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Ion channels and transporters in lymphocyte function and immunity

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Preface

Lymphocyte function is regulated by a network of ion channels and transporters in the plasma membrane of T and B cells. They modulate the cytoplasmic concentrations of diverse cations such as calcium, magnesium and zinc, which function as second messengers to regulate critical lymphocyte effector functions including cytokine production, differentiation and cytotoxicity. The repertoire of ion conducting proteins includes calcium release-activated calcium (CRAC) channels, P2X receptors, transient receptor potential (TRP) channels, potassium channels as well as magnesium and zinc transporters. This review discusses the roles of several ions channels and transporters in lymphocyte function and immunity.

Ion channels and transporters function as gateways for charged ions that cannot freely diffuse across lipid membrane barriers. They regulate the intracellular concentration of a variety of ions such as calcium (Ca^{2+}), magnesium (Mg^{2+}) or zinc (Zn^{2+}). The movement of these cations across the plasma membrane depends on electrical gradients that are maintained in turn by potassium (K^+), sodium (Na^+) and chloride (Cl^-) channels. In the past couple of years, fundamental progress has been made towards identifying the molecules that control the function of CRAC channels (the predominant antigen receptor-activated calcium channels in lymphocytes) and channels that mediate magnesium and zinc influx in T cells. We discuss the mechanisms regulating the function of these ion channels in lymphocytes and review their roles in immunity and their emerging potential for therapeutic immunomodulation.

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Further information
ONLINE LINKS for gene expression data:
BioGPS: http://biogps.gnf.org
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Several other ion channels, pumps and organelles are also required for the regulation of ion homeostasis in lymphocytes. For example, transient increases in the intracellular Ca^{2+} concentration are mediated by the release of Ca^{2+} from endoplasmic reticulum (ER) stores via Ca^{2+} permeable inositol 1,4,5 triphosphate ($InsP_3$) receptor and ryanodine receptor (RyR) channels. Conversely, Ca^{2+} is cleared from the cytoplasm by uptake into mitochondria and the ER via sarco/endoplasmic reticulum Ca^{2+} ATPases (SERCA) and Ca^{2+} export through plasma membrane Ca^{2+} ATPases (PMCA). Due to space limitations, these intracellular ion channels and transporters are not discussed here.

Store-operated Ca^{2+} channels

Ca^{2+} is a well-established second messenger in lymphocytes regulating proliferation, gene expression, motility and other functions. Similar to other mammalian cell types, the intracellular Ca^{2+} concentration, or $[Ca^{2+}]_i$, in unstimulated T and B cells is kept at ~ 50–100 nM, which is ~ 10^4-fold lower than the $[Ca^{2+}]$ in the serum. Following antigen binding to the T cell receptor (TCR) or B cell receptor (BCR), $[Ca^{2+}]_i$ can rise to ~ 1 μM [1]. Several ion channels have been identified in lymphocytes that mediate Ca^{2+} influx [1] (Fig. 1, Table 1). In the following sections, we discuss Ca^{2+} release-activated Ca^{2+} (CRAC) channels, P2X purinoreceptor channels, transient receptor potential (TRP) channels and voltage-gated Ca^{2+} (Ca_V) channels.

CRAC channels

Antigen binding by the TCR and BCR is coupled – via protein tyrosine kinases – to the activation of PLCγ1 in T cells and PLCγ2 in B cells and the generation of the lipid metabolite $InsP_3$. $InsP_3$ promotes the release of Ca^{2+} from ER stores, resulting in Ca^{2+} influx across the plasma membrane, a process termed store-operated Ca^{2+} entry (SOCE) (Fig. 1,2) [2]. The store-operated Ca^{2+} (SOC) channels of T cells, known as CRAC channels, have been extensively characterized [3, 4] and are distinguished by an extremely high selectivity for Ca^{2+} and low conductance [5] (Table 1). CRAC channels are activated through the binding of the ER Ca^{2+} sensors, stromal interaction molecule 1 (STIM1) and STIM2 to the CRAC channel proteins, ORAI1-ORAI3 (also known as CRACM1-CRACM3) [6].

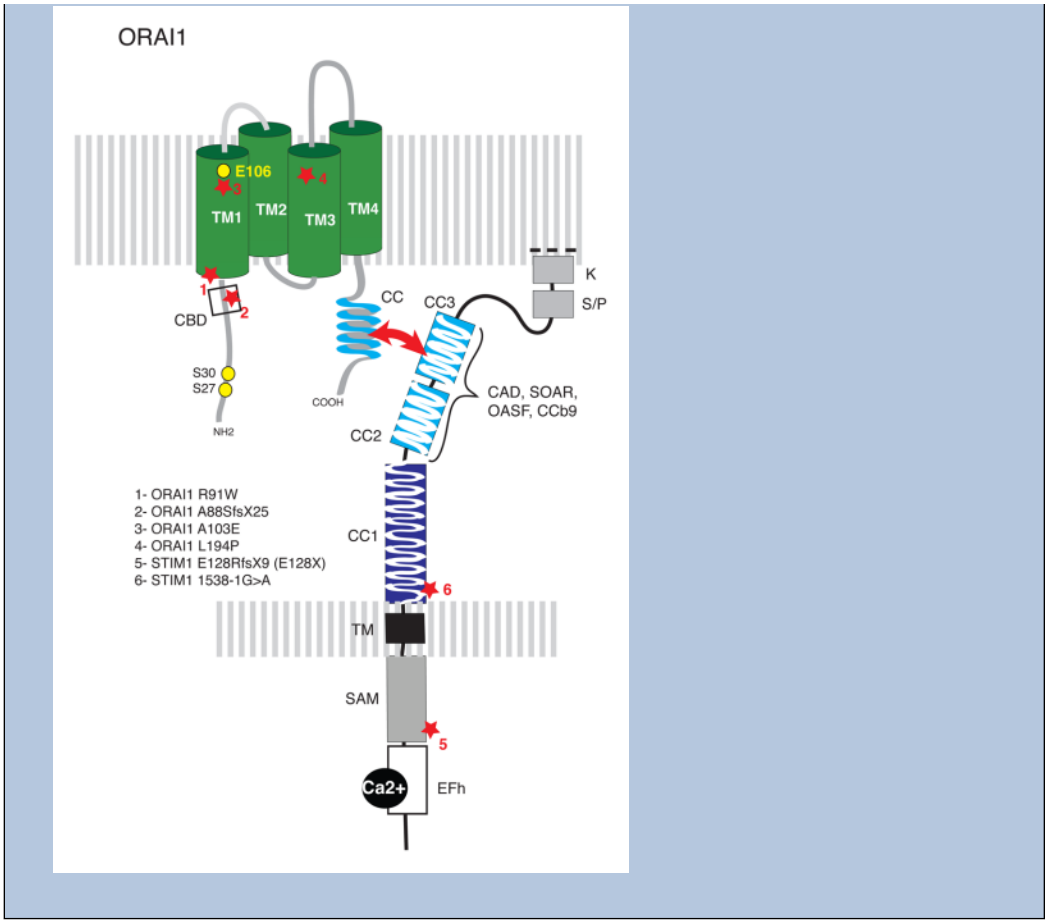
Identification of ORAI1 proteins

An important milestone in the identification of ORAI1 as the prototypic CRAC channel was the discovery that human patients with a severe form of combined immunodeficiency (CID) lack functional CRAC channels and SOCE in T cells [7–11]. *ORAI1* (or *CRACM1*) was identified nearly simultaneously by three laboratories as the gene encoding the CRAC channel by linkage analysis in CID patients and RNAi screens for regulators of SOCE and NFAT function [12–14]. ORAI1 is a widely expressed surface glycoprotein with four predicted transmembrane domains, intracellular N- and C- termini (Fig. 2, Box 1), and no sequence homology to other ion channels. CID arises from a single point mutation in ORAI1 (R91W) that abrogates CRAC channel activity [12]. All three ORAI isoforms (ORAI1-3) form Ca^{2+} channels with broadly similar functional properties when ectopically expressed, although they differ in their inactivation characteristics, pharmacological properties and tissue expression [15, 16]. ORAI1 remains the best-studied CRAC channel homologue and appears to be the predominant isoform mediating SOCE in lymphocytes [6, 17]. By contrast, there is no direct functional or genetic evidence for a role of ORAI2 and ORAI3 channels in immune cells yet.

Box 1

Molecular structure of the CRAC channel components ORAI1 and STIM1

ORAI1 is localized in the plasma membrane and constitutes the pore-forming subunit of the CRAC channel. The channels is formed by assembly of four ORAI1 subunits [6], of which the first transmembrane domain (TM1) line the channel pore [178, 179]. The selectivity filter of the CRAC channel is formed by a quartet of glutamate (E) 106 residues that form a high-affinity Ca^{2+} binding site to furnish the CRAC channel with high Ca^{2+} selectivity [180–183]. Analysis of other pore-lining residues in TM1 indicate that the CRAC channel pore is narrow [178, 179], potentially explaining its low conductance (i.e. a small number of Ca^{2+} ions passing through it), which limits the increase in $[Ca^{2+}]_i$ following channel opening. The intracellular C-terminus of ORAI1 features a coiled-domain (CC) domain that comprises a STIM1 binding site. The ORAI1 N-terminus contains a calmodulin binding domain (CBD) and two inhibitory phosphorylation sites (S27, S30) [184, 185]. STIM1 and STIM2 (not shown) are single-pass membrane proteins in the ER. Their N-terminus is located in the lumen of the ER and contains an EF-hand Ca^{2+} binding domain that allows them to sense the ER Ca^{2+} concentration. Mutations in the EF-hand domain of STIM1 result in impaired Ca^{2+} binding and constitutive CRAC channel activation independently of ER Ca^{2+} store depletion [18, 21]. The second and third coiled-coil domains (CC2, CC3) in the C-terminus of STIM1 are part of a minimal CRAC channel activation domain (CAD, also called SOAR, OASF, CCb9 [29, 184, 186, 187]), which binds directly to ORAI1 to activate CRAC channels. Autosomal recessive mutations in *ORAI1* and *STIM1* genes identified in patients with CRAC channelopathy are indicated by stars [12, 35–38]. These mutations abolish CRAC channel function and SOCE, either by eliminating channel function (1) or by abolishing ORAI1 and STIM1 protein expression (2–6). SAM, sterile alpha motif.



Activation of CRAC channels

Activation of ORAI CRAC channels involves a complex series of coordinated steps during which STIM proteins fulfill two critical roles: first, they sense the depletion of ER Ca^{2+} stores, and second, they communicate store depletion to CRAC channels [18–20] (Fig .2). In resting cells with replete Ca^{2+} stores, STIM proteins are diffusely distributed throughout the ER membrane [18, 21]. Upon depletion of Ca^{2+} stores, STIM1 is activated, oligomerizes and redistributes into discrete puncta located in junctional ER sites that are in close proximity to the plasma membrane [22–25]. In these puncta, STIM1 co-localizes with and interacts directly with the C- and N-termini of ORAI1 to activate CRAC channels [26]. The formation of overlapping STIM1-ORAI1 puncta involves direct binding of a cytoplasmic domain of STIM1 to the C- and N-termini of ORAI1 [27–29]. Lymphocytes express two closely related STIM isoforms, STIM1 and STIM2, and both mediate SOCE in T and B cells [30, 31]. Like STIM1, STIM2 also binds to and activates ORAI1-CRAC channels, but it does so upon smaller decreases in $[Ca^{2+}]_{ER}$ and with slower kinetics compared to STIM1 [32, 33]. This and the higher expression levels of STIM1 compared to STIM2 in naive mouse T cells may explain why STIM2-deficient T cells have initially normal Ca^{2+} levels after TCR stimulation but fail to sustain Ca^{2+} influx. By contrast, STIM1-deficient T cells display a near-complete lack of SOCE [31].

Control of lymphocyte function by CRAC channels

Genetic studies in patients with mutations in *ORAI1* or *STIM1* genes and in mice lacking functional *Orai1*, *Stim1* and/or *Stim2* genes have established important and non-redundant

roles for CRAC channels in lymphocytes and other immune cells (reviewed in [34]). Autosomal recessive mutations in human *ORAI1* (12q24) and *STIM1* (11p15) abolish CRAC channel function and Ca^{2+} influx in T cells, B cells and NK cells, resulting in CID with increased susceptibility to severe infections with viruses (especially herpes viruses [35, 36]), bacteria and fungal pathogens (*Candida albicans* [36, 37]) (Box 1, Table 1). The combination of CID with associated non-immunological clinical symptoms is referred to as CRAC channelopathy [38, 39].

$CD4^+$ and $CD8^+$ T cells from ORAI1- and STIM1-deficient patients and mice show defective production of many cytokines including interleukin-2 (IL-2), IL-4, interferon-γ (IFNγ), tumour necrosis factor (TNF)α and IL-17 [7, 40], which is partly due to the impaired activation of the Ca^{2+}-dependent transcription factor nuclear factor of activated T cells (NFAT) [7, 31]. SOCE-deficient human T cells also fail to proliferate in response to TCR or mitogen stimulation [8, 10, 36, 37]. This dependence of lymphocyte effector functions on SOCE is not limited to T cells. NK cells from an ORAI1-deficient patient showed impaired production of IFNγ, TNFα and CC-chemokine ligand 2 (CCL2) and failed to degranulate and kill tumour target cells [41]. B cells of mice lacking ORAI1 or STIM1/STIM2 exhibit diminished BCR-induced (but not anti-CD40- or LPS-dependent) proliferative responses [30, 42]. SOCE is also required for the production of IL-10, especially by $CD1d^{hi}$ $CD5^+$ regulatory B cells. Impaired expression of this anti-inflammatory cytokine in mice with B cell-specific deletion of *Stim1* and *Stim2* genes was associated with exacerbated autoimmune CNS inflammation in the EAE model of multiple sclerosis [30]. By contrast, CRAC channels do not play a major role in antibody production despite profoundly impaired SOCE in B cells from ORAI1- and STIM1-deficient patients and mice. Serum immunoglobulin levels in patients are not reduced, and T-dependent and T-independent antibody responses following immunization were normal in $Stim1^{-/-}$ [43] and $Stim1^{fl/fl}$ $Stim2^{fl/fl}$ *Mb1Cre* mice [30]. It is noteworthy however that in some ORAI1- and STIM1-deficient patients titers of specific antibodies against the recall antigens diphtheria and tetanus toxoid (DT) were absent [44]. Collectively, these studies emphasize the critical importance of CRAC channels for T cell- (and to a lesser degree B cell) mediated immunity.

Immunopathologies resulting from CRAC channel deficiencies

CRAC channels in T cells are not only critical for host defense to infection, but also for T cell-mediated hypersensitivity, allotransplant rejection and autoimmune inflammation. $CD4^+$ T cell-dependent skin contact hypersensitivity responses are abolished in ORAI1-deficient mice and these mice also failed to efficiently reject MHC-mismatched skin allografts [45]. Likewise, $CD4^+$ T cells from $Stim1^{-/-}$ mice exhibit slower and attenuated acute graft versus host disease (GvHD) compared to wildtype T cells when transferred to fully allogeneic mice [43]. When investigated for their ability to mediate autoimmunity in animal models of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE) and inflammatory bowel disease (IBD), CRAC-deficient T cells from ORAI1-, STIM1- and STIM2-deficient mice failed to induce disease [45–47]. Th1 and Th17 cells are critical mediators of inflammation in these models as in their human disease counterparts [48, 49]. Accordingly, IFNγ and IL-17 production by CRAC-deficient T cells isolated from central nervous system (CNS)-draining lymph nodes and mesenteric lymph nodes of mice was severely impaired, indicating that SOCE is required for the function of Th1 and Th17 cells [45–47]. Disease severity correlated with residual SOCE in T cells as STIM1-deficient mice were completely protected from developing EAE, whereas mice lacking STIM2 showed either delayed onset [47] or reduced severity of disease [46].

In humans, immunodeficiency in STIM1-deficient (and to a lesser degree ORAI-deficient) patients is associated with autoimmunity characterized by hemolytic anemia, thrombocytopenia and lymphoproliferative disease. Autoimmunity is most likely due to

reduced numbers of $CD25^+$ $Foxp3^+$ regulatory T (T_{Reg}) cells found in these patients [37]. A more profound reduction in T_{Reg} numbers is observed in the thymus and secondary lymphoid organs of mice with combined T cell-specific deletion of *Stim1* and *Stim2* [31]. STIM1/2-deficient Treg cells in addition show severely impaired suppressive function [31]. Accordingly, these mice develop massive splenomegaly, lymphadenopathy and pulmonary inflammation. The dependence of T_{Reg} cell development and function on SOCE is likely explained by the Ca^{2+}-dependent activation of NFAT and its role in FOXP3 expression [50, 51]. By contrast, the development and suppressive function of T_{Reg} cells from ORAI1- and STIM1-deficient mice were only moderately impaired [43, 45], indicating that residual Ca^{2+} influx, likely mediated by ORAI2/3 and STIM2 respectively, is sufficient for T_{Reg} cell development and function. Taken together, CRAC channels emerge as important regulators of T cell-dependent self-tolerance and autoimmunity.

Other Ca^{2+} permeable ion channels

P2X purinoreceptor channels

P2X receptors are a family of non-selective ion channels (Fig. 3) that are activated by extracellular ATP and allow the influx of Na^+, Ca^{2+} and other cations (reviewed in [52]). At least three different P2X receptors have been implicated in Ca^{2+} influx in human T cells: P2X1, P2X4 [53] and P2X7 [54]. Their opening, especially that of P2X7, causes Ca^{2+} influx and activation of downstream signalling molecules such as calcineurin, resulting in the proliferation of T and B cells [55, 56] and IL-2 production [53, 57]. RNAi-mediated depletion of P2X1, P2X4 and P2X7 or their pharmacological inhibition with P2X receptor antagonists results in impaired Ca^{2+} influx, NFAT activation and IL-2 production following TCR stimulation in Jurkat T cells and human $CD4^+$ T cells [53, 54]. Potential sources of ATP required for P2X receptor activation include T cells themselves, which are reported to release ATP in an autocrine manner through pannexin-1 hemichannels that colocalize with P2X7 receptors at the immunological synapse (IS) [53, 58] (Fig. 3). It has been suggested that autocrine ATP signalling in T cells via P2X receptors serves to amplify weak TCR signals, gene expression and T cell effector functions [52].

Several lines of evidence suggest that P2X receptors regulate T cell immune responses *in vivo.* Inhibition of all P2X receptors with oxidized ATP (oATP) protects mice from diabetes following adoptive transfer of pancreatic β-cell-specific T cells and colitis in an adoptive T cell transfer model of IBD [58]. Protection was associated with impaired production of IL-17, IFNγ and TNFα, suggesting that P2X7 signalling is required for the function of pro-inflammatory T cells [58]. Further analysis revealed that P2X7 also controls the function of $CD4^+$ $CD25^+$ T_{Reg} cells. Stimulation of T_{Reg} cells with the P2X7 agonist BzATP inhibited expression of FOXP3 but enhanced the levels of the Th17 cell-specific transcription factor RORγt [59]. A similar ATP-dependent conversion into Th17 cells was not observed in T_{Reg} cells from *P2x7*$^{-/-}$ mice. P2X7 signalling in T cells therefore appears to be pro-inflammatory by mediating the differentiation and function of Th17 cells and by inhibiting the stability of T_{Reg} cells [59]. The effects of P2X7 on adaptive immune responses are not always this unambiguous as several studies using *P2x7*$^{-/-}$ mice have alternatively shown an increased [60] or decreased [61] susceptibility to autoimmune CNS inflammation in the EAE model of multiple sclerosis. The cause of these discrepancies is not known. Future studies will need to carefully address which P2X receptors contribute to the influx of Ca^{2+} and other cations in T cells at physiological ATP concentrations and and which P2X receptors regulate adaptive immune responses *in vivo*, taking advantage of the various P2X receptor knock-out mice that have been generated [62–64].

Voltage-gated Ca^{2+} (Ca_V) channels

Ca_V channels are highly Ca^{2+} selective channels that mediate Ca^{2+} influx in response to depolarization of excitable cells such as myocytes, cardiomyocytes and neurons [65]. All members of the L-type family of Ca_V channels (Ca_V1.1, 1.2, 1.3 and 1.4) and their regulatory β3 and β4 subunits were found to be expressed in human and mouse T cells with several studies reporting the presence of truncated or alternatively spliced Ca_V isoforms [66–68]. Recent genetic studies in mice have implicated Ca_v channels in T cell function. CD^{4+} and CD^{8+} T cells from mice with mutations in β3 and β4 subunits had partially reduced Ca^{2+} influx in response to TCR stimulation and impaired IL-4, IFNγ and TNFα production [66, 69]. Impaired Ca^{2+} influx in β3-deficient $CD8^+$ T cells was associated with absent Ca_V1.4 protein expression [69]. Likewise, naive $CD4^+$ and $CD8^+$ T cells from Ca_V1.4-deficient (*Cacna1f*$^{-/-}$) mice had impaired TCR-induced Ca^{2+} influx. *Cacna1f*$^{-/-}$ mice failed to mount an effective T cell response to infection with *Listeria monocytogenes* [70] that was associated with reduced cytotoxic function of $CD8^+$ T cells [70]. Ca_V1.4-deficient T cells also showed enhanced cell death [70] consistent with a role of the β3 subunit in $CD8^+$ T cell survival reported previously [69]. In addition to Ca_V1.4, the RNAi-mediated depletion of Ca_V1.2 and Ca_V1.3 in T cells reduced TCR-induced Ca^{2+} influx in Th2 cells, attenuated IL-4 production and reduced airway inflammation in mouse model of allergic asthma [71].

Despite these intriguing findings, the role of Ca_v channels in lymphocytes remains highly controversial. A major gap in our understanding of the role of L-type Ca_V channels in lymphocytes is whether they function as Ca^{2+} channels or facilitate Ca^{2+} influx by other mechanisms. Cell depolarization, the canonical mechanism to activate Ca_v channels, fails to evoke typical Ca_V channel currents in the hands of most investigators. Although it is theoretically possible that Ca_v channels in T cells are activated by other, depolarization-independent pathways, this remains speculative. Complicating the picture further are recent studies that report the inhibition of Ca_V channels by STIM1 [72, 73], raising the possibility that the function of CRAC and Ca_V channels might be reciprocally regulated. Evidence against a significant role of Ca_v channels in T cells comes from loss-of-function mutations in Ca_V channels in human patients, which are not associated with an overt immunological phenotype [74] and pharmacological inhibitors of L-type Ca_v channels such as nifedipine and verapamil, which are in wide clinical use for cardiovascular diseases, yet have no reported effects on immune function. In the absence of a thorough validation of Ca_v channel currents by patch-clamp measurements and a molecular mechanism of Ca_v channel activation in T cells, the effects of genetic deletion of Ca_v channel components on T cell function and immune responses remain difficult to interpret.

Channels controlling membrane potential and Ca^{2+} influx

Ca^{2+} influx in lymphocytes is dependent on a negative membrane potential (V_m) that provides the electrical driving force for Ca^{2+} entry [75]. Two classes of channels regulate V_m in lymphocytes: K^+ channels and TRPM4 channels.

K^+ channels

K^+ channels protect against membrane depolarization by mediating the efflux of K^+ to hyperpolarize the plasma membrane [76]. The best-studied K^+ channels that predominately regulate V_m in lymphocytes are the voltage-activated K^+ channel Kv1.3 and the Ca^{2+}-activated K^+ channel KCa3.1 (or KCNN4, $IKCa^{2+}$ and SK4). Kv1.3 is a homotetramer of four α-subunits, each composed of six transmembrane segments (S1–S6) and is activated by membrane depolarization [77]. Depolarization of the membrane potential is sensed by four arginine residues localized in the S4 segment, which results in a conformational change

causing channel opening [78]. KCa3.1, by contrast, is a Ca^{2+}-activated K^+ channel that has a similar membrane topology and pore architecture to Kv1.3. However, rather than containing a voltage sensor, the carboxy-terminus of KCa3.1 is constitutively bound to calmodulin and channel opening occurs after Ca^{2+} binding to calmodulin [79]. KCa3.1 channels powerfully hyperpolarize the membrane following $[Ca^{2+}]_i$ elevations and help to sustain the driving force for Ca^{2+} entry. In addition to Ca^{2+}, KCa3.1 channel activity depends on the class II phosphatidylinositol 3-kinase (PI3K) PI3K-C2β, which increases the plasma membrane PI(3)P concentration. This allows the histidine kinase nucleoside diphosphate kinase B (NDPK-B, also known as nm23H2) to activate KCa3.1 by phosphorylating histidine 358 in the C-terminus of KCa3.1 [80, 81]. In agreement with the finding that both PI(3)P and histidine phosphorylation of KCa3.1 are critical for activation, the PI(3)P phosphatase myotubularin related protein 6 and the histidine phosphatase phosphohistidine phosphatase 1 inhibit KCa3.1, TCR-stimulated Ca^{2+} influx and proliferation of activated naïve human $CD4^+$ T cells by dephosphorylating PI(3)P and KCa3.1, respectively [80, 82]. In addition, the E3 ubiquitin ligase tripartite motif-containing protein 27 inhibits KCa3.1 and TCR-stimulated Ca^{2+} influx and cytokine production by ubiquitylating and inhibiting PI3K-C2β [83].

The relative contribution of Kv1.3 and KCa3.1 in lymphocyte Ca^{2+} influx is determined primarily by their expression levels, which is related to lymphocyte subsets and their state of activation. Under resting conditions, $CCR7^+CD45RA^+$ naïve human T cells predominantly express Kv1.3 channels and depend on Kv1.3 for activation [84]. Following activation, naïve human T cells upregulate KCa3.1 [85] and KCa3.1 inhibition in pre-activated T cells inhibits TCR-stimulated Ca^{2+} influx and proliferation[86, 87]. Furthermore, mouse Th1 and Th2 cells predominantly express KCa3.1 and depend on KCa3.1 for TCR-stimulated Ca^{2+} influx and cytokine production, whereas Th17 cells mainly express Kv1.3 and depend on Kv1.3 for activation and production of IL-17 [88]. Differential use of K^+ channels is also observed in effector (T_{EM}) and central (T_{CM}) memory T cells [76, 80, 89, 90]. T_{EM} cells ($CCR7^-CD62L^{low}CD45RA^-$) activated at sites of inflammation produce cytokines including IFNγ, IL-4 and IL-5 and exclusively upregulate Kv1.3. By contrast, T_{CM} cells ($CCR7^+CD62L^{hi}CD45RA^-$) present in lymph nodes and mucosal lymphoid organs upregulate KCa3.1 upon activation. As a result, Kv1.3 blockers are effective inhibitors of T_{EM} cells, whereas KCa3.1 blockers are effective at inhibiting T_{CM} cells.

Given their prominent role in regulating Ca^{2+} signalling, Kv1.3 and KCa3.1 have emerged as important drug targets [91, 92]. Several potent peptide toxins, such as ShK derived from sea anenome venom, and oral small molecule inhibitors, such as Psora-4 and PAP-1, specifically inhibit Kv1.3 [93–95]. Several specific inhibitors of KCa3.1 channels have also been developed and include TRAM34 and ICA-17043 [96, 97]. Inhibitors of Kv1.3 and KCa3.1 have been very useful to study the role of K^+ channels for immune responses *in vivo*, especially as Kv1.3 knockout mice showed no overt T cell defect due to the upregulation of a Cl^- channel that compensates for the loss of Kv1.3 [98].

The finding that Kv1.3 and KCa3.1 function to activate distinct lymphocyte subsets provides an opportunity to more selectively target lymphocyte subsets for therapeutic purposes. Studies in a rat model of multiple sclerosis reveal that Kv1.3 expression is upregulated and required for the proliferation of encephalogeneic T cells, and treatment of rats with Kv1.3 blockers in models of EAE markedly ameliorated disease[89]. The relevance of these findings to humans was demonstrated by findings of high levels of Kv1.3 expression on myelin-reactive T cells isolated from patients with multiple sclerosis [99]. Similar studies have shown an increase in disease-associated T_{EM} cells in patients with type 1 diabetes, rheumatoid arthritis and psoriasis, and treatment of these diseases with Kv1.3 blockers ShK or PAP1 led to the amelioration of disease [90, 100–102]. Consistent with a role for Kv1.3 in the activation of Th17 cells [88], Kv1.3 blockers may have a therapeutic benefit in autoimmune diseases driven

by Th17 cells[103, 104]. By contrast, Th1 and Th2 cells depend on KCa3.1 for their activation [88]. Inhibition of KCa3.1 protected mice from developing colitis in two mouse models of IBD[88], suggesting that KCa3.1 may be a novel therapeutic target to treat patients with Crohn's disease or ulcerative colitis.

TRPM4 channels

TRPM4 channels are expressed by T cells and many other immune cells. Unlike most other TRP channels, their role in lymphocyte function is well documented. TRPM4 channels mainly conduct Na^+ and K^+ and, in contrast to other TRP channels, are only weakly permeable to Ca^{2+} [105]. Activation of TRPM4 channels – accomplished by increases in $[Ca^{2+}]_i$ – results in Na^+ influx, membrane depolarization and a reduction in the electrical driving force for Ca^{2+} influx. TRPM4 channels thus provide a negative feedback mechanism for the regulation of SOCE and were proposed to prevent Ca^{2+} overload of cells. Given that activation of TRPM4 and Kv channels elicit opposing effects on V_m, it remains to be elucidated precisely how TRPM4 works together with Kv1.3 and KCa3.1 channels to regulate changes in V_m and intracellular Ca^{2+} levels. Overexpression of a dominant-negative mutant of TRPM4 or depletion of TRPM4 by RNAi in Jurkat T cells resulted in enhanced Ca^{2+} signalling and increased IL-2 production [106]. Similar effects were observed in mouse Th2 cells, in which TRPM4 regulates Ca^{2+} levels, motility and the production of IL-2 and IL-4 by controlling the nuclear translocation of NFAT [107]. Mast cells from *Trpm4*$^{-/-}$ mice showed increased Ca^{2+} influx, degranulation and histamine release after FcεRI stimulation; accordingly these mice had a more severe IgE-mediated acute passive cutaneous anaphylactic response [108]. How enhanced Ca^{2+} influx in the absence of TRPM4 affects lymphocyte-dependent immune responses *in vivo* remains to be elucidated.

TRP channels

TRP channels form a large superfamily of 28 cation channels in humans, which can be divided into 7 subfamilies [109]. T cells predominantly express channels belonging to the TRPC and TRPM subfamilies including TRPC1, TRPC3, TRPC5, TRPM2, TRPM4 and TRPM7 [110] (Table 1). Most TRP channels are non-selective and permeable to several cations including Ca^{2+} and Na^+ [111, 112]. We will briefly discuss the role of TRPC and TRPM2 channels; TRPM7 channels will be discussed further below in the context of Mg^{2+} signaling.

TRPC channels

TRPC1-7 form non-selective cation channels whose activation is generally linked to stimulation of plasma membrane receptors coupled to PLCγ [111, 113]. Prior to the identification of ORAI1 as the CRAC channel [12–14], there was avid interest in the possibility that TRPC channels contribute to SOCE in T cells. A recent study showed that RNAi-mediated depletion of TRPC3 has a moderate effect on SOCE and T cell proliferation [110]. Expression of TRPC5 was reported to increase after activation of mouse $CD4^+$ and $CD8^+$ T cells and to mediate Ca^{2+} influx after crosslinking of GM1 ganglioside with the B subunit of cholera toxin [114]; whether TRPC5 mediates TCR-induced Ca^{2+} influx has not been examined. While these studies were generally interpreted as supporting a role for TRPC channels in SOCE, recent evidence has questioned whether TRPC channels are activated by store depletion [115]. Overall, the biophysical and immunological evidence for a significant role of TRPC channels in lymphocytes and adaptive immune responses awaits further evaluation.

TRPM2 channels

TRPM2 is a non-selective Ca^{2+} permeable channel that is activated by intracellular adenosine diphosphate ribose (ADPR) and regulated by several intracellular factors including Ca^{2+}, cyclic ADPR (cADPR), H_2O_2, NAADP and AMP [116, 117]. TRPM2 channels mediate stress induced Ca^{2+}signals in a diverse group of immune cells including myeloid cells and T cells [116, 117]. In T cells, TRPM2 expression was found to increase after TCR stimulation [110] and endogenous TRPM2 currents could be activated by cADPR, ADPR and NAADP [118]. Although there is no direct evidence that TRPM2 is required for Ca^{2+} influx in lymphocytes and T cell function, TCR stimulation has been reported to evoke the release of cADPR from the ER [119], thus potentially activating TRPM2 in T cells. Studies in myeloid cells indicate that cADPR and hydrogen peroxide (H_2O_2)synergize in the activation of TRPM2 (reviewed in [116, 117]). Since H_2O_2 is produced by several immune cell types under inflammatory conditions, Ca^{2+} influx through TRPM2 has been investigated as a potential mediator of reactive oxygen species (ROS)-induced pathologies [120, 121]. TRPM2-deficient mice were largely resistant to dextran sulphate sodium (DSS)-induced colitis due to impaired Ca^{2+} influx, nuclear factor-κB (NF-κB) activation and production of CXCL2 by monocytes [122]. Conversely, TRPM2 inhibited ROS production in phagocytic cells by attenuating NADPH oxidase function and prevented endotoxin-induced lung inflammation in mice [120]. Whether TRPM2 channels are modulated by ROS in T cells and regulate T cell responses during inflammation *in vivo* remains to be elucidated.

Magnesium channels and transporters

Mg^{2+} is the most abundant divalent cation in eukaryotic cells. It binds to and regulates the function of many polyphosphate-containing molecules such as DNA, RNA and ATP. While > 90% of all cellular Mg^{2+} is in the form of Mg-ATP [123], ~ 5% is free and can potentially function as a second messenger similar to Ca^{2+}. Mg^{2+} is required for the proliferation of mitogen-stimulated T cells [124, 125], and stimulation of T cells through the TCR results in a transient increase in $[Mg^{2+}]_i$ [126]. Recent studies provide evidence for an important role of TRPM7, a Mg^{2+} permeable channel, and MagT1, a Mg^{2+} transporter, in T cell function and development [126, 127].

TRPM7 channels

TRPM7 is a ubiquitously expressed nonselective cation channel that exhibits nearly equal permeabilities for Mg^{2+} and Ca^{2+} [128] (Fig. 4). TRPM7 channels are believed to regulate cellular and whole body Mg^{2+} homeostasis because of their high Mg^{2+} permeability. This is supported by evidence indicating that mutations in the closely related TRPM6 channel cause hypomagnesemia due to impaired renal and intestinal Mg^{2+} absorption [129–131]. Direct evidence for a role of TRPM7 in immune function came from genetic deletion of TRPM7 in DT-40 chicken B cells, which failed to proliferate, showed increased cell death and had reduced total cellular Mg^{2+} [132]. These defects could partially be rescued by growing cells in medium containing high extracellular Mg^{2+} (10 mM) [132].

An important role for TRPM7 in T cell development was shown using mice with T cell-specific deletion of TRPM7. *Trpm7$^{fl/-}$ Lck-Cre* mice had a severe block in T cell development at the $CD4^-CD8^-$ double negative stage, resulting in reduced numbers of $CD4^+$ and DP T cells in the thymus and $CD3^+$ T cells in the spleen [127]. Lack of TRPM7 in T cells was associated with impaired expression of growth factors such as FGF7, FGF13 and midkine, and consequently a progressive loss of medullary thymic epithelial cells (mTECs) [127]. It remains unclear, however, whether the observed defects are intrinsic to TRPM7-deficient T cells or secondary to the loss of mTECs. Another question is whether the primary role of TRPM7 in T cells is in Mg^{2+} homeostasis. Although Mg^{2+} currents in

thymocytes from *Trpm7$^{fl/-}$Lck-Cre* mice were markedly reduced, Mg^{2+} influx and total cellular Mg^{2+} content were normal, suggesting that TRPM7 may not be required for Mg^{2+} influx in T cells [127]. How does TRPM7 then control T cell development and function? Possible explanations are that Mg^{2+} influx through TRPM7 may cause highly localized increases in $[Mg^{2+}]_i$ close to the mouth of the channel resulting in the activation of signal transduction (Fig. 4) or, alternatively, that the main function of TRPM7 in T cells is not to promote Mg^{2+} but Ca^{2+} influx consistent with the channel's documented Ca^{2+} permeability [133]. Finally, TRPM7 could regulate T cell development through its C terminal kinase domain independent of channel function [128]. While the mechanisms by which TRPM7 controls lymphocyte function remain in debate, it is noteworthy that this is the only ion channel identified so far (with the exception of the Mg^{2+} transporter MagT1 discussed below) that is required for lymphocyte development.

MagT1

MagT1 is a Mg^{2+} transporter essential for Mg^{2+} signalling in T cells (Fig. 4). It was discovered in two independent screens and has little sequence similarity to other ion channels or transporters [134, 135]. MagT1 is highly selective for Mg^{2+} and does not conduct Ca^{2+}, Zn^{2+}, Ni^{2+} or other divalent cations when expressed in Xenopus oocytes [134]. It mediates Mg^{2+} influx in T cells and RNAi-mediated depletion of MagT1 resulted in a moderate decrease in cytoplasmic Mg^{2+} concentrations [135]. The importance of MagT1 and Mg^{2+} signalling in T cells is emphasized by patients with inherited mutations in *MAGT1* who suffer from a rare form of immunodeficiency (Table 1) [126]. Patients with XMEN disease (for X-linked immunodeficiency with magnesium defect and EBV infection and neoplasia) suffer from $CD4^+$ lymphocytopenia and increased susceptibility to viral infections, particularly with Epstein-Barr virus (EBV) due to abolished MagT1 protein expression and Mg^{2+} influx following TCR stimulation. In contrast to T cells, B cell development and function are normal in these patients, consistent with the lack of Mg^{2+} influx in control B cells stimulated by anti-IgM or anti-CD40 antibodies.

One of the main functions of Mg^{2+} influx by MagT1 is the activation of PLCγ1 as TCR crosslinking of MagT1-deficient T cells resulted in delayed activation of PLCγ1 and abolished SOCE [126]. By contrast, proximal TCR signalling events such as the phosphorylation of CD3ε, ζ-chain-associated protein kinase of 70 kDa (ZAP70) and linker for activation of T cells (LAT) occurred normally [126]. The mechanisms by which Mg^{2+} influx through MagT1 regulates PLCγ1 activation are not understood (Fig. 4). Another open question is how TCR stimulation activates MagT1 and thus Mg^{2+} influx, especially since MagT1 only contains two short intracellular domains available to interact with cytoplasmic signalling molecules (Fig. 4) [135]. Despite these unresolved questions, the profound immunological effects of MagT1 deficiency validate the important role of Mg^{2+} ions in T cell function.

Zinc transporters

Zinc is an essential trace element and a structural component of numerous metalloproteins such as zinc finger-containing transcription factors through which it contributes to immune function (reviewed in [136–138]). In addition, emerging evidence suggests that Zn^{2+} regulates lymphocyte function directly as a second messenger. The free $[Zn^{2+}]_i$ in lymphocytes is very low (~ 0.35 nM) [139], whereas that in the serum is ~16 μM [140], establishing a > 10^4-fold gradient between extracellular and intracellular $[Zn^{2+}]$. Stimulation of human and mouse T cells by IL-2 [141] or incubation with DCs results in rapid increases in $[Zn^{2+}]_i$ accompanied by T cell proliferation and cytokine production, suggesting a potentially important role for Zn^{2+} in lymphocyte signal transduction [141–143].

The role of Zn^{2+} in immunity is highlighted by the inherited Zn^{2+} malabsorption syndrome acrodermatitis enteropathica (AE) caused by impaired Zn^{2+} uptake through the zinc transporter Zrt-Irt like protein 4 (ZIP4) in the intestinal epithelium [144, 145]. The AE phenotype includes immunodeficiency with recurrent infections in ~ 30% of patients. Immunodeficiency is associated with thymus atrophy and lymphopenia ([146, 147] and references therein), which have been attributed to increased glucocorticoid production and apoptosis of immature T and B cells [147]. Several *in vitro* studies have shown that Zn^{2+} is required for T cell functions, for instance proliferation [143, 148] and the production of cytokine like IL-2 and IFNγ [149]. At higher concentrations, however, Zn^{2+} was shown to exert an inhibitory effect on the proliferation of mouse T cells [150] and the expression of cytokines by Jurkat T cells [151] and human $CD4^+$ T cells [142]. The molecular mechanisms underlying these concentration-dependent effects of Zn^{2+} are only partially understood. Fig. 5 shows some of the signalling pathways in lymphocytes that are either activated or inhibited by Zn^{2+} (reviewed in [136–138]). For instance, increases in $[Zn^{2+}]_i$ were reported to enhance activation of kinases such as Lck and PKC [152], but to inhibit the phosphatase calcineurin (Fig. 5) [153, 154]. More recently, Zn^{2+} influx was reported to mediate T cell activation by enhancing the phosphorylation of ZAP70 and decreasing the recruitment of the tyrosine phosphatase SHP1 to the TCR, thereby prolonging Ca^{2+} influx [143].

The proteins that mediate Zn^{2+} levels in lymphocytes and their molecular regulation are still poorly defined. Two classes of Zn^{2+} transporters have been described to regulate intracellular Zn^{2+} concentrations: ZIP transporters (or solute carrier family 39, SLC39A) and Zinc transporters (ZnT or SLC30A). ZIP and ZnT proteins are localized either in the plasma membrane or intracellular organelles where they mediate Zn^{2+} influx (ZIP) or Zn^{2+} efflux (ZnT) into or from the cytoplasm respectively (Fig. 5) (reviewed in [155]). 14 mammalian ZIP genes have been identified [137]. *ZIP3* is highly expressed in $CD34^+$ human haematopoietic stem cells and genetic deletion of *Zip3* in mice resulted in the depletion of $CD4^+CD8^+$ T cells in the thymus under zinc-limiting conditions. By contrast, the numbers of single-positive $CD4^+$ or $CD8^+$ thymocytes were increased suggesting accelerated T cell maturation [156]. In T cells, two Zn^{2+} transporters, ZIP6 and ZIP8, were reported to mediate Zn^{2+} influx across the plasma membrane [143] and release from lysosomal Zn^{2+} stores [142], respectively. When primary human $CD4^+$ T cells were stimulated by incubation with DCs, $[Zn^{2+}]_i$ increased within 1 minute after formation of the IS between T cells and DCs. This increase in $[Zn^{2+}]_i$ and the subsequent expression of CD25 and CD69 depended on ZIP6. Interestingly, increases in $[Zn^{2+}]_i$ were spatially restricted to the IS [143], potentially due to rapid sequestration by zinc-binding proteins such as metallothionein. Another mechanism to limit increases in $[Zn^{2+}]_i$ is provided by ZnT transporters that mediate uptake of Zn^{2+} into intracellular organelles or promote Zn^{2+} export across the plasma membrane. Of the 10 ZnT transporters in mammalian cells, only a few are known to be functional in immune cells. ZnT5 is required for mast cell function [157, 158], but the ZnT molecules controlling $[Zn^{2+}]_i$ in lymphocytes remain to be elucidated. It is noteworthy that primary human T and B cells express ZnT1, ZnT4, ZnT6 and ZnT7 [157] and that mRNA expression in T cells was strongly reduced following phytohaemagglutinin stimulation. Thus, downregulation of ZnT levels may be a means to maintain elevated $[Zn^{2+}]_i$ during T cell activation. Despite these leads, the overall role of Zn^{2+} transporters in immune function, development and adaptive immunity remains poorly understood.

Chloride channels

Several chloride channels that allow the influx of Cl^- anions across the plasma membrane were reported tobe active in lymphocytes and control their function. Volume-regulated Cl^- (or Cl_{swell}) channels open upon swelling of T cells in a hypotonic environment, resulting in the efflux of Cl^- and, ultimately, water from the cell, and thus a return to normal cell

volume [76, 159, 160]. The osmotic activation of chloride channels in Jurkat T cells depends on the Src kinase Lck [161]. Interestingly, the induction of apoptosis in T cells by crosslinking of Fas (CD95) induces Cl^- currents in a Lck-dependent manner [162], suggesting that Cl^- channels may regulate apoptosis in T cells. A further analysis of the physiological role of volume-regulated Cl^- channels in lymphocytes is hampered, however, by the fact that their molecular identity is unknown (reviewed in [76]).

Several studies have demonstrated the expression of γ-aminobutyric acid (GABA) receptors in human, mouse and rat T cells [163, 164]. $GABA_A$ receptors are heteropentameric ligand-gated Cl^- channels whose inhibitory role in neuronal function in the CNS is well established [165]. GABA-activated Cl^- currents were reported in mouse and rat T cells and macrophages [164, 166, 167]. GABA administration inhibited T cell proliferation, the production of IL-2 and IFNγ as well as the cytotoxic function of $CD8^+$ T cells *in vitro* [163, 164, 168, 169]. *In vivo*, administration of GABA or GABAergic agents ameliorated disease outcome in a number of animal models of autoimmunity such as type 1 diabetes [163], rheumatoid arthritis [170] and multiple sclerosis [166], suggesting that $GABA_A$ receptors may inhibit the activation of T cells to protect GABA-secreting cells from T cell-mediated inflammatory tissue damage. How GABA receptor-mediated Cl^- influx inhibits T cell function has not been elucidated. Unlike excitable cells, in which GABA receptors inhibit Cav channels through membrane hyperpolarization, this mechanism is unlikely to account for the effects of GABA on T cells.

Another chloride channel that has been reported to regulate T cell function is the cystic fibrosis transmembrane conductance regulator (CFTR), mutations of which cause cystic fibrosis (CF). cAMP-activated Cl^- currents were originally reported in Jurkat T cells, $CD4^+$ T cell clones and EBV-transformed B cells and shown to be defective in T and B cells from CF patients [171, 172]. The effects of the (F508 CFTR mutation, the most common mutation in CF patients, on murine and human T cells were however very different. Whereas T cell clones from CF patients showed impaired production of IL-5 and IL-10 after stimulation with anti-CD3 and PMA[171], $CD4^+$ T cells from *Cftr-F508* (*Cftr−/−*) mice showed increased IL-4 and IL-13 production when stimulated with congenic monocytes and OVA peptide [173]. The cause of this discrepancy between human and mouse T cells is not clear. Further studies are required to corroborate a role for CFTR in lymphocyte function and to provide a better mechanistic understanding how CFTR may regulate T cell function.

Summary and perspectives

Lymphocytes express an abundance of ion channels that are critical for their development and function. While the importance of individual ion channels and transporters for T-cell effector function is now well-recognized, how different ion transport mechanisms interact with each other to fine-tune overall cellular responses for the most the most optimal outcome still remains poorly understood. It seems likely that interactions among the various ion transport mechanisms could help to generate complex signal transduction patterns and generate specificity by enhancing the dynamic range of the individual signaling pathways and by improving signal-to-noise. Examples of cross-talk include the regulation of Ca^{2+} influx by the MagT1 Mg^{2+} transport proteins [126], Zn^{2+} influx [143], and the well-known modulation of Ca^{2+} influx by K^+ channels through control of the membrane potential. Such cross-talk could permit more finely tuned regulation of cell signalling than may be possible through the action of individual independent pathways. In most cases, the molecular foundations of cross-talk are unclear, but possible explanations include the colocalization of ion transport proteins, as suggested for CRAC (ORAI) and K^+ channels [9] and CRAC channels and P2X receptors [53]. It is tempting to speculate that different types of ion channel

aggregate in signalling complexes in lymphocytes where they modulate each others function, but more in-depth studies are needed to investigate this possibility.

Many ion channels discussed here contribute to T cell-mediated autoimmune and/or inflammatory responses and therefore are attractive targets for pharmacological immune modulation. Whereas drugs acting on ion channels have successfully been used for the treatment of neurological and cardiovascular disorders [174], ion channels have not been systematically exploited as drug targets for immune therapy. Plasma membrane channels are readily accessible to small molecule compounds and biological reagents such as blocking antibodies and peptides. Inhibitory antibodies against TRPC5 and TRPM3 channels have been developed that target an extracellular loop in close proximity to the ion channel pore [175, 176]. It is possible that these approaches could be extended to TRPM2 channels (given its pro-inflammatory role in monocytes [122]) and ORAI Ca^{2+} channels. As described above, genetic deletion of ORAI1 and STIM1 in mice abolishes the expression of several pro-inflammatory cytokines [7, 46, 47] and protects mice from autoimmune CNS inflammation, colitis, allograft rejection and GvHD [43, 45–47]. Inhibition of SOCE can be achieved by directly targeting ORAI1 CRAC channels, or indirectly by inhibiting the function of K^+ channels. As discussed above, considerable progress has been made in developing K^+ channel blockers [92, 177]. Similarly, P2X7 receptor antagonists may provide a multipronged approach to anti-inflammatory therapy given the role of these channels in the pro-inflammatory function of lymphocytes and innate immune cells [58, 59]. It will therefore be an important long-term goal to develop safe, selective and potent inhibitors of ion channels for the treatment of inflammation, autoimmunity, allergy and transplant rejection.

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GLOSSARY

inositol 1,4,5 triphosphate ($InsP_3$) receptor	a Ca^{2+}-permeable channel located in the membrane of the endoplasmic reticulum (ER) which mediates the release of Ca^{2+} from ER stores upon binding by the second messenger $InsP_3$
ryanodine receptor (RyR)	a Ca^{2+}-permeable channel located in the membrane of the sarcoplasmic reticulum (SR) and ER which mediates the release of Ca^{2+} from the SR/ER stores upon binding by the second messenger cyclic ADP ribose (cADPR) or Ca^{2+} itself
sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA)	located in the membrane of the ER, is a Ca^{2+} pump that moves Ca^{2+} from the cytoplasm into the ER through hydrolysis of ATP
plasma membrane Ca^{2+} ATPases (PMCA)	a family of ion transport ATPases located in the plasma membrane that export Ca^{2+} from the cytoplasm
store-operated Ca^{2+} entry (SOCE)	Ca^{2+} influx process triggered by the depletion of ER Ca^{2+} stores and activation of plasma membrane ORAI calcium channels by STIM proteins
Ca^{2+} release-activated Ca^{2+} (CRAC) channel	a highly Ca^{2+} selective ion channel located in the plasma membrane that is encoded by ORAI proteins

ion selectivity	the specificity of the channel for a particular species of ions, for example Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} etc; non-selective channels do not discriminate between different types of ions
conductance	a measure of the ability of an ion channel to carry electric charge, measured by the ratio of current divided by the potential difference (voltage); measured in siemens (S)
(Severe) combined immunodeficiency (SCID and CID)	SCID is caused by inherited defects in T cell (and in some cases B cell) development, whereas CID is due to inherited defects in T cell function (but not T cell development). SCID and CID result in severe, often lethal infections in early infancy
nuclear factor of activated T cells (NFAT)	a family of Ca^{2+}-dependent transcription factors that are activated via dephosphorylation by the phosphatase calcineurin; mediate expression of many cytokine genes in lymphocytes
CRAC channelopathy	CRAC channel dysfunction caused by autosomal recessive mutations in *ORAI1* and *STIM1* genes that results in a pathognomonic clinical combination of immunodeficiency, autoimmunity, congenital muscular hypotonia and ectodermal dysplasia with impaired dental enamel calcification and sweat gland dysfunction
membrane potential	difference between the electrical potential between the inside and outside of a cell; typically −60 to −80 mV in resting cells
ion channels	pore-forming transmembrane proteins that enable the flow of ions down an electrochemical gradient
ion transporters	pore-forming transmembrane proteins that carry ions against a concentration gradient using energy, typically in the form of ATP

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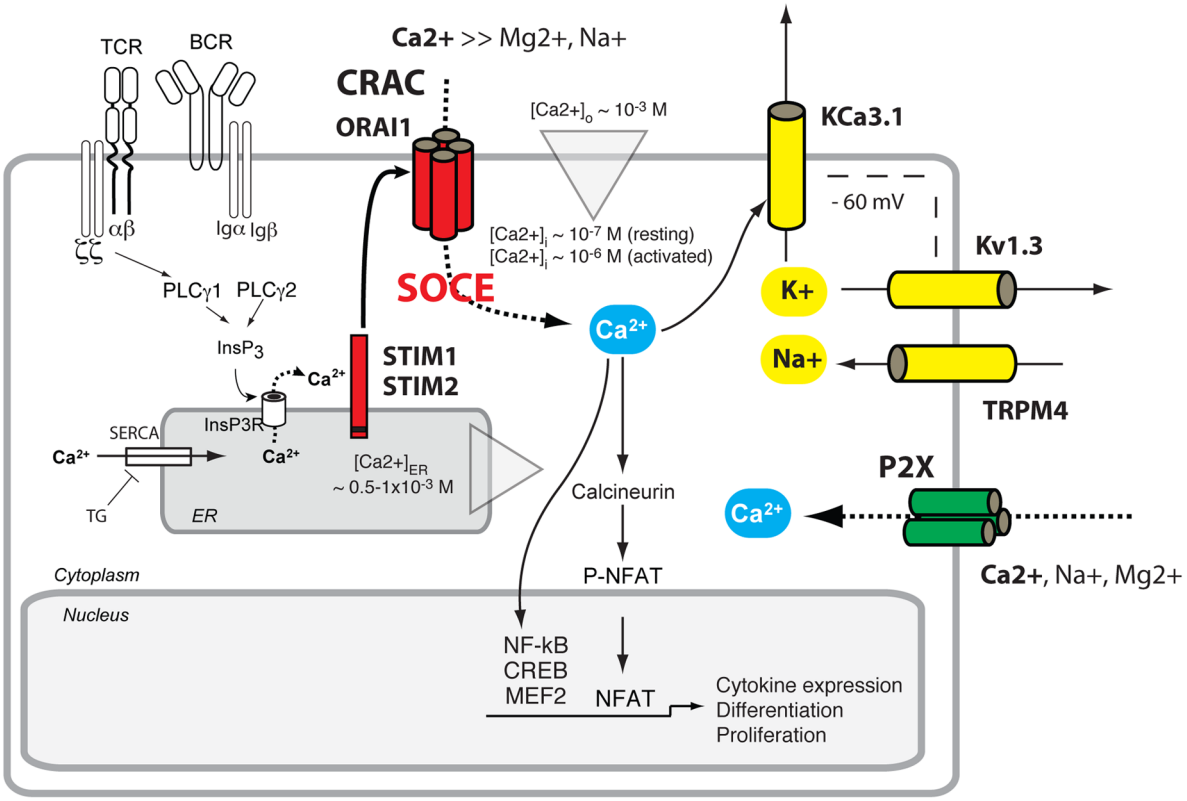

Figure 1. Ion channels regulating Ca^{2+} signalling in lymphocytes

CRAC channels are activated following antigen receptor (TCR, BCR) engagement, which results in the activation of phospholipase Cγ (PLCγ), production of inositol 1,4,5 triphosphate (InsP3) and release of Ca^{2+} from ER Ca^{2+} stores [1, 6, 17]. The ensuing activation of STIM1 and STIM2 results in the opening of ORAI1 CRAC channels and store-operated Ca^{2+} entry (SOCE) (for details see Fig. 2, 3). Sustained Ca^{2+} influx through CRAC channels leads to the activation of Ca^{2+}-dependent enzymes and transcription factors, including calcineurin and NFAT. P2X receptors such as P2X4 and P2X7 are non-selective Ca^{2+} channels activated by extracellular ATP originating, for instance, from autocrine ATP release through pannexin hemichannels (Panx1) [52]. Ca^{2+} influx in lymphocytes depends on the gradient between Ca^{2+} concentrations in the extracellular (~ 1 mM) and intracellular (~ 0.1 μM) compartments and on an electrical gradient established by two K^+ channels, Kv1.3 and KCa3.1, and the Na^+-permeable channel TRPM4 [76, 92]. Abbreviations: SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase.

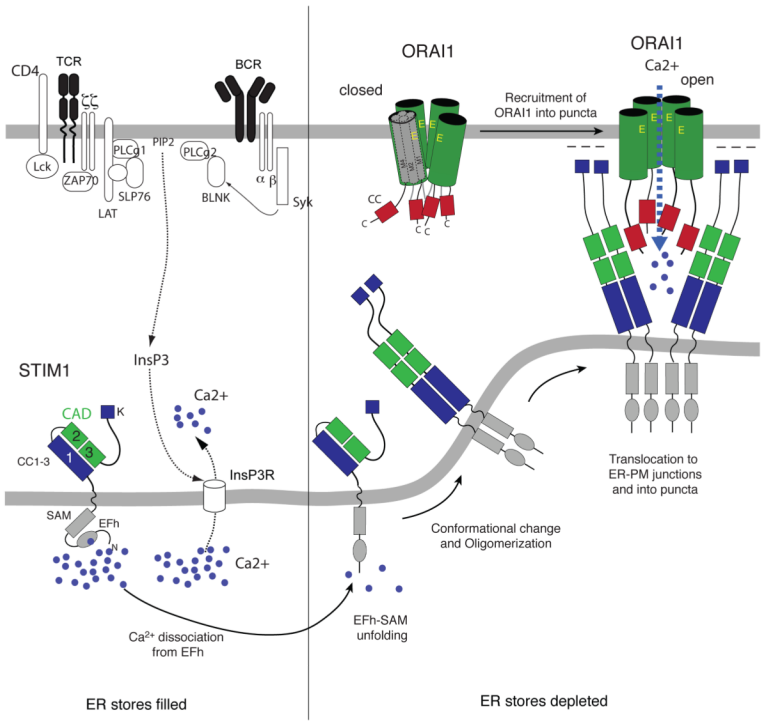

Figure 2. The molecular choreography of CRAC channel activation

In resting lymphocytes, ER Ca^{2+} stores are filled with Ca^{2+} bound to the EF hand Ca^{2+} binding domain in the N-terminus of STIM1. Antigen receptor stimulation causes the activation TCR/BCR-proximal signalling cascades and the production of $InsP_3$, resulting in the release of Ca^{2+} from the ER through $InsP_3$ receptors, which are non-selective ion channels. The fall in ER Ca^{2+} concentration leads to the dissociation of Ca^{2+} from the EF hand domain in STIM1, unfolding of the STIM1 N-terminus and the multimerization of STIM1 proteins [6]. STIM1 multimers translocate to junctional ER sites in which the ER membrane is juxtaposed to the plasma membrane. STIM1 multimers form large clusters (or puncta) into which they recruit ORAI1 CRAC channels. A minimal CRAC channel activation domain (variously referred to as the CAD, SOAR, OASF or CCb9 domain) in the C terminus of STIM1 (green boxes) is necessary and sufficient for ORAI1 binding, CRAC channel activation, and SOCE [29, 184, 186, 187]. This domain contains two coiled (CC) domains, which interact with a CC domain in the C-terminus (red boxes) and additional domains in the N-terminus (not shown) of ORAI1 [27]. Abbreviations: SAM, sterile-alpha motif.

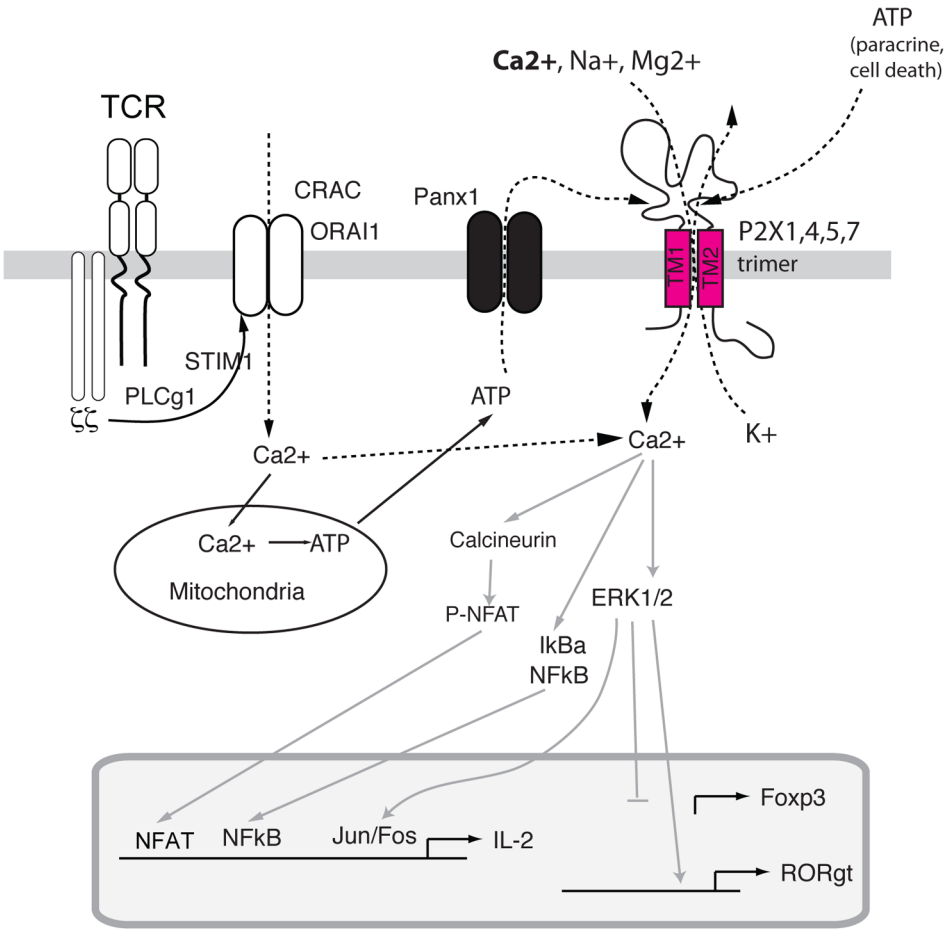

Figure 3. P2X receptors are non-selective Ca^{2+} channels mediating T cell activation

P2X receptors are homotrimeric ion channels located in the plasma membrane of lymphocytes. They form non-selective ion channels that allow influx of Ca^{2+}, Na^{+} and other cations [52, 55]. P2X1, P2X4 and P2X7 are activated by extracellular ATP, for which they have distinct affinities [52]. P2X7 is unusual among P2X receptors, as it functions as a non-selective cation channel at low extracellular [ATP], but forms large pores following prolonged exposure to high extracellular [ATP]. In addition, it was reported to mediate K^{+} efflux required for NLRP3 inflammasome activation in innate immune cells [188]. The ATP required for P2X receptor opening in T cells originates from dying cells, ATP secreting cells (paracrine) or T cells themselves (autocrine). T cells were shown to release ATP through pannexin 1 hemichannels following TCR stimulation and mitochondrial ATP production [58]. Opening of P2X receptors results in Ca^{2+} influx that has been suggested to synergizes with SOCE to activate Ca^{2+} dependent signalling molecules and transcription factors resulting in enhanced cytokine expression. P2X7 dependent ERK1/2 activation was shown to repress FOXP3 transcription in favor of RORγt expression, thereby promoting the differentiation of CD4 T cells into Th17 cells [59].

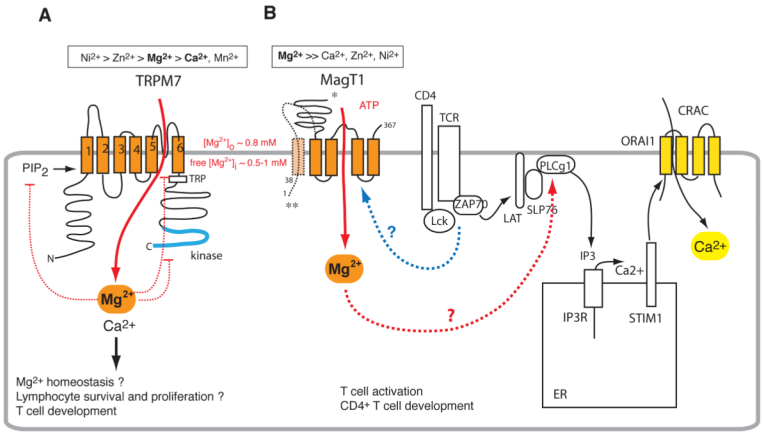

Figure 4. Mg^{2+} channels and transporters in lymphocytes

A, TRPM7 is a Mg^{2+}-permeable channel that is a "chanzyme" because it functions as both an ion channel and an enzyme through its C-terminal serine/threonine kinase domain. As with other TRP channels, its ion channel pore is located between transmembrane (TM) domains 5 and 6. TRPM7 is a non-selective cation channel and conducts Mg^{2+} and Ca^{2+} with near equal permeabilities. One of the defining features of TRPM7 channels is inhibition by intracellular Mg^{2+} but the mechanism of Mg^{2+} regulation is incompletely understood [76]. TRPM7 function further depends on PIP_2 and is regulated by extracellular pH [133]. **B**, MagT1 belongs to a family of recently identified Mg^{2+} transporters. It is highly selective for Mg^{2+} compared to Ca^{2+}, Zn^{2+}, Ni^{2+} and other divalent cations [134]. MagT1 opening in response to TCR stimulation results in a global increase in $[Mg^{2+}]_i$, activation of PLCγ1 and Ca^{2+} influx, presumably via CRAC channels. The mechanisms by which TCR signalling causes MagT1 to open and how Mg^{2+} activates PLCγ1 are not understood. Two MagT1 isoforms have been described: a short one (335 aa) with a confirmed tetraspanning membrane topology (*) [135] and a longer version (367 aa) [126] predicted to contain five TM domains and an intracellular N terminus (**), which may facilitate TCR-dependent activation of MagT1.

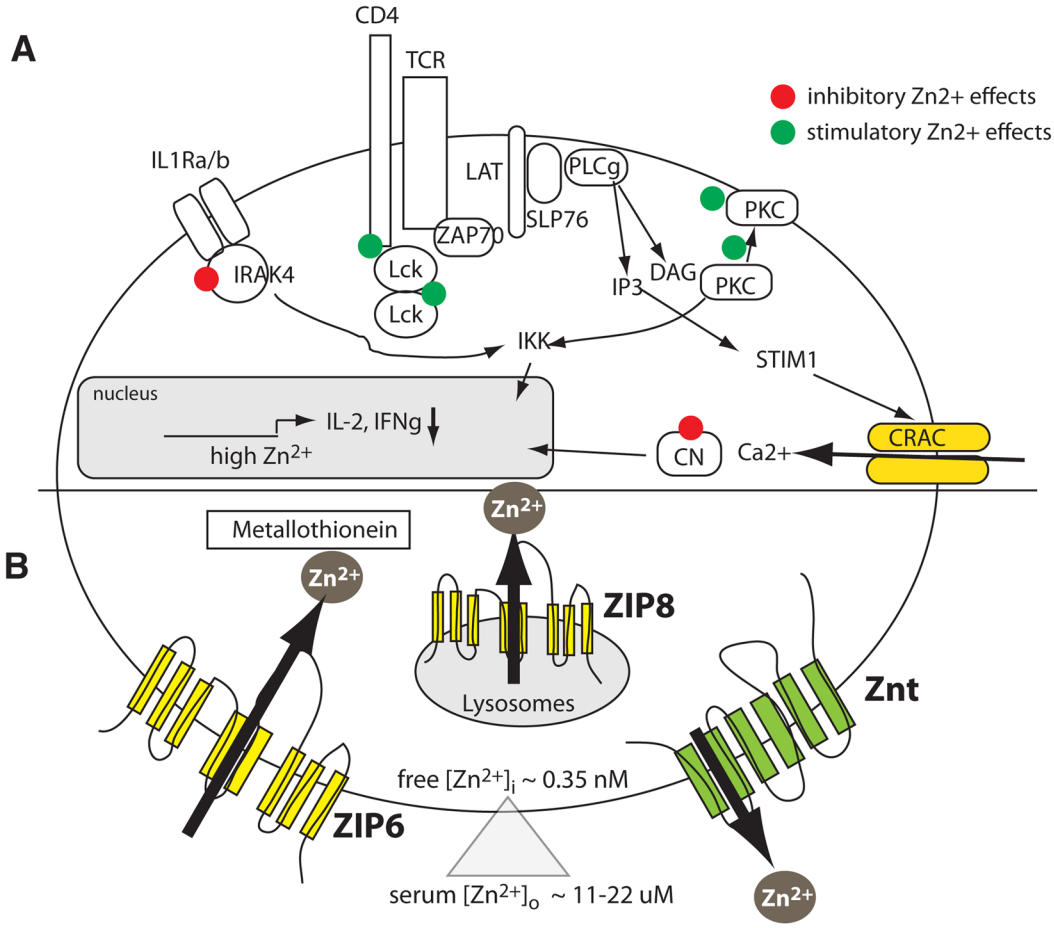

Figure 5. Zinc signalling and Zinc transporters in T cells

A, Zn^{2+} ions have activating and inhibitory effects on signal transduction in T cells [136–138]. Zn^{2+} mediates the recruitment of the src kinase Lck to CD4 and CD8 and promotes Lck dimerization, resulting in enhanced TCR signalling [152]. Zn^{2+} also promotes protein kinase C (PKC) signalling, likely by recruiting PKC to the plasma membrane. By contrast, Zn^{2+} inhibits the activity of the phosphatase calcineurin, thus preventing nuclear translocation of the transcription factor NFAT [153, 154]. Furthermore, Zn^{2+} inhibits the function of interleukin-1 receptor-associated kinase (IRAK) 4, thereby restraining signalling through the IL-1R and activation of NF-kB. Inhibitory effects of Zn^{2+} on both NFAT and NF-kB may explain the reduced production of cytokines such as IL-2 and IFNg in the presence of increasing extracellular [Zn^{2+}]. **B**, Increases in intracellular [Zn^{2+}] in lymphocytes are mediated by Zinc influx from the extracellular space or efflux from intracellular organelles that are mediated by Zrt-Irt like proteins (ZIP). These Zn^{2+} transporters contain eight transmembrane domains (TM) with an aqueous pore predicted to be formed by TM4 and TM5 [189]. Zn^{2+} is exported from the cytoplasm by ZnT transporters resulting in decreased intracellular [Zn^{2+}]. In T cells, the Zinc transporters ZIP3, ZIP6 and ZIP8 have been implicated in Zn^{2+} influx [142, 143, 156], whereas the nature of ZnT proteins mediating Zn^{2+} efflux in lymphocytes are presently unknown. In addition to Zn^{2+} transport, intracellular Zn^{2+} levels are modulated by Zn^{2+} binding to metallothionein and other proteins.

Table 1

Properties and functions of ion channels and transporters in lymphocytes

This table includes the majority of ion channels and transporters reported to be functional or expressed in lymphocytes. Some molecules such as CRAC channels and K^+ channels are well-studied and widely recognized to mediate important roles in lymphocyte function. By contrast, our understanding of the properties and roles of other channels (including TRPC, Ca_V and Cl^- channels as well as Zn^{2+} transporters) is still in infancy and requires further clarification. Nevertheless, the data to date illustrate that lymphocytes use a diverse set of ion transport mechanisms to fine-tune the overall immune response.

Channel	Selectivity	Activation	Function in lymphocytes	Associated channelopathies
Calcium				
CRAC				
ORAI1	Ca^{2+}	Antigen receptor stimulation, depletion of ER Ca^{2+} stores by IP_3, activation of STIM1 and STIM2.	T, B and NK cells *in vitro*: proliferation, cytokine production, cytotoxicity. *In vivo*: immunity to infection, T cell-mediated autoimmunity and inflammation, allogeneic T cell responses; Treg development.	CRAC channelopathy with immunodeficiency and autoimmunity caused by mutations in *STIM1* and *ORAI1*.
ORAI2, ORAI3	Ca^{2+}	(see ORAI1)	t.b.d.	
TRP				
TRPC	Ca^{2+}, Na^+	t.b.d.	t.b.d.	
TRPM2	Ca^{2+}, Na^+	ADPR, cADRP, H_2O_2, NAADP	t.b.d.	
TRPM4	Na^+	Intracellular Ca^{2+}	Depolarization of V_m. Cytokine production.	
Cav				
$Ca_V1.2$, 1.3, 1.4	Ca^{2+}	Activation mechanism following TCR stimulation unknown. Activation not mediated by depolarization. Ca_V function inhibited by STIM1.	Cytokine production, CD8 T cell survival, CD8 T cell immunity to infection, Th2 function in asthma.	
P2X				
P2X7	Ca^{2+}, Na^+, other	Extracellular ATP	T cell proliferation, cytokine production, promotes Th17 and inhibits Treg differentiation.	
P2X1,4	Ca^{2+}, Na^+	Extracellular ATP	T cell proliferation, cytokine production; thymocyte apoptosis.	
Magnesium				
TRPM7	$Ni^{2+} > Zn^{2+} > Mg^{2+}$, Ca^{2+}	Upstream cellular activation mechanism unknown. Regulators include intracellular Mg^{2+}, PIP_2 and extracellular pH	Thymocyte development, production of thymocyte growth factors. Proliferation and survival of DT40 B cells.	
MagT1	Mg^{2+}	TCR stimulation. Activation mechanism unknown.	$CD4^+$ T cell development and activation. Immunity to infection (EBV).	XMEN syndrome caused by X-linked mutations in *MAGT1*.

Channel	Selectivity	Activation	Function in lymphocytes	Associated channelopathies
Zinc				
ZIP3, ZIP6, ZIP8	Zn^{2+} permeable	Activation mechanism unknown. TCR stimulation (ZIP6).	T cell activation (ZIP6). T cell development (ZIP3)?	Acrodermatitis enteropathica with immunodeficiency caused by mutations in intestinal *ZIP4* transporter.
ZnT	Zn^{2+} permeable	t.b.d.	t.b.d.	
Potassium				
$K_v1.3$	K^+	Membrane depolarization	Regulation of V_m. T cell activation (Th17, T_{EM}), cytokine production, T cell-mediated autoimmunity and inflammation.	
$K_{Ca}3.1$	K^+	Intracellular Ca^{2+}	Hyperpolarization of V_m. T cell activation (Th1, Th2, T_{CM}), cytokine production, autoimmune colitis.	
Chloride				
Cl_{swell}	Cl^- (I^-, Br^-)	Molecular identity of channel unknown. Cell swelling activates Cl_{swell} currents.	Apoptosis in T cells.	
CFTR	Cl^-	cAMP	Cytokine production by T cells ?	
$GABA_A$	Cl^-	Extracellular GABA	Inhibition of T cell proliferation, cytokine production, cytotoxicity and T cell-mediated autoimmunity.	

Abbreviations: ADPR, ADP ribose; cADPR, cyclic ADP ribose; cAMP, cyclic adenosine monophosphate; Cav, voltage-gated Ca^{2+} channel; CFTR, Cystic fibrosis transmembrane conductance regulator; CRAC, Ca^{2+} release activated Ca^{2+} channel; GABA, γ-aminobutyric acid; K_V, voltage-gated K^+ channel; K_{Ca}, Ca^{2+} gated K^+ channel; MagT, Mg^{2+} transporter; NAADP, nicotinic acid adenine dinucleotide phosphate; PLC, phospholipase C; STIM, stromal interaction molecule; t.b.d., to be determined; TRP, transient receptor potential; V_m, membrane potential; XMEN, X-linked immunodeficiency with Mg^{2+} defect and EBV infection and neoplasia; ZIP, Zrt-Irt like protein; ZnT, Zinc transporter.