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Calcium signaling in immune cells

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Abstract

Calcium acts as a second messenger in many cell types, including lymphocytes. Resting lymphocytes maintain a low concentration of Ca^{2+} . However, engagement of antigen receptors induces calcium influx from the extracellular space by several routes. A chief mechanism of Ca^{2+} entry in lymphocytes is through store-operated calcium (SOC) channels. The identification of two important molecular components of SOC channels, CRACM1 (the pore-forming subunit) and STIM1 (the sensor of stored calcium), has allowed genetic and molecular manipulation of the SOC entry pathway. In this review, we highlight advances in the understanding of Ca^{2+} signaling in lymphocytes with special emphasis on SOC entry. We also discuss outstanding questions and probable future directions of the field.

In lymphocytes, crosslinking of antigen receptors typically activates phosphoinositide-specific phospholipase C. Phospholipase C breaks down phosphatidylinositol-4,5-bisphosphate to generate inositol-1,4,5-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$) and diacylglycerol. $\text{Ins}(1,4,5)\text{P}_3$ binds its receptor located on the surface of internal Ca^{2+} stores, mainly the endoplasmic reticulum, and activates the release of Ca^{2+} into the cytoplasm. This event, known as ‘store depletion’, in turn activates store-operated calcium (SOC) channels in the plasma membrane to recruit Ca^{2+} . Lymphocytes are believed to use SOC entry (SOCE) as the main mode of Ca^{2+} influx. The best characterized SOC channels in lymphocytes are known as ‘calcium release-activated calcium’ (CRAC) channels¹. CRAC channels are highly Ca^{2+} -selective, low-conductance channels with a characteristic inwardly rectifying current-voltage relationship. The past few years have brought substantial progress in understanding the molecular composition of the CRAC signaling complex. High-throughput screens based on RNA-mediated interference have identified STIM1 (stromal interaction molecule 1) as the endoplasmic reticulum-resident Ca^{2+} sensor and CRACM1 (calcium release-activated calcium modulator 1; also called Orai1) as the pore-forming subunit of CRAC channels^{2–6}. STIM1 has one homolog, STIM2, whereas CRACM1 has two homologous proteins, CRACM2 and CRACM3, in mice and humans.

The canonical transient receptor potential (TRPC) channels have also been reported to increase intracellular Ca^{2+} concentrations either directly through coupled plasma membrane receptor stimulation or, arguably, through ‘store depletion’, in different cell types^{7–9}. Of the seven mammalian TRPC channels (TRPC1–TRPC7), TRPC1 has most often been reported to form diverse channels, ranging from relatively Ca^{2+} selective to nonselective, in a variety of cell types by selective homomeric or heteromeric interactions with TRPC3, TRPC4 and TRPC7 (refs. ^{10,11}). In their non-store-operated mode, TRPC3, TRPC6 and TRPC7 can also be activated by diacylglycerol^{8,12,13}. Direct involvement of TRPCs in SOCE remains

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controversial with no conclusive reports of store-operated TRPC Ca^{2+} currents in lymphocytes. The evidence and controversies surrounding the function of TRPC channels in SOCE have been discussed^{1,11}. There are many other non-store-operated routes of lymphocyte Ca^{2+} entry and modulators of lymphocyte cytosolic Ca^{2+} concentration (summary, Table 1 and Fig. 1).

SOC influx during antigen receptor signaling

Antigen recognition in lymphocytes typically results in the tyrosine phosphorylation of immunoreceptor tyrosine-based activation motifs and the recruitment and activation of protein tyrosine kinases. These initial signaling events, which are more or less similar in T and B lymphocytes and mast cells, trigger the phosphorylation and activation of phospholipase C, which generates $\text{Ins}(1,4,5)\text{P}_3$ and diacylglycerol. $\text{Ins}(1,4,5)\text{P}_3$ -induced release of Ca^{2+} from the endoplasmic reticulum results in a transient increase in the cytosolic free Ca^{2+} concentration, and diacylglycerol activates protein kinase C. Protein kinase C and Ca^{2+} together initiate certain rapid cellular responses, such as rearrangement of the actin cytoskeleton to promote T cell motility, adhesion and formation of the immunological synapse, although maintenance of the immunological synapse probably requires a prolonged increase in the cytosolic Ca^{2+} concentration as a result of SOCE¹⁴. Diacylglycerol can accomplish several other functions, including directly activating non-store-operated Ca^{2+} influx through certain TRPC channels and stimulating the GTPase Ras–mitogen-activated protein kinase signaling cascade, which subsequently activates the AP-1 transcriptional complex^{8,12}. In lymphocytes, AP-1 acts together with the transcription factors NFAT and NF- κB to modulate gene expression¹⁵. Ca^{2+} influx mediated by CRAC channels may also directly activate the Ras–mitogen-activated protein kinase cascade and thus also promote AP-1 activation¹⁶. Other transcriptional regulators that depend on Ca^{2+} signaling for lymphocyte activation include NFAT, NF- κB , the kinase Jnk and calmodulin-dependent kinase, although the duration and amplitude of cytosolic Ca^{2+} flux required for activation of each transcription factor varies^{17, 18}. For example, a prolonged increase in cytosolic Ca^{2+} concentration via the CRAC channels is crucial for the activation of calcineurin, a serine-threonine phosphatase needed for the dephosphorylation and nuclear localization of NFAT. Even after its translocation into the nucleus, NFAT requires a sustained increase in Ca^{2+} concentration to prevent its ejection¹⁹. Several other cytosolic and nuclear factors modulate the intracellular localization of NFAT. These include the calcineurin inhibitors AKAP, CABIN, CHP and DSCR1, the newly identified Homer family proteins and the NFAT kinases GSK3, CK1 and DYRK^{19,20}. Thus, the SOCE-calcineurin-NFAT pathway acts together with several other signaling cascades to promote lymphocyte antigen receptor-induced gene transcription.

STIM proteins

STIM1 is a 77-kilodalton single-spanning transmembrane protein that resides mainly in the endoplasmic reticulum and to some extent also in the plasma membrane. STIM1 has been proposed to sense the depletion of Ca^{2+} stores through its amino-terminal Ca^{2+} -binding EF hand domain. Store depletion triggers the formation of oligomers of STIM1 in the endoplasmic reticulum through the EF-SAM region and subsequent translocation to discrete ‘puncta’ at endoplasmic reticulum–plasma membrane junctions^{21,22}. Structural insights into the mechanism of the formation of oligomers have shown that in resting, Ca^{2+} -replete conditions, the EF-SAM domain exists as a well-folded monomer. Ca^{2+} depletion induces partial unfolding and exposure of hydrophobic residues in the EF-SAM region, which results in its formation of oligomers²³. Consistent with those findings, forced formation of heterodimers of STIM1 results in Ca^{2+} -independent punctae formation and activation of CRAC^{2,24,25}. The coiled-coil domains of STIM1, which mediate constitutive homotypic interactions, may further stabilize the EF-SAM-triggered oligomers and promote the translocation of STIM1 to endoplasmic reticulum–plasma membrane junctions²⁶. The exact mechanism by which STIM1 oligomers

activate CRAC channels once located in the endoplasmic reticulum–plasma membrane junctional region still remains to be determined.

STIM1 has a closely related homolog, STIM2. The function of STIM2 has remained somewhat controversial, with some early reports assigning it a positive function in SOCE and others suggesting a negative regulatory function^{2,27}. A subsequent report has confirmed that STIM1 and STIM2 are activators of SOCE in HeLa cervical cancer cells, human embryonic kidney 293 cells and human umbilical vein endothelial cells²⁸. More notably, knockdown of STIM1 or STIM2 expression mediated by small interfering RNA has shown that STIM2, but not STIM1, regulates mainly basal cytosolic and endoplasmic reticulum Ca^{2+} concentrations in these cells²⁸. The authors suggest that to maintain basal Ca^{2+} concentrations, STIM2 has a Ca^{2+} -binding EF hand domain with a lower affinity for Ca^{2+} than that of STIM1. As a result, even a small decrease in the endoplasmic reticulum Ca^{2+} concentration results in the translocation of STIM2 to endoplasmic reticulum–plasma membrane junctions to activate CRAC channels. In contrast to that report²⁸, no obvious changes in the basal cytosolic Ca^{2+} or endoplasmic reticulum Ca^{2+} pools have been detected in *ex vivo* naive or *in vitro*–differentiated T cells isolated from *Stim2*^{−/−} mice, whereas naive *Stim1*^{−/−} T cells seem to have less release of endoplasmic reticulum Ca^{2+} induced by antibody to CD3, which indicates that *Stim1*^{−/−} cells have depleted resting endoplasmic reticulum Ca^{2+} pools²⁹.

Similarly, mast cells derived from *Stim1*^{−/−} mice have much less antigen- or thapsigargin-stimulated release of endoplasmic reticulum Ca^{2+} than do *Stim1*^{+/+} mast cells¹⁶. Although the size of resting endoplasmic reticulum Ca^{2+} pools in *Stim1*^{−/−} and *Stim1*^{+/+} mast cells was not specifically assessed in that study¹⁶, as mentioned above, it seems likely that STIM1 is crucial for the replenishment of endoplasmic reticulum Ca^{2+} during the resting state in mast cells and T cells. Thus, although it is clear that both STIM1 and STIM2 contribute to the maintenance of intracellular Ca^{2+} concentrations, their exact physiological functions seem to be complex and may differ depending on the cell type and mechanism of activation.

CRACM proteins

CRACM1 is a small protein of 32.7 kilodaltons with four transmembrane domains and amino and carboxyl ends that face the cytosol^{4–6}. One of the first experiments to test whether CRACM1 could form the CRAC channel involved expressing CRACM1 together with STIM1 in human embryonic kidney and Jurkat cell lines. This resulted in an enormous amplification of 50- to 100-fold in CRAC currents^{6,30–32}. Further biochemical analysis, including coimmunoprecipitation and site-directed mutagenesis of negatively charged transmembrane glutamate residues, has shown that CRACM1 creates the pore of the CRAC channel by forming homo-oligomers^{33–35}. In experiments with heterologous expression systems, CRACM1 can also form heteropolymers with CRACM2 (Orai2) and CRACM3 (Orai3) and possibly some TRPC channel subunits^{36,37}. A functional CRAC channel pore requires the tetrameric assembly of CRACM subunits. This has been shown by coexpression of preassembled tandem CRACM1 multimers composed of varying numbers of subunits along with a dominant negative CRACM1 mutant^{38,39}.

Communication between STIM1 and CRACM1

A strong functional interaction between STIM1 and CRACM1 was demonstrated by many laboratories^{30–32}. Such studies suggest that STIM1 and CRACM1 are necessary and sufficient to generate CRAC-like currents *in vitro*. Additional interacting molecules are either not required or are not limiting for the *in vitro* reconstitution of CRAC currents. After store depletion, STIM1 forms oligomers, moves to the endoplasmic reticulum–plasma membrane junctions and localizes within 10–25 nm of the plasma membrane⁴⁰. Furthermore, the STIM1 clusters in the endoplasmic reticulum–plasma membrane junctions are present near the regions

of Ca^{2+} influx from the plasma membrane. Although much insight has been gained from heterologous expression studies of CRACM1 and STIM1, many basic questions remain unanswered. For example, does STIM1 associate directly with CRACM1 to activate Ca^{2+} influx or is the interaction mediated by accessory proteins? Is plasma membrane STIM1 involved in SOCE? Using coimmunoprecipitation and fluorescence resonance energy transfer, various groups have shown that drosophila as well as mammalian homologs of STIM1 and CRACM1 can localize together and associate with each other^{33,34,41–43}, with only a few exceptions that have failed to detect any interaction⁴⁴. In addition, store depletion can enhance the association between drosophila STIM and CRACM proteins³⁴. Using chemically inducible bridges of varying lengths between endoplasmic reticulum and plasma membrane, one study has shown that the estimated protrusion of CRACM1 into the cytosol is between 11 nm and 14 nm, whereas the STIM1 cytosolic tail measures only 6 nm. Although those data do not rule out the possibility of direct interaction between CRACM1 and STIM1, it is possible that unlike STIM1, CRACM1 is part of a larger molecular complex and that accessory proteins serve the function of connecting the two molecules⁴⁵. One candidate for this could be Ca^{2+} -independent phospholipase A_2 , a component of the Ca^{2+} -influx factor pathway⁴⁶. In fact, a drosophila ortholog of Ca^{2+} -independent phospholipase A_2 , CG6718, has shown up as a fairly strong ‘hit’ in a genome-wide RNA-mediated interference screen for genes involved in regulating SOCE⁴. However, the hypothesis noted above can be reconciled with earlier reports^{6,30–32} showing amplification of about 100-fold in CRAC currents induced by coexpression of only CRACM1 and STIM1 when other intracellular components contributing to SOCE are present in nonlimiting quantities.

In attempts to elucidate the molecular mechanism of activation of CRAC channels, extensive delineation of the domains of STIM1 has identified regions involved in targeting STIM1 to the endoplasmic reticulum–plasma membrane junction versus those involved in activation or CRACM1 binding^{22,26,41,47}. Some data indicate that the STIM1 carboxy-terminal cytosolic tail containing a conserved ezrin-radixin-moesin domain and a lysine-rich region is sufficient to constitutively activate CRAC currents in human embryonic kidney cells⁴¹. However, another study found the polycationic region to be dispensable for CRAC activation⁴⁷. That study also reported that the coiled-coil domain is essential for STIM1 aggregation and the serine-proline domain is essential for targeting to the endoplasmic reticulum–plasma membrane junction⁴⁷. Furthermore, others have proposed that the carboxy-terminal domain of STIM1 dynamically binds a putative coiled-coil domain in the carboxy-terminal tail of CRACM1 in a Ca^{2+} store-dependent way⁴³. One group used chemical crosslinking and single-molecule imaging of green fluorescent protein–tagged CRACM1 to show that CRACM1 exists as a dimer in resting conditions. Furthermore, expression of green fluorescent protein–tagged CRACM1 together with the carboxyl terminus of STIM1 induces the ‘dimerization’ of CRACM1 dimers, resulting in active tetrameric CRAC channels in a store-independent way⁴⁸. Further studies in this direction will delineate the precise molecular domains and steps involved in CRAC channel gating.

Functions of CRAC in mast cells

Mast cells are the key effectors that work at the interface of innate and adaptive immune responses⁴⁹. Although mast cells can be activated in many different ways and thus serve many different functions, crosslinking of the high-affinity immunoglobulin E receptor FcεRI is the main mechanism of mast cell activation. Crosslinking of FcεRI initiates two parallel pathways activated by the Src-family protein tyrosine kinases Lyn and Fyn. Until recently it was generally accepted that in mast cells the Lyn–Syk–membrane adaptor Lat–phospholipase C axis is the main contributor to Ca^{2+} mobilization, whereas the Fyn–adaptor Gab2–phosphatidylinositol-3-OH kinase–protein kinase C axis controls degranulation. Notably, Syk-deficient mast cells show defects in Ca^{2+} mobilization as well as degranulation, which indicates

that the two pathways are not entirely independent of each other⁵⁰. One distinct point of convergence between the two pathways seems to be at the level of protein kinase C activation and the increase in the cytosolic Ca^{2+} concentration, both of which probably act in concert to activate degranulation. The importance of the increase in cytosolic Ca^{2+} concentrations to mast cell degranulation is well documented. However, a specific requirement for CRAC channel-mediated Ca^{2+} influx has been evaluated only recently with mast cells derived from CRACM1- and STIM1-knockout mice^{16,51}.

Mast cells lacking either STIM1 or CRACM1 show a considerable defect in degranulation. FcεRI-induced *in vivo* anaphylaxis is also strongly inhibited in CRACM1-knockout mice and is much lower in *Stim1*^{+/-} mice^{16,51}. As the release of Ca^{2+} from the endoplasmic reticulum is similar in wild-type and CRACM1-knockout mast cells, CRAC-mediated Ca^{2+} influx is crucial for mast cell degranulation. Further support for the idea that CRAC channels are involved in mast cell degranulation and anaphylaxis comes from *Trpm4*^{-/-} mice. TRPM4 (transient receptor potential, melastatin, member 4) is a Ca^{2+} -activated nonselective cation channel that decreases the driving force for Ca^{2+} entry through CRAC channels by modulating the membrane potential^{52,53}. *Trpm4*^{-/-} bone marrow-derived mast cells show more SOCE than their *Trpm4*^{+/+} counterparts do in response to FcεRI stimulation. Furthermore, bone marrow-derived mast cells from *Trpm4*^{-/-} mice show augmented degranulation and a more severe immunoglobulin E-mediated acute passive cutaneous anaphylactic response⁵⁴. Degranulation involves the translocation, docking and fusion of granules with the plasma membrane. Therefore, it is possible that CRAC channel-mediated sub-plasmalemmal increases in the cytosolic Ca^{2+} concentration activate or amplify certain 'downstream' signaling pathways involved in granule secretion⁵⁵ or simply promote granule exocytosis by binding to Ca^{2+} -sensing proteins, such as synaptotagmins, which are part of the SNARE complex involved in membrane fusion⁵⁶. Unexpectedly, leukotriene secretion is also strongly inhibited in CRACM1-knockout mice, which suggests the involvement of CRAC channels in the secretion of proinflammatory lipid mediators⁵¹. Increases in cytosolic Ca^{2+} concentrations activate NFAT and NF-κB, which regulate the transcription of genes encoding cytokines. It is therefore not unexpected that overall cytokine secretion is lower in CRACM1-knockout and *Stim1*^{-/-} mast cells and augmented in *Trpm4*^{-/-} mast cells. However, careful analysis shows that individual cytokines are affected differently. For example, secretion of tumor necrosis factor is much lower in *Stim1*^{-/-} and CRACM1-knockout mast cells and higher in *Trpm4*^{-/-} mast cells. However, secretion of interleukin 6 is much lower, less affected or unaffected in *Stim1*^{-/-}, CRACM1-knockout or *Trpm4*^{-/-} mast cells, respectively^{16,51,54}. Secretion of interleukin 6 in mast cells depends on nuclear translocation of NF-κB⁵⁷, which in turn depends on a large transient increase in the cytosolic Ca^{2+} concentration. NFAT translocation, however, requires a more sustained increase in Ca^{2+} . It is likely that the smaller basal endoplasmic reticulum Ca^{2+} pools in *Stim1*^{-/-} mast cells fail to provide the required large transient increase in cytosolic Ca^{2+} concentrations and thus result in less secretion of interleukin 6 (ref. 16). In agreement with that hypothesis, CRACM1-deficient and *Trpm4*^{-/-} mast cells show no substantial change in resting endoplasmic reticulum Ca^{2+} pools^{16,51,54}. Therefore, it is possible that other CRACM homologs or store-operated channels are involved in maintaining the basal cytosolic and resting endoplasmic reticulum Ca^{2+} concentrations in mast cells. Such a scenario would also be consistent with the general idea that STIM1 acts as a global store sensor and activator of all store-operated channels.

Notably, CRACM1-knockout and *Stim1*^{-/-} mast cells show no defects in proliferation or differentiation *in vitro*, and tissue mast cell numbers in CRACM1-knockout and *Stim1*^{-/-} mice are similar to those in wild-type mice. These data suggest that a non-store-operated mode of Ca^{2+} entry may be crucial for the differentiation and proliferation of mast cells, but SOCE is not.

Calcium signaling in thymocytes

Calcium signaling has long been proposed to be crucial for thymocyte maturation. Early studies using cyclosporine A (a calcineurin inhibitor) showed impaired development of CD4⁺CD8⁺ double-positive thymocytes into CD4⁺CD8⁻ or CD4⁻CD8⁺ single-positive thymocytes, as well as defects in thymocyte negative selection^{58,59}. In addition, targeted disruption of the catalytic subunit (calcineurin A β)⁶⁰ as well as the regulatory subunit (calcineurin B1)⁶¹ of calcineurin results in a distinct defect in positive selection, with fewer single-positive cells, although negative selection seems normal⁶¹. Similarly, disruption of NFATc3, which is 'preferentially' expressed in double-positive thymocytes, results in fewer single-positive thymocytes and is associated with defects in expression of the antiapoptosis protein Bcl-2 by double-positive cells⁶². Notably, CRACM1-deficient and *Stim1*^{-/-} *Stim2*^{-/-} mice show no defect in the thymic development of conventional T cells^{29,51,63}. These observations are unexpected, given the reports described above linking calcium-calcineurin-NFAT signaling to T cell development.

As CRACM1 has two closely related homologs (CRACM2 and CRACM3), one obvious issue that arises from the studies discussed above is whether CRACM2 and/or CRACM3 form functional channels to recruit Ca²⁺ in thymocytes. Both CRACM2 and CRACM3 have been shown to be able to conduct CRAC currents in *in vitro* overexpression systems^{36,64}. Indeed, CRACM2 mRNA is much more abundant than CRACM1 mRNA in wild-type mouse thymocytes⁵¹. Moreover, the lymphoid regions of thymus and spleen in a 'gene-trap' CRACM1-deficient mouse are devoid of CRACM1 protein expression, as indicated by β -galactosidase reporter activity⁵¹, whereas another report has detected faint expression of CRACM1 protein in wild-type thymus and lymph nodes⁶³. Thus, CRACM2 may be the dominant homolog in mouse thymocytes, different CRACM homologs may be expressed and function during different stages of T cell development and maturation, and/or thymocytes may use a non-store-operated method of sustaining Ca²⁺ signaling during positive selection. Further studies with CRACM2- and CRACM3-deficient mouse models should be able to test these various possibilities.

Notably, deletion of TRPM7 results in a block in thymocyte development at the CD4⁻CD8⁻ double-negative stage⁶⁵. TRPM7 is a non-store-operated cation channel that conducts Ca²⁺ and Mg²⁺ ions and has an intrinsic kinase activity. Although the exact mechanism of this defect remains to be demonstrated, the Mg²⁺ homeostasis of TRPM7-deficient thymocytes seems to be normal⁶⁵.

CRAC channels in naive and differentiated T cells

Since the discovery of the CRAC current, crucial functions for SOCE in all aspects of lymphocyte activation, including proliferation and cytokine secretion, have been proposed^{66,67}. Moreover, analysis of peripheral blood lymphocytes⁶⁸ and T cell lines⁶⁹ derived from patients with severe combined immunodeficiency (SCID) who have a loss-of-function mutation in the gene encoding CRACM1 has identified defective T cell proliferation and cytokine secretion. Two independent analyses of CRACM1-deficient mice have provided several new insights into the function of CRAC channels in T cell activation. One group has used gene-trap technology to 'knock down' CRACM1 expression⁵¹, whereas the other group has generated CRACM1-deficient mice by targeted gene ablation⁶³. Unexpectedly, naive T cell populations analyzed *ex vivo* show either no defect⁵¹ or an insubstantial decrease⁶³ in SOC influx in the presence of physiological concentrations of extracellular Ca²⁺. Furthermore, proliferation is unaffected, although cytokine secretion by naive T lymphocytes is impaired in CRACM1-deficient mice^{51,63}. In addition, thymocyte development is normal in CRACM1-deficient mice^{29,51,63}. Therefore, the phenotype of thymocytes and naive T cells from

CRACM1-deficient mice contrasts considerably with the phenotype of T cells from patients with SCID who have mutations in the gene encoding CRACM1 (refs. ^{68,69}). These differences may be due to species-specific dependency on CRACM1 and/or CRACM2 and CRACM3. The other important difference is the activation status of the cells when naive T cells from CRACM1-deficient mice are compared with immortal T cell lines developed *in vitro* from human patients with SCID. Indeed, analysis of *in vitro*-differentiated helper T cell populations has reconciled some (but not all) of these phenotypic differences^{5,63,68,69}. Differentiated helper T cells from CRACM1-deficient mice have much less SOC influx as well as cytokine release, although T cell proliferation remains unaffected, in contrast to the earlier reported proliferation defects in T cells from patients with SCID^{63,68,69}.

It is unlikely that both of the strains of CRACM1-deficient mice described above are hypomorphic; thus, the other logical possibilities are discussed here. The higher expression of CRACM2 than of CRACM1 and CRACM3 mRNA in mouse thymocytes and naive T cells suggests that these populations may use CRACM2 as the dominant CRACM homolog to form the Ca²⁺-recruiting SOC channel⁵¹. In agreement with that hypothesis, relatively less CRACM2 mRNA is detected in the *in vitro*-differentiated helper T cell subsets⁶³. Another possibility is that, as reported for TRPC channels, CRACM homologs may form distinct heteromultimeric CRAC channels *in vivo* with variable cell- or tissue-specific composition. Thus, the expression of CRACM homologs and/or the composition of CRAC channels in T cells may vary with the differentiation status of the cell. Although CRACM2 has been reported to result in store-operated currents when overexpressed with STIM1 (refs. ^{36,64}), it is important to note that the biophysical and pharmacological properties of the resulting currents are distinct from those of native CRAC channels. CRACM homologs also presumably have different activation thresholds and Ca²⁺-dependent inactivation properties^{36,64}. As these differences may not be apparent in assays using fluorescence-based Ca²⁺ indicators (such as Fura-2), direct recording from developing and naive T cells, although challenging, is crucial and may provide insights into the exact route of Ca²⁺ influx in these T cell populations.

Another independent observation that supports the hypothesis outlined above is that STIM2 expression, although negligible in naive T cells, is higher in helper T cells differentiated *in vitro*²⁹. Moreover, the hypothesis that STIM2 translocates to endoplasmic reticulum-plasma membrane junctions to activate CRAC channels after sensing even a slight depletion in endoplasmic reticulum Ca²⁺ stores²⁸ fits well with the finding that antigen-experienced, differentiated or memory T cell populations require a relatively suboptimal stimulus to trigger cytokine release. We have provided a summary of the proposed expression pattern for STIM and CRACM homologs on the basis of published and unpublished data (Fig. 2). Notably, *Stim1*^{-/-} *Stim2*^{-/-} mice show no obvious defect in the thymic differentiation of conventional T cells and only a partial defect in the proliferation of naive T cells²⁹. Given that STIM proteins are considered global activators of SOCE, these data also suggest that a non-store-operated method of Ca²⁺ influx may contribute to the regulation of Ca²⁺ signaling in naive T cells. However, *Stim1*^{-/-} *Stim2*^{-/-} mice do have a defect in regulatory T cell numbers and develop lymphoproliferative disorders²⁹. Such observations are consistent with the lymphocyte hyperproliferation reported in mice lacking both NFATc1 and NFATc2 (ref. ⁷⁰).

Non-store-operated Ca²⁺ signaling in T cells

T lymphocytes also express voltage-gated Ca²⁺ channels (Cav channels), but whether these channels are functional in lymphocytes remains debatable^{71,72}. Cav channels are expressed and functional mainly in excitable cells such as neurons and muscle cells, where they are activated in response to membrane depolarization. CD4⁺ T cells lacking the Cav regulatory β 4 or β 3 subunits show impaired Ca²⁺ responses in response to stimulation of the T cell antigen receptor⁷². These Ca²⁺ responses seemed to be independent of the release of Ca²⁺ from

endoplasmic reticulum stores, as no defect is found in response to thapsigargin, which mediates passive release of Ca^{2+} from the endoplasmic reticulum and activates CRAC channels. Nuclear translocation of both NFAT subtypes, NFATc2 and NFATc1, is inhibited in both β -subunit-deficient T cell strains, which shows that the entire calcium-calcineurin-NFAT pathway is affected. Although the proliferation of β 4-mutant CD4 T lymphocytes is intact, cytokine secretion from these cells is lower. These data collectively suggest that Cav channels are necessary for a normal T cell antigen receptor-mediated Ca^{2+} response in CD4⁺ T cells.

However, membrane depolarization does not result in the activation of Cav channels in T lymphocytes, which suggests the existence of an alternative gating mechanism for these channels⁷². An independent study has shown that a carboxy-terminal fragment of Cav1.2 (also called the 'L-type voltage-gated calcium channel') can translocate to the nucleus and regulate transcription⁷³. This calcium channel-associated transcription regulator can bind to a nuclear protein and an endogenous promoter and to regulate the expression of a wide variety of genes essential for signaling and excitability in neurons. The nuclear localization of the calcium channel-associated transcription regulator is regulated by changes in intracellular Ca^{2+} concentrations, among other parameters. In summary, the operation and functional importance of store-independent modes of Ca^{2+} entry in lymphocytes remains to be determined.

Conclusions

Molecular and genetic approaches have demonstrated that components of the SOCE signaling pathway are crucial for the function of lymphocytes. However, several alternative routes of Ca^{2+} influx exist and may be implemented at various stages of the development and maturation of lymphocytes. Future studies of how individual lymphocyte types use different sources of Ca^{2+} influx during their lifespan should provide useful insights.

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Figure 1.

Routes of Ca^{2+} influx and efflux. Routes with similar mechanisms of activation are grouped together here. The probability of activation of a particular mechanism and its eventual contribution toward an increase in cytosolic Ca^{2+} may vary, and all routes may not be active at a given time. Red dots, Ca^{2+} ; blue dots, Na^+ ; green dots, K^+ ; ?, controversial route. ROCE, receptor-operated Ca^{2+} entry; K_v , voltage-gated K^+ channel; K_{Ca} , Ca^{2+} -activated K^+ channel; PMCA, plasma membrane Ca^{2+} ATPase; Ins(1,4,5) P_3 R, Ins(1,4,5) P_3 receptor; TRPV6, transient receptor potential, vanilloid, member 6; ARC, arachidonate-regulated, Ca^{2+} -selective; P2 receptors, purinergic receptors; RyR, ryanodine receptor; SERCA, sarco-endoplasmic reticulum Ca^{2+} ATPase; ER, endoplasmic reticulum.

Figure 2.

Hypothetical model of the various modes of Ca^{2+} influx in developing, mature and activated T lymphocytes. Top (yellow shaded region), unknown chief source of Ca^{2+} influx; bottom (blue shaded region), SOCE is the main mode of Ca^{2+} influx; +, ++ and +++, reported changes (increases) in the expression of CRACM and STIM mRNA; ?, untested and unknown; -, undetectable; orange ovals, store-independent channels; black ovals, store-dependent channels. TCR, T cell antigen receptor; DP, double-positive.

Table 1 Routes of cytosolic Ca^{2+} modulation in lymphocytes

Route	Activation	Physiological outcome	Reference
Pumps			
SERCA	ATP	Pumps Ca^{2+} from cytosol to ER	74
PMCA	ATP	Prevents cytosolic Ca^{2+} accumulation and contributes to Ca^{2+} signaling dynamics	75,76
Intracellular Ca^{2+} channels			
Ins(1,4,5) P_3 R, ER	Ins(1,4,5) P_3	Transient increase in cytosolic Ca^{2+}	77
RyR	Ca^{2+} , cADPR, L-type Ca^{2+} channels	Transient increase in cytosolic Ca^{2+}	78,79
Ca^{2+}-selective store-operated channels			
CRAC	Store-operated	Sustained increase in cytosolic Ca^{2+}	4-6
Nonselective (receptor- or store-operated) channels			
TRPC	Diacylglycerol or store-operated?	Sustained increase in cytosolic Ca^{2+}	7-9
Ion exchangers			
Dual-mode sodium-calcium exchanger	Forward mode: high cytosolic Ca^{2+} concentrations	Prevents accumulation of Ca^{2+} in the cytosol	80,81
	Reverse mode: high cytosolic Na^+ concentrations	Extrudes Na^+ and contributes to increase in cytosolic Ca^{2+}	76,81
Mitochondrial Ca^{2+} buffering			
Ca^{2+} -uptake channel: mitochondrial Ca^{2+} uniporter	High cytosolic Ca^{2+} concentrations	Buffers sub-plasmalemmal Ca^{2+}	82,83
Ca^{2+} -extrusion channel: sodium-calcium exchanger	High mitochondrial Ca^{2+} concentrations	Buffers sub-plasmalemmal Ca^{2+} , facilitates ER refilling	82,83
Indirect modulators of cytosolic Ca^{2+}			
TRPM4 and TRPM5 (Na^+ channels)	Membrane depolarization-activated	Inhibits the driving force for CRAC-mediated Ca^{2+} influx	52,54
Kv1.3 (K^+ channel)	Membrane depolarization-activated	Potentiates the driving force for CRAC-mediated Ca^{2+} influx	84
KCa3.1 (K^+ channel)	Calcium-activated	Potentiates the driving force for CRAC-mediated Ca^{2+} influx	84
Non-store-operated Ca^{2+} influx			
Purinergic P2 receptors	Nucleotides	Nonselective cation influx	85
TRPV6 (CaT1)	Constitutively active; inhibited by cytosolic increase in Ca^{2+}	Selective Ca^{2+} influx	1,86
TRPM2 (LTRPC2)	Pyrimidine nucleotides, ADPR and NAD	Nonselective cation influx	87,88
TRPM7 (MIC or LTRPC7)	Intracellular Mg^{2+} concentrations	Nonselective cation influx	89,90
ARC channel	Phosphorylation	Selective Ca^{2+} influx	91
Ins(1,4,5) P_3 R, PM (controversial)	Ins(1,4,5) P_3	Unknown	92
Cav (controversial)	Unclear in lymphocytes	Contributes to increases in cytosolic Ca^{2+} and NFAT translocation	71,72

CaT1, Ca^{2+} transport protein; ADPR, adenosine 5' diphosphoribose; NAD, nicotinamide adenine dinucleotide; LTRPC, long transient receptor potential channel; MIC, Mg^{2+} inhibited cation channel; PM, plasma membrane.