between mitochondrial metabolism, reactive oxygen species (ROS) generation, and CRAC channel activity. As a target of mitochondrial ROS and Ca^{2+} regulation, we describe the involvement of the serine/threonine kinase DRAK2 in the context of these processes. Given the important roles for Ca^{2+} dependent signaling and cellular metabolism in adaptive immune responses, the crosstalk between these pathways is likely to be important for the regulation of T cell activation, tolerance, and homeostasis.

Keywords: calcium, metabolism, T cells, immune system, CRAC, reactive oxygen, DRAK2

INTRODUCTION

The adaptive immune system exhibits a wide potential for responding to infectious agents encountered throughout a lifetime. Notably, T cells play a critical role in mounting an appropriate response through the activation of their T cell receptor (TCR) upon the recognition of specific antigen presented through the major histocompatibility complex (MHC). This foreign peptide: MHC complex activates a T cell with specificity for the foreign antigen, culminating in proliferation, and differentiation into effector cells. In order to stimulate naïve T cells, ligation of the TCR by antigen must be accompanied by a costimulatory signal. Costimulation serves as a mechanism to modulate the strength of the TCR signal and promote higher gene expression of immunomodulatory cytokines like interleukin-2, as well as other factors that facilitate T cell proliferation and differentiation (1, 2). When a naïve T cell becomes activated, in the context of proper costimulation, it executes developmental reprograming characterized by rapid growth, proliferation, and acquisition of specialized effector functions. Given that this is an energetically demanding process, T cells undergo biochemical and biophysical reprograming to meet these requirements (3).

A key mediator of T cell signaling is the divalent cation calcium (Ca^{2+}). Indeed, this second messenger is so important to T cell biology that its downstream signaling serves as a vital pharmacological target for the treatment of autoimmune disease and the prevention of chronic allograft rejection (4). As with many cell types, ligation of surface molecules that induce phospholipase C leads to mobilization of intracellular calcium, with depletion of Ca^{2+} from the endoplasmic reticulum (ER). However, it has long been known that for sustained signaling downstream of Ca^{2+} release, T cells bear special plasma membrane Ca^{2+} channels that promote the influx of extracellular Ca^{2+} (5). While beyond the scope of the present review, recent work in several laboratories has revealed that T cells and a select few other cell types, express plasma membrane channels that are activated in response to depletion of intracellular stores of Ca^{2+} (6). These calcium release activated calcium (CRAC) channels are activated by Stim1, an ER membrane protein that itself is induced upon depletion of Ca^{2+} stores in ER. Major efforts are currently underway to understand the biophysics of CRAC activation, and importantly, to determine if manipulation of CRAC signaling may be of therapeutic benefit.

In the present review, we examine the link between cellular metabolism and calcium signaling in the context of T cells. As described, Ca^{2+} modulates a variety of signaling cascades downstream of the TCR and costimulatory receptors, many of which impinge upon bioenergetic and biosynthetic pathways needed for T cell clonal expansion, differentiation, and homeostasis. First, we briefly outline several important control points that orchestrate cellular metabolic changes following TCR stimulation. Next, we discuss the crosstalk between mitochondrial

metabolism and CRAC activity, describing the influence of the metabolic status of a given T cell on Ca^{2+} mobilization and signaling. Finally, the impact of the release of mitochondrial reactive oxygen species (ROS) on Ca^{2+} signaling is considered, with a focus on the immunoregulatory serine/threonine kinase DRAK2 in this process.

MODULATION OF CELLULAR METABOLISM FOLLOWING T CELL ACTIVATION

Although one might consider metabolic regulation a mundane housekeeping function of cells, it is becoming apparent that specific metabolic programs are induced in distinct T cell subsets and developmental stages. For example, the metabolic status of naïve T cells is significantly different from their activated counterparts. Naïve T cells actively maintain a quiescent state through engagement of both intracellular signaling pathways and cell extrinsic signals (7), resulting in efficient use of available energy sources (8). These quiescent lymphocytes maintain a catabolic state and do not actively take up nutrients, nor do they secrete effector cytokines (9). Once committed to a T cell fate in the thymus, cellular metabolism plays a vital role in the development and proliferation of double negative (DN) thymocyte precursors (11). In DN thymocytes, both interleukin-7 (IL-7) and Notch prevent cell death and promote pre-T cell development via activation of glycolytic metabolism and the Akt signaling pathway (12). Following release from the thymus, antigenic stimulation of mature T cells facilitates metabolic changes that support various bioenergetically dependent processes needed for rapid clonal expansion (9). It is proposed that T cells must shift from catabolic to anabolic metabolism in order to rapidly proliferate, likely allowing them to respond to microbial infection [(10) p. 2313]. Indeed, CD8 T cells have the capacity to divide once every 4–6 h (13), a process that is highly energy dependent. Naïve T cells appear to favor energetically efficient processes such as the tricarboxylic acid (TCA) cycle linked to the generation of ATP via oxidative phosphorylation (OXPHOS), which results in roughly 30–32 ATP units per molecule of glucose. In contrast, antigenically stimulated T cells rapidly shift to a dependence on aerobic glycolysis, a less efficient process that yields only two ATP units per molecule of glucose (14, 15). Activated T cells that fail to switch metabolic processes are rendered anergic or undergo apoptosis (16), likely a consequence of failing to accommodate the specific bioenergetic demands of proliferation and differentiation (17, 18). Thus, it is clear that the metabolic status must match the needs of distinct T cell subsets and developmental stages in order for appropriate immune responses to be generated.

With regard to the intracellular signaling involved in metabolic regulation, it has long been appreciated that the mechanistic target of rapamycin (mTOR) has a critical role in T cell activation and metabolism (19). In the context of metabolism, mTOR serves to integrate nutrient and immune signals, including the availability of amino acids and oxygen, as well as the presence of extracellular growth factors. mTOR then acts as an effector to modulate downstream cellular metabolic processes needed to meet the demands of the cell upon stimulation (20). Such processes include protein translational initiation via phosphorylation of S6K1 and eIF4E (21), and lipid biosynthesis through the transcription factor SREBP1 and the nuclear protein receptor PPARγ (22). It is crucial, however, that mTOR does not become prematurely activated until T cells are antigenically stimulated, as the quiescent metabolic state of resting T cells appears to be important for their homeostatic proliferation (23). As detailed later, the energy sensing protein kinase AMPK acts as a master regulator of the metabolic status in resting T cells. Induced by high levels of AMP, AMPK influences mTOR activity through the tumor suppressor tuberous sclerosis complex (24). Comprised of TSC1 and TSC2, the tuberous sclerosis complex itself negatively regulates mTOR activation (25) and is crucial to maintaining homeostatic proliferation of naïve T cells. Supporting this, Yang and colleagues observed that TSC1 deficient naïve T cells possess hyperactive mTOR activity, a resulting loss in quiescence, and a predisposition to undergo apoptosis (26). Though naïve T cells do not initially require mTOR for TCR induced activation, its absence by genetic deletion in mouse CD4 T cells yields a skewed differentiation toward induced T regulatory cells over other effector T cell subsets (27). In addition, mTOR has a central role in the regulation of both activated and long-lived memory T cells as its genetic deletion or pharmacologic inactivation leads to diminished memory T cell activation and function (22, 28, 29).

METABOLIC STATES IN DISTINCT T CELL SUBSETS

Naïve T cells utilize efficient oxidative metabolism to maintain their quiescent state. Conversely, once T cells are stimulated by antigen, they must quickly expand their numbers to eliminate an antigenic challenge. This view is reminiscent of the Warburg hypothesis, in which heightened glycolysis observed in cancer cells is thought to allow for rapid tumor cell proliferation, particularly under limiting oxygen tension (30). Alternatively, it has been recently proposed that the switch to aerobic glycolysis is instead necessary to support effector T cell differentiation. Pearce and colleagues demonstrated that blockade of glycolysis prevented the expression of interferon gamma in activated T cells, but did not impair clonal expansion (31). Moreover, the rapid recall response observed in memory T cells, cells that often must respond quite rapidly to antigenic rechallenge, is supported by enhanced respiratory and glycolytic capacity (32, 33). It remains to be determined if this may reflect differential survival of unique subpopulations during clonal expansion. Regardless, distinct metabolic processes are clearly involved in providing for the energetic demands of unique T cell subpopulations, with fatty acid oxidation (FAO) and aerobic glycolysis playing significant roles.

FATTY ACID OXIDATION

In resting T cells that circulate in the periphery, FAO is the default metabolic state, and the metabolism of these quiescent cells is characterized by a need for basal energy utilization over macromolecular biosynthesis (34). These naïve T cells utilize high energy yielding OXPHOS processes, involving β-oxidation of fatty acids and oxidation of glutamine and pyruvate via the TCA cycle (34). Both naïve CD4 and CD8 T cells also rely on intrinsic IL-7 to maintain homeostasis and quiescent survival (35). Loss of IL-7 receptor (IL-7R) signaling results in defective T cell physiology, characterized by decreased cell size/growth and markedly impaired cell survival (36). The involvement of IL-7R signaling in

the maintenance of peripheral T cell homeostasis is complex; it has been recently found that IL-7R signaling must be intermittent and not continuous, as the latter results in sensitization of naïve T cells to cytokine induced cell death (37). While IL-7R signaling promotes survival of quiescent peripheral T cells, and is required for homeostatic proliferation under lymphopenic conditions, it alone is not sufficient to induce naïve T cell activation and the metabolic changes associated with this (38). Instead, with minimal mTOR activity, resting T cells utilize other signaling factors to regulate metabolic pathways. These factors include the nuclear receptors PPARα and PPARγ, both of which regulate fatty acid metabolism and inhibit activation upon TCR engagement (12, 39).

While an increase in glycolysis generally is observed in activated T cells, this is not always the case. Induced regulatory T cells (iT_{reg}), differentiated from peripheral $CD4^+$ T cells, are a unique subset of $CD4^+$ T cells that suppress effector T cells and are vital to immune peripheral tolerance (40). Following a lag phase heavily dependent on glycolysis and glutaminolysis (28), T cells activated in the context of extracellular signals that favor iT_{reg} differentiation (e.g., IL-2 and TGF-β) acquire a metabolic phenotype similar to naïve T cells (41). Relying on lipid oxidation as their primary source of energy, peripherally differentiated iT_{regs} and their thymically derived nT_{reg} counterparts have intermediate to low mTOR activity (18). The distinct metabolic profile of T_{reg} can be replicated through *in vitro* culture with addition of glycolytic or mTOR inhibitors, such as 2-deoxyglucose or rapamycin, respectively (12). As with naïve T cells, PPARα and PPARγ are important for T_{regs}, serving as fatty acid sensors, and promoting Foxp3 expression in $CD4^+$ T cells activated in the presence of TGF-β (42).

Fatty acid oxidation also plays a vital role in the maintenance of memory T cell pools. Following clearance of an acute viral infection, the antiviral $CD8^+$ effector T cell pool is radically depleted, with a loss of 90–95% of virus specific $CD8^+$ T cells (43). The surviving cells in turn become long-lived memory T cells (44), possessing unique metabolic characteristics when compared with effector cells (45). Memory $CD8^+$ T cells must be able to withstand periods of both antigenic neglect and rapid antigen specific recall through the acquisition of increased spare respiratory capacity (SRC) through biogenesis of mitochondria and increased glycolytic flux (32). Thus, in contrast to their effector counterparts, these long-lived $CD8^+$ T cells have significantly enhanced SRC. Memory $CD8^+$ T cells share an analogous metabolic profile with resting T cells and T_{regs}, primarily engaging in FAO to maintain their survival and homeostasis (46). These metabolic processes are maintained by IL-15 signaling, which facilitates the biogenesis of mitochondria and expression of CPT1A, an enzyme responsible for the rate-limiting step of FAO (32).

GLYCOLYSIS

As noted above, activated T cells switch their metabolic programing to aerobic glycolysis upon antigenic stimulation (15, 47). This may seem counterproductive, as the effective ATP output per glucose molecule taken into the cell is roughly one fifteenth of the units generated via OXPHOS (12). Instead, it has been proposed that this switch is necessary to facilitate the rapid clonal expansion required to eliminate a microbial infection (45). Growth factor stimulation results in enhanced uptake of glucose through the upregulation of the glucose transporter Glut1 on the surface of cells, along with increased expression of the glycolytic enzymes hexokinase and phosphofructokinase (14), processes activated in T cells by TCR ligation (48). Costimulation through CD28 leads to the induction of Akt, thereby enhancing glycolytic activity in T cells (15), and the prevention of growth factor withdrawal induced cell death (17). Supporting a crucial role for Akt in promoting metabolic changes and the survival of activated T cells, ectopic expression of an active form of Akt leads to increased rates of glycolysis and T cell survival, even in the absence of CD28 signaling (49).

The AMP-dependent protein kinase AMPK serves a critical regulator of cellular metabolism, both in naïve and newly activated T cells (**Figure 1A**). In resting cells, a high ratio of AMP to ATP leads to elevated AMPK activity and diminished mTOR function. TCR engagement activates LKB1 and in parallel, increases intracellular Ca^{2+} stores, both leading to an increase in the expression of AMPK (50). LKB1 positively regulates AMPK (51, 52), the latter of which serves as an upstream regulator of TSC1 (52). As TSC1 inhibits mTOR activity in naïve T cells through the tuberous sclerosis complex, AMPK restricts the engagement of metabolic programs associated with clonal expansion. Deletion of the *Tsc1* gene leads to metabolic alterations in T cells, most notably, increases in glucose uptake and glycolytic flux (53). AMPK activity is positively influenced by calcium mobilization via its impact on Ca^{2+} – calmodulin-dependent protein kinase kinase (CaMKK) activity (50, 54). Thus, while AMPK may be associated with a quiescent T cell state, the TCR induced increase of cytosolic Ca^{2+} enhances AMPK activity (**Figure 1B**). This effectively conserves ATP by inhibiting mTOR associated anabolic processes, and by promoting OXPHOS (50, 55). With time, the resulting increase in the ATP to AMP ratio leads to diminished AMPK activity, and downstream restriction of mTOR is overcome, allowing the T cell to shift its metabolism from FAO to OXPHOS (**Figure 1C**).

Upon successful engagement of their TCR and the costimulation molecule CD28, the T cell begins to switch toward an activated metabolic program, utilizing aerobic glycolysis, the phosphate pentose pathway (PPP), and glutaminolysis (18). Two transcriptions factors that coordinate metabolic status following T cell activation are Myc and HIF1α (28, 56). Induction of Myc expression in activated T cells is largely responsible for metabolic reprograming at a global gene transcriptome level, as its acute deletion inhibited glycolysis, glutaminolysis, and provoked an overall failure in cell growth and proliferation (28). Myc is also found to intersect with the mTOR pathway, as its loss in activated T cells led to reduced expression and phosphorylation of the downstream mTOR targets S6 and 4E-BP (28). mTOR itself, specifically the mTOR complex 1 (mTORC1), is responsible for activating two key metabolic transcriptional targets, HIF1α and SREBP1/2. Specifically, HIF1α activates downstream targets involved in glycolysis and glucose uptake, while SREBP1/2 activates the PPP and lipid biosynthesis (57). However, while SREBP activity is essential for metabolic reprograming in activated T cells (58), HIF1α appears to play a more selective role in T cell subsets such as Th17 cells (56, 59). It is likely that HIF1β serves a compensatory role in HIF1α-deficient T cells, as loss of HIF1β leads to defective glycolysis, survival, trafficking, and function of $CD8^+$ T cells (60).

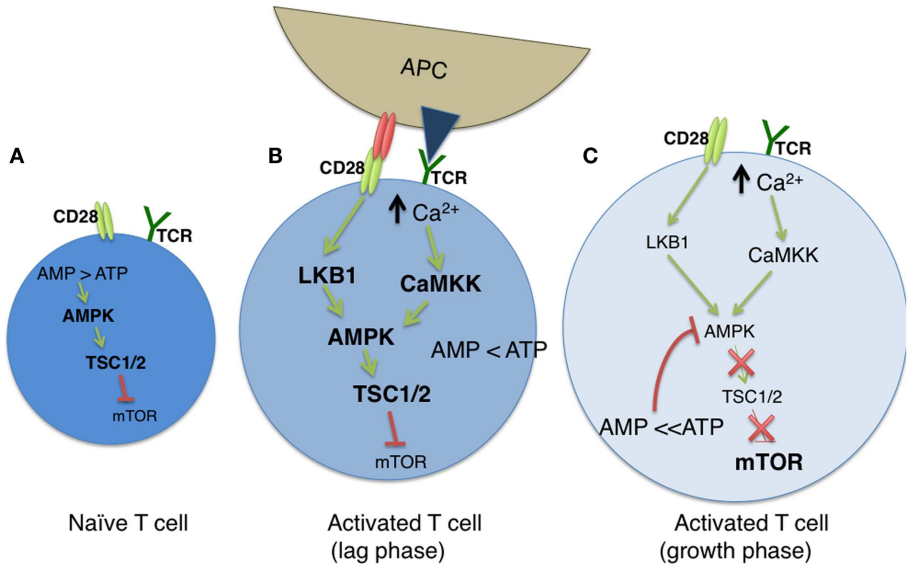

FIGURE 1 | The modulation of cellular metabolism via T cell receptor (TCR) signaling. **(A)** In naïve T cells, fatty acid oxidation (FAO) is used to maintain basal cellular metabolism. In this quiescent state, the absence of TCR signaling leads to an elevated AMP to ATP ratio, resulting in sustained AMPK and diminished mTOR activities. **(B)** Upon TCR stimulation by antigen presenting cells (APC), along with CD28-mediated costimulation, glycolysis is greatly enhanced, leading to a diminished AMP:ATP ratio. During a lag phase that precedes T cell clonal expansion, LKB1 and CaMKK (itself induced by high cytosolic Ca^{2+} levels) promote AMPK function despite reduced AMP levels, and mTOR activity remains low via TSC1/2 mediated inhibition. **(C)** As T cells exit the lag phase, heightened levels of ATP block AMPK activity, which results in the loss of TSC1/2 activity. This allows for sustained mTOR function, and subsequent mTOR driven clonal expansion and cell growth.

CALCIUM MOBILIZATION FOLLOWING TCR LIGATION

Having considered the modulation of cellular metabolism via TCR proximal signaling pathways, we now turn our attention to calcium mobilization, and how this signaling platform shapes the metabolic response in T cells. As described in detail in this compendium of reviews, TCR stimulation engages numerous signaling cascades, resulting in cellular activation and proliferation. One such molecule that is activated is phospholipase Cγ1 (PLCγ1), which mediates the hydrolysis of phosphatidylinositol-3,4-bisphospahte (PIP_2) into inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Ca^{2+} release from ER stores is triggered upon the binding of IP_3 to inositol trisphosphate receptor (IP_3R) found on the ER membrane. The two major ion channels that are known to participate in Ca^{2+} release from the ER in response to agonist stimulation are IP_3Rs (61) and ryanodine receptors (RyRs) (62). Mobilization of intracellular Ca^{2+} triggers store-operated Ca^{2+} entry (SOCE), primarily through calcium release activated Ca^{2+} (CRAC) channels (63). The binding of antigen/MHC to the TCR complex results in the phosphorylation and activation of PLCγ1, which then hydrolyzes phospholipids at the plasma membrane to produce DAG and IP_3. IP_3 then binds to its respective receptors in the ER, triggering calcium release into the cytosol. This secondarily activates CRAC channels within the plasma membrane, causing a rapid influx of extracellular calcium that sustains high calcium levels required for T cell activation (64). Upon accumulation, cytosolic calcium binds calmodulin (CaM), inducing a conformational change in CaM that promotes its ability to interact with and activate the protein phosphatase calcineurin (65). Calcineurin dephosphorylates the cytoplasmic subunits (NFATc) of nuclear factor of activated T cells (NFAT) transcription complexes, exposing a nuclear localization sequence that results in their import into the nucleus (66).

Cytosolic calcium is a universal second messenger affecting a variety of cellular processes extending from short- to long-term responses in immune cells (67). Unlike other intracellular messengers, Ca^{2+} is neither synthesized nor metabolized. Rather, Ca^{2+} storage and release are carefully regulated by a series of channels and pumps that maintain precise Ca^{2+} concentrations within distinct cellular compartments. In lymphocytes, activation of immune receptors initiates a signaling cascade culminating in the depletion of intracellular Ca^{2+} stores. Upon depletion, store-operated calcium channels are activated, allowing Ca^{2+} to enter into the cell, primarily through CRAC channels. The increase in intracellular free calcium is essential for lymphocyte activation, since the transcription factor families NFAT, NF-κB, and AP-1 are all targets of Ca^{2+} mediated signaling (68). Remarkably, 75% of all activation-regulated genes in T cells show a dependence on Ca^{2+} influx (68). Supporting this, loss of Ca^{2+} mobilization dramatically reduces, and often prevents, T cell activation and proliferation (68–70). Below, we detail the regulation of intracellular calcium oscillation by CRAC channels and mitochondria, and describe how Ca^{2+} influences the generation of and response to cellular oxidants.

STORE-OPERATED CA^{2+} CHANNELS

Calcium release activated calcium channels comprise a widespread and highly conserved Ca^{2+} entry pathway in cells such as lymphocytes, and are activated following the depletion of Ca^{2+} within the

ER. This phenomenon is referred to as SOCE and CRAC activity is proportional to the amount of Ca^{2+} depletion within the ER. Over the last decade, the key components of the CRAC channel machinery were identified as STIM1, the ER Ca^{2+} sensor and Orai1/2, pore forming subunits of the CRAC channel (71). Depletion of Ca^{2+} within the ER is detected by the Ca^{2+} sensors STIM1 and STIM2, resulting in the activation of store-operated calcium channels. STIM1 and STIM2 are EF-hand containing single-pass transmembrane proteins with both EF hands capable of binding Ca^{2+}. STIM2 is thought to have a lower affinity for Ca^{2+} since STIM1-deficient mouse T cells and fibroblasts bear a severe impairment in store-operated Ca^{2+} influx whereas a deficiency in STIM2 has a less dramatic effect (72).

The luminal EF-hand of STIM1 senses Ca^{2+} depletion within the ER and subsequently causes its oligomerization and translocation toward the plasma membrane (73). STIM1 binds through specialized ER-PM junctions, located within 25 nm of the plasma membrane, to a component of the CRAC channel known as Orai1 (74). Once activated, CRAC channels have a remarkably selective single channel conductance for Ca^{2+}, and sensitive Ca^{2+} dependent feedback regulation (75). Association of STIM1 and Orai1 depends on the involvement of STIM1 with the phosphoinositides (PIs) of Orai1. Within STIM1, the cytosolic SOAR/CAD domain contains an alpha-helical domain necessary for Orai1 binding (76, 77). Deletion of the SOAR/CAD domain abolishes store depletion-induced clustering of Orai1 monomers and dramatically reduces SOCE, suggesting this domain is required for the assembly of the SOCE complex at ER-PM junctions (76, 77).

MITOCHONDRIAL CALCIUM BUFFERING AND HOMEOSTASIS

Mitochondria are recognized as essential calcium signaling organelles. Through calcium buffering, mitochondria influence CRAC channel activity (78). With the ability to sense Ca^{2+} microdomains, mitochondria are able to translocate to the immunological synapse upon Ca^{2+} influx, leading to maximal Ca^{2+} uptake. The positioning of mitochondria correlates with the magnitude of local calcium entry via the reduction of Ca^{2+} dependent channel inactivation (79). By co-localizing near ER, mitochondria are better suited to buffer cytosolic Ca^{2+}.

Isolated mitochondria take up Ca^{2+} when supported by exogenous electron transport chain (ETC) substrates (80). Although it has been well known that mitochondria are endowed with a complex array of Ca^{2+} transporters, the function of these transporters was not well appreciated until the early 1990s when Pozzan, Rizzuto and colleagues examined aequorin, a Ca^{2+} sensitive bioluminescent protein. They found that agonist-stimulated elevation of cytosolic free Ca^{2+} results in a rapid and transient increase in mitochondrial Ca^{2+}, an increase blocked by pretreatment with a mitochondrial uncoupler (81). Prior to this discovery, it was believed that mitochondria failed to release much Ca^{2+} into the cytosol and had little effect on $InsP_3$-mediated Ca^{2+} release. It has since become appreciated that mitochondrial matrix Ca^{2+} concentrations are crucial regulators of the Ca^{2+} dependent enzymes of the TCA cycle (82). Mitochondria also play a vital role in the gating of CRAC channels and do so through Ca^{2+} buffering (83).

Mitochondria act as Ca^{2+} buffers through uptake of Ca^{2+} released by IP_3R on proximal ER membranes (**Figure 2**). Mitochondria accomplish this through the ability to detect changes in Ca^{2+} microdomains (84); Ca^{2+} uptake by mitochondria occurs by mitochondrial Ca^{2+} uniporter (MCU) activity across the inner mitochondrial membrane (85). This Ca^{2+} buffering is functionally significant as it modifies the Ca^{2+} dependent inactivation of IP_3R, ultimately leading to greater Ca^{2+} mobilization. Mitochondria also deplete Ca^{2+} adjacent to the ER, resulting in less available Ca^{2+} for transport into the ER via SERCA pumps. Together, these events result in robust activation of CRAC channels (86, 87). It has been estimated that between 25 and 50% of the Ca^{2+} released from ER is taken up by mitochondria, dependent on cell type, and the Ca^{2+} release channel involved (88). This Ca^{2+} uptake influences mitochondrial metabolic processes, as three rate-limiting enzymes of the Krebs cycle are activated by a rise in Ca^{2+} concentration, subsequently causing an increase in mitochondrial ATP generation (89). These enzymes include pyruvate dehydrogenase (PDH), NAD^+-isocitrate dehydrogenase (NAD-IDH), and 2-oxoglutarate dehydrogenase (82).

In addition to influencing CRAC channel gating, mitochondria also influence STIM1 trafficking (90). Formation of STIM1 puncta below the plasma membrane is diminished, independently of STIM1 oligomerization, upon strong mitochondrial depolarization. This is accomplished by the mitochondrial protein mitofusin 2 (MFN2), an outer mitochondrial membrane GTPase that couples mitochondrial energetic status to STIM1 migration (90). In MFN2-deficient cells, mitochondrial depolarization fails to influence STIM1 trafficking and Ca^{2+} entry, suggesting that MFN2 confers sensitivity to mitochondrial depolarization. MFN2 assists in tethering mitochondria to the ER and is abundant in the contact sites between the two organelles, though the exact mechanism through which these two organelles interact remains unclear (91). It is speculated that the most likely function of the tethering is to allow mitochondria to localize proximal to Ca^{2+} released from ER. However, the question as to why STIM1 overexpression partially rescues Ca^{2+} entry in MFN2 overexpressing cells has yet to be answered.

While both ER and mitochondria serve as important intracellular reservoirs of Ca^{2+}, mitochondria utilize functionally distinct Ca^{2+} transport mechanisms. Mitochondria possess a large electrochemical gradient across their inner membrane ($\Delta\Psi_m$), which acts as a driving force for the uptake of Ca^{2+} (92). Unlike with ER, mitochondrial Ca^{2+} release occurs via ion exchangers, and Ca^{2+} uptake is mediated via a selective ion channel (93). High calcium levels, in combination with other signals, have been known to trigger the opening of channels within the inner mitochondrial membrane, ultimately resulting in cell death (94). The combined action of these mechanisms allows for the rapid cycling of Ca^{2+} across the inner mitochondrial membrane (95).

REGULATION OF STIM1/ORAI SIGNALING BY OXIDATIVE STRESS

Numerous physiological processes, including proliferation, and cell death, are influenced by ROS (96, 97). To prevent oxidative stress, the cell must maintain a balance between superoxide generation, a byproduct of oxidative energy production, and rapid clearance of ROS. The latter is achieved through circulating antioxidants that act as ROS scavengers. Out of the ∼20 types of ROS,

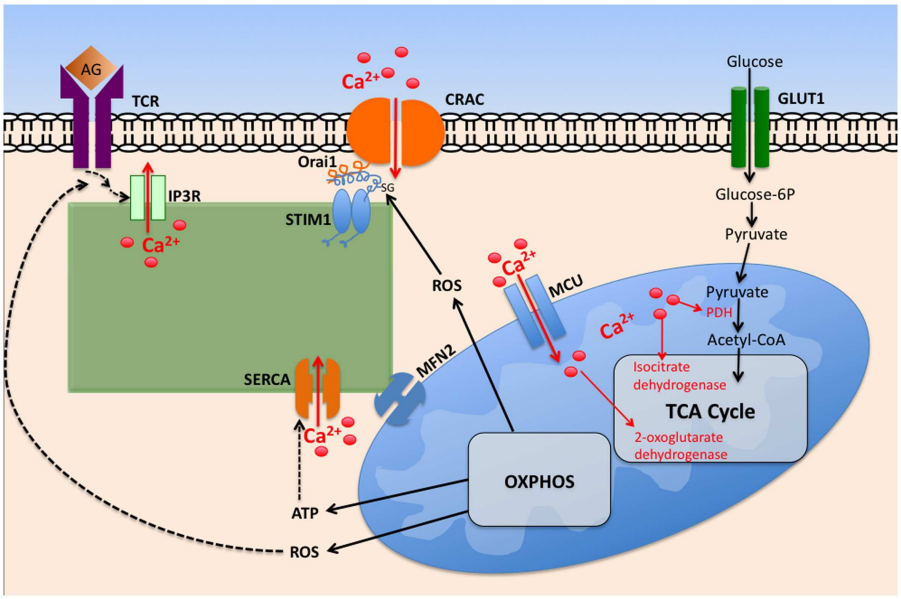

FIGURE 2 | The interplay between intracellular calcium mobilization and cellular metabolism in T cells. Ligation of the T cell receptor (TCR) by antigen (Ag) promotes opening of IP_3R and depletion of ER Ca^{2+} via IP3, itself generated by phospholipase Cγ1. Depletion of ER Ca^{2+} stores promotes STIM1 interaction with Orai1, promoting oligomerization of Orai monomers, thus forming CRAC channels. The resultant increase in cytosolic Ca^{2+} promotes its entry into the mitochondrial matrix via the mitochondrial calcium uniporter (MCU). Elevated matrix Ca^{2+} enhances the activity of key tricarboxylic acid cycle (TCA) enzymes, leading to elevated oxidative phosphorylation (OXPHOS), and ATP generation. Reactive oxygen species (ROS) byproducts of OXPHOS enhance TCR proximal signaling by inhibiting tyrosine phosphatase activity. Elevated cytosolic ROS governs CRAC activity by ROS mediated modification of cysteine residues exposed on Orai1.

hydrogen peroxide (H_2O_2) appears to be the major contributor to oxidative damage (96). Of interest here, it has been found that oxidative stress regulates STIM1/Orai1 signaling (98–100). CRAC channels, under the action of STIM1, are stimulated by micromolar concentrations of H_2O_2 (98–100). Altered calcium signaling during oxidant stress is attributed to a reduction in the Ca^{2+} binding affinity of STIM1 due to a modification involving its cysteine 56, ultimately resulting in constitutive CRAC activation (100). Upon exposure to ROS, STIM1 becomes S-glutathionylated, thereby triggering its oligomerization and translocation to the plasma membrane (100). Alternatively, Grupe and colleagues demonstrated that H_2O_2 enhanced the CRAC current, I_{CRAC}-mediated Ca^{2+} influx by activating IP_3R independent of Orai1 (99). Regardless of the route of entry, both groups demonstrate that an increase an ROS leads to enhanced Ca^{2+} entry into the cell. While several studies support the hypothesis that ROS positively stimulate CRAC channel activity by triggering the oligomerization of STIM1, Bogeski and colleagues report that oxidation via H_2O_2 specifically blocks the activation of Orai1 channels in human T helper lymphocytes (98). It is speculated that the oxidation of the cysteine, found within the extracellular loop of Orai1, may lock the channel in the closed conformation. Nevertheless, cells with an SOCE deficiency are more susceptible to oxidative stress. Despite having an up-regulated basal antioxidant response, STIM1-deficient mouse embryonic fibroblasts experience an imbalance of ROS production and detoxification upon addition of exogenous oxidants (101). Henke and colleagues conclude that functional SOCE machinery is required to balance ROS production and the cellular antioxidant defense system. Collectively, these studies support the notion that the cellular oxidative stress response is influenced through a dynamic interplay between ROS balance and Ca^{2+} influx.

OXIDANT DEPENDENCE OF T CELL ACTIVATION

Oxidative stress is provoked by an imbalance between the production of mitochondrial superoxide and insufficient scavenging of ROS, ultimately resulting in a wide range of pathological conditions (102). The dynamics of ROS production and scavenging can be detected using several fluorescent dyes, including reduced forms of ethidium bromide (DHE) (103), fluorescein (DCFDA) (104, 105), rhodamine (DHR) (106), and hydroethidine (HE) (107). These dyes remain relatively non-fluorescent until they are oxidized by ROS, and their unquenched fluorescence correlates with increased oxidation. These intracellular dyes provide sensitive probes for multiple reactive species and allow for the detection of fluorescent products using fluorimetry and flow cytometry. In T cells, DCFDA, DHR, and DHE have been utilized to examine ROS production from a variety of stimuli including mitogens (108), viral and bacterial superantigen (109, 110), and TCR peptide agonists (111, 112). ROS generation has been observed following stimulation of T cells by other ligands including TGF-β, insulin, angiotensin II, and EGF (113–116). Collectively, these studies suggest that ROS function as secondary messengers necessary for complete T cell activation. Superoxide is generated from the mitochondrial ETC through complexes I, II, and III (117). Complexes I and II emit superoxide into the mitochondrial matrix, upon which

superoxide dismutase 2 (SOD2) converts it into hydrogen peroxide (H_2O_2). In contrast, complex III emits superoxide into both the matrix and intermembrane spaces. Intermembrane-space superoxide is capable of reaching the cytosol through voltage-dependent anion channels (VDAC) without conversion into H_2O_2 (118, 119).

Recently, Sena et al. reported that blocking mitochondrial ROS, specifically generated at complex III, results in the inhibition of CD3/CD28 induced IL-2 expression. Additionally, extracellular calcium influx and subsequent Ca^{2+} uptake by mitochondria were both required for mitochondrial ROS production (120). These findings support the hypothesis that complex III produced mitochondrial ROS, a byproduct of mitochondrial metabolism, facilitate T cell activation and functionality. However, the mechanisms through which oxidants may regulate T cell responses upon stimulation remain poorly defined. Importantly, the timing and subcellular localization of ROS generation are likely of greater influence in T cell responses than overall redox balance (102, 121, 122). ROS clearly play key roles in modulating T cell activation and differentiation, but vital details regarding the influence of these oxidants on specific signaling molecules remain to be clarified.

DRAK2 AS A AN INTERMEDIARY IN CALCIUM AND METABOLIC SIGNALING IN T CELLS

One molecule expressed in T cells that may potentially link calcium and ROS signaling to cellular metabolic regulation is DRAK2/STK17B. DRAK2 is a serine/threonine kinase of the death associated protein kinase (DAPK) family (123), which is comprised of five known members (DAPK1, DAPK2, DAPK3, DRAK1, and DRAK2). All of these kinases are capable of inducing cell death when ectopically expressed in cells, and the prototype DAPK1 possesses a calmodulin regulatory domain that, through binding to calmodulin/Ca^{2+}, regulates its catalytic activity (124). While DRAK2 lacks a calmodulin binding domain, like other DAPK family members, DRAK2 has been shown to induce apoptosis upon overexpression in cell lines (125), and its phosphorylation and nuclear translocation participates in ultraviolet light induced cell death (126).

Although broadly expressed at low levels (127), DRAK2 expression is enriched in cells of hematopoietic origin (128). Loss of expression by virtue of a germline deletion of the *Drak2* gene leads to hyperactive Ca^{2+} mobilization in T cells, especially under suboptimal TCR stimulus conditions (128, 129), supporting the hypothesis that DRAK2 acts as a negative regulator of TCR signaling (130). The notion that DRAK2 serves as a rheostat in calcium signaling following TCR signaling is supported by the finding that it is itself activated by calcium mobilization, and that its ectopic expression in double positive thymocytes raises the threshold for both negative and positive selection (131, 132). Enigmatically, loss of the *Drak2* gene leads to diminished autoimmune susceptibility, with reduced clinical severity observed in animal autoimmune models for multiple sclerosis (experimental autoimmune encephalomyelitis) and type I diabetes-prone non-obese diabetic (NOD) mice (129, 133–135). The reduced EAE susceptibility is due, at least in part, to diminished survival of activated effector T cells, perhaps a consequence of impaired calcium homeostasis (133–135).

Using a mass spectrometry based approach, we determined that DRAK2 possesses several autophosphorylation sites, most notably Ser10, Ser12, and Ser351. Using antibodies specific for phosphorylation at Ser12, it was found that TCR stimulation induces DRAK2 Ser12 autophosphorylation (131), and this requires entry of extracellular Ca^{2+} into T cells. The SERCA inhibitor thapsigargin was found to potently induce DRAK2 autophosphorylation on Ser12, supporting the hypothesis that ER store-operated calcium entry modulates DRAK2 catalytic activity. Recent studies have revealed that TCR induced DRAK2 activation is highly dependent on CRAC function, as DRAK2 was poorly activated following antigenic stimulation in Orai2-deficient T cells (136). Using a panel of small molecule inhibitors and RNA interference approaches, it was found that DRAK2 activation by antigenic stimulation requires protein kinase D (PKD).

Curiously, DRAK2 catalytic activity in activated T cells also depends on mitochondrial ROS, as antioxidants blocked Ser12 autophosphorylation (136). Since the electron transport complex III inhibitor FCCP led to Ser12 phosphorylation independent of antigenic stimulation of T cells, these findings demonstrate that ROS are both necessary and sufficient to promote DRAK2 catalytic activity. PKD activity is itself subject to activation by mitochondrial ROS (137, 138), and thus ROS induced by enhanced mitochondrial respiration that results from TCR stimulation likely participates in PKD-mediated DRAK2 activation (**Figure 3**). The specific nature of the interaction between mitochondrial ROS and DRAK2's substrates is poorly understood.

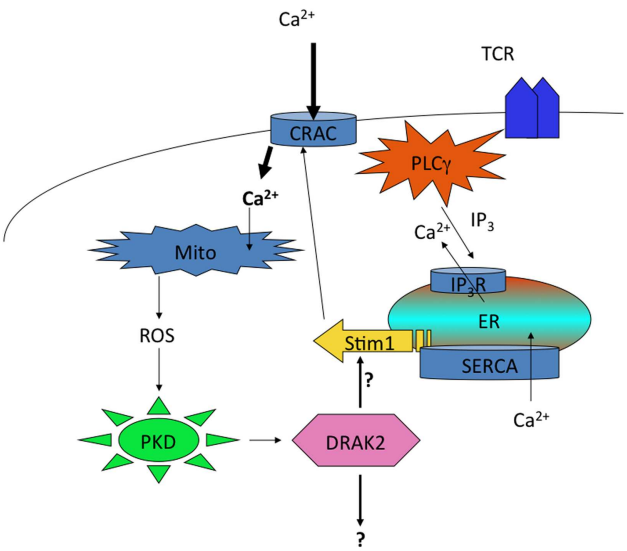

FIGURE 3 | Regulation of DRAK2 activity in T cells via Ca^{2+}-induced respiratory burst. TCR induced Ca^{2+} depletion from ER stores results in opening of CRAC channels. This high cytosolic [Ca^{2+}] promotes the induction of TCA cycle and OXPHOS in mitochondria, the latter of which release ROS as an OXPHOS byproduct. In addition to inhibiting phosphatases, ROS activate PKD, which itself binds to and activates DRAK2 (likely through transphosphorylation on DRAK2). DRAK2 then impacts Ca^{2+} signaling by altering SERCA activity (hypothetical), or other unknown substrates.

However, as DRAK2-deficient T cells bear enhanced CRAC activity (136), and DRAK2-transgenic thymocytes have diminished Ca^{2+} mobilization following TCR stimulation (132), it is likely that DRAK2 may phosphorylate an intermediate in CRAC signaling. DRAK2 has been shown to phosphorylate S6K1 (139), an important target of mTOR and of TCR signaling that impacts cellular metabolism (22, 57, 140). Thus, DRAK2 may itself serve as an important link between calcium and mTOR signaling, impacting the differentiation and survival of T cell subsets selectively required for immune responsiveness.

CONCLUSION

Research into the regulation of CRAC activity, and the unique involvement of metabolic signaling in T cells, has been intense over the last several years. It is becoming quite evident that both signaling paradigms are highly regulated and vital to T cell activation, differentiation, and homeostasis. These pathways also appear to play selective roles in distinct T cell subsets, with the potential that manipulation of such pathways could influence the balance between immunity vs. self-tolerance in different disease states. A significant challenge to immunologists is to develop selective therapies to influence this balance. In the case of autoimmune disorders, the difficulty is to block autoreactive T cell function without promoting general immunosuppression. In the case of chronic microbial pathogen infections and cancer, therapeutic approaches to reinvigorate antimicrobial or anti-tumor T cell responsiveness are highly sought after. It is thus imperative that novel methods are considered to influence the appropriate immune response. Given the unique crosstalk between calcium mobilization and bioenergetic metabolism, there may be an opportunity to uniquely affect the outcome of immune therapies by targeting the molecules that mediate this crosstalk.

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REFERENCES

1. Michel F, Attal-Bonnefoy G, Mangino G, Mise-Omata S, Acuto O. CD28 as a molecular amplifier extending TCR ligation and signaling capabilities. *Immunity* (2001) **15**:935–45. doi:10.1016/S1074-7613(01)00244-8
2. Riley JL, June CH. The CD28 family: a T-cell rheostat for therapeutic control of T-cell activation. *Blood* (2005) **105**:13–21. doi:10.1182/blood-2004-04-1596
3. Gerriets VA, Rathmell JC. Metabolic pathways in T cell fate and function. *Trends Immunol* (2012) **33**:168–73. doi:10.1016/j.it.2012.01.010
4. Schreiber S, Crabtree G. The mechanism of action of cyclosporin A and FK506. *Immunol Today* (1992) **13**(4):136–42. doi:10.1016/0167-5699(92)90111-J
5. Lewis RS, Cahalan MD. Mitogen-induced oscillations of cytosolic Ca2+ and transmembrane Ca2+ current in human leukemic T cells. *Cell Regul* (1989) **1**:99–112.
6. Cahalan MD, Chandy KG. The functional network of ion channels in T lymphocytes. *Immunol Rev* (2009) **231**:59–87. doi:10.1111/j.1600-065X.2009.00816.x
7. Hua X, Thompson CB. Quiescent T cells: actively maintaining inactivity. *Nat Immunol* (2001) **2**:1097–8. doi:10.1038/ni1201-1097
8. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. *Nat Rev Immunol* (2005) **5**:844–52. doi:10.1038/nri1710
9. Jones RG, Thompson CB. Revving the engine: signal transduction fuels T cell activation. *Immunity* (2007) **27**:173–8. doi:10.1016/j.immuni.2007.07.008
10. Jones RG, Bui T, White C, Madesh M, Krawczyk CM, Lindsten T, et al. The proapoptotic factors Bax and Bak regulate T Cell proliferation through control of endoplasmic reticulum Ca(2+) homeostasis. *Immunity* (2007) **27**:268–80. doi:10.1016/j.immuni.2007.05.023
11. Robey EA, Bluestone JA. Notch signaling in lymphocyte development and function. *Curr Opin Immunol* (2004) **16**:360–6. doi:10.1016/j.coi.2004.03.009
12. Waickman AT, Powell JD. mTOR, metabolism, and the regulation of T-cell differentiation and function. *Immunol Rev* (2012) **249**:43–58. doi:10.1111/j.1600-065X.2012.01152.x
13. van Stipdonk MJ, Lemmens EE, Schoenberger SP. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat Immunol* (2001) **2**:423–9.
14. Vander Heiden MG, Plas DR, Rathmell JC, Fox CJ, Harris MH, Thompson CB. Growth factors can influence cell growth and survival through effects on glucose metabolism. *Mol Cell Biol* (2001) **21**:5899–912. doi:10.1128/MCB.21.17.5899-5912.2001
15. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* (2002) **16**:769–77. doi:10.1016/S1074-7613(02)00323-0
16. Zheng Y, Delgoffe GM, Meyer CF, Chan W, Powell JD. Anergic T cells are metabolically anergic. *J Immunol* (2009) **183**:6095–101. doi:10.4049/jimmunol.0803510
17. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. *Mol Cell Biol* (2003) **23**:7315–28. doi:10.1128/MCB.23.20.7315-7328.2003
18. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* (2012) **13**:907–15. doi:10.1038/ni.2386
19. Delgoffe GM, Powell JD. mTOR: taking cues from the immune microenvironment. *Immunology* (2009) **127**:459–65. doi:10.1111/j.1365-2567.2009.03125.x
20. Heikamp EB, Powell JD. Sensing the immune microenvironment to coordinate T cell metabolism, differentiation & function. *Semin Immunol* (2012) **24**:414–20. doi:10.1016/j.smim.2012.12.003
21. von Manteuffel SR, Dennis PB, Pullen N, Gingras AC, Sonenberg N, Thomas G. The insulin-induced signalling pathway leading to S6 and initiation factor 4E binding protein 1 phosphorylation bifurcates at a rapamycin-sensitive point immediately upstream of p70s6k. *Mol Cell Biol* (1997) **17**:5426–36.
22. Powell JD, Delgoffe GM. The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. *Immunity* (2010) **33**:301–11. doi:10.1016/j.immuni.2010.09.002
23. Yang K, Chi H. mTOR and metabolic pathways in T cell quiescence and functional activation. *Semin Immunol* (2012) **24**:421–8. doi:10.1016/j.smim.2012.12.004
24. Ojuka EO. Role of calcium and AMP kinase in the regulation of mitochondrial biogenesis and GLUT4 levels in muscle. *Proc Nutr Soc* (2004) **63**:275–8. doi:10.1079/PNS2004339
25. Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* (2004) **23**:3151–71. doi:10.1038/sj.onc.1207542
26. Yang K, Neale G, Green DR, He W, Chi H. The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. *Nat Immunol* (2011) **12**:888–97. doi:10.1038/ni.2068
27. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* (2009) **30**:832–44. doi:10.1016/j.immuni.2009.04.014
28. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* (2011) **35**:871–82. doi:10.1016/j.immuni.2011.09.021
29. Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, et al. mTOR regulates memory CD8 T-cell differentiation. *Nature* (2009) **460**:108–12. doi:10.1038/nature08155

30. Warburg O. The chemical constitution of respiration ferment. *Science* (1928) **68**:437–43. doi:10.1126/science.68.1767.437
31. Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* (2013) **153**:1239–51. doi:10.1016/j.cell.2013.05.016
32. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* (2012) **36**:68–78. doi:10.1016/j.immuni.2011.12.007
33. van der Windt GJ, O'Sullivan D, Everts B, Huang SC, Buck MD, Curtis JD, et al. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. *Proc Natl Acad Sci U S A* (2013) **110**:14336–41. doi:10.1073/pnas.1221740110
34. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* (2013) **31**:259–83. doi:10.1146/annurev-immunol-032712-095956
35. Surh CD, Sprent J. Regulation of mature T cell homeostasis. *Semin Immunol* (2005) **17**:183–91. doi:10.1016/j.smim.2005.02.007
36. Jacobs SR, Michalek RD, Rathmell JC. IL-7 is essential for homeostatic control of T cell metabolism in vivo. *J Immunol* (2010) **184**:3461–9. doi:10.4049/jimmunol.0902593
37. Kimura MY, Pobezinsky LA, Guinter TI, Thomas J, Adams A, Park JH, et al. IL-7 signaling must be intermittent, not continuous, during CD8(+) T cell homeostasis to promote cell survival instead of cell death. *Nat Immunol* (2013) **14**:143–51. doi:10.1038/ni.2494
38. Surh CD, Sprent J. Homeostatic T cell proliferation: how far can T cells be activated to self-ligands? *J Exp Med* (2000) **192**:F9–14. doi:10.1084/jem.192.4.F9
39. Bain G, Cravatt CB, Loomans C, Alberola-Ila J, Hedrick SM, Murre C. Regulation of the helix-loop-helix proteins, E2A and Id3, by the Ras-ERK MAPK cascade. *Nat Immunol* (2001) **2**:165–71. doi:10.1038/84273
40. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* (2008) **133**:775–87. doi:10.1016/j.cell.2008.05.009
41. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* (2011) **186**:3299–303. doi:10.4049/jimmunol.1003613
42. Lei J, Hasegawa H, Matsumoto T, Yasukawa M. Peroxisome proliferator-activated receptor alpha and gamma agonists together with TGF-beta convert human CD4+CD25- T cells into functional Foxp3+ regulatory T cells. *J Immunol* (2010) **185**:7186–98. doi:10.4049/jimmunol.1001437
43. Araki K, Youngblood B, Ahmed R. The role of mTOR in memory CD8 T-cell differentiation. *Immunol Rev* (2010) **235**:234–43. doi:10.1111/j.0105-2896.2010.00898.x
44. Kaech SM, Ahmed R. Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat Immunol* (2001) **2**:415–22.
45. Michalek RD, Rathmell JC. The metabolic life and times of a T-cell. *Immunol Rev* (2010) **236**:190–202. doi:10.1111/j.1600-065X.2010.00911.x
46. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* (2009) **460**:103–7. doi:10.1038/nature08097
47. Brand K, Netzker R, Aulwurm U, Hermfisse U, Fabian D, Weigert C, et al. Control of thymocyte proliferation via redox-regulated expression of glycolytic genes. *Redox Rep* (2000) **5**:52–4.
48. Jacobs SR, Rathmell JC. Lymphocyte selection by starvation: glucose metabolism and cell death. *Trends Immunol* (2006) **27**:4–7. doi:10.1016/j.it.2005.11.002
49. Rathmell JC, Elstrom RL, Cinalli RM, Thompson CB. Activated Akt promotes increased resting T cell size, CD28-independent T cell growth, and development of autoimmunity and lymphoma. *Eur J Immunol* (2003) **33**:2223–32. doi:10.1002/eji.200324048
50. Tamas P, Hawley SA, Clarke RG, Mustard KJ, Green K, Hardie DG, et al. Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca2+ in T lymphocytes. *J Exp Med* (2006) **203**:1665–70. doi:10.1084/jem.20052469
51. Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, et al. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* (2003) **13**:2004–8. doi:10.1016/j.cub.2003.10.031
52. Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc Natl Acad Sci U S A* (2004) **101**:3329–35. doi:10.1073/pnas.0308061100
53. MacIver NJ, Blagih J, Saucillo DC, Tonelli L, Griss T, Rathmell JC, et al. The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J Immunol* (2011) **187**:4187–98. doi:10.4049/jimmunol.1100367
54. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. *Circ Res* (2007) **100**:328–41. doi:10.1161/01.RES.0000256090.42690.05
55. Blagih J, Krawczyk CM, Jones RG. LKB1 and AMPK: central regulators of lymphocyte metabolism and function. *Immunol Rev* (2012) **249**:59–71. doi:10.1111/j.1600-065X.2012.01157.x
56. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) **208**:1367–76. doi:10.1084/jem.20110278
57. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* (2010) **39**:171–83. doi:10.1016/j.molcel.2010.06.022
58. Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, Argus JP, et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat Immunol* (2013) **14**:489–99. doi:10.1038/ni.2570
59. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* (2011) **146**:772–84. doi:10.1016/j.cell.2011.07.033
60. Finlay DK, Rosenzweig E, Sinclair LV, Feijoo-Carnero C, Hukelmann JL, Rolf J, et al. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J Exp Med* (2012) **209**:2441–53. doi:10.1084/jem.20112607
61. Foskett JK, White C, Cheung KH, Mak DO. Inositol trisphosphate receptor Ca2+ release channels. *Physiol Rev* (2007) **87**:593–658. doi:10.1152/physrev.00035.2006
62. Fill M, Copello JA. Ryanodine receptor calcium release channels. *Physiol Rev* (2002) **82**:893–922.
63. Zhou Y, Meraner P, Kwon HT, Machnes D, Oh-hora M, Zimmer J, et al. STIM1 gates the store-operated calcium channel ORAI1 in vitro. *Nat Struct Mol Biol* (2010) **17**:112–6. doi:10.1038/nsmb.1724
64. Lewis RS. Calcium signaling mechanisms in T lymphocytes. *Annu Rev Immunol* (2001) **19**:497–521. doi:10.1146/annurev.immunol.19.1.497
65. Vetter SW, Leclerc E. Novel aspects of calmodulin target recognition and activation. *Eur J Biochem* (2003) **270**:404–14. doi:10.1046/j.1432-1033.2003.03414.x
66. Gallo EM, Cante-Barrett K, Crabtree GR. Lymphocyte calcium signaling from membrane to nucleus. *Nat Immunol* (2006) **7**:25–32. doi:10.1038/ni1295
67. Carafoli E. The calcium-signalling saga: tap water and protein crystals. *Nat Rev Mol Cell Biol* (2003) **4**:326–32. doi:10.1038/nrm1073
68. Feske S, Giltnane J, Dolmetsch R, Staudt LM, Rao A. Gene regulation mediated by calcium signals in T lymphocytes. *Nat Immunol* (2001) **2**:316–24. doi:10.1038/86318
69. Fanger CM, Hoth M, Crabtree GR, Lewis RS. Characterization of T cell mutants with defects in capacitative calcium entry: genetic evidence for the physiological roles of CRAC channels. *J Cell Biol* (1995) **131**:655–67. doi:10.1083/jcb.131.3.655
70. Le Deist F, Hivroz C, Partiseti M, Thomas C, Buc HA, Oleastro M, et al. A primary T-cell immunodeficiency associated with defective transmembrane calcium influx. *Blood* (1995) **85**:1053–62.
71. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, et al. STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J Cell Biol* (2005) **169**:435–45. doi:10.1083/jcb.200502019
72. Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, et al. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T

cell activation and tolerance. *Nat Immunol* (2008) **9**:432–43. doi:10.1038/ni1574
73. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol* (2006) **174**:815–25. doi:10.1083/jcb.200604015
74. Muik M, Fahrner M, Schindl R, Stathopulos P, Frischauf I, Derler I, et al. STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation. *EMBO J* (2011) **30**:1678–89. doi:10.1038/emboj.2011.79
75. Parekh AB, Putney JW Jr. Store-operated calcium channels. *Physiol Rev* (2005) **85**:757–810. doi:10.1152/physrev.00057.2003
76. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, et al. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* (2009) **136**:876–90. doi:10.1016/j.cell.2009.02.014
77. Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, Muallem S. SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol* (2009) **11**:337–43. doi:10.1038/ncb1842
78. Hoth M, Button DC, Lewis RS. Mitochondrial control of calcium-channel gating: a mechanism for sustained signaling and transcriptional activation in T lymphocytes. *Proc Natl Acad Sci U S A* (2000) **97**:10607–12. doi:10.1073/pnas.180143997
79. Schwindling C, Quintana A, Krause E, Hoth M. Mitochondria positioning controls local calcium influx in T cells. *J Immunol* (2010) **184**:184–90. doi:10.4049/jimmunol.0902872
80. Deluca HF, Engstrom GW. Calcium uptake by rat kidney mitochondria. *Proc Natl Acad Sci U S A* (1961) **47**:1744–50. doi:10.1073/pnas.47.11.1744
81. Rizzuto R, Simpson AW, Brini M, Pozzan T. Rapid changes of mitochondrial Ca2+ revealed by specifically targeted recombinant aequorin. *Nature* (1992) **358**:325–7. doi:10.1038/358325a0
82. Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. Decoding of cytosolic calcium oscillations in the mitochondria. *Cell* (1995) **82**:415–24. doi:10.1016/0092-8674(95)90430-1
83. Rizzuto R, Bernardi P, Pozzan T. Mitochondria as all-round players of the calcium game. *J Physiol* (2000) **529**(Pt 1):37–47. doi:10.1111/j.1469-7793.2000.00037.x
84. Rizzuto R, Brini M, Murgia M, Pozzan T. Microdomains with high Ca2+ close to IP3-sensitive channels that are sensed by neighboring mitochondria. *Science* (1993) **262**:744–7. doi:10.1126/science.8235595
85. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* (2004) **427**:360–4. doi:10.1038/nature02246
86. Gilabert JA, Parekh AB. Respiring mitochondria determine the pattern of activation and inactivation of the store-operated Ca(2+) current I(CRAC). *EMBO J* (2000) **19**:6401–7. doi:10.1093/emboj/19.23.6401
87. Gilabert JA, Bakowski D, Parekh AB. Energized mitochondria increase the dynamic range over which inositol 1,4,5-trisphosphate activates store-operated calcium influx. *EMBO J* (2001) **20**:2672–9. doi:10.1093/emboj/20.11.2672
88. Pacher P, Csordas P, Schneider T, Hajnoczky G. Quantification of calcium signal transmission from sarco-endoplasmic reticulum to the mitochondria. *J Physiol* (2000) **529**(Pt 3):553–64. doi:10.1111/j.1469-7793.2000.00553.x
89. Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. *Proc Natl Acad Sci U S A* (1999) **96**:13807–12. doi:10.1073/pnas.96.24.13807
90. Singaravelu K, Nelson C, Bakowski D, de Brito OM, Ng SW, Di Capite J, et al. Mitofusin 2 regulates STIM1 migration from the Ca2+ store to the plasma membrane in cells with depolarized mitochondria. *J Biol Chem* (2011) **286**:12189–201. doi:10.1074/jbc.M110.174029
91. de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* (2008) **456**:605–10. doi:10.1038/nature07534
92. Nicholls DG. Mitochondria and calcium signaling. *Cell Calcium* (2005) **38**:311–7. doi:10.1016/j.ceca.2005.06.011
93. Prins D, Michalak M. Organellar calcium buffers. *Cold Spring Harb Perspect Biol* (2011) **3**:1–16. doi:10.1101/cshperspect.a004069
94. Halestrap AP. Calcium, mitochondria and reperfusion injury: a pore way to die. *Biochem Soc Trans* (2006) **34**:232–7. doi:10.1042/BST20060232
95. Carafoli E. Mitochondrial uptake of calcium ions and the regulation of cell function. *Biochem Soc Symp* (1974) **39**:89–109.
96. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* (2002) **82**:47–95.
97. Rhee SG. Cell signaling. H2O2, a necessary evil for cell signaling. *Science* (2006) **312**:1882–3. doi:10.1126/science.1130481
98. Bogeski I, Kummerow C, Al-Ansary D, Schwarz EC, Koehler R, Kozai D, et al. Differential redox regulation of ORAI ion channels: a mechanism to tune cellular calcium signaling. *Sci Signal* (2010) **3**:ra24. doi:10.1126/scisignal.2000672
99. Grupe M, Myers G, Penner R, Fleig A. Activation of store-operated I(CRAC) by hydrogen peroxide. *Cell Calcium* (2010) **48**:1–9. doi:10.1016/j.ceca.2010.05.005
100. Hawkins BJ, Irrinki KM, Mallilankaraman K, Lien YC, Wang Y, Bhanumathy CD, et al. S-glutathionylation activates STIM1 and alters mitochondrial homeostasis. *J Cell Biol* (2010) **190**:391–405. doi:10.1083/jcb.201004152
101. Henke N, Albrecht P, Pfeiffer A, Toutzaris D, Zanger K, Methner A. Stromal interaction molecule 1 (STIM1) is involved in the regulation of mitochondrial shape and bioenergetics and plays a role in oxidative stress. *J Biol Chem* (2012) **287**:42042–52. doi:10.1074/jbc.M112.417212
102. Finkel T. Oxidant signals and oxidative stress. *Curr Opin Cell Biol* (2003) **15**:247–54. doi:10.1016/S0955-0674(03)00002-4
103. Rothe G, Valet G. Flow cytometric analysis of respiratory burst activity in phagocytes with hydroethidine and 2',7'-dichlorofluorescin. *J Leukoc Biol* (1990) **47**:440–8.
104. Szejda P, Parce JW, Seeds MS, Bass DA. Flow cytometric quantitation of oxidative product formation by polymorphonuclear leukocytes during phagocytosis. *J Immunol* (1984) **133**:3303–7.
105. Crow JP. Dichlorodihydrofluorescein and dihydrorhodamine 123 are sensitive indicators of peroxynitrite in vitro: implications for intracellular measurement of reactive nitrogen and oxygen species. *Nitric Oxide* (1997) **1**:145–57. doi:10.1006/niox.1996.0113
106. Rothe G, Oser A, Valet G. Dihydrorhodamine 123: a new flow cytometric indicator for respiratory burst activity in neutrophil granulocytes. *Naturwissenschaften* (1988) **75**:354–5. doi:10.1007/BF00368326
107. Diez B, Cordo Russo R, Teijo MJ, Hajos S, Batlle A, Fukuda H. Ros production by endogenously generated protoporphyrin IX in murine leukemia cells. *Cell Mol Biol* (2009) **55**:15–9.
108. Williams MS, Henkart PA. Role of reactive oxygen intermediates in TCR-induced death of T cell blasts and hybridomas. *J Immunol* (1996) **157**:2395–402.
109. Weber GF, Abromson-Leeman S, Cantor H. A signaling pathway coupled to T cell receptor ligation by MMTV superantigen leading to transient activation and programmed cell death. *Immunity* (1995) **2**:363–72. doi:10.1016/1074-7613(95)90144-2
110. Hildeman DA, Mitchell T, Teague TK, Henson P, Day BJ, Kappler J, et al. Reactive oxygen species regulate activation-induced T cell apoptosis. *Immunity* (1999) **10**:735–44. doi:10.1016/S1074-7613(00)80072-2
111. Kwon J, Devadas S, Williams MS. T cell receptor-stimulated generation of hydrogen peroxide inhibits MEK-ERK activation and lck serine phosphorylation. *Free Radic Biol Med* (2003) **35**:406–17. doi:10.1016/S0891-5849(03)00318-6
112. Devadas S, Zaritskaya L, Rhee SG, Oberley L, Williams MS. Discrete generation of superoxide and hydrogen peroxide by T cell receptor stimulation: selective regulation of mitogen-activated protein kinase activation and fas ligand expression. *J Exp Med* (2002) **195**:59–70. doi:10.1084/jem.20010659
113. Thannickal VJ, Day RM, Klinz SG, Bastien MC, Larios JM, Fanburg BL. Ras-dependent and -independent regulation of reactive oxygen species by mitogenic growth factors and TGF-beta1. *FASEB J* (2000) **14**:1741–8. doi:10.1096/fj.99-0878com
114. Mahadev K, Zilbering A, Zhu L, Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1b in vivo and enhances the early insulin action cascade. *J Biol Chem* (2001) **276**:21938–42. doi:10.1074/jbc.C100109200

115. Ushio-Fukai M, Alexander RW, Akers M, Griendling KK. p38 Mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy. *J Biol Chem* (1998) **273**:15022–9. doi:10.1074/jbc.273.24.15022
116. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, et al. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem* (1997) **272**:217–21. doi:10.1074/jbc.272.1.217
117. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* (2003) **552**:335–44. doi:10.1113/jphysiol.2003.049478
118. Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J Biol Chem* (2004) **279**:49064–73. doi:10.1074/jbc.M407715200
119. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J* (2009) **417**:1–13. doi:10.1042/BJ20081386
120. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* (2013) **38**:225–36. doi:10.1016/j.immuni.2012.10.020
121. Pani G, Colavitti R, Borrello S, Galeotti T. Redox regulation of lymphocyte signaling. *IUBMB Life* (2000) **49**:381–9. doi:10.1080/152165400410227
122. Reth M. Hydrogen peroxide as second messenger in lymphocyte activation. *Nat Immunol* (2002) **3**:1129–34. doi:10.1038/ni1202-1129
123. Bialik S, Kimchi A. The death-associated protein kinases: structure, function, and beyond. *Annu Rev Biochem* (2006) **75**:189–210. doi:10.1146/annurev.biochem.75.103004.142615
124. Cohen O, Feinstein E, Kimchi A. DAP-kinase is a Ca2+/calmodulin-dependent, cytoskeletal-associated protein kinase, with cell death-inducing functions that depend on its catalytic activity. *EMBO J* (1997) **16**:998–1008. doi:10.1093/emboj/16.5.998
125. Sanjo H, Kawai T, Akira S. DRAKs, novel serine/threonine kinases related to death-associated protein kinase that trigger apoptosis. *J Biol Chem* (1998) **273**:29066–71. doi:10.1074/jbc.273.44.29066
126. Kuwahara H, Nishizaki M, Kanazawa H. Nuclear localization signal and phosphorylation of Serine350 specify intracellular localization of DRAK2. *J Biochem* (2008) **143**:349–58. doi:10.1093/jb/mvm236
127. Mao J, Qiao X, Luo H, Wu J. Transgenic drak2 overexpression in mice leads to increased T cell apoptosis and compromised memory T cell development. *J Biol Chem* (2006) **281**:12587–95. doi:10.1074/jbc.M600497200
128. Friedrich ML, Wen BG, Bain G, Kee BL, Katayama C, Murre C, et al. DRAK2, a lymphoid-enriched DAP kinase, regulates the TCR activation threshold during thymocyte selection. *Int Immunol* (2005) **17**:1379–90. doi:10.1093/intimm/dxh315
129. McGargill MA, Wen BG, Walsh CM, Hedrick SM. A deficiency in Drak2 results in a T cell hypersensitivity and an unexpected resistance to autoimmunity. *Immunity* (2004) **21**:781–91. doi:10.1016/j.immuni.2004.10.008
130. Honey K. DRAK2 puts the brakes on T-cell responses. *Nat Rev Immunol* (2005) **5**:98. doi:10.1038/nri1555
131. Friedrich ML, Cui M, Hernandez JB, Weist BM, Andersen HM, Zhang X, et al. Modulation of DRAK2 autophosphorylation by antigen receptor signaling in primary lymphocytes. *J Biol Chem* (2007) **282**:4573–84. doi:10.1074/jbc.M606675200
132. Gatzka M, Newton RH, Walsh CM. Altered thymic selection and increased autoimmunity caused by ectopic expression of DRAK2 during T cell development. *J Immunol* (2009) **183**:285–97. doi:10.4049/jimmunol.0803530
133. Ramos SJ, Hardison JL, Stiles LN, Lane TE, Walsh CM. Antiviral effector T cell responses and trafficking are not dependent upon DRAK2 signaling following viral infection of the central nervous system. *Autoimmunity* (2007) **40**:54–65. doi:10.1080/08916930600996700
134. Ramos SJ, Hernandez JB, Gatzka M, Walsh CM. Enhanced T cell apoptosis within Drak2-deficient mice promotes resistance to autoimmunity. *J Immunol* (2008) **181**:7606–16.
135. McGargill MA, Choy C, Wen BG, Hedrick SM. Drak2 regulates the survival of activated T cells and is required for organ-specific autoimmune disease. *J Immunol* (2008) **181**:7593–605.
136. Newton RH, Leverrier S, Srikanth S, Gwack Y, Cahalan MD, Walsh CM. Protein kinase D orchestrates the activation of DRAK2 in response to TCR-induced Ca2+ influx and mitochondrial reactive oxygen generation. *J Immunol* (2011) **186**:940–50. doi:10.4049/jimmunol.1000942
137. Waldron RT, Rozengurt E. Oxidative stress induces protein kinase D activation in intact cells. Involvement of Src and dependence on protein kinase C. *J Biol Chem* (2000) **275**:17114–21. doi:10.1074/jbc.M908959199
138. Storz P, Doppler H, Toker A. Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. *Mol Cell Biol* (2005) **25**:8520–30. doi:10.1128/MCB.25.19.8520-8530.2005
139. Mao J, Luo H, Han B, Bertrand R, Wu J. Drak2 is upstream of p70S6 kinase: its implication in cytokine-induced islet apoptosis, diabetes, and islet transplantation. *J Immunol* (2009) **182**:4762–70. doi:10.4049/jimmunol.0802255
140. Arechiga AF, Bell BD, Leverrier S, Weist BM, Porter M, Wu Z, et al. A Fas-associated death domain protein/caspase-8-signaling axis promotes S-phase entry and maintains S6 kinase activity in T cells responding to IL-2. *J Immunol* (2007) **179**:5291–300.

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Modulation of T lymphocyte calcium influx patterns via the inhibition of Kv1.3 and IKCa1 potassium channels in autoimmune disorders

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Currently available immunotherapies have improved the treatment of autoimmune diseases; however, these therapies are known to have considerable side-effects, such as increasing the susceptibility to infections. Therefore, there is an unmet need for novel immunosuppressive strategies with different mechanisms of action and higher specificity for disease-causing autoreactive T lymphocytes from existing immunomodulators.

The increase of the cytoplasmic calcium concentration from intra- and extracellular sources (i.e., the endoplasmic reticulum and store-operated calcium entry through the plasma membrane) is the cornerstone of T lymphocyte activation and functionality. In the course of lymphocyte activation, potassium channels maintain the driving force for sustained calcium influx from the extracellular milieu as they grant the efflux of potassium from the cytoplasm, thus conserving an electrochemical potential gradient between the intra- and extracellular spaces. There are two major types of potassium channels in T cells: the voltage-gated Kv1.3 and the calcium-activated IKCa1 channels. The relation between the calcium currents through calcium release-activated calcium (CRAC) channels and the efflux of potassium makes the proliferation and activation of lymphocytes sensitive to pharmacological modulation of Kv1.3 and IKCa1 channels, and provides an opportunity for targeted intervention. Specific inhibition of these channels results in a diminished calcium influx in lymphocytes and a lower level of lymphocyte activation.

Previous data suggest that selective modulation of lymphocyte activation through specific inhibition of potassium channels may be a possible therapeutic approach for the treatment of autoimmune disease (1–6). Beeton et al. showed that terminally differentiated effector memory T (TEM) cells play a pivotal role in the pathogenesis of autoimmunity (4). Wulff et al. described that the characteristic potassium channel phenotype of TEM cells in multiple sclerosis (MS) is Kv1.3high IKCa1low, contrasting naïve, and central memory T (TCM) cells, which exhibit a Kv1.3low IKCa1high channel phenotype (1). Therefore the therapeutic relevance of specific Kv1.3 channel inhibitors is of outstanding interest, as they may offer the possibility for selective modulation of pathogenic TEM cells, while naïve and TCM cells (needed for physiological immune responses) would escape the inhibition through upregulation of IKCa1 channel expression. Beeton et al. demonstrated that the symptoms of experimental autoimmune encephalitis, a murine model of MS, significantly improved after treatment with selective Kv1.3 inhibitors (5).

Although results from animal models are promising, limited data is available on the effects of potassium channel inhibition on T cell function in humans. Furthermore, besides naïve and memory T cells, alterations in the activation pattern of effector (CD4+ helper and CD8+ cytotoxic) T lymphocytes have not been described upon Kv1.3 and IKCa1 inhibition. Although these cells might have a less-specific role in the maintenance of autoreactivity compared to TEM cells, their inhibition have important consequences on the overall immune response. Therefore, over the recent years, we have investigated calcium influx characteristics in effector T cell subsets in a number of autoimmune disorders.

We isolated peripheral blood mononuclear cells from MS, rheumatoid arthritis (RA) and type 1 diabetes mellitus (T1DM) patients and applied a novel flow cytometry-based approach for the detection of calcium influx (7–10). Until the recent past, single-cell techniques were used for the investigation of calcium influx during lymphocyte activation. There has been no high-throughput method available to study the kinetics of lymphocyte activation in more subsets at the same time. Single-cell techniques are restricted by not being capable of characterizing this process in complex cellular systems, thus ignoring the interaction between the different lymphocyte subsets that may modulate the course of their activation. Therefore, over the recent years we have developed a novel algorithm that allows simultaneous monitoring of calcium influx in several lymphocyte subsets. Our software (FacsKin) fits functions to median values of the data of interest and calculates relevant parameters describing each function. By selecting the best fitting function, this approach provides an opportunity for the mathematical analysis and statistical comparison of kinetic flow cytometry measurements of distinct samples (more details available at www.facskin.com). Our findings indicate important differences in calcium influx kinetics in the studied autoimmune disorders compared to healthy controls.

MULTIPLE SCLEROSIS

Multiple sclerosis is an autoimmune disease affecting the central nervous system (CNS). The autoimmune reaction primarily destroys the myelin sheath covering the nerve cells. T lymphocytes play a key role in the pathogenesis of MS. They regulate the ongoing inflammatory process of the CNS which leads to the damage of the myelin sheath and axons. However, only a small part of T lymphocytes are myelin-specific autoreactive cells. Besides the demyelinating action of these cells of the CNS, the activation of peripheral lymphocytes also contributes to the pathogenesis and disease progression (11). In our investigations we

measured samples of healthy individuals and MS patients with no immunomodulatory therapy, as well as MS patients treated with interferon beta (IFN-b), currently regarded as the most effective therapy of MS. We discovered increased sensitivity of CD8 cells to Kv1.3 channel inhibition in MS. Therefore, from the CD4-CD8 point of view, we demonstrated specific immunomodulation that may be beneficial in the therapy of MS via the selective suppression of CD8 cytotoxic lymphocytes over CD4 helper cells. However, this specificity is not present within the CD4 subset, since our results suggest that Th1 and Th2 cells are similarly suppressed upon the inhibition of Kv1.3 channels. Since the cytokine balance is of utmost importance in the regulation of the autoimmune reaction, the inhibition of the Th2 subset would probably result in a setback of therapeutic efforts in MS. Our findings are relevant in the light of observations regarding the contribution of TEM cells to the development of MS, as described above. Although the Kv1.3high IKCa1low pattern is found in both CD4+ and CD8+ TEM cells, we can assume that the majority of TEM cells are CD8+, since TEM cells express immediate effector function. This might provide an explanation for the increased sensitivity of CD8 cells to Kv1.3 channel inhibition in MS in our study.

We have also demonstrated important differences in calcium influx kinetics and lymphocyte potassium channel function in MS patients with and without IFN-b therapy. Selective blockers of the Kv1.3 channel might be promising drugs in combination therapy, supplementing the presently used IFN-b treatment. Our results indicate that IFN-b therapy is related to compensatory changes only in the Th1 subset in MS regarding calcium influx kinetics and the function of potassium channels. However, the increased function of the Th2 subset, and therefore the production of anti-inflammatory cytokines are less affected. This might contribute to a more effective treatment of the autoimmune process in this disorder (9).

RHEUMATOID ARTHRITIS

The short-term activation of peripheral blood and synovial fluid T lymphocytes, especially that of autoreactive T cells plays a crucial role in initiating and maintaining the chronic inflammation in the joints of patients suffering from RA. These cells regulate the inflammatory process resulting in the destruction of the articular cartilage and also play a role in extra-articular damage. We investigated T lymphocyte calcium influx kinetics following activation in peripheral blood of recently diagnosed RA patients compared to healthy individuals. Our results indicate that margatoxin (MGTX), a specific blocker of the Kv1.3 channel acts differentially on calcium influx kinetics in major peripheral blood effector lymphocyte subsets of RA patients. The immunomodulatory effect of Kv1.3 channel inhibition is predominantly seen in cytotoxic (CD8) T cells in RA. However, this effect does not seem to be as specific as reported before by Beeton and colleagues in case of TEM cells (2), since anti-inflammatory Th2 cells are also affected to a noteworthy extent. This subset has an important role in counterbalancing the ongoing inflammatory process, and therefore its inhibition is not useful in the treatment of RA. A reason for limited specificity of Kv1.3 inhibition in the peripheral lymphocytes might be the differential distribution of disease-associated autoreactive T cells in RA patients on local and systemic levels. In the synovial fluid (locally), autoreactive TEM cells, expressing high numbers of Kv1.3 channels are abundantly present. However, this Kv1.3 pattern was not detected in peripheral blood T cells, because autoreactive TEM cells are infrequent in the circulation. Peripheral blood T cells were predominantly found to be naive and TCM cells (2).

TYPE 1 DIABETES MELLITUS

Our results indicate that the sensitivity of T1DM lymphocytes to the inhibition of Kv1.3 channels is increased compared to healthy individuals. It has been hypothesized that beneficial effects of Kv1.3 channel inhibition by MGTX are due to the dominance of Kv1.3 channels on activated TEM cells (1, 2). It was also presumed that MGTX would inhibit the activation of CD8 lymphocytes responsible for cytotoxic destruction of pancreatic beta cells. Nevertheless, our findings support that Kv1.3 channels have an important role in each investigated lymphocyte subset in T1DM, including Th2 lymphocytes acting as counterbalancing factors in the development of T1DM through the production of anti-inflammatory cytokines (12). Therefore, administration of Kv1.3 channel inhibitors would not have an exclusive effect on cells responsible for the autoimmune response in T1DM, but may have an impact on the activation characteristics of immune cells in general (8).

SUMMARY

Based on our results, a number of dominant features were identified that were present in the investigated autoimmune diseases. First, the time when the peak of calcium influx was reached decreased in autoimmune patients compared to healthy individuals, indicating that these cells are in a state of sustained reactivity due to the ongoing autoimmune reaction.

Earlier studies were limited to the investigation of potassium channels in naive and memory lymphocytes. We have extended these findings to significant effector T lymphocyte subsets, and found a different pattern of sensitivity to the inhibition of lymphocyte potassium channels in Th1 cells of autoimmune patients compared to healthy individuals. In healthy controls the inhibition of the IKCa1 channel decreased calcium influx in Th2 and CD4 cells to a lower extent than in Th1 and CD8 cells. On the contrary, the inhibition of Kv1.3 channels resulted in a larger decrease of calcium entry in Th2 and CD4 than in Th1 and CD8 cells. In the investigated autoimmune patients a greater decrease of calcium influx upon the inhibition of the Kv1.3 channel than that of the IKCa1 channel was observed in Th1 cells. This finding is of special interest, since Th1 cells are regarded as key players in the mediation of pro-inflammatory responses.

However, the selectivity of the investigated inhibitors was limited in our experiments, as they did not only affect a single subset, as previously suggested. Although in earlier observations the inhibition of Kv1.3 channels specifically blocked the function of TEM cells, our investigations extending to significant effector T lymphocyte subsets demonstrated that the inhibitory effect is present not only in disease-associated CD8 and Th1 cells, but also in the anti-inflammatory Th2 subset. The induced decrease in their function could lead to unwanted side-effects and in a setback of therapy *in vivo*.

Therefore, further studies, including the analysis of functional consequences (such as cytokine production or proliferation) of

lymphocyte potassium channel inhibition are needed on human samples and experimental models to judge the usefulness of this approach in the fight against autoreactive lymphocyte subsets and harmful cellular responses in autoimmune patients.

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REFERENCES

1. Wulff H, Calabresi PA, Allie R, Yun S, Pennington M, Beeton C, et al. The voltage-gated Kv1.3 K(+) channel in effector memory T cells as new target for MS. *J Clin Invest* (2003) **111**:1703–13. doi: 10.1172/JCI16921
2. Chandy KG, Wulff H, Beeton C, Pennington M, Gutman GA, Cahalan MD. K+ channels as targets for specific immunomodulation. *Trends Pharmacol Sci* (2004) **25**:280–9. doi: 10.1016/j.tips.2004.03.010
3. Rus H, Pardo CA, Hu L, Darrah E, Cudrici C, Niculescu T, et al. The voltage-gated potassium channel Kv1.3 is highly expressed on inflammatory infiltrates in multiple sclerosis brain. *Proc Natl Acad Sci U S A* (2005) **102**:11094–9. doi: 10.1073/pnas.0501770102
4. Beeton C, Wulff H, Standifer NE, Azam P, Mullen KM, Pennington MW, et al. Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. *Proc Natl Acad Sci U S A* (2006) **103**:17414–9. doi: 10.1073/pnas.0605136103
5. Rangaraju S, Chi V, Pennington MW, Chandy KG. Kv1.3 potassium channels as a therapeutic target in multiple sclerosis. *Expert Opin Ther Targets* (2009) **13**:909–24. doi: 10.1517/14728220903018957
6. Varga Z, Csepany T, Papp F, Fabian A, Gogolak P, Toth A, et al. Potassium channel expression in human CD4+ regulatory and naive T cells from healthy subjects and multiple sclerosis patients. *Immunol Lett* (2009) **124**:95–101. doi: 10.1016/j.imlet.2009.04.008
7. Kaposi AS, Veress G, Vasarhelyi B, Macardle P, Bailey S, Tulassay T, et al. Cytometry-acquired calcium-flux data analysis in activated lymphocytes. *Cytometry A* (2008) **73**:246–53. doi: 10.1002/cyto.a.20518
8. Toldi G, Vasarhelyi B, Kaposi A, Meszaros G, Panczel P, Hosszufalusi N, et al. Lymphocyte activation in type 1 diabetes mellitus: the increased significance of Kv1.3 potassium channels. *Immunol Lett* (2010) **133**:35–41. doi: 10.1016/j.imlet.2010.06.009
9. Toldi G, Folyovich A, Simon Z, Zsiga K, Kaposi A, Meszaros G, et al. Lymphocyte calcium influx kinetics in multiple sclerosis treated without or with interferon beta. *J Neuroimmunol* (2011) **237**:80–6. doi: 10.1016/j.jneuroim.2011.06.008.
10. Toldi G, Bajnok A, Dobi D, Kaposi A, Kovacs L, Vasarhelyi B, et al. The effects of Kv1.3 and IKCa1 potassium channel inhibition on calcium influx of human peripheral T lymphocytes in rheumatoid arthritis. *Immunobiology* (2013) **218**:311–6. doi: 10.1016/j.imbio.2012.05.013
11. Martino G, Furlan R, Brambilla E, Bergami A, Ruffini F, Gironi M, et al. Cytokines and immunity in multiple sclerosis: the dual signal hypothesis. *J Neuroimmunol* (2000) **109**:3–9. doi: 10.1016/S0165-5728(00)00295-2
12. Yoon JW, Jun HS. Cellular and molecular pathogenic mechanisms of insulin-dependent diabetes mellitus. *Ann N Y Acad Sci* (2001) **928**:200–11. doi: 10.1111/j.1749-6632.2001.tb05650.x

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A mathematical model of T lymphocyte calcium dynamics derived from single transmembrane protein properties

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Fate decision processes of T lymphocytes are crucial for health and disease. Whether a T lymphocyte is activated, divides, gets anergic, or initiates apoptosis depends on extracellular triggers and intracellular signaling. Free cytosolic calcium dynamics plays an important role in this context. The relative contributions of store-derived calcium entry and calcium entry from extracellular space to T lymphocyte activation are still a matter of debate. Here we develop a quantitative mathematical model of T lymphocyte calcium dynamics in order to establish a tool which allows to disentangle cause-effect relationships between ion fluxes and observed calcium time courses. The model is based on single transmembrane protein characteristics which have been determined in independent experiments. This reduces the number of unknown parameters in the model to a minimum and ensures the predictive power of the model. Simulation results are subsequently used for an analysis of whole cell calcium dynamics measured under various experimental conditions. The model accounts for a variety of these conditions, which supports the suitability of the modeling approach. The simulation results suggest a model in which calcium dynamics dominantly relies on the opening of channels in calcium stores while calcium entry through calcium-release activated channels (CRAC) is more associated with the maintenance of the T lymphocyte calcium levels and prevents the cell from calcium depletion. Our findings indicate that CRAC guarantees a long-term stable calcium level which is required for cell survival and sustained calcium enhancement.

Keywords: T lymphocytes, calcium dynamics, mathematical model, CRAC, endoplasmic reticulum

1. INTRODUCTION

The dynamics of the free cytosolic calcium concentration upon stimulation of T lymphocytes (TCs) is crucial for TC activation and fate decision processes. While it is clear that calcium rises upon stimulation of the TC receptor (TCR), the calcium pattern associated with different fates of TCs has not been deciphered (1–4). However, it is likely that the calcium signal is correlated with the later fate of the activated TC, i.e., anergy, division, acquisition of the regulatory phenotype or apoptosis (3, 5, 6). Dysregulation in TC calcium signaling has been linked to inflammatory and autoimmune diseases as well as to allograft rejection (2). A relevant player in calcium dynamics is the calcium-release activated channel (CRAC) which is located in the plasma-membrane and activated by calcium depletion in the intracellular calcium stores like the endoplasmic reticulum (ER). As ion-gating transmembrane proteins in the plasma-membrane (PM) are possible targets of drug applications in the context of various clinical settings (2, 7, 8), insight into the specific calcium dynamics is essential for an efficient control of TC behavior.

The relative contribution of ER-derived calcium entry versus CRAC to the calcium signal following TC stimulation is a matter of ongoing debate (9–12). While a considerable number of scientists argue for CRAC being the major player of TC calcium dynamics (12, 13), others argue for a dominant role of calcium-induced calcium-release (CICR) (10, 14, 15) or for a dominant role of second messenger-induced calcium-release from ER (16, 17). All three contributions are required for a functional TC calcium signal, however, the sequence of the contributions might be essential. Second messenger-induced activity appears as a very early signal (18), which might act as triggering event for CICR and subsequent CRAC activation (2, 19–21). Quantitative analysis of the components of calcium signaling during TC activation is essential for the development of strategies for an efficient control of TC responses. A mathematical analysis of the calcium dynamics in a model including calcium stores and CRAC may shed light on the relation and relevance of both calcium sources (12, 22). This is the major motivation for the present work.

T lymphocytes are non-excitable cells in the sense that they do not exhibit bursts like pancreatic beta-cells, spikes like neurons, or comparable fast dynamics (23–25) even though single cell measurements detected calcium oscillations (10, 19, 26). The non-excitability of TCs might have prevented a larger interest of mathematical modelers in lymphocyte calcium dynamics. The few existing models (22, 27–29) mostly focused on modeling of CRAC-channel dynamics or a special part of the store-operated-calcium-entry signaling pathway (27) and its contribution to intracellular

calcium dynamics. Also the dependence of ORAI1 assembly to a tetrameric CRAC on calcium oscillations was considered (28). In one approach a spatial resolution of CRAC currents and of the calcium dynamics in TCs was considered in the context of immunological synapse formation (22). Two different models for inositol 1,4,5-tris-phosphate-receptor (IP3R) activity were compared and it was shown that they differ in their impact on TC calcium dynamics (27), a result that will be used in the present model as well. The plasma-membrane calcium-ATPase (PMCA) was modeled in Jurkat TCs and a reversible modulation of PMCA activity was postulated (12), which is a further topic addressed in this investigation.

The aforementioned theoretical studies on TC calcium dynamics were all based on whole cell current models. In the context of excitable cells we have shown that it is possible to derive the whole cell currents from the single transmembrane properties (30). To achieve this goal, specific quantitative measurements of protein activation, inactivation, dependencies and conductance were used. The big advantage of this approach is that most parameters of the model are determined by independent experiments, which increases the predictive power of the model. The present paper applies this strategy to calcium dynamics of TCs. The model also includes the dynamics of transmembrane channel expression kinetics in order to represent CRAC recruitment upon calcium store depletion. The mathematical model was validated using dynamic calcium data measured under specific experimental conditions. The validated model allowed to reassess the relative role of store-derived and CRAC-mediated calcium entry on a quantitative basis. A new role of the CRAC-channel is postulated, which is associated with maintenance of TC calcium levels rather than TC activation.

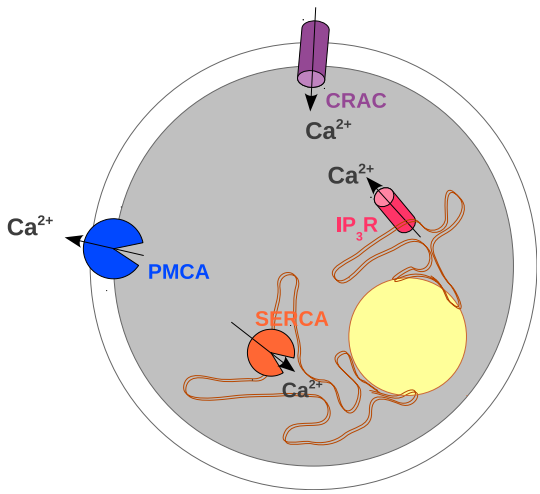

FIGURE 1 | Scheme of the transmembrane proteins included in the mathematical model. The elements of a TC considered in the mathematical model are shown with particular emphasis on calcium-conducting transmembrane proteins. The outer border of the cell represents the PM. In the PM CRAC and PMCA are located which induce calcium influx and extrusion, respectively. The intracellular organelle around the nucleus (yellow) depicts the ER. The membrane of the ER contains IP3R and SERCA (sarco/endoplasmic reticulum calcium-ATPase) which control the exchange of calcium between the ER and the cytosol.

2. MATERIALS AND METHODS

The modeling framework is presented in this section. Three compartments, extracellular space (ES), cytosol, and ER are distinguished, each being represented by ordinary differential equations. The nucleus is only included as an object which reduces intracellular space (Section 2.4). The compartments are encased by the PM and the membrane of the ER. Both membranes contain transmembrane proteins (**Figure 1**), which allow for a flow of ions from one compartment to the other. The surface densities and the properties of these proteins in terms of conductance and control parameters determine the resting and activated states of the TC. While the protein properties are derived from measured single protein data, which are independent of the whole cell calcium experiments used for validation, the protein densities are in parts derived from steady state conditions (Section 2.9) and in parts determined by fitting to whole cell calcium dynamics (Section 2.10).

In the following, the equations for calcium dynamics, calcium-buffering, and for the second messenger D-myo-inositol-1,4,5-tris-phosphate (IP3) are introduced. The details of the compartment sizes and the surface between the compartments are explained in Section 2.4. Particular emphasis is put on the geometry of the Jurkat TC, the specific cell line, which is used in the subsequently described experiments. The single transmembrane protein characteristics will be described and the corresponding mathematical models introduced. Wherever possible we implemented data from experiments performed with Jurkat TCs. Finally, all model pieces are merged to the proposed model of TC calcium dynamics in Section 2.10. This includes the determination of the remaining unknown parameters.

2.1. CALCIUM DYNAMICS AND BUFFERING

Two major players determine exchange of calcium through the PM (**Figure 1**): PMCAs actively transport calcium from the cytosol into ES (12, 31), while CRAC-channels allow for a passive electrochemical current of calcium ions into the cell (21, 32, 33). The density of active CRAC-channels ρ_{CRAC} in the PM is increased in dependence on ER calcium (C_{ER}) depletion (34). The free cytosolic calcium concentration (C) is further affected by modulations of the calcium flow between cytosol and ER (**Figure 1**). Sarco/endoplasmic reticulum calcium-ATPase (SERCA) transports calcium ions from the cytosol into the ER (10, 35) and by this maintains a chemical gradient of calcium from the ER to the cytosol. Conversely, calcium can passively leave the ER into the cytosol when IP3R channels open in dependence on calcium and the second messenger IP3 (1, 36, 37) associated with calcium-induced-calcium-release (CICR) (38). Furthermore other second messengers like cyclic ADP ribose (cADPR) (16) and nicotinic acid adenine dinucleotide phosphate (NAADP) (39) were found to influence calcium dynamics. Their effect is mediated by activation of the ryanodine receptor (RyR) which leads to calcium-release from intracellular stores (17, 40–43). In order to avoid an over-parametrization of whole cell calcium curves, which the present model focusses on, we restrict ourselves to the dynamics of IP3. The inclusion of cADPR and NAADP and their effect on RyR

requires a more detailed data basis and should be addressed with a more complex model in the future.

2.1.1. Calcium in the cytosol

The four sources and sinks of free cytosolic calcium C are described as

$$\frac{dC}{dt} = \frac{-1}{z_{\mathrm{Ca}} F (1 + B_{\mathrm{C}})} \left(\xi \rho_{\mathrm{PMCA}} I_{\mathrm{PMCA}} + \xi \rho_{\mathrm{CRAC}} I_{\mathrm{CRAC}} + \xi_{\mathrm{ERC}} \rho_{\mathrm{SERCA}} I_{\mathrm{SERCA}} + \xi_{\mathrm{ERC}} \rho_{\mathrm{IP3R}} I_{\mathrm{IP3R}}\right), \quad (1)$$

where $z_{\mathrm{Ca}} = 2$ is the valence of calcium ions, F the Faraday constant, ξ and ξ_{ERC} geometrical surface to volume ratios for PM [equation (16)] and ER membrane [equation (17)], respectively. ρ_{X} is the surface density and I_{X} the single transmembrane protein current, which are defined in the subsequent sections. By convention, positive ions that enter the cytosol are represented by negative electrical currents. B_{C} represents the cytosolic calcium-buffer in the rapid buffer approximation

$$B_{\mathrm{C}} = \frac{b_0 K_{\mathrm{b}}}{(C + K_{\mathrm{b}})^2} \quad , \quad (2)$$

where b_0 is the total buffer concentration, and K_{b} the calcium-buffer dissociation constant. The fraction of free calcium in the cytosol then reads

$$f_{\mathrm{C}} = \left[1 + \frac{b_0}{C + K_{\mathrm{b}}}\right]^{-1} \quad . \quad (3)$$

The main buffer within the cytosol is calmodulin (CaM) with 4 calcium binding sites per CaM. There is a diversity of measured CaM concentrations depending on cell type and organ (44–46). A realistic average value is 25 μM of CaM, corresponding to $b_0 = 100\,\mu$M of calcium binding sites. The dissociation constant K_{b} was determined by the required fraction of free calcium of $f_{\mathrm{C}} \approx 0.1\%$ in non-excitable cells (47), leading to $K_{\mathrm{b}} = 0.1\,\mu$M.

2.1.2. Calcium in the ER

The dynamics of the calcium concentration in the ER C_{ER} is described by an equation analogous to equation (1)

$$\frac{dC_{\mathrm{ER}}}{dt} = \frac{\xi_{\mathrm{ER}} \left(\rho_{\mathrm{SERCA}} I_{\mathrm{SERCA}} + \rho_{\mathrm{IP3R}} I_{\mathrm{IP3R}}\right)}{z_{\mathrm{Ca}} F (1 + B_{\mathrm{C,ER}})} \quad , \quad (4)$$

with a different geometrical factor ξ_{ER} [equation (18)], and a different calcium-buffer $B_{\mathrm{C,ER}}$ defined by

$$B_{\mathrm{C,ER}} = \frac{b_{\mathrm{ER},0} K_{\mathrm{ER,b}}}{\left(C_{\mathrm{ER}} + K_{\mathrm{ER,b}}\right)^2} \quad . \quad (5)$$

The fraction of free calcium in the ER reads

$$f_{\mathrm{C,ER}} = \left[1 + \frac{b_{\mathrm{ER},0}}{C_{\mathrm{ER}} + K_{\mathrm{ER,b}}}\right]^{-1} \quad . \quad (6)$$

The resting ER calcium level is $C_{\mathrm{ER},0} = 400\,\mu$M (2) which holds true for Jurkat TCs considered here (34).

In the ER calcium is buffered by calsequestrin, with three high and three low-affinity binding sites (48), and by calreticulin, with two distinct domains, one with high-affinity ($K = 0.01$ mM) but low capacity (0.6–1 mol Ca^{2+}/mol protein), and one with low-affinity ($K = 2$ mM) but high capacity (18 mol Ca^{2+}/mol protein) (49). As calreticulin binds more than 50% of the luminal ER calcium (50) only this buffer is considered here. In pancreatic acinar cells it was estimated that 20-times more calcium would be free in the ER compared to the cytosol (47), suggesting $f_{\mathrm{C,ER}} \approx 0.02$. This is achieved by using $b_{\mathrm{ER},0} = 30$ mM and $K_{\mathrm{ER,b}} = 0.1$ mM, which corresponds to an intermediate dissociation constant of both calcium binding domains.

2.2. IP3 DYNAMICS

IP3 (P) is generated in a TCR- and calcium-dependent way described by

$$\frac{dP}{dt} = \beta_{\mathrm{P}} H(C, C_{\mathrm{P}}, n_{\mathrm{P}}) T(t) - \gamma_{\mathrm{P}} P \quad , \quad (7)$$

where β_{P} is the production and γ_{P} the degradation rate. The Hill-function is defined as

$$H(X, K, n) \equiv \frac{X^n}{X^n + K^n} \quad , \quad (8)$$

where K is the concentration of X at which the Hill-function reaches its half value, and n the Hill-coefficient which determines the steepness of the Hill-function.

The degradation rate in equation (7) is determined by steady state conditions for IP3 in equation (35). The production rate is the tonic production rate and is modulated by increased calcium with the Hill-function in equation (7), leading to a positive feedback loop between calcium and IP3. β_{P} is fitted as described in Section 2.10 and mainly influences the speed of the early calcium response after TC stimulation.

The production is further modified by the time-dependent input function $T(t)$ representing the degree of TCR stimulation of the cell. $T = 1$ is assumed in the resting state.

The resting concentration of IP3 P_0 is identified as critical parameter. It strongly determines the responsivity of the cell via activation of IP3R (see Section 2.6). It was fitted as described in Section 2.10.

2.3. MEMBRANE AND REVERSAL POTENTIALS

The resting membrane potential is set to $V = -60$ mV (51–53). It is assumed that the membrane potential is not changed by the calcium currents ($V = V_0$) and that the electrical current corresponding to calcium fluxes in or out of the cell is equilibrated by other ions.

Further it is assumed that $V_{\mathrm{ER},0} = V_0 = -60\,mV$, thus, the ER and the cytosol are electrically equilibrated (54). ER calcium efflux may lead to small fluctuations (55) which are neglected. Thus, $V_{\mathrm{ER}} = V$ is assumed at all times.

In this approximation, the reversal potentials depend on the chemical gradient only. The Nernst-equation is used to calculate the reversal potential during dynamical changes of calcium

concentrations:

$$\overline{V_C} = \frac{RT}{z_{Ca}F} \ln\left(\frac{C_{ext}}{C}\right) - \Delta V_C$$
$$\overline{V_{C,ER}} = \frac{RT}{z_{Ca}F} \ln\left(\frac{C_{ER}}{C}\right) - \Delta V_{C,ER} \quad , \tag{9}$$

where $R = 8.315\,\mathrm{J/(K\ mol)}$ the Rydberg (molar) gas constant, $T = 310\,\mathrm{K}$, and F the Faraday constant.

For many channels, the real I-V-relationship is not linear as assumed for the currents I_X in equations (23) and (28). Therefore, the reversal potential is corrected for the CRAC-channel by a shift $\Delta V_C = 78\,\mathrm{mV}$ in order to achieve the correct linear extrapolation of the I-V-relation of CRAC-channels with a zero around $\overline{V_C} \approx 50$ mv [(34) Figure 1, (33) Figure 2]. This approximation is only valid for depolarization below $V = 50\,\mathrm{mV}$.

The reversal potential for ER calcium $\overline{V_{C,ER}}$ is treated in complete analogy to the cytosolic case which leads to a correction of $\Delta V_{C,ER}$. As the value is not known it is derived using the fitting routine in Section 2.10.

2.4. TC GEOMETRY

Most measurements on TC calcium dynamics are performed in Jurkat TCs which are small but still larger than normal human blood derived TCs. In an approach based on ordinary differential equations the effect of an ion current onto the concentration of the ion in the cytosol or ER is not spatially resolved. While local calcium entry can induce transient high concentrations of calcium (22) the comparably small cytosolic volume of TCs justifies this approach for the description of whole cell calcium dynamics because local inhomogeneities will quickly equilibrate. In the model this is reflected as change of the average concentration. How an ion current changes the average calcium concentration depends on the geometry of the cell. In the dynamic equations for the ion concentrations the current I_X through an individual ion-conducting protein X is multiplied by the surface density ρ_X. Thus the concentration change is derived from a current surface density. The latter has to be translated to the actual change in concentration by a surface to volume ratio, which is considered here.

Given a cell radius R_{cell}, the cell volume V_{cell} and cell surface A_{cell} are known as well. However, the volume relevant to changes in concentration is not the cell volume V_{cell} but the cytosolic volume V_{cyt} which can be approximated as

$$V_{cyt} = V_{cell} - \tilde{V}_{ER} - V_{nucleus} \quad , \tag{10}$$

using the volumes of ER and nucleus. This is important because the nucleus, with a radius of

$$R_{nucleus} = f_R R_{cell} \tag{11}$$

is substantially reducing the resulting cytosolic volume. $f_R \approx 0.8$ is assumed for human TCs (56), and $f_R \approx 0.25$ for Jurkat TCs. The volume of the ER is expressed as a fraction of the total cell volume

$$\tilde{V}_{ER} = f_V V_{cell} \quad , \tag{12}$$

with $f_V \approx 0.1$ (57). However, electron micrographs of TCs suggest that $f_V \approx 0.01$ (25) which is used here. Taking this together, the cytosolic volume becomes

$$V_{cyt} = V_{cell}\left(1 - f_V - f_R^3\right) \quad . \tag{13}$$

The surface of the ER is also needed in order to translate the current surface densities calculated on the ER surface into concentration changes in cytosol and ER. While the TC itself is approximated as a sphere, the ER is absolutely non-spherical. The exact surface of the ER is difficult to be measured and accordingly approximated as

$$A_{ER} = f_A \tilde{A}_{ER} \equiv 4\pi f_A \left(\frac{3\tilde{V}_{ER}}{4\pi}\right)^{2/3} \quad , \tag{14}$$

where $\tilde{A}_{ER}$ is the surface of a spherical ER with volume $\tilde{V}_{ER}$ [determined in equation (12)], and f_A is the fold increase of the ER surface with respect to the surface of a spherical ER. $f_A = 30$ was roughly estimated from the folding degree of the ER in electron micrographs of TCs (25). Note that only the product of f_A with $f_V^{2/3}$ enters the model, such that both parameters are redundant and were only separated because of their physiological meaning.

The size of human blood TCs can be estimated starting from the capacity of $C_m = 0.028\,\mathrm{pF}/\mu\mathrm{m}^2$ (58, 59) and using the whole cell capacitance of $C_{cell} = 2$ pF [(60), p. 606]. $C_{cell} = 1.7$ pF was found in Fomina et al. (14). Using $C_{cell}/A_{cell} = C_m$ this yields a radius

$$R_{cell} = \sqrt{\frac{C_{cell}}{4\pi\, C_m}} \tag{15}$$

and the resulting $R_{cell} \approx 2.4\,\mu\mathrm{m}$ corresponds to $A_{cell} = 72.4\,\mu\mathrm{m}^2$. The experiments described below were performed with Jurkat TCs and the same authors determined the cell volume to $V_{cell} = 2\,\mathrm{pl}$ (12). This determines the values $R_{cell} = 8\,\mu\mathrm{m}$ and $A_{cell} = 804.2\,\mu\mathrm{m}^2$ used in the present simulations.

Given the cell radius R_{cell}, the fractions f_V and f_R, as well as the factor f_A, the surface to volume ratios required in equations (1) and (4) can be calculated by

$$\xi = \frac{A_{cell}}{V_{cyt}} \tag{16}$$

$$\xi_{ERC} = \frac{A_{ER}}{V_{cyt}} \tag{17}$$

$$\xi_{ER} = \frac{A_{ER}}{\tilde{V}_{ER}} \tag{18}$$

with

$$A_{cell} = 4\pi R_{cell}^2 \tag{19}$$

$$V_{\text{cyt}} = \frac{4}{3}\pi R_{\text{cell}}^3 \left(1 - f_{\text{V}} - f_{\text{R}}^3\right) \tag{20}$$

$$\tilde{V}_{\text{ER}} = \frac{4}{3}\pi f_{\text{V}} R_{\text{cell}}^3 \tag{21}$$

$$A_{\text{ER}} = 4\pi f_{\text{A}} \left(\frac{3\tilde{V}_{\text{ER}}}{4\pi}\right)^{2/3} \quad . \tag{22}$$

2.5. CRAC-CHANNEL

The open CRAC-channel current is determined by the electrochemical gradient

$$I_{\text{CRAC}} = \overline{g_{\text{CRAC}}}\left(V - \overline{V_{\text{C}}}\right) \quad . \tag{23}$$

This approach closely follows the model of Martin et al. (22). The validity of the Ohm's law approximation is only guaranteed within limited ranges of membrane potentials.

2.5.1. Single channel conductance

The single channel CRAC conductance was found to be extremely small in the order of $\overline{g_{\text{CRAC}}} = 2$ fs (61).

2.5.2. CRAC recruitment

The density of active CRAC-channels, estimated by the steady state CRAC-channel current, is a dynamic function of the ER-calcium concentration [(34) Figure 1C] described by

$$\frac{d\rho_{\text{CRAC}}}{dt} = \frac{\overline{\rho_{\text{CRAC}}} - \rho_{\text{CRAC}}}{\tau_{\text{CRAC}}} \quad , \tag{24}$$

where

$$\overline{\rho_{\text{CRAC}}} = \rho_{\text{CRAC}}^- + \left(\rho_{\text{CRAC}}^+ - \rho_{\text{CRAC}}^-\right) \left(1 - H\left(C_{\text{ER}}, C_{\text{CRAC}}, n_{\text{CRAC}}\right)\right), \tag{25}$$

with $C_{\text{CRAC}} = 169\,\mu$M and $n_{\text{CRAC}} = 4.2$. To our knowledge, this is the first time that the surface density of active CRAC-channels in the PM is modeled as a dynamic quantity.

When estimating the same quantity from the degree of STIM1-redistribution, a rather similar relation is found with $C_{\text{CRAC}} = 187\,\mu$M and $n_{\text{CRAC}} = 3.8$ [(34), Figure 2]. The uniformity of both curves supports the view that CRAC-channels are recruited and open in response to ER calcium depletion (34). It can be deduced from the similarity of both curves that the opening dynamics is substantially faster than the redistribution of STIM1. As no opening dynamics of the CRAC-channel is included in the model, the dynamics of the current itself and not of STIM1-redistribution is used.

2.5.3. CRAC density

In equation (25) $\rho_{\text{CRAC}}^{\pm}$ are the upper and lower limits of possible active CRAC densities. The resting value $\rho_{\text{CRAC},0}$ is not known from experiment and is determined by parameter fitting to calcium dynamics upon TCR stimulation (Section 2.10).

The density of CRAC-channels upon activation with PHA increased about 9-fold (33) which constraints ρ_{CRAC}^+. A 10-fold increase has been reported for the whole cell CRAC current in response to stepwise reduced C_{ER} [Figure 1C in Luik et al. (34)]. These findings translate into the condition

$$\rho_{\text{CRAC}}^+ = f_{\text{CRAC}}\rho_{\text{CRAC},0} \quad . \tag{26}$$

where ρ_{CRAC}^+ was determined by parameter fitting within the experimental boundaries in Section 2.10. A value for ρ_{CRAC}^- is not known and is determined by the steady state condition equation (36).

2.5.4. CRAC time scales

The time scale of CRAC recruitment can be estimated from the rising time of calcium curves which provides an upper bound of $\tau_{\text{CRAC}} < 100$ s for the activation time. It is likely that this time is associated with CRAC recruitment rather than with CRAC opening because opening time scales are typically much shorter. The time scale of CRAC recruitment is set to $\tau_{\text{CRAC}} = 5$ s. Larger values could also be used as the fit was insensitive to τ_{CRAC}. Inactivation of CRAC-channels is difficult to be assessed (62). As the time scale of inactivation is in the order of 1000 s (14) and thus longer than the typical experimental durations used here, the present model ignores CRAC inactivation and assumes that the reduction of active CRAC-channels is a secondary effect of C_{ER} recovery.

2.6. IP3R IN THE ER

The ER releases its calcium content if activated by IP3 and cytosolic calcium (63, 64). The release of calcium from intracellular stores is based on the opening dynamics of RyR and IP3R in the membrane of the ER. TCs express both, RyR and IP3R and even different subtypes of them.

IP3Rs have binding sites for IP3 and calcium and exhibit complex forms of cooperativity (65). For the present purpose the heuristic description of IP3R activation and inhibition is sufficient. The characteristic feature of the IP3R conductance is a calcium-dependent log-bell-shaped opening probability curve (63) which has been measured for ER vesicles from canine cerebellum and further reviewed in Foskett et al. (38). The open probability curve was best fitted by the product of an activating and an inhibiting Hill-function, both with Hill-coefficient 2 (63).

2.6.1. Open probability

TCR signaling leads to the generation of IP3, the ligand of the IP3R, which modulates the open probability of IP3R and is described as the product of an activation term g_{IP3R} and an inactivation term h_{IP3R}. The properties of single channel openings were quantitatively determined in *Xenopus laevis* oocytes (37, 38) and we assume that the single channel properties are transferable to TCs. The measured dynamics are well described by the previously published Mak–McBride–Foskett model (37, 38).

$$\begin{aligned} g_{\text{IP3R}} &= g_{\text{IP3R,max}}\, H\left(C, C_{\text{IP3R,act}}, n_{\text{IP3R,act}}\right) \\ h_{\text{IP3R}} &= H\left(C_{\text{IP3R,inh}}, C, n_{\text{IP3R,inh}}\right) \\ C_{\text{IP3R,inh}} &= \overline{C_{\text{IP3R,inh}}}\, H\left(P, P_{\text{IP3R,C}}, n_{\text{IP3R,C}}\right). \end{aligned} \tag{27}$$

The according parameters were obtained from a data fit (37) to be $g_{\text{IP3R,max}} = 0.81$, $C_{\text{IP3R,act}} = 0.21\,\mu$M, $n_{\text{IP3R,act}} = 1.9$,

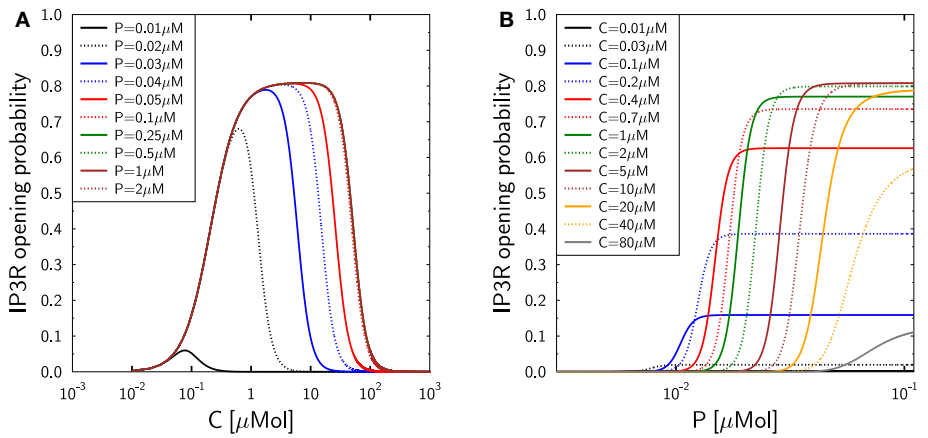

FIGURE 2 | Activation and inactivation of IP3R in dependence on calcium and IP3. Reproduction of the experimental data (37) with the Mak–McBride–Foskett model equation (27). **(A)** IP3R opening probability $g_{IP3R}h_{IP3R}$ in dependence on free cytosolic calcium C for different IP3-concentrations P. **(B)** The same IP3R opening probability in dependence on IP3-concentration P for different free cytosolic calcium concentrations C.

$n_{IP3R,inh} = 3.9$, $\overline{C_{IP3R,inh}} = 52\,\mu M$, $n_{IP3R,C} = 4$, and the IP3-concentration of half-activation $P_{IP3R,C} = 0.050\,\mu M$.

The dependencies of the model equation (27) on calcium and IP3 are depicted in **Figure 2** and correctly reproduce the measurements in Mak et al. (37) suggesting that the modulating effect of IP3 is mediated by IP3R inactivation (38). At low calcium this effect is hardly visible and IP3R activation remains unaffected by changes in IP3 for resting concentrations beyond 50 nM. The dynamic IP3 range is between 100 nM and 1 μM (see (66), Table 1), a regime of IP3 at which the IP3R-type1 exhibits saturation (27, 67). We do not aim at resolving whether the resting IP3 is lower in TCs or whether the IP3R characteristics are different in TCs, such that the DeYoung–Keizer model should be employed instead (27, 65). It is assumed that the resting concentration of IP3 is in the range of 5–10 nM which ensures that an increased IP3-concentration has an impact on the IP3R opening probability as depicted in **Figure 2**.

2.6.2. IP3R calcium current

The steady state activation function (**Figure 2**) can be used to define the calcium current through the IP3R which follows the electrochemical gradient between the cytosol and the ER

$$I_{IP3R} = \overline{g_{IP3R}}\, g_{IP3R}\, h_{IP3R}(V - V_{ER} - \overline{V_{C,ER}}), \tag{28}$$

with $\overline{g_{IP3R}} = 0.064pS$. Note that the conductance differs between tissues (38). $V - V_{ER}$ is the potential difference between ER and cytosol, and $\overline{V_{C,ER}}$ is the ER-reversal potential for calcium, calculated from the Nernst-equation equation (9). We assume an electrical equilibrated relation of cytosol and ER such that $V = V_{ER}$ holds true.

2.6.3. (In)activation dynamics

The activation and inactivation factors g and h are treated dynamically and approach equation (27) in steady state, while the adaptation of $C_{IP3R,inh}$ in equation (27) is treated in quasi steady state:

$$\begin{aligned} \frac{dg_{IP3R}}{dt} &= \frac{g_{IP3R,max}H(C, C_{IP3R,act}, n_{IP3R,act}) - g_{IP3R}}{\tau_{IP3R}} \\ \frac{dh_{IP3R}}{dt} &= \frac{H(C_{IP3R,inh}, C, n_{IP3R,inh}) - h_{IP3R}}{\theta_{IP3R}} \end{aligned} . \tag{29}$$

2.6.4. Activation time

Activation time scales can be determined from Mak and Foskett (68), Figure 5, and are in the range of less than 5 and 20 ms for depolarizations to 20 and 40 mV. Two exponentials were needed to fit the opening frequency. Using rat hepatocytes the activation and inactivation time scales were found to depend on the IP3-levels (69): activation varied between 100 and 500 ms for 10 μM and 400 nM of IP3, respectively (see Figure 1 therein). The time delay reported in Marchant and Taylor (69) is consistent with the IP3-dependent time delay of channel opening of $1\,s > \tau_{IP3R} > 100\,ms$ using basophilic leukemia cells from rats (70). As the model focusses on calcium dynamics on the scale of minutes, a constant $\tau_{IP3R} = 100$ ms is assumed.

2.6.5. Inactivation time

Onset of inactivation happens in less than 2 min (68). A slow and a fast current were distinguished (69). The fast current inactivates on a time scale of 200–450 ms, the slow one between 1 and 6 s (see Figure 2 in the same publication). The authors attribute the fast time scale to inactivation of IP3R and the slow one to the depletion of the calcium content in the ER. Accordingly, only the fast time scales are relevant for the single IP3R, and $\theta_{IP3R} = 300$ ms is assumed.

2.6.6. Calmodulin dependence

It was found that the calcium-release from ER is reduced for high concentrations of the calcium-buffer calmodulin (71). Such a dependence is neglected in the present model.

2.6.7. IP3R density

The IP3R density on Jurkat TCs is not known and is determined using steady state condition equation (34).

2.7. PLASMA-MEMBRANE CALCIUM-ATPase

Plasma-membrane calcium-ATPase is an ATP-driven calcium pump which extrudes calcium from the cell to the ES. It was characterized in TCs (12). In a first attempt the dependence on the ATP concentration is ignored and assumed to be large enough in order to make the pump work optimally. In this case the activity is mainly dependent on the calcium concentration in the cell. A suitable modeling approach is

$$I_{\mathrm{PMCA}} = \overline{I_{\mathrm{PMCA}}} g_{\mathrm{PMCA}} \tag{30}$$

with

$$\frac{dg_{\mathrm{PMCA}}}{dt} = \frac{H\left(C, C_{\mathrm{PMCA}}, n_{\mathrm{PMCA}}\right) - g_{\mathrm{PMCA}}}{\tau_{\mathrm{PMCA}}}. \tag{31}$$

The current $\overline{I_{\mathrm{PMCA}}}$ is positive as it carries calcium out of the cell. The Hill-coefficient was determined to be $n_{\mathrm{PMCA}} = 2$ (72).

2.7.1. Turn-over rate

The turn-over rate of PMCA is approximately 30 Hz (73) which corresponds to an activity rate of $k_{\mathrm{a}} = 0.03/\mathrm{ms}$ which is also used in Sherman et al. (74). This turn-over rate can be translated into an electrical current by using that every pumping event corresponds to the flow of 2 electrical charges which yields $\overline{I_{\mathrm{PMCA}}} = z_{\mathrm{Ca}} e k_{\mathrm{a}} = 60 \cdot 1.6 \cdot 10^{-19}\,\mathrm{C/s} \approx 10^{-17}\,\mathrm{A} = 10^{-5}\,\mathrm{pA}$.

2.7.2. Calcium-dependent activation

Typical values for the half-activation calcium concentration are $C_{\mathrm{PMCA}} = 0.1\,\mu\mathrm{M}$ (at 540 nM calmodulin, see e.g., (75), Figure 3). The isoforms 2a and 2b exhibit $C_{\mathrm{PMCA2ab}} < 0.1\,\mu\mathrm{M}$. The predominant isoform of PMCA in Jurkat TCs is 4b [(76), Figure 6B]. $C_{\mathrm{PMCA4b}} \approx 0.2\,\mu\mathrm{M}$ was found in Jurkat TCs [Figure 2 in Caride et al. (76)] and is used here.

2.7.3. Calmodulin-dependent activation

Note that the values of half-activation also depend on the calmodulin concentration (75–77). For calmodulin concentrations above $1\,\mu\mathrm{M}$ (which is exclusively the case in all present simulations) full activation of all isoforms is ensured (75, 78). Hence, the dependence on calmodulin is weak in this regime and is neglected.

2.7.4. Delay of activation

Binding of calcium to PMCA is a comparably fast process with a rate constant >3 per second (79). However, the activity of PMCA is delayed in some isoforms including the isoform 4b (76) which is relevant for TCs. The rate constant of PMCA activation upon stimulation with 500 nM of free calcium was in the range of 0.02 per second (76), which leads to a delay of PMCA activation in the range of $\tau_{\mathrm{PMCA}} = 50\,\mathrm{s}$ in equation (31). This delay was associated with a calmodulin and calcium-dependent activation (76). However, as the exact mechanism is not known this delay is modeled in equation (31) on a phenomenological basis.

2.7.5. PMCA density

For TCs no precise value of the protein density is known and the value is determined by the steady state condition equation (33).

2.8. SARCO/ENDOPLASMIC RETICULUM CALCIUM-ATPase

The calcium level in ER is kept high with the help of SERCA calcium pumps. The activity of SERCA is assumed to rapidly adapt to the present calcium concentration in the cytosol and can be described by a Hill-function.

$$I_{\mathrm{SERCA}} = \overline{I_{\mathrm{SERCA}}} H\left(C, C_{\mathrm{SERCA}}, n_{\mathrm{SERCA}}\right). \tag{32}$$

In every turn-over cycle two calcium ions are transported per ATP (80). There are different subtypes of SERCA whose properties differ. In Jurkat TCs as well as in human tonsil lymphocytes the dominant isoform is SERCA2b (81).

2.8.1. Turn-over rate

The turn-over rates of most isoforms are in the range of $k = 10\,\mathrm{Hz}$ (i.e., $\overline{I_{\mathrm{SERCA}}} = \alpha_{\mathrm{SERCA}} z_{\mathrm{Ca}} e k = 6 \cdot 10^{-6}\,\mathrm{pA}$). For SERCA2b $k_{2\mathrm{b}} = 5\,\mathrm{Hz}$ was reported (82) which implies the value $\overline{I_{\mathrm{SERCA}}} = 3 \cdot 10^{-6}\,\mathrm{pA}$ used in the model.

2.8.2. Calcium-dependent activation

For the SERCA isoforms 1, 2a, and 2b the half-activation $C_{\mathrm{SERCA}} = 0.4\,\mu\mathrm{M}$ and the Hill-coefficient $n_{\mathrm{SERCA}} = 2$ are a good approximation [(82) Figure 4]. The half-activation of SERCA3 is around $1\mu\mathrm{M}$ with the same Hill-coefficient (82). SERCA2b is an isoform active at relatively low calcium concentrations (82). Specifically, in Jurkat TCs as well as in human tonsil lymphocytes, the dominant isoform SERCA2b was characterized with $n_{\mathrm{SERCA2b}} = 2.0$ and $C_{\mathrm{SERCA2b}} = 0.25\,\mu\mathrm{M}$ (81), which is used here.

2.8.3. SERCA density

Even though the expression of SERCA was shown to be modulated upon activation (83), the expression density of SERCA within ER is not known and is determined by parameter fitting in Section 2.10.

2.9. STEADY STATE DETERMINES PROTEIN DENSITIES

The resting state of the TC is determined by setting the dynamics in equations 1, 4, 7, and 24 to zero. Accordingly, the equations for C, C_{ER}, P, and ρ_{CRAC}, read:

$$\rho_{\mathrm{PMCA}} = -\frac{\rho_{\mathrm{CRAC},0} I_{\mathrm{CRAC},0}}{I_{\mathrm{PMCA},0}} \tag{33}$$

$$\rho_{\mathrm{IP3R}} = -\frac{\rho_{\mathrm{SERCA}} I_{\mathrm{SERCA},0}}{I_{\mathrm{IP3R},0}} \tag{34}$$

$$\gamma_{\mathrm{P}} = \frac{\beta_{\mathrm{P}} H(C_0, C_P, n_P)}{P_0} \tag{35}$$

$$\rho_{\mathrm{CRAC}}^{-} = \frac{\rho_{\mathrm{CRAC},0}}{H\left(C_{\mathrm{ER},0}, C_{\mathrm{CRAC}}, n_{\mathrm{CRAC}}\right)} \left[1 - f_{\mathrm{CRAC}}\left(1 - H\left(C_{\mathrm{ER},0}, C_{\mathrm{CRAC}}, n_{\mathrm{CRAC}}\right)\right)\right], \tag{36}$$

where $I_{\mathrm{X},0}$ denotes the currents with all quantities X in the resting configuration X_0. The parameters of the model are summarized in **Table 1**.

Table 1 | List of parameters, values, and references.

Parameter	Meaning	Value	ΔQI (%)	Comments and reference
CELL GEOMETRY				
R_{cell}	TC radius	8 μm	4.0	Fixed from Jurkat TC (12)
f_R	Nucleus radius fraction of R_{cell}	0.25	0.1	Fixed, Section 2.4
f_V	ER volume fraction	0.01	2.8	Fixed (25)
f_A	ER surface, fold of spherical	30	3.5	Fixed, Section 2.4
C_m	Membrane capacitance	28 fF/μm^2	0.0	Fixed (58, 59)
IONS AND POTENTIAL				
T	Temperature	310 K	–	Fixed, used in equation (9)
V_0	Resting membrane potential	−60 mV	–	Fixed (51, 52, 53)
$V_{ER,0}$	Resting ER potential	−60 mV	–	Fixed = V_0 (54)
C_0	Resting calcium	0.1 μM	–	Fixed (12)
$C_{ER,0}$	Resting ER calcium	0.4 mM	4.0	Fixed in range 0.1–0.8 mM (2)
C_{ext}	Extracellular calcium	2 mM	0.0	Fixed by experimental settings
ΔV_C	Reversal potential shift	78 mV	0.3	Fixed (33, 34)
$\Delta V_{C,ER}$	ER-reversal potential shift	63 mV	176	Variable
CALCIUM-BUFFER				
b_0	Cytosolic calcium-buffer	100 μM	3.6	Fixed (44, 45, 46)
K_b	Buffer dissociation constant	0.1 μM	0.2	Fixed by $f_C = 0.1\%$ in equation (3)
$b_{ER,0}$	ER calcium-buffer	30 mM	4.3	Fixed by $f_{C,ER} = 20 f_C$ (47)
$K_{ER,b}$	ER buffer dissociation constant	0.1 mM	1.2	Fixed (49)
SECOND MESSENGERS				
P_0	Resting IP3	8.7 nM	318	Variable
β_P	IP3 production rate	0.6 nM/s	6.5	Variable
γ_P	IP3 degradation rate	0.01149/s	–	Fixed by steady state equation (35)
C_P	Calcium of half IP3 production	0.5 μM	19.4	Fixed (85)
n_P	IP3 production Hill-coefficient	1	21.2	Fixed
TRANSMEMBRANE PROTEIN DENSITIES				
ρ_{IP3R}	ER-IP3R density	11.35/μm^2	–	Fixed by steady state equation (34)
ρ_{SERCA}	ER-SERCA density	700/μm^2	3.7	Variable
ρ_{PMCA}	PMCA density	68.57/μm^2	–	Fixed by steady state equation (33)
$\rho_{CRAC,0}$	Resting active CRAC density	0.6/μm^2	1.0	Variable
ρ^+_{CRAC}	Max active CRAC density	3.9/μm^2	6.5	Variable, range from Luik et al. (34)
ρ^-_{CRAC}	Min active CRAC density	0.5115/μm^2	–	Fixed by steady state equation (36)

Fixed parameters were determined either by direct measurement, by indirect constraints, or using steady state conditions. Variable parameters were subject to the fitting algorithm described in Section 2.10. ΔQI measures the sensitivity of QI in equation (37) for changed parameter values: each parameter is increased by 10% and the percentage of the change in QI is provided. Values below 0.05% are given as 0.0%.

2.10. NUMERICAL SOLUTION AND PARAMETER FITTING

The model defined by the equations (1, 2, 4, 5, 7, 8, 16–25, 27–32) was implemented as C^{++}-code and solved using a self-written 4th-order Runge–Kutta algorithm with adaptive stepsize control.

As not all parameters could be determined by steady state conditions or by experimental constraints, Figure 1A in Bautista et al. (12) was used to determine the remaining free parameters. We used a two-step fitting procedure: at first, the differential evolution algorithm defined in Storn and Price (84) was incorporated into the C^{++}-code of the model on the basis of all parameters in **Table 1** that were not determined by steady state conditions. The parameters were varied within hard-coded boundaries dictated by experimental constraints (when available). The quality of the fit to the calcium data in Bautista et al. (12) was measured as the mean square deviation

$$QI = \frac{1}{N}\sqrt{\sum_{i=1}^{N}\frac{(X_i - E_i)^2}{E_i^2}}, \tag{37}$$

with X_i and E_i representing the simulation and experimental values, respectively. In a second step, the first approximative fit was subject to a sensitivity analysis in which each parameter was varied by 10% while monitoring the effect on QI. The three unknown protein densities ρ_{SERCA}, $\rho_{CRAC,0}$, and ρ^+_{CRAC}, two sensitive IP3 related parameters P_0 and β_P, as well as the very sensitive parameter $\Delta V_{C,ER}$ were used for fine-tuning the initial parameter fit with the same differential evolution

algorithm. These fit parameters are marked *variable* in **Table 1**. The final fit reached QI = 0.100197 with $N = 22$. The sensitivity analysis was repeated for the final fit and the impact of each parameter on QI in equation (37) is provided in **Table 1**.

All subsequently described simulations are started with the cell in steady state as defined by the hard-coded equations (33–36). Starting from these initial conditions, the respective stimulation protocols are applied as described in the results section.

3. RESULTS

In the methods section single protein characteristics were summarized and specific mathematical models capturing their main properties were proposed or cited. The models for the single proteins were combined to a whole cell model and the unknown parameters were determined using steady state conditions or by data fitting as described in Section 2.10. In this section, we replicate specific experimental setups described in the literature *in silico* and analyze the calcium dynamics from the perspective of the model.

3.1. TCR STIMULATION

The introduced TC-model is used to investigate the experiments of Bautista et al. (12) in Jurkat TCs. TC activation by stimulation of TCR induces an intracellular rise of second messengers like cADPR, NAADP, and IP3. In the present model this rise is collectively reflected in equation (7) for IP3. The IP3 signal activates IP3R and by this induces a calcium-release from the ER. The positive feedback loop of CICR leads to even more calcium-release from the ER, which in turn reduces the ER calcium concentration C_{ER}. CRAC is activated in a C_{ER}-dependent manner, as represented by equation (24). The rising cytosolic calcium is cleared by PMCA and the ER is refilled by SERCA, both being ATP-dependent processes.

Using 2 mM of external calcium a cell in resting state was activated with OKT3 via TCR [see Figure 1A in Bautista et al. (12)]. This induced a calcium peak to more than 1 μM within 50–100 s, which subsequently relaxed to a plateau level of $\approx$0.7 μM over the following 200 s. This behavior is well reproduced by the model (**Figure 3A**) and was used to determine the unknown parameters (**Table 1**). The peak height relies on both, on a proper activation of IP3R and CRAC. The choice of P_0 turned out to be rather important in order to guarantee a proper activation of IP3R. The maximum activated CRAC density was essential for the height of the plateau. A value of $f_{CRAC} = 6.5$ was found to correctly reproduce the plateau height as measured in Bautista et al. (12).

Bautista et al. reported that the delay of PMCA activation is responsible for the calcium overshoot (12). Accordingly, we investigated whether this conclusion is supported by the model. For that purpose we reduced τ_{PMCA} from 50 s (76) to 1 millisecond in the otherwise unaltered simulation. This modification does not touch the steady state configuration, such that all other parameters remained unchanged. The model still generated an overshoot of calcium, however, with a reduced amplitude (**Figure 3A**, blue dotted line). The height of the plateau as well as the relaxation time from peak to plateau remained unchanged. Thus, the simulation supports an influence of the PMCA delay onto the overshoot, but it turned out to not be essential for its existence.

This surprising result led to the question whether a different delay in the model could explain the calcium overshoot. All delays were tested and the only delay with impact on the overshoot was IP3R inactivation, i.e., the parameter θ_{IP3R} (**Figure 3A**, green dashed line). In conclusion, the model suggests that intrinsic properties of the IP3R and not the delay of PMCA activation are responsible for the calcium overshoot upon TCR stimulation.

Next, the model is used for an analysis of the currents which are associated with the different phases of the calcium dynamics (**Figure 3B**). The IP3-current (red full line) is clearly the largest and also persists beyond the overshoot of calcium due to SERCA activity (red dotted line). The calcium peak induces a PMCA current (black dotted line) that drives the calcium out of the cell (black dashed line). Thus, the CRAC current (black full line), induced by the loss of calcium in the ER, does not even induce a net flow of calcium into the cell (black dashed line) but just prevents the cell from running out of calcium. This model result suggests that the role of CRAC is the stabilization of the cell rather than its activation, which is mostly mediated by calcium from the ER.

The single protein currents (**Figure 3C**) show that in the model IP3R currents react much more dynamic than CRAC currents. The increased cytosolic calcium even reduces the CRAC currents on the single channel level. However, the reduced ER calcium level induces a strong increase in the active CRAC-channel density (**Figure 3D**). Thus, the increased whole cell CRAC current seen in **Figure 3B** is not a result of single channel responses but of a changed channel density.

3.2. TCR STIMULATION WITH ZERO EXTERNAL CALCIUM

Zero external calcium experiments aim at suppressing CRAC currents in order to investigate ER calcium currents in response to TCR stimulation. TCR stimulation of Jurkat TCs hold at zero external calcium results in an intracellular calcium peak after 50–100 s with reduced amplitude in comparison to stimulation with normal external calcium [Figure 2A in Bautista et al. (12)]. The increased calcium is cleared below baseline level within 100–200 s.

In the model, the Nernst-equation prohibits the use of zero external calcium conditions. At very low calcium concentrations the Nernst-equation loses its validity and the reversal potential diverges. Therefore, external calcium is set to the concentration C^*_{ext} at which the CRAC current vanishes in resting state:

$$C^*_{\text{ext}} = C_0 \exp\left\{\frac{(V_0 - \Delta V_C)\, z_{Ca} F}{RT}\right\} \quad . \tag{38}$$

This mimicks the suppression of CRAC currents as intended in the experiment. The *in silico* result is shown in **Figure 4**. The CRAC current vanishes at resting state (**Figure 4B**, black full line). This is approximately true during the whole experiment, such that the method for mimicking the zero external calcium experiment appears appropriate.

The calcium peak (**Figure 4A**) is lower than the one found in **Figure 3** which is qualitatively consistent with the experimental result (12). Furthermore, the time scales of calcium rise and clearance are perfectly matched between simulation and experiment. Even the overshoot of clearance below the baseline calcium level is fully reproduced. However, quantitatively, the peak is higher in

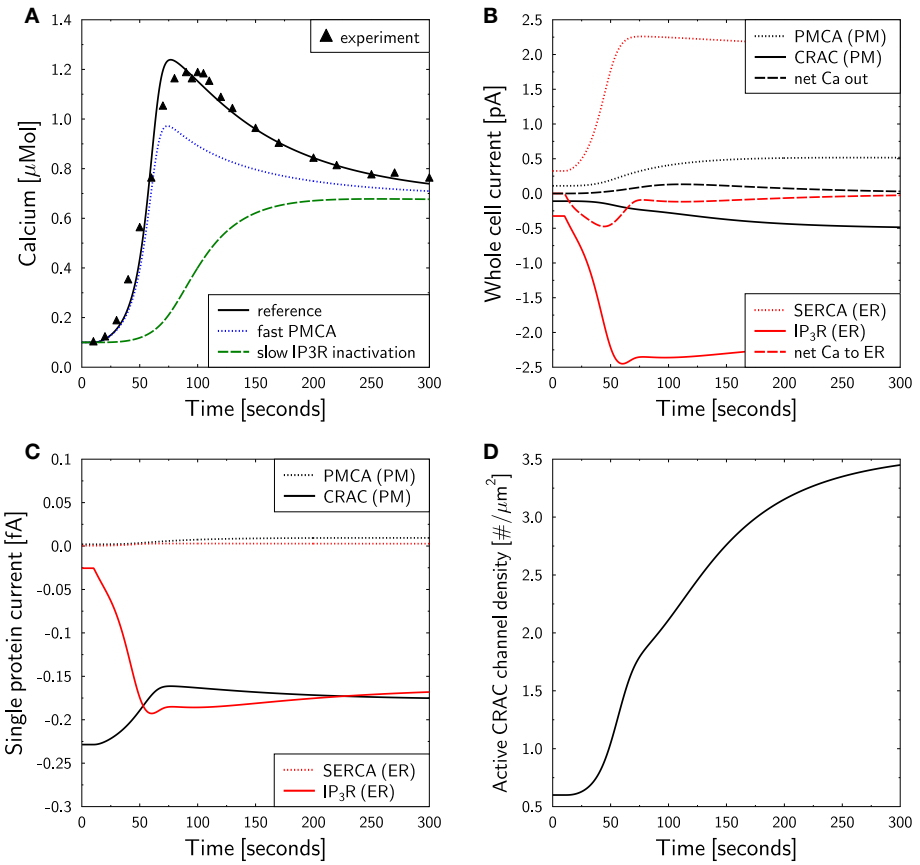

FIGURE 3 | Calcium dynamics in response to TCR stimulation. Simulation of the experiment in Figure 1A in Bautista et al. (12). **(A)** The cell is in steady state until $t = 10$ s when stimulation is started by setting $T(t) = 1.6$ in equation (7). Stimulation is kept constant throughout the simulation. *Reference* is the simulation used as a basis for all other simulations in the paper and is compared to the experimental result [black triangles read off Figure 1A in Bautista et al. (12)]. For *fast PMCA* a value of 1 ms was used for τ_{PMCA}. For *slow IP3R inactivation* a value of 300 s was used for θ_{IP3R}. **(B)** Whole cell currents show an initial release of calcium from the ER which is followed by a CRAC inward current. Negative currents are calcium currents into the cytosol. **(C)** Single transmembrane protein currents. **(D)** Dynamic response of active CRAC-channel density.

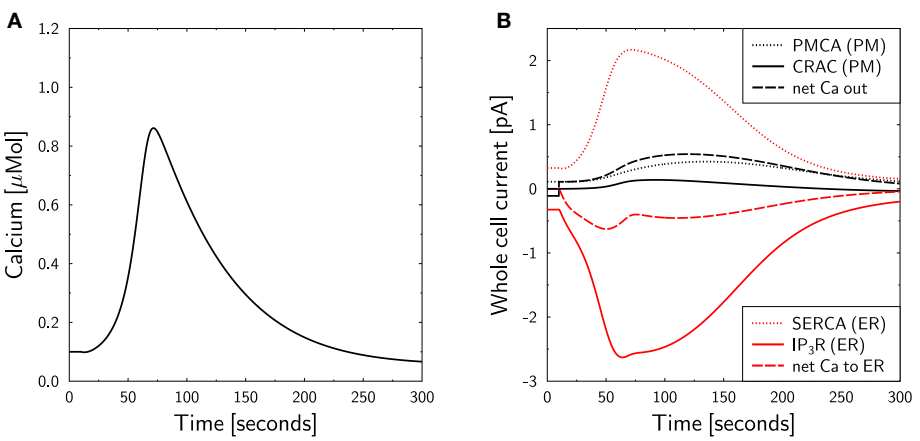

FIGURE 4 | Calcium dynamics in response to TCR stimulation at zero external calcium. Simulation of the experiment in Figure 2A in Bautista et al. (12). **(A)** The cell is in steady state until $t = 10$ s, when stimulation is started by setting $T(t) = 1.6$ in equation (7) and external calcium is set to equation (38). Stimulation and external calcium are kept constant throughout the simulation. **(B)** Whole cell currents show the almost complete inhibition of CRAC currents. The calcium peak (left) is generated by ER calcium only. Negative currents are calcium currents into the cytosol.

theory than in experiment. As the *in silico* peak is exclusively generated by the ER, one might hypothesize that the ER is too big or its calcium content is too high. As these parameters were already chosen comparably low (**Table 1**), it is more likely that the lack of external calcium influences the stimulation of the cell. The peak size can be reduced to the measured amplitude by reducing the stimulation T from 1.6 to 1.25 (not shown).

3.3. BLOCK OF SERCA AT ZERO EXTERNAL CALCIUM

Thapsigargin (TG) is frequently used to block SERCA activity as it prevents calcium uptake by the ER and leads to a continuous reduction of ER calcium. As low ER calcium recruits and activates CRAC-channels, this would lead to a strong influx of extracellular calcium. In order to prevent this according ER depletion and CRAC activation experiments are performed in zero calcium medium. This strategy was used in Jurkat TCs to generate a cell state in which the ER is mostly void of calcium and CRAC-channels are recruited and activated to a maximum (12, 13, 25, 86). It was reported that this procedure leads to a transient calcium peak of about 0.5 μM after more than 100 s which is slowly cleared and reaches calcium levels below the resting level [Figure 1A in Quintana et al. (86)].

Having established a strategy [equation (38)] for mimicking a medium with zero calcium, a SERCA block is performed *in silico* by setting $I_{\mathrm{SERCA}} = 0$ at $t = 10$ s. No TCR stimulation was applied. The measured dynamics are well reproduced without any further parameter fitting (**Figure 5A**, black full line). Furthermore, the intended depletion of ER is achieved (**Figure 5A**, red dashed line): a continuous reduction of ER calcium is observed. Note that also IP3 exhibits some dynamics (**Figure 5A**, blue dotted line) which further accelerates ER calcium loss by activation of IP3R. As expected, the reduced ER calcium leads to the recruitment of active CRAC-channels (**Figure 5B**).

3.4. BLOCK OF PMCA IN A TG TREATED TC

As the TG-mediated SERCA block works *in silico* (**Figure 5**), the role of PMCA in the clearance of cytosolic calcium is investigated. Following Figure 6 in Bautista et al. (12) TG was applied in a zero calcium medium as in **Figure 5**. Then a pulse of 2 mM external calcium was applied for 50 s. This induces a steep rise in calcium which is also steeply cleared again. Together with Lanthan (La^{3+}), a PMCA, and CRAC inhibitor (2), the clearance of such a calcium peak was substantially slower (12). A block of PMCA alone by carboxyeosin led to an only weakly modified clearance time, without a return to baseline levels within 300 s.

The same protocol is applied in the model (**Figure 6**). As before, ER calcium is depleted (**Figure 6A**, red dashed line) and the active CRAC density is increased correspondingly (as in **Figure 5B**). The initial free cytosolic calcium peak (**Figure 6A**, black full line) is the same as in **Figure 5A**. Upon increasing external calcium to 2 mM for 50 s at $t = 300$ s, a strong CRAC current is induced (**Figure 6B**, black full line), which steeply increases cytosolic calcium (**Figure 6A**, black full line). This calcium is also quickly cleared upon return to the mimicked zero calcium medium. Calcium clearance is dominated by the PMCA current (**Figure 6B**, black dotted line). However, in the model a small CRAC current is observed supporting extrusion of calcium out of the cell in the case of zero external calcium concentration. Upon permanent block of PMCA and repetition of the transient stimulation by external calcium, it is this backward CRAC current that clears calcium from the cytosol. The time course of the clearance is slower than without PMCA block and the calcium baseline is not reached after 350 s (**Figure 6B**, black full line). This is a similar behavior as in the corresponding experiment with carboxyeosin [Figure 6D in Bautista et al. (12)]. However, it is not known whether the real CRAC allows for such inverse current under zero calcium conditions. The return of calcium to the baseline might also be supported by uptake of calcium by other organelles like mitochondria, which is not covered by the present model.

In the case of PMCA- and CRAC-block with La^{3+} a rather slow calcium clearance is observed in experiments which apply the same stimulation protocol [Figure 6C in Bautista et al. (12)]. *In silico* no clearance is observed at

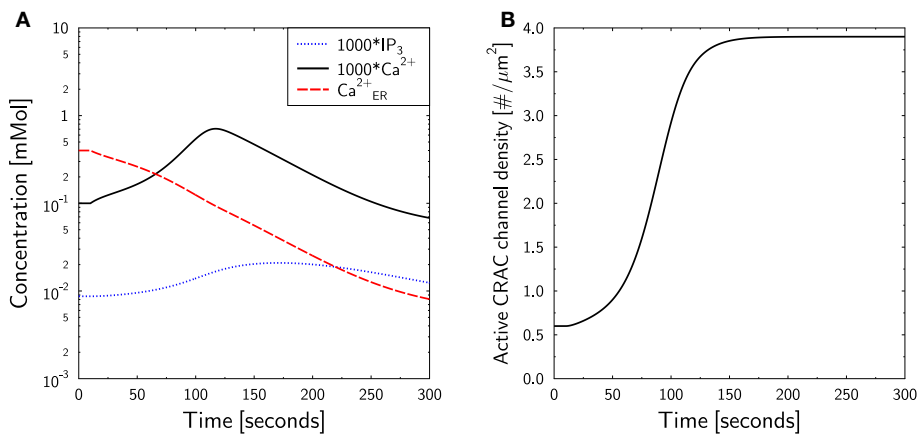

FIGURE 5 | Calcium dynamics in response to TG SERCA block at zero external calcium. Simulation of the experiment in Figure 1A in Bautista et al. (86). **(A)** The cell is in steady state until $t = 10$ s, when SERCA block is applied and external calcium is set to equation (38). Block and external calcium are kept constant throughout the simulation. P (blue dotted line), C (black full line), and C_{ER} (red dashed line) are shown. Note the factor 1000 applied to P and C. **(B)** The time course of active CRAC density ρ_{CRAC}.

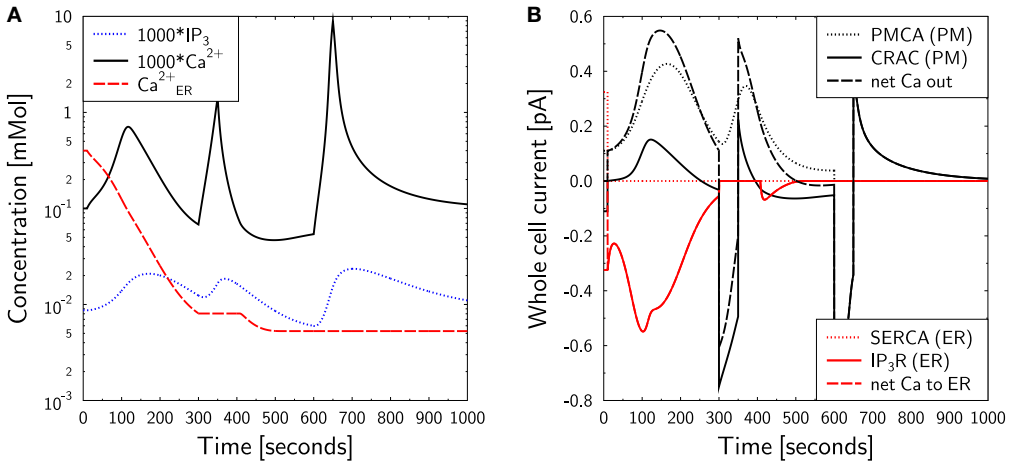

FIGURE 6 | Calcium dynamics in response to SERCA and PMCA block at zero external calcium. Simulation of the experiment in Figures 6C,D of Bautista et al. (12). **(A)** The cell is in steady state until $t = 10$ s, when SERCA block is applied and external calcium is set to equation (38). At $t = 300$ s 2 mM external calcium are applied for 50 s followed by a switch back to equation (38). The procedure is repeated at $t = 600$ s. In addition, I_{PMCA} is set to zero for the remaining simulation time. SERCA block is kept throughout the simulation. P (blue dotted line), C (black full line), and C_{ER} (red dashed line) are shown. Note the factor 1000 applied to P and C. **(B)** The CRAC density was increased during the first 300 s by ER calcium depletion with TG at zero external calcium [**(A)** red dashed line]. The whole cell currents show a sudden CRAC current (black full line) upon restoration of external calcium to 2 mM, which is cleared by PMCA activity (black dotted line) after return to zero external calcium. When PMCA is blocked in addition (at $t = 600$ s), the calcium clearance is slower (black full line). Negative currents are calcium currents into the cytosol.

all. SERCA is blocked and positive I_{IP3R}-currents are not allowed in the model, such that an uptake of calcium into the ER is excluded. A full block of PMCA and CRAC also excludes any expulsion of calcium out of the cell. Thus, the model suggests that the slow clearance of cytosolic calcium is either due to incomplete block of PMCA or to leakage currents.

3.5. BLOCK OF PMCA IN AN UNTREATED TC

The model suggests that the role of CRAC for TC activation is mainly the maintenance of the integrity of the TCs during stimulation in the sense that it prevents the activated TC from running out of calcium. If we block PMCA *in silico* at the time of TCR stimulation (protocol as in **Figure 3**) in an otherwise untreated TC, the TC would be prevented of losing calcium. According to our interpretation of the role of CRAC we would expect that CRAC activity is strongly reduced in comparison to **Figure 3**.

T lymphocytes receptor stimulation of PMCA-blocked TCs is predicted to induce a strong increase of cytosolic calcium (**Figure 7**, full black line). Thereby, the steady state CRAC current is not increased (as in **Figure 3B**, full black line) but reduced (not shown). However, the CRAC current is not reduced to zero such that the total block of PMCA infers a persistent net influx of calcium into the cell and, thus, to a persistent increase of cytosolic calcium. This would ultimately destroy a real TC. As SERCA activity is normal in this *in silico* experiment, calcium in the ER is only transiently reduced (not shown), leading to a transient and weak increase in the CRAC density (**Figure 7**, dotted red line). The amplitude is less than twofold instead of sixfold in **Figure 3D**.

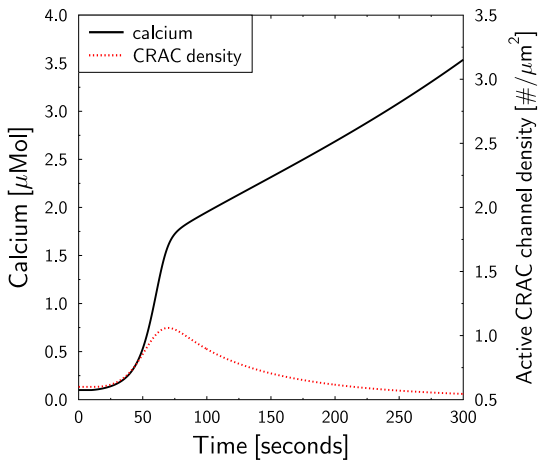

FIGURE 7 | Block of PMCA in untreated TCs. The TCR stimulation protocol in **Figure 3** is repeated. At the time of stimulation ($t = 10$ s), PMCA activity is blocked preventing calcium efflux from the cell. Cytosolic calcium (full black line, left axis) and the response of the CRAC density (dotted red line, right axis) are shown. The axis for the CRAC density was scaled as in **Figure 3D** for better comparison.

This result supports the interpretation of the role of CRAC-channels *in silico* and may be tested in experiment. It further shows, that the calcium level in the ER may be used as an indicator for the overall calcium status of TCs.

4. DISCUSSION

Within the presented investigation whole cell calcium dynamics were derived from models of single transmembrane

ion-conducting proteins. The model successfully described a number of experimental settings and captured important characteristics of calcium dynamics upon TCR stimulation. In particular, the role of store-derived calcium-release versus CRAC activation was well represented. We conclude, that we have generated a modeling framework suitable for the quantitative analysis of calcium dynamics of TCs.

The value of using measured single transmembrane protein characteristics is twofold: at first, it substantially reduces the number of free parameters in an otherwise very complex model. All measured single protein properties were just implemented and not altered in the fine-tuning of the model. The reduced number of free parameters increases the predictive power of the model. Secondly, it can be considered as a multi-scale approach which links single proteins to whole cell behavior. This allows the analysis of the dynamics on the single protein level and its implications onto the cellular properties. The drawback of this approach is that not all parameters could be derived from TCs such that we had to assume that single transmembrane properties are universal, which is a correct assumption in many cases (30). Within this framework, cell-specific properties are controlled by the protein densities and the cell-specific properties, both determining the activity range of the respective proteins. However, the predictive power of the model would benefit from corresponding TC related data.

The model of Jurkat TC calcium dynamics as supported by the mathematical model starts from a TCR-derived increase in second messengers like IP3. This ultimately activates IP3R currents and induces an initial calcium current from the ER into the cytosol which triggers CICR. However, calcium uptake via SERCA activity equilibrates the calcium loss in the ER, which leads to a zero net flux on long-term – despite ongoing TCR stimulation. The ER calcium loss, according to the model, was the major contribution to the free cytosolic calcium peak. However, as PMCA activity leads to an overall loss of calcium in the cell, a compensation mechanism is required for a sustained elevation of free cytosolic calcium. The model suggests that the CRAC activity essentially contributes to this compensation mechanism. The initiation of CRAC currents by the depletion of ER calcium levels is in line with this interpretation. The model predicts, that a standard stimulation of TCs together with a block of PMCA activity would lead to a strong calcium rise together with a minor and transient increase of the CRAC density and on long-term to a reduced CRAC current (see **Figure 7**). This prediction may be tested in experiment in order to validate this interpretation of the role of CRAC-channels.

The emerging hypothesis that the role of CRAC is the stabilization of the TC calcium level, needs to be further strengthened by more detailed modeling work. In particular, early events after stimulation like NAADP generation (87) and subsequent RyR calcium currents from the ER are essential for the early calcium rise (11, 18) and will have to be included in the mathematical model for a proper time-resolved coverage of calcium dynamics. The need for additional mechanisms is also underpinned by the strong sensitivity of the model behavior to changes in the IP3 resting concentration P_0 (**Table 1**). In the present simulation the long-term calcium plateau height mostly relies on the maximum possible CRAC activation by store-operated calcium depletion. For the long-lasting calcium rise, cADPR was proven relevant (16, 40) and has to be considered in the context of the present hypothesis on the role of CRAC. Also the transferability to human blood derived TCs, which exhibit a different geometry, has to be assessed.

As the PMCA block experiment at zero external calcium has shown, it might be important to include leakage currents into the model. However, it should be noted that the postulated role of delayed PMCA activity for the calcium overshoot after TCR stimulation (12) could only partially be confirmed by the mathematical model. With fast activation of PMCA, an overshoot was still observed in our model, and the overshoot could only be suppressed by lack of IP3R inactivation. It should be noted that this result might rely on the Mak–McBride–Foskett model (37), which was used for IP3R dynamics and which exhibits inactivation at rather low IP3. The result might differ if the TC calcium dynamics would be based on the DeYoung–Keizer model for IP3R activity (65).

The proposed model has limitations in its range of applicability. For example, the usage of the Nernst-equation for the chemical gradient and Ohm's law for the current-voltage relationship of ion-conducting pores is justified only in narrow limits. Experiments with zero external calcium drive the model to the very limits of this range of applicability which was circumvented here using a phenomenological approximation. The model may be reformulated in terms of the Fokker-Planck-equation in order to describe ion transport through the pores in more detail. Furthermore, it is known that calcium entrance points lead to spatially inhomogeneous calcium dynamics (22) which are not covered by the present space-averaged model. The value of the present approach lies in the surprising result that quantitative characteristics of single transmembrane proteins are sufficient to determine the cell behavior in the framework of an ordinary-differential-equation based model. The model has proven its predictive power, as it was fitted to data of one experiment in **Figure 3** and could be used to predict and explain further data of calcium dynamics generated under other experimental conditions in **Figures 4** and **6**. It is planned to elaborate the potential and the limits of the model predictions by application to further experimental settings.

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REFERENCES

1. Parekh A, Putney J. Store-operated calcium channels. *Physiol Rev* (2005) **85**:757–810. doi:10.1152/physrev.00057.2003
2. Feske S. Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol* (2007) **7**: 690–702. doi:10.1038/nri2152
3. Arrol H, Church L, Bacon P, Young S. Intracellular calcium signalling patterns reflect the differentiation status of human T cells. *Clin Exp Immunol* (2008) **153**: 86–95. doi:10.1111/j.1365-2249.2008.03677.x
4. Feske S. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca2+ entry in the immune system and beyond. *Immunol Rev* (2009) **231**:189–209. doi:10.1111/j.1600-065X.2009.00818.x
5. Oh-hora M, Rao A. Calcium signaling in lymphocytes. *Curr Opin Immunol* (2008) **20**: 250–8. doi:10.1016/j.coi.2008.04.004
6. Quintana A, Pasche M, Junker C, Al-Ansary D, Rieger H, Kummerow C, et al. Calcium microdomains at the immunological synapse: how ORAI channels, mitochondria and calcium pumps generate local calcium signals for efficient T-cell activation. *EMBO J* (2011) **30**:3895–912. doi:10.1038/emboj.2011.289
7. McCarl C-A, Khalil S, Ma J, Oh-hora M, Yamashita M, Jens Roether J, et al. Store-operated Ca2+ entry through ORAI1 is critical for T cell-mediated autoimmunity and allograft rejection. *J Immunol* (2010) **185**:5845–58. doi:10.4049/jimmunol.1001796
8. Jin S, Chin J, Kitson C, Woods J, Majmudar R, Carvajal V, et al. Natural regulatory T cells are resistant to calcium release-activated calcium (CRAC/ORAI) channel inhibition. *Int Immunol* (2013) **25**:1–10. doi:10.1093/intimm/dxt013
9. Putney J. A model for receptor-regulated calcium entry. *Cell Calcium* (1986) **7**:1–12. doi:10.1016/0143-4160(86)90026-6
10. Dolmetsch R, Lewis R. Signaling between intracellular Ca2+ stores and depletion-activated Ca2+ channels generates $[Ca2+]_i$ oscillations in T lymphocytes. *J Gen Physiol* (1994) **103**:365–88. doi:10.1085/jgp.103.3.365
11. Guse AH, Tsygankov AY, Weber K, Mayr GW. Transient tyrosine phosphorylation of human ryanodine receptor upon T cell stimulation. *J Biol Chem* (2001) **276**:34722–7. doi:10.1074/jbc.M100715200
12. Bautista D, Hoth M, Lewis R. Enhancement of calcium signalling dynamics and stability by delayed modulation of the plasma-membrane calcium-ATPase in human T cells. *J Physiol* (2002) **541**: 877–94. doi:10.1113/jphysiol.2001.016154
13. Bautista D, Lewis R. Modulation of plasma membrane calcium-ATPase activity by local calcium microdomains near CRAC channels in human T cells. *J Physiol* (2004) **556**:805–17. doi:10.1113/jphysiol.2003.060004
14. Fomina A, Fanger C, Kozak A, Cahalan M. Single channel properties and regulated expression of Ca2+ release-activated Ca2+ (CRAC) channels in human T cells. *J Cell Biol* (2000) **150**:1435–44. doi:10.1083/jcb.150.6.1435
15. Dadsetan S, Zakharova L, Molinski T, Fomina A. Store-operated Ca2+ influx causes Ca2+ release from the intracellular Ca2+ channels that is required for T cell activation. *J Biol Chem* (2008) **283**:12512–9. doi:10.1074/jbc.M709330200
16. Guse AH, da Silva CP, Berg I, Skapenko AL, Weber K, Heyer P, et al. Regulation of calcium signalling in T lymphocytes by the second messenger cyclic ADP-ribose. *Nature* (1999) **398**:70–3. doi:10.1038/18024
17. Guse A. Linking NAADP to ion channel activity: a unifying hypothesis. *Sci Signal* (2012) **5**. doi:10.1126/scisignal.2002890
18. Gasser A, Bruhn S, Guse A. Second messenger function of nicotinic acid adenine dinucleotide phosphate revealed by an improved enzymatic cycling assay. *J Biol Chem* (2006) **281**:16906–13. doi:10.1074/jbc.M601347200
19. Lewis R. Calcium oscillations in T-cells: mechanisms and consequences for gene expression. *Biochem Soc Trans* (2003) **31**:925–9. doi:10.1042/BST0310925
20. Wu M, Buchanan J, Luik R, Lewis R. Ca2+ store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J Cell Biol* (2006) **174**:803–13. doi:10.1083/jcb.200604014
21. Parekh A. Store-operated CRAC channels: function in health and disease. *Nat Rev Drug Discovery* (2010) **9**:399–410. doi:10.1038/nrd3136
22. Martin G, Yun Y, Conforti L. Modulation of T cell activation by localized K+ accumulation at the immunological synapse: a mathematical model. *J Theor Biol* (2012) **300**:173–82. doi:10.1016/j.jtbi.2012.01.018
23. Lewis R, Cahalan M. Mitogen-induced oscillations of cytosolic Ca2+ and transmembrane Ca2+ current in human leukemic T cells. *Cell Regul* (1989) **1**:99–112.
24. Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* (1992) **355**:353–6. doi:10.1038/355353a0
25. Bergling S, Dolmetsch R, Lewis R, Keizer J. A fluorometric method for estimating the calcium content of internal stores. *Cell Calcium* (1998) **23**:251–9. doi:10.1016/S0143-4160(98)90123-3
26. Hess S, Oortgiesen M, Cahalan M. Calcium oscillations in human T and natural killer cells depend upon membrane potential and calcium influx. *J Immunol* (1993) **150**:2620–33.
27. Kowalewski J, Uhlen P, Kitano H, Brismar H. Modeling the impact of store-operated Ca2+ entry on intracellular Ca2+ oscillations. *Math Biosci* (2006) **204**:232–49. doi:10.1016/j.mbs.2006.03.001
28. Chen X, Li C, Wang P, Li M, Wang W. Dynamic simulation of the effect of calcium-release activated calcium channel on cytoplasmic Ca2+ oscillation. *Biophys Chem* (2008) **136**:87–95. doi:10.1016/j.bpc.2008.04.010
29. Liu W, Tang F, Chen J. Designing dynamical output feedback controllers for store-operated Ca2+ entry. *Math Biosci* (2010) **228**:110–8. doi:10.1016/j.mbs.2010.08.013
30. Meyer-Hermann M. The electrophysiology of the beta-cell based on single transmembrane protein characteristics. *Biophys J* (2007) **93**:2952–68. doi:10.1529/biophysj.107.106096
31. Zylinska L, Soszynski M. Plasma membrane Ca^{2+}-ATPase in excitable and nonexcitable cells. *Acta Biochim Pol* (2000) **47**:529–39.
32. Kozak A, Kerschbaum H, Cahalan M. Distinct properties of CRAC and MIC channels in RBL cells. *J Gen Physiol* (2002) **120**:221–35.
33. Prakriya M, Lewis R. Separation and characterization of currents through store-operated CRAC channels and Mg2+-inhibited cation. *J Gen Physiol* (2002) **119**:487–507. doi:10.1085/jgp.20028551
34. Luik R, Wang B, Prakriya M, Wu M, Lewis R. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature* (2008) **454**:538–42. doi:10.1038/nature07065
35. Wuytack F, Papp B, Verboomen H, Raeymaekers L, Dode L, Bobe R, et al. A sarco/endoplasmic reticulum Ca(2+)-ATPase 3-type Ca2+ pump is expressed in platelets, in lymphoid cells, and in mast cells. *J Biol Chem* (1994) **14**:1410–6.
36. Meyer T, Holowka D, Stryer L. Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate. *Science* (1988) **240**: 653–6. doi:10.1126/science.2452482
37. Mak D-OD, McBride S, Foskett JK. Inositol 1,4,5-tris-phosphate activation of inositol tris-phosphate receptor Ca2+ channel by ligand tuning of Ca2+ inhibition. *Proc Natl Acad Sci U S A* (1998) **95**:15821–5. doi:10.1073/pnas.95.26.15821
38. Foskett K, White C, Cheung K-H, Mak D-OD. Inositol trisphosphate receptor Ca2+ release channels. *Physiol Rev* (2007) **87**:593–658. doi:10.1152/physrev.00035.2006
39. Guse AH. Second messenger signaling: multiple receptors for NAADP. *Curr Biol* (2009) **19**:521–3. doi:10.1016/j.cub.2009.05.045
40. Schwarzmann N, Kunerth S, Weber K, Mayr G, Guse A. Knock-down of the type 3 ryanodine receptor impairs sustained Ca2+ signaling via the T cell receptor/CD3 complex. *J Biol Chem* (2002) **277**:50636–42. doi:10.1074/jbc.M209061200
41. Hohenegger M, Suko J, Gscheidlinger R, Drobny H, Zidar A. Nicotinic acid-adenine dinucleotide phosphate activates the skeletal muscle ryanodine receptor. *Biochem J* (2002) **367**:423–31. doi:10.1042/BJ20020584
42. Kunerth S, MF L, Schwarzmann N, Gu X, Huang L, Yang Z, et al. Amplification and propagation of pacemaker Ca2+ signals by cyclic ADP-ribose and the type 3 ryanodine receptor in T cells. *J Cell Sci* (2004) **117**:2141–9. doi:10.1242/jcs.01063
43. Dammermann W, Guse A. Functional ryanodine receptor expression is required for NAADP-mediated local Ca2+ signaling in T-lymphocytes. *J Biol Chem* (2005) **280**:21394–9. doi:10.1074/jbc.M413085200
44. Persechini A, Cronk B. The relationship between the free concentrations of Ca2+ and Ca2+-calmodulin in intact cells. *J Biol Chem* (1999) **274**:6827–30. doi:10.1074/jbc.274.11.6827

45. Tansey M, Luby-Phelps K, Kamm K, Stull J. Ca(2+)-dependent phosphorylation of myosin light chain kinase decreases the Ca2+ sensitivity of light chain phosphorylation within smooth muscle cells. *J Biol Chem* (1994) **269**:9912–20.
46. Kakiuchi S, Yasuda S, Yamazaki R, Teshima Y, Kanda K, Kakiuchi R, et al. Quantitative determinations of calmodulin in the supernatant and particulate fractions of mammalian tissues. *J Biochem* (1982) **92**:1041–8.
47. Mogami H, Gardner J, Gerasimenko O, Camello P, Petersen O, Tepikin A. Calcium binding capacity of the cytosol and endoplasmic reticulum of mouse pancreatic acinar cells. *J Physiol* (1999) **15**:463–7. doi:10.1111/j.1469-7793.1999.0463p.x
48. Sanchez E, Lewis K, Danna B, Kang C. High-capacity Ca2+ binding of human skeletal calsequestrin. *J Biol Chem* (2012) **287**:11592–601. doi:10.1074/jbc.M111.335075
49. Baksh S, Michalak M. Expression of calreticulin in *Escherichia coli* and identification of its Ca2+ binding domains. *J Biol Chem* (1991) **266**:21458–65.
50. Vandekaetsbeek I, Vangheluw P, Raeymaekers L, Wuytack F. The Ca2+ pumps of the endoplasmic reticulum and Golgi apparatus. *Cold Spring Harbor Perspect Biol* (2011) **3**.
51. Ishida Y. Lack of voltage sensitive potassium channels and generation of membrane potential by sodium potassium ATPase in murine T lymphocytes. *J Immunol* (1993) **151**:610–20.
52. Verheugen J, Vijverberg H, Oortgiesen M, Cahalan M. Voltage-gated and Ca(2+)-activated K+ channels in intact human T lymphocytes. Noninvasive measurements of membrane currents, membrane potential, and intracellular calcium. *J Gen Physiol* (1995) **105**:765–94. doi:10.1085/jgp.105.6.765
53. Launay P, Cheng H, Srivatsan S, Penner R, Fleig A, Kinet J-P. TRPM4 regulates calcium oscillations after T cell activation. *Science* (2004) **306**:1374–7. doi:10.1126/science.1098845
54. Taylor C, Dale P. Intracellular Ca2+ channels – a growing community. *Mol Cell Endocrinol* (2012) **353**:21–8. doi:10.1016/j.mce.2011.08.028
55. Mahrl M, Schuster S, Brumen M, Heinrich R. Modelling the interrelations between calcium oscillations and ER membrane potential oscillations. *Biophys Chem* (1997) **1622**:221–39.
56. Kono M, Takagi Y, Kawauchi S, Wada A, Mirikawa T, Funakoshi K. Non-activated T and B lymphocytes become morphologically distinguishable after detergent treatment. *Cytometry A* (2013) **83A**:396–402. doi:10.1002/cyto.a.22262
57. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Walter P. *The Molecular Biology of the Cell*. 3rd ed. New York: Garland (1994).
58. Gentet LJ, Stuart GJ, Clements JD. Direct measurement of specific membrane capacitance in neurons. *Biophys J* (2000) **79**:314–20. doi:10.1016/S0006-3495(00)76293-X
59. Goepel S, Zhang Q, Eliasson L, Ma X, Galvanovskis J, Kanno T, et al. Capacitance measurements of exocytosis in mouse pancreatic alpha-, beta- and delta-cells within intact islets of Langerhans. *J Physiol* (2004) **556**:711–26. doi:10.1113/jphysiol.2003.059675
60. Grissmer S, Nguyen A, Cahalan M. Calcium-activated potassium channels in resting and activated human T lymphocytes. *J Gen Physiol* (1993) **102**:601–30. doi:10.1085/jgp.102.4.601
61. Zweifach A, Lewis R. Mitogen-regulated Ca2+ current of T lymphocytes is activated by depletion of intracellular Ca2+ stores. *Proc Natl Acad Sci U S A* (1993) **90**:6295–9. doi:10.1073/pnas.90.13.6295
62. Zweifach A, Lewis R. Slow calcium-dependent inactivation of depletion-activated calcium current. *J Biol Chem* (1995) **270**:14445–51. doi:10.1074/jbc.270.24.14445
63. Bezprozvanny Y, Watras J, Ehrlich BE. Bell-shaped calcium-response curves of Ins(1,4,5)P3- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* (1991) **351**:751–4. doi:10.1038/351751a0
64. Taylor CW, Tovey SC. IP(3) receptors: toward understanding their activation. *Cold Spring Harb Perspect Biol* (2010) **2**:a004010. doi:10.1101/cshperspect.a004010
65. De Young GW, Keizer J. A single-pool ionositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillations in Ca2+ concentration. *Proc Natl Acad Sci U S A* (1992) **89**:9895–9. doi:10.1073/pnas.89.20.9895
66. Guse AH, Roth E, Emmrich F. D-myo-inositol 1,3,4,5-tetrakisphosphate releases Ca2+ from crude microsomes and enriched vesicular plasma membranes, but not from intracellular stores of permeabilized T-lymphocytes and monocytes. *Biochem J* (1992) **288**:489–95.
67. Sneyd J, Falcke M. Models of the inositol trisphosphate receptor. *Prog Biophys Mol Biol* (2005) **89**:207–45. doi:10.1016/j.pbiomolbio.2004.11.001
68. Mak D-OD, Foskett JK. Single-channel kinetics, inactivation, and spatial distribution of inositol trisphosphate (IP3) receptors in xenopus oocyte nucleus. *J Gen Physiol* (1997) **109**:571–87. doi:10.1085/jgp.109.5.571
69. Marchant JS, Taylor CW. Rapid activation and partial inactivation of inositol trisphophate receptors by inositol trisphophate. *Biochemistry* (1998) **37**:11524–33. doi:10.1021/bi980808k
70. Meyer T, Wensel T, Stryer L. Kinetics of calcium channel opening by inositol 1,4,5-trisphosphate. *Biochemistry* (1990) **29**:32–7. doi:10.1021/bi00453a004
71. Missiaen L, Parys JB, Weidema AF, Sipma H, Vanlingen S, De Smet P, et al. The bell-shaped Ca2+ dependence of the inositol 1,4,5-trisphosphate-induced Ca2+ release is modulated by Ca2+/calmodulin. *J Biol Chem* (1999) **274**:13748–51. doi:10.1074/jbc.274.20.13748
72. Caride AJ, Penheiter AR, Filoteo AG, Bajzer Z, Enyedi A, Penniston JT. The plasma membrane calcium pump displays memory of past calcium spikes. Differences between isoforms 2b and 4b. *J Biol Chem* (2001) **276**:39797–804.
73. Juhaszova M, Church P, Blaustein MP, Stanley EF. Location of calcium transporters at presynaptic terminals. *Eur J Neurosci* (2000) **12**:839–46. doi:10.1046/j.1460-9568.2000.00974.x
74. Sherman A, Rinzel J, Keizer J. Emergence of organized bursting in clusters of pancreatic beta-cells by channel sharing. *Biophys J* (1988) **54**:411–25. doi:10.1016/S0006-3495(88)82975-8
75. Elwess NL, Filoteo AG, Enyedi A, Penniston JT. Plasma membrane Ca2+ pump isoforms 2a and 2b are unusually responsive to calmodulin and Ca2+. *J Biol Chem* (1997) **272**:17981–6. doi:10.1074/jbc.272.29.17981
76. Caride AJ, Filoteo AG, Penheiter AR, Paszty K, Enyedi A, Penniston JT. Delayed activation of the plasma membrand calcium pump by a sudden increase in Ca2+: fast pumps reside in fast cells. *Cell Calcium* (2001) **30**:49–57. doi:10.1054/ceca.2001.0212
77. Cox JA, Comte M, Stein EA. Activation of human erythrocyte Ca2+-dependent Mg2+-activated ATPase by calmodulin and calcium: quantitative analysis. *Proc Natl Acad Sci U S A* (1982) **79**:4265–9. doi:10.1073/pnas.79.14.4265
78. Graupner M, Erler F, Meyer-Hermann M. A theory of plasma membrane calcium pump stimulation and activity. *J Biol Phys* (2005) **31**:183–206. doi:10.1007/s10867-005-4472-2
79. Forge V, Mintz E, Guillain F. Ca2+ binding to sarcoplasmic reticulum ATPase revisited. II. equilibrium and kinetic evidence for a two-route mechanism. *J Biol Chem* (1993) **268**:10961–8.
80. Wolosker H, Engelender S, Meis LD. Reaction mechanism of the sarcoplasmic reticulum Ca2+-ATPase. *Adv Mol Cell Biol* (1998) **23A**:1–31.
81. Chandrasekera P, Kargacin M, Deans J, Lytton J. Determination of apparent calcium affinity for endogenously expressed human sarco(endo)plasmic reticulum calcium-ATPase isoform SERCA3. *Am J Physiol Cell Physiol* (2009) **296**:C1105–14. doi:10.1152/ajpcell.00650.2008
82. Lytton J, Westlin M, Burk SE, Shull GE, MacLennan DH. Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J Biol Chem* (1992) **267**:14483–9.
83. Launay S, Bobe R, Lacabaratz-Porret C, Bredoux R, Kovàcs T, Enouf J, et al. Modulation of endoplasmic reticulum calcium pump expression during T lymphocyte activation. *J Biol Chem* (1997) **272**:10746–50. doi:10.1074/jbc.272.16.10746
84. Storn R, Price K. Differential evolution – a simple and efficient heuristic for global optimization over continuous spaces. *J Glob Opt* (1997) **11**:341–59. doi:10.1023/A:1008202821328
85. Fridlyand LE, Tamarina N, Philipson LH. Modeling of Ca2+ flux in pancreatic beta-cells: role of the plasma membrane and intracellular stores. *Am J Phyisol Endocrinol Metab* (2003) **285**:E138–54.
86. Quintana A, Schwarz E, Schwindling C, Lipp P, Kaestner L, Hoth M. Sustained activity of calcium release-activated calcium channels requires translocation of mitochondria to the plasma membrane. *J Biol Chem* (2006) **281**:40302–9. doi:10.1074/jbc.M607896200

87. Berg I, Potter B, Mayr G, Guse A. Nicotinic acid adenine dinucleotide phosphate (NAADP+) is an essential regulator of T-lymphocyte Ca2+-signaling. *J Cell Biol* (2000) **150**:581–8. doi:10.1083/jcb.150.3.581

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