What’s Bred in the Bone: Calcium Channels in Lymphocytes
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Calcium (Ca$^{2+}$) is an important second messenger in lymphocytes and is essential in regulating various intracellular pathways that control critical cell functions. Ca$^{2+}$ channels are located in the plasma membrane and intracellular membranes, facilitating Ca$^{2+}$ entry into the cytoplasm. Upon Ag receptor stimulation, Ca$^{2+}$ can enter the lymphocyte via the Ca$^{2+}$ release-activated Ca$^{2+}$ channel found in the plasma membrane. The increase of cytosolic Ca$^{2+}$ modulates signaling pathways, resulting in the translocation of target genes implicated in differentiation, activation, proliferation, survival, and apoptosis of lymphocytes. Along with Ca$^{2+}$ release-activated Ca$^{2+}$ channels, several other channels have been found in the membranes of T and B lymphocytes contributing to key cellular events. Among them are the transient receptor potential channels, the P2X receptors, voltage-dependent Ca$^{2+}$ channels, and the inositol 1,4,5-trisphosphate receptor as well as the N-methyl-D-aspartate receptors. In this article, we review the contributions of these channels to mediating Ca$^{2+}$ currents that drive specific lymphocyte functions. The Journal of Immunology, 2019, 202: 1021–1030.

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Abbreviations used in this article: Ca$^{2+}$, calcium; cADPR, cyclic ADP-ribose; Ca$^{2+}$, voltage-dependent Ca$^{2+}$; CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis; CRAC, Ca$^{2+}$ release-activated Ca$^{2+}$; EAE, experimental autoimmune encephalomyelitis; ER, endoplasmic reticulum; IP3, inositol 1,4,5-trisphosphate; KO, knockout; Na$^+$, sodium; NMDA, N-methyl-D-aspartate; RyR, ryanodine receptor; SOCE, store-operated Ca$^{2+}$ entry; STM, stratal interaction molecule; TRP, transient receptor potential.

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plethora of Ca\(^{2+}\) channel regulation comes together to control lymphocyte development and effector functions, which will be explored further in this review.

**CRAC channels (Orai and stromal interaction molecules)**

The best-described mechanism of extracellular Ca\(^{2+}\) entering lymphocytes is through the CRAC channel. The CRAC channel is comprised of two components that act together: Orai1, the pore-forming subunit, which is found in the plasma membrane, and stromal interaction molecule (STIM) 1, the regulatory subunit located in the endoplasmic reticulum (ER) membrane. TCR/BCR engagement triggers a signaling cascade that leads to the transport of Ca\(^{2+}\) from the ER into the cytoplasm via the Ca\(^{2+}\) channel inositol 1,4,5-trisphosphate (IP3) receptor upon binding of its ligand IP3. The Ca\(^{2+}\) levels in the ER are monitored by the Ca\(^{2+}\) sensing protein STIM1, the regulatory subunit of the CRAC channel. STIM1 proteins, which are usually readily spread out in the ER membrane, oligomerize in certain puncta during low levels of Ca\(^{2+}\). These areas are in close proximity to the plasma membrane so that STIM1 can interact with and activate Orai1, the pore-forming unit of the CRAC channel, and trigger a Ca\(^{2+}\) influx from the extracellular space. The process is termed store-operated Ca\(^{2+}\) entry (SOCE), and although the CRAC channel is the prime example of it, other channels, such as transient receptor potential (TRP) and Ca\(_V\) channels, are thought to also participate in SOCE and will be discussed later (9, 10).

The existence of the CRAC channel in T cells was recognized well before its constituents Orai1 and STIM1 were discovered (11). Interestingly, the importance of CRAC channels was first demonstrated in patients with SOCE and CRAC channel deficiencies, which presented with SCID (12–14). Although the T cells of these patients underwent normal development, in mature cells, TCR-induced Ca\(^{2+}\) flux was impaired along with effector functions, resulting in life-threatening recurrent infections, such as pneumonia and severe CMV infections (12–14). A decade after the initial pathogenic characterization of these patients, genomic linkage analysis of more CRAC channel–deficient patients (15–17), as well as RNA interference screens in *Drosophila* cells (18, 19), identified Orai1 and STIM1 as the main components of the CRAC channel. The CRAC channel–deficient patients harbored mutations in either the Orai1- or STIM1-encoding genes that led to an abrogation of their T cell Ca\(^{2+}\) influx and CRAC channel function. Soon after, it was confirmed in a mouse model that Orai1 and STIM1 were components of the CRAC channel and that their deficiency led to a reduced SOCE (20, 21). In mice, Orai1 deficiency led to a less drastic phenotype, as T cells exhibited residual SOCE, which was attributed to the
upregulation of the Orai1 paralog Orai2, partially compensating for Orai1 deficiency (22). Along with this compensatory role in the absence of Orai1, an interesting function for Orai2 was recently described by Vaeth et al. (23). Although Orai1/Orai2 double-deficient T cells were completely devoid of SOCE, the deletion of the paralog Orai2 alone surprisingly increased SOCE into mouse cells. This demonstrates an inhibitory role of Orai2 in Ca^{2+} flux, which is thought to fine-tune immune responses. The authors suggest that Orai2 can confer its inhibitory role by forming a heteromeric complex with Orai1 (23).

STIM2, in contrast, was found to have a similar activating role to that of its paralog STIM1. However, its absence did not cause severe defects equal to the STIM1 deficiency, and it interfered mostly with sustained Ca^{2+} influx (21). In STIM1-deficient T cells, STIM2 could also partially compensate for the absence of STIM1, and on a molecular level, STIM1 and STIM2 deficiency both impaired the downstream NFAT pathway (21).

In Orai1- or STIM1/STIM2-deficient T cells, the reduced SOCE led to decreased effector functions such as impaired cytokine production and proliferation (20, 21, 24, 25). Because of these deficits in cytokine production, particularly lower production of TNF-α and IFN-γ along with impaired degranulation of cytotoxic T cells, STIM1/2 was required for the suppression of invasive tumors. As such, STIM1/2-deficient mice succumbed to induced melanoma and adenocarcinoma (26). Similarly, STIM1/2-deficient production of IL-2 and IFN-γ was critical during early antiviral responses of CD8 T cells (27). Also, during acute secondary viral infection, STIM1/2 was required for memory responses and specifically CD4 T cell helper function, which reactivates cytotoxic CD8 T cells upon re-exposure (27). Although Orai1 and STIM1 were important for proper effector functions of T cells, they were dispensable for their maturation, as demonstrated in traditional murine TCRαβ T cells (20). Conversely, agonist-selected T cells, which include regulatory T cells (Tregs) and invariant NKT cells, exhibited a defective development in the absence of STIM1 (21, 28). It is believed that this occurred because of reduced NFAT translocation, which is necessary for the induction of FOXP3, a transcription factor required for differentiation and function of Tregs (29). Because Tregs are important for suppressing T effector function (T_{E,R}) function and potentially deleterious immune responses, STIM1 and STIM2 deficiency provoked the development of a type of lymphoproliferative disorder, a condition characterized by the uncontrolled expansion of T cells (21).

Orai1 was also indispensable for differentiation of naive CD4 T cells to T_{H17} cells because of impaired NFAT signaling, which would normally activate the T_{H17} lineage transcription factor retinoic-acid-receptor-related-orphan-receptor (ROR) (30). T_{H1} and T_{H2} cell differentiation, in contrast, was not affected by the absence of Orai1 (30). Given the proinflammatory role of T_{H17} cells, in the absence of Orai1, the suppression of T_{H17} differentiation resulted in the reduced severity of experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis. Similar to the phenotype exhibited by Orai1 deficiency, T cell–specific STIM1 and STIM2 deficiencies dampened EAE severity by impairing T_{H1} and T_{H17} proinflammatory cytokine production (31). Intriguingly, graft-versus-host disease was also attenuated following adoptive transfer of STIM1-deficient CD4 T cells into MHC-mismatched recipient mice (24).

Finally, a functional CRAC channel was also necessary for T cell homing. T cell homing involves the migration of lymphocytes from the thymus into the secondary lymphoid organs such as spleen and lymph nodes, where they encounter dendritic cells that present Ag for their activation (32). In transgenic mice expressing a dominant-negative Orai1 mutant (E106A), T cell migration into the peripheral organs was impaired (33). However, another study found no defects in homing when examining T cells expressing a similar Orai1 mutant (E106Q) (34), leaving this aspect controversial.

Along with T lymphocytes, STIM1/2 and Orai1/2 also play a role in B cells. For example, it was previously demonstrated that Orai1-deficient B cells exhibited an impaired BCR-induced Ca^{2+} flux, as well as defects in cell proliferation (20). In addition, STIM1/2 double deficiency led to a complete abrogation of Ca^{2+} flux and severe proliferative defects (35). Furthermore, upon BCR cross-linking, this impairment in Ca^{2+} flux dampened NFAT signaling, resulting in the reduced production of anti-inflammatory cytokine IL-10 (35). As IL-10 is a negative regulator of autoimmunity, its absence resulted in the heightened severity of EAE in mice (35). Intriguingly, despite these cellular and regulatory defects, the Ab responses of these B cells were normal, suggesting the mechanisms controlled by STIM1/2 do not regulate this process (35).

Analogous to T cells, B cells developed normally in the absence of STIM1 (36). However, STIM1 overexpression increased the Ca^{2+} entry into maturing B cells and was sufficient to activate a newly discovered proapoptotic ERK signaling pathway predisposing the cells to negative selection (36, 37). Despite these findings, T cells are more dependent on CRAC channels than B cells, as the mediated Ca^{2+} flux is not required for Ab production and other B cell related immune responses (38).

Although the CRAC channel is the best-studied contributor to the TCR/BCR-induced Ca^{2+} flux, several additional Ca^{2+} channels have been detected in the plasma membrane of lymphocytes (39).

**TRPs**

The TRP channels are permeable for Ca^{2+} and sodium (Na^+) and are best known for their role as pain receptors in sensory neurons (40). They contain six transmembrane domains, of which the two most C-terminal ones encompass the pore-forming domain (41). Currently, 28 mammalian TRP channel homologs have been described and can be divided into six subfamilies based on their amino acid sequence: TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPML (mucolipin), and TRPP (polycystin). Of these TRP channels, TRPC, TRPM, TRPV, and TRPA have previously been shown to play roles in lymphocyte development or function.

TRPV1, for example, is known to contribute to the TCR-induced Ca^{2+} flux as a non-SOCE channel in CD4 T cells and is gated by phosphorylation, which is dependent on the lymphocyte-specific protein tyrosine kinase (LCK). Using a TRPV1−/− mouse model, Bertin et al. showed that the reduced Ca^{2+} flux translated to impaired TCR signaling, resulting in reduced NFAT and NF-κB translocation and
subsequent defects in CD4 T cell activation and proinflammatory cytokine production (42). The role of TRPV1 in cytokine production was also confirmed using TRPV1 antagonists in murine splenic T cells (43). Because of lower proinflammatory mediator production, TRPV1 deficiency was also shown to be protective in a mouse model of T cell-mediated colitis (42).

TRP channels can also inhibit Ca\(^{2+}\) flux by modulating the activity of one another. An example of this is TRPA1, an ankyrin TRP channel that was found to inhibit the activity of TRPV1. It is thought that this inhibition is mediated by the formation of heteromeric complex between TRPA1 and TRPV1, similar to what was suggested for Orai1 and Orai2 earlier. In a mouse model of colitis, TRPA1 deficiency resulted in increased TRPV1 activation and therefore amplified TCR-induced Ca\(^{2+}\) flux and subsequent hyperactivation of inflammatory mediators, exacerbating the disease (44).

TRPC channels (specifically TRPC5) have been found to be important in mediating Treg-influenced inhibition of T\(_{\text{eff}}\) cells. Specifically, once bound to a Treg, TRPC5 becomes overexpressed in the interacting T\(_{\text{eff}}\) cell, triggering a Ca\(^{2+}\) flux and inhibiting the proliferation of that cell (45). The exact mechanism of how this Ca\(^{2+}\) flux can inhibit TCR-induced proliferation, however, remains elusive. In addition to TRPC5, other TRPC channels like TRPC3 and TRPC6 are also involved in T cell Ca\(^{2+}\) flux, and in the case of TRPC3, this Ca\(^{2+}\) flux modulated cell proliferation (46–48).

Another TRP channel important for T cell effector function is TRPM2 (49). A mouse model deficient in TRPM2 exhibited reduced T cell proliferation, proinflammatory cytokine secretion, and thus reduced EAE severity after TCR stimulation (49). In another study using Jurkat cells, it was shown that Ca\(^{2+}\) flux by the TRPM2 channel could be activated with the second messenger molecules cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) (50). Upon TCR stimulation and Ca\(^{2+}\) entry, cADPR is released from the ER (51), from which it potentially binds to TRPM2, resulting in additional and sustained Ca\(^{2+}\) release. Another member of the TRPM family, TRPM4 is a monovalent channel that can flux Na\(^{+}\) into T cells. This, however, has profound impacts on Ca\(^{2+}\) signaling because the entry of Na\(^{+}\) depolarizes the plasma membrane, thereby reducing the driving force for Ca\(^{2+}\) influx during SOCE (52). In a TRPM4 Jurkat cell mutant, PHA treatment induced a prolonged Ca\(^{2+}\) influx and led to increased IL-2 production (52). A similar effect was seen in mouse T\(_{\text{H}2}\) cells, in which small interfering RNA-mediated knockdown of TRPM4 amplified Ca\(^{2+}\) flux as well as NFAT translocation and IL-2 production (53). TRPM4 knockdown in T\(_{\text{H}1}\)-polarized cells, however, showed the opposite effect (53). It is hypothesized that the differences in the two T\(_{\text{H}}\) subsets are due to different expression levels of TRPM4 as well as distinct Ca\(^{2+}\) clearance dynamics.

In B cells, very little is known about the role of TRP channels. Although TRPC1, 3, and 7 were all shown to be involved in BCR-induced signaling, these observations have so far been limited to the DT40 chicken B cell line (54–56). Recently, however, a clinical study of patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) sheds some light into the role of TRPM3 in lymphocytes (57). Interestingly, multiple single-nucleotide polymorphisms in the TRPM3 gene from PBMCs appear to correlate with the disease, and although TRPM3 is expressed on B as well as NK cells of healthy donors, its expression is reduced on those of CFS/ME patients (57). Additionally, the Ca\(^{2+}\) influx upon BCR stimulation or thapsigargin treatment (which induces SOCE) was significantly reduced in the patients' B cells compared with healthy controls, suggesting that TRPM3 is a store-operated Ca\(^{2+}\) channel (57). Although immunological dysfunctions have been reported in CFS/ME patients, it is unknown if they are causative of the disease.

Different types of Ca\(^{2+}\) channels can influence one another, as a recent study linking a TRP and the CRAC channel suggests (58). The authors demonstrated that SOCE was reduced in the absence of TRPM7. Interestingly, TRPM7 is not a store-operated Ca\(^{2+}\) channel itself but rather activates SOCE by phosphorylation of CRAC components. One can hypothesize that this mechanism also applies to the previously mentioned TRPM3 channel.

P2X receptors

Purinergic P2X receptors include seven different channels, of which the foremost receptors, P2X7 as well as P2X1, P2X4, and P2X5, were shown to be functional in T lymphocytes. These receptors are cation channels that can flux Ca\(^{2+}\) and Na\(^{+}\) into the cell and potassium (K\(^{+}\)) out of the cell. They do so upon the binding of the energy metabolite and nucleic acid building block ATP, giving it a role as a signaling molecule (59). ATP is usually released from the mitochondria through membrane channels, such as pannexin-1, by stressed or apoptotic cells that are sheared or under osmotic pressure. Consequently, ATP release is amplified and spread in an autocrine/paracrine fashion by binding to P2X receptors on the cells from which the ATP was released, as well as neighboring cells, to induce apoptosis (60). This ATP-mediated apoptosis was found to play an important role in negative selection of thymocytes. Negative selection takes place in the thymus during T cell maturation and is a critical event as it results in the deletion of thymocytes with a self-reactive TCR. Apoptosis of cells due to negative selection causes the release of ATP and, in a paracrine fashion, induces apoptosis of neighboring thymocytes (60). Importantly, the P2X7 receptor was found to be essential to this ATP-mediated induction of apoptosis (61). Additionally, in mature T cells, P2X7 was crucial for induction of cell death not only by ATP but also NAD (62).

Despite this clear role of ATP signaling in apoptosis, a baseline mitochondrial ATP production and signaling are necessary for T cell homeostasis and for the cells' ability to recognize Ag. This basal purinergic signaling is transmitted via P2X1 (63). Upon activation, T cells upregulate their mitochondrial activity and therefore ATP secretion, which causes further autocrine activation via P2X receptors. The increased metabolism of T cells requires augmented cytosolic Ca\(^{2+}\) that is transported into the mitochondria to fuel ATP production. This Ca\(^{2+}\) is provided via the CRAC channel discussed earlier (64) and therefore demonstrates an interesting link between CRAC and P2X channels. The concentration of ATP seems to play a role in whether a T cell is resting, becomes activated, or induces apoptosis (65). P2X activation by ATP was found to be essential for sustained...
ERK signaling in murine T cells, and antagonizing P2X receptors resulted in decreased IL-2 expression and T cell proliferation (66). Furthermore, inhibiting P2X receptors also led to a reduced TCR-mediated Ca\textsuperscript{2+} flux and induced T cell anergy (66). Similarly, in Jurkat cells the use of ATP scavengers or P2X\textsuperscript{7} inhibitors caused a reduction of TCR-mediated Ca\textsuperscript{2+} flux and impaired NFAT translocation and IL-2 production (67). Furthermore, P2X\textsuperscript{7} was essential for ATP-induced shedding of CD62L, CD27, and CD23, as well as IL-6R, from T cells via the activation of metalloproteases. This shedding is usually induced upon T cell activation and converts the membrane proteins into soluble effector proteins (68–71).

Although P2X\textsuperscript{7} receptors were always uniformly distributed on the cell surface, P2X1 and P2X4 receptors have also been detected in human peripheral blood CD4 T cells but were shown, together with pannexin-1, to translocate to the immune synapse upon T cell activation (72). This might be important for the formation of Ca\textsuperscript{2+} microdomains and the increase of local Ca\textsuperscript{2+} levels for TCR signaling next to the immunological synapse. Similar to P2X\textsuperscript{7}, P2X1 and P2X4 were also important during T cell activation, as their pharmacological antagonism and genetic mutation reduced TCR-mediated Ca\textsuperscript{2+} flux as well as NFAT translocation and IL-2 synthesis (72). Given this generally inflammatory role of P2X receptors, it was also shown that P2X antagonism had a protective role in a diabetic and inflammatory bowel syndrome mouse model due to the suppression of cytokine production and T cell proliferation.

Apart from autocrine signaling during T cell activation, ATP signaling can have paracrine effects on surrounding lymphocytes, particularly on their motility by inducing Ca\textsuperscript{2+} flux through P2X4 and P2X7. During T cell priming, T cells reduce their velocity, allowing them to better interact with cognate dendritic cells. Interestingly, ATP released from these T cells can reduce the motility of bystander T cells, allowing for the creation of lymphocyte clusters that can effectively tackle infection (73).

Another study demonstrated that P2X signaling can also convert T cells into different subsets. In Tregs, P2X\textsuperscript{7} activation inhibited the immunosuppressive role of the cells and instead promoted their conversion to T\textsubscript{11}17 cells in vivo. P2X\textsuperscript{7} inhibition, in contrast, promoted the differentiation of CD4 T cells into Tregs (74). Finally, a novel P2X\textsuperscript{5} transcript was discovered in human CD4 T cells, and its expression was upregulated upon T cell activation (75). Its small interfering RNA-mediated knockdown led to an increased production of IL-10 (75).

### Ca\textsubscript{V} channels

The Ca\textsubscript{V} channels are expressed in neuronal and muscle cells, where they flux Ca\textsuperscript{2+} in response to membrane depolarization (76). They are grouped into three major families, which are further divided into different subtypes based on their amino acid sequence: the Ca\textsubscript{V}1 family (Ca\textsubscript{V}1.1–Ca\textsubscript{V}1.4) contains L (long-lasting and large)-type channels; the Ca\textsubscript{V}2 family consists of P/Q (Purkinje-type) (Ca\textsubscript{V}2.1), N (neuronal)-type (Ca\textsubscript{V}2.2), and R (toxin-resistant)-type (Ca\textsubscript{V}2.3) channels; and the Ca\textsubscript{V}3 family (Ca\textsubscript{V}3.1–Ca\textsubscript{V}3.3) is also referred to as the T (transient and tiny)-type channels (77).

The fully assembled Ca\textsubscript{V} channels are structurally comprised of the α, α2β, β, and γ subunits. The α1 subunit forms the pore in the membrane, which consists of four homologous repeated domains (I–IV), each containing six transmembrane segments (S1–S6). S5 and S6 are separated by a pore-forming loop containing an ion-selectivity filter, whereas the S4 region contains the voltage sensor. The auxiliary subunits α2β and β do not take part in pore formation but instead modulate the expression and biophysical properties of the channel. The γ subunit was found to constitute the t-type channel complex, but little is known about its function (77). Several studies have now demonstrated that the pore-forming Ca\textsubscript{V}1 α1 as well as the β regulatory subunits are, along with neuronal and muscle cells, expressed in lymphocytes (78–80).

The first Ca\textsubscript{V} channel that was found to be expressed in lymphocytes was Ca\textsubscript{V}1.4, whose α1 subunit is encoded by the Ca\textsubscript{V}1.4a wire-gated channel subunit α1 F gene (CACNA1F). Ca\textsubscript{V}1.4 is known for its role in the retina, where it mediates Ca\textsuperscript{2+} entry into photoreceptors. Mutations in CACNA1F have been linked to congenital stationary night blindness (81). Interestingly, the splice variants first found in the Jurkat T cell leukemia line and human peripheral blood T lymphocytes differ from those in the retina (78). One of them, called Ca\textsubscript{V}1.4a, misses exons 31–34 and 37, which translates to a deletion of the transmembrane segments S3, S4, S5, and half of S6 of motif IV. The affected region includes the voltage-sensing domain and might impact voltage gating and reaction to depolarization (78). Additionally, a frameshift caused a change in the amino acid sequence of the C terminus, resulting in a 40% homology with Ca\textsubscript{V}1.1. Ca\textsubscript{V}1.4b, another novel splice variant, is missing the exons 32 and 37, resulting in a deletion of the extracellular loop between S3 and S4 and part of the transmembrane segment S6 in motif IV. Although the voltage sensor is still present in this splice variant, loss of the S3–S4 extracellular loop might still have an impact on the voltage gating characteristics, as it is in close proximity (78).

Also, Ca\textsubscript{V}1.1 was recently shown to exist as a splice variant in activated T cells. In this spliceform, exon 29 was excised, and the initial two N-terminal exons that are expressed in muscle cells were replaced with five new exons. Paralleling the earlier study describing the novel Ca\textsubscript{V}1.4b splice variant reported by Kotturi et al. (78), skipping exon 29 of Ca\textsubscript{V}1.1 resulted in the deletion of the linker region between S3 and S4 next to the voltage sensor in domain IV. In human embryonic kidney cells, the transfection with this splice variant increased the basal Ca\textsuperscript{2+} levels, which was not the case in cells transfected with a variant in which exon 29 was restored (82). Various splice variants of Ca\textsubscript{V}1.4 were also found during the extensive examination of retinal tissues, likely contaminated with blood cells like B and T lymphocytes. The discovery of 19 splice variants of Ca\textsubscript{V}1.4 suggests that splicing has a very significant role in tuning Ca\textsubscript{V}-dependent Ca\textsuperscript{2+} currents (83). As already mentioned, novel splice variants have been shown to possess altered gating characteristics (79) and are sometimes completely insensitive to membrane depolarization (84). It therefore has been suggested that Ca\textsubscript{V} channels in lymphocytes can be gated by Ag receptor signaling. Pharmacological studies by Kotturi et al. (85) have shown that this is indeed the case. Treating Jurkat cells as well as human peripheral blood T cells with the Ca\textsubscript{V}1 channel antagonist nifedipine severely reduced their TCR-induced Ca\textsuperscript{2+} flux.
ERK phosphorylation, and IL-2 production. In Jurkat cells, the transcriptional activity of NFAT was reduced by ndefidine after TCR cross-linking. The CaV1 agonist Bay K8644, in contrast, increased intracellular Ca^{2+} levels and phosphorylation of ERK. Later, the role of CaV1 channels in T cells was also confirmed using genetically engineered mouse models (79). Badou et al. (79) showed that mice lacking the β3 or β4 regulatory subunit exhibit an impaired TCR-mediated Ca^{2+} response. This further resulted in reduced NFAT translocation and compromised cytokine production of CD4 T cells. Additionally, the absence of the β regulatory subunits also led to a decreased expression of the pore-forming unit CaV1.1, which suggests that it might assemble with the regulatory subunits and likely has a role in T cells (79). Eventually, CaV1.1 was shown to be required for TCR-induced Ca^{2+} entry by the same group in knockout experiments using lentiviral short hairpin RNA (82).

Whereas the β3 subunit is important for effector functions of CD4 T cells, CD8 T cells require it for survival. The number of CD8 T cells was significantly reduced in β3-deficient mice because of spontaneous apoptosis induced by high expression of the apoptosis-inducing cell surface receptor Fas (first apoptosis signal receptor). This suggests that CaV1 channels provide a tonic survival signal that prevents CD8 T cells from apoptosing (80). The remaining CD8 T cells exhibited an activated memory T cell phenotype along with defects in TCR-induced Ca^{2+} flux and NFAT translocation and proliferation (80). The authors also showed that the β3 subunit formed a complex with CaV1.4 in naive CD8 T cells and suggested that the observed phenotype in the β3-deficient mice can be attributed to impaired CaV1.4–β3 channel formation (80).

By using a CaV1.4-α1-deficient mouse model, our laboratory has previously shown that CaV1.4 is essential for TCR-induced SOCE into naive CD4 and CD8 T cells and subsequent activation of the ERK and NFAT pathways (86). The T cells in CaV1.4-deficient mice also displayed a memory T cell phenotype and upregulated activation markers, suggesting that CaV1.4 is necessary for naïve T cell maintenance. Upon Listeria monocytogenes infection, the CaV1.4 knockout (KO) mice exhibited severe immune deficiencies, as reflected in a reduced number of functional Ag-specific CD4 and CD8 T cells (86). Interestingly, CaV1.4-deficient T cells were still sensitive to membrane depolarization. Because of the low activation threshold of CaV1.4 and its relatively small current (87), it is possible that the channel is active in resting cells and contributes to tonic filling of intracellular Ca^{2+} stores. In addition to its role in the murine immune system, our laboratory has recently demonstrated that CaV1.4 deficiency can also lead to a new form of X-linked immunodeficiency in humans, thereby establishing its important function in the human immune system (F. Fenninger and W.A. Jefferies, manuscript submitted for publication).

Although it has become clear that Ag receptor signaling can regulate CaV channels, an exact signaling pathway remains to be elucidated. However, a very intriguing regulatory mechanism for CaV1.2 and potentially other T-type Ca^{2+} channels in general was described by Wang et al. (88) and Park et al. (89). Apart from the activation of the CRAC channel described earlier, the Ca^{2+} sensor STIM1 was also found to inhibit CaV1.2 by internalization upon TCR stimulation.

This suggests that Ca^{2+} currents mediated by CaV1.2 are important in resting T cells and crucial for T cell survival, whereas the CRAC channel is important for T cell activation and effector function (80). As the role of CaV1.2 is similar to the previously proposed function of CaV1.4, the question arises whether STIM1 can also inhibit CaV1.4. It remains to be seen if other CaV channels can be gated by STIM1 interaction.

Apart from the different subunits that are essential to form a CaV channel, it was found that the protein AHNAK1 acts as a scaffold protein for the channel. It is thought to physically interact with the β regulatory subunits and thereby stabilize the channel complex in the plasma membrane. Consequently, AHNAK1 was also required for CaV1.1 α1 subunit expression, and its deficiency caused a reduced TCR-induced Ca^{2+} flux. This furthermore led to improper effector function of CD4 T cells as well as cytolysis of target cells by cytotoxic T cells (90, 91).

Additional studies highlight the role of CaV channels in leukocyte biology. CaV1.2 is expressed in human Th2 cells, where its antisense and pharmacological inhibition decreased Ca^{2+} and cytokine responses in a protein kinase C-dependent manner (92). Recently, it has also been demonstrated that the inhibition of the α2δ2 auxiliary subunit in CaV channels in T112 cells is also sufficient to disrupt TCR-induced Ca^{2+} flux and cytokine production (93).

Finally, T-type channels also play role in lymphocyte physiology. Recently, CaV3.1 was shown to be expressed in murine T lymphocytes (94). Interestingly, it retained its voltage-gated characteristics and conducted a substantial current at resting membrane potential in CD4 T cells but did not contribute to SOCE. The authors went on to demonstrate that CaV3.1 deficiency had a protective role in an EAE mouse model, most likely because of reduced GM-CSF production by T111 and T1117 cells (94).

Other channels: IP3Rs, ryanodine receptors, and N-methyl-D-aspartate receptors

IP3Rs are well described in the ER membrane, where they release Ca^{2+} from the ER stores into the cytoplasm upon binding of their cytosolic ligand IP3 (95). There have also been a few reports of IP3R presence in the plasma membrane of T cells, although their role is not quite clear (96, 97). In DT40 chicken and mouse B cells, it was reported that only two functional IP3Rs are located in the plasma membrane, where they conduct, despite their small number, a substantial BCR-induced Ca^{2+} current (98, 99). If true, these two high-conductance IP3Rs can flux a similar amount of Ca^{2+} upon BCR stimulation as thousands of low-conductance Orai proteins (100). It would be interesting to identify the exact sites of influx of these plasma membrane IP3Rs to see what proteins and signaling pathways are modulated by it.

In addition to IP3Rs, ryanodine receptors (RyRs) are located in the ER membrane. In cardiac and muscle cells, they release Ca^{2+} from intracellular stores upon excitation (101). The pharmacogenetic disorder malignant hyperthermia is caused by RyR1 gain-of-function mutations that lead to an increased release of Ca^{2+} in myotubes of muscle. For easier diagnosis of the disease, which usually involves the surgical excision of muscle, studies investigated whether the increased Ca^{2+} release can also be observed in B cells of affected individuals. Indeed,
stimulating B cells with a RyR1 agonist showed a greater Ca\(^{2+}\) response in B cells of malignant hypothermia patients, which harbor a RyR1 mutation, compared with controls and also correlated with a greater metabolic activity (102, 103). Another study demonstrated that caffeine, which is also used to activate RyRs, increased intracellular Ca\(^{2+}\) concentrations in naive splenic lymphocytes from mice (104), further supporting the notion that RyRs transport Ca\(^{2+}\) in lymphocytes.

Similar to the aforementioned TRPM2 channel, RyR activity was found to be ligand-modulated by the second messenger molecule cADPR (105). In T cells, cADPR has Ca\(^{2+}\)-mobilizing properties, and pharmacological studies in Jurkat T cells have confirmed that these properties can also be conferred through RyRs (106). As another study showed that the flux of Ca\(^{2+}\) from RyRs was induced upon SOCE (107), it is likely that cADPR release after SOCE activates RyRs to sustain the induced Ca\(^{2+}\) influx.

The functional role that RyR-mediated Ca\(^{2+}\) flux plays in lymphocytes is not very well explored, but one recent report claims that RyR inhibitors reduced the amount of T\(_{H1}\) and T\(_{H17}\) cells in cultured primary human T cells (108). The authors further reported that a gain-of-function mutation of RyR1 in an EAE mouse model exacerbated the disease and a RyR1 inhibitor reduced CNS inflammation and neurologic symptoms (108). Although exact signaling mechanisms have not been explored, this suggests a proinflammatory role for RyR-mediated Ca\(^{2+}\) currents.

N-Methyl-D-aspartate (NMDA) receptors are ionotropic glutamate receptors that permeate Ca\(^{2+}\) upon binding of the neurotransmitter L-glutamate. They are best described in neurobiology, where their dysfunction has been associated with several neurologic disorders such as stroke, epilepsy, and Alzheimer disease (109). However, NMDARs have also been identified in lymphocytes, and there is some evidence that they regulate lymphocyte function (9). Pharmacological studies, for example, demonstrated that NMDAR antagonists can inhibit thapsigargin-induced Ca\(^{2+}\) flux in T cells, suggesting a role for NMDARs during SOCE (110). Another study also found that NMDAR antagonists reduced TCR-mediated Ca\(^{2+}\) mobilization, resulting in an impairment of NFAT and ERK signaling as well as reduced effector functions like cytokine production and proliferation (111).

Additionally, the spliceoforms that are expressed in lymphocytes are different from those found in neuronal and muscle cells, where these channels were originally identified. Often the lymphocyte-specific splicing mechanisms alter the conductivity of the K\(^+\) channels Kv1.3 and KCa3.1 (111). These channels also play a crucial role during T cell activation, and importantly, the role of NMDARs during T cell activation demonstrated using MK801 could not be replicated when using a NMDAR-deficient mouse model (111). Therefore, the effect of MK801 on K\(^+\) channels should be taken into consideration when evaluating results using this drug as an NMDAR inhibitor.

**Conclusions**

Although the best-known contributor to Ag receptor–mediated Ca\(^{2+}\) flux in lymphocytes is the CRAC channel, the role of many other Ca\(^{2+}\) channels that are found in the plasma membrane remains poorly understood. In fact, not long ago, there was doubt that additional plasma membrane Ca\(^{2+}\) channels resided in the membranes of lymphocytes. Studies on Ca\(_{\text{V}}\) channels in lymphocytes eventually settled this debate, and the literature has been expanding since this time to include a constellation of leukocyte Ca\(^{2+}\) channels.

The expression of many of these channels may be restricted to specific developmental stages and subsets of lymphocytes, giving rise to the hypothesis that they all control very specific mechanisms. Indeed, we have seen that Ca\(^{2+}\) channels play key roles during lymphocyte development, particularly negative selection, homeostasis, and T\(_{H1}\) cell polarization. However, the most prominent role of Ca\(^{2+}\) channels appears to be in T cell inflammation, in which we have seen the involvement of many different channels, including the CRAC channel, TRP channels, P2X receptors, and Ca\(_{\text{V}}\) channels. Although some channels might act in parallel and have to some extent redundant roles, it is perplexing how the KO of a single channel sometimes almost completely abrogates the TCR/BCR-induced Ca\(^{2+}\) flux and effector functions and raises the question of what role is left for other Ca\(^{2+}\) channels in these processes. One possible explanation for this phenomenon is the formation of heteromeric channels, as we have seen with Orai1/Orai2 and TRPV1/TRPA1. Although in specific examples one subunit has an inhibitory role, it would also be possible that both subunits have activating properties and the absence of either one will disrupt the Ca\(^{2+}\) signaling. We have also seen that STIM1 not only regulates Ca\(^{2+}\) flux through Orai but it also regulates Ca\(_{\text{V}}\)1.2. It is likely that STIM1/2 also control other channels, which would be a reason why a STIM1/2 double-KO B cell exhibits an almost complete absence of BCR-induced flux. Furthermore, ligand-gated Ca\(^{2+}\) channels like the P2X receptors are activated by second messenger molecules to amplify or sustain Ca\(^{2+}\) currents.

In many cases, the activating ligands, however, are only secreted upon lymphocyte activation so that the P2X receptors are only activated downstream of an initial Ca\(^{2+}\) flux. Therefore, CRAC channel or other SOCE deficiencies will most likely also lead to a reduced flux in these downstream channels.

Additionally, the spliceoforms that are expressed in lymphocytes are different from those found in neuronal and muscle cells, where these channels were originally identified. Often the lymphocyte-specific splicing mechanisms alter the gating characteristics of the channels, particularly that of Ca\(_{\text{V}}\) channels. Although most channels directly or indirectly are responsive to Ag receptor cross-linking, the mechanisms that lead to the opening/closing of the channels remain for the most part elusive.

Despite many unanswered questions, because of the mostly inflammatory role of Ca\(^{2+}\) channels, blocking them is a promising avenue for therapeutic drug intervention for autoimmune disorders and complications due to transplant rejection. By using small molecules or Abs that bind to the extracellular portion of a channel, it should be possible to...
modulate their activity and, for example, block the production of harmful cytokines. Particularly Orai, P2X receptors, and CaV channels, whose ablation decreases lymphocyte effector function, have therapeutic potential. The fact that the inhibition or genetic deletion of many of these channels is protective during autoimmune CNS inflammation, inflammatory bowel syndrome, colitis, and graft-versus-host disease highlights their suitability as druggable targets. Finally, if it becomes possible to inhibit the specific splice variants that exist in lymphocytes, therapies could target lymphocyte subset-specific isoforms while leaving other cells untouched and thereby prevent adverse side effects.

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