



Published in final edited form as:

Annu Rev Immunol. 2015 ; 33: 291–353. doi:10.1146/annurev-immunol-032414-112212.

Ion Channels in Innate and Adaptive Immunity

Stefan Feske¹, Heike Wulff⁶, and Edward Y. Skolnik^{2,3,4,5}

Edward Y. Skolnik: Edward.Skolnik@nyumc.org

¹Department of Pathology, New York University School of Medicine, New York, NY 10016

²Division of Nephrology, New York University School of Medicine, New York, NY 10016

³Department of Molecular Pathogenesis, New York University School of Medicine, New York, NY 10016

⁴Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY 10016

⁵The Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute for Biomolecular Medicine, New York University School of Medicine, New York, NY 10016

⁶Department of Pharmacology, School of Medicine, University of California, Davis, California 95616

Abstract

Ion channels and transporters mediate the transport of charged ions across hydrophobic lipid membranes. In immune cells, divalent cations such as calcium, magnesium, and zinc have important roles as second messengers to regulate intracellular signaling pathways. By contrast, monovalent cations such as sodium and potassium mainly regulate the membrane potential, which indirectly controls the influx of calcium and immune cell signaling. Studies investigating human patients with mutations in ion channels and transporters, analysis of gene-targeted mice, or pharmacological experiments with ion channel inhibitors have revealed important roles of ionic signals in lymphocyte development and in innate and adaptive immune responses. We here review the mechanisms underlying the function of ion channels and transporters in lymphocytes and innate immune cells and discuss their roles in lymphocyte development, adaptive and innate immune responses, and autoimmunity, as well as recent efforts to develop pharmacological inhibitors of ion channels for immunomodulatory therapy.

Keywords

ion channels; transporters; calcium; magnesium; zinc; potassium; sodium; chloride; CRAC; ORAI; STIM; $K_V1.3$; $K_{Ca3.1}$; TRP; T cells; B cells; macrophages; mast cells; DC; disease; therapy

DISCLOSURE STATEMENT

S. Feske is a cofounder of Calcimedica. H. Wulff is an inventor on the University of California patent claiming PAP-1 for immunosuppression. This patent has been licensed by Airmid, Inc., a start-up company for which H. Wulff is a scientific founder. Airmid has licensed on the PAP-1 patent to Circassia Ltd. (Oxford, UK) for development as a topical treatment for psoriasis.

I. INTRODUCTION

Cells of the innate and adaptive immune systems express various ion channels and ion transporters whose opening allows the influx and efflux of ions across the plasma membrane (PM) or their release from intracellular organelles such as the endoplasmic reticulum (ER), mitochondria, or lysosomes. This review focuses on PM channels and transporters that regulate the influx (Na^+ , Ca^{2+} , Mg^{2+} , Zn^{2+}) or efflux (K^+ , Cl^-) of ions (Table 1; see below). Ion fluxes through channels depend on concentration gradients between the intra- and extracellular space and on the electrical gradient that is established by the negative membrane potential (V_m) in immune cells. By contrast, transporters move ions against concentration gradients using energy, such as in the form of ATP. Here, we review the role of ion channels and transporters in innate and adaptive immune responses by focusing on channels whose function in immune cells in vitro and in vivo is supported by genetic (e.g., knockout mice, RNAi in mammalian cells, and mutations in human patients) or strong pharmacological evidence.

II. ION CHANNELS AND TRANSPORTERS REGULATING IMMUNE FUNCTION

Like all other excitable and nonexcitable cells, lymphocytes express ion channels and transporters to regulate V_m and signaling by Ca^{2+} and other divalent cations as well as physiological cell functions, such as gene expression, apoptosis, proliferation, development, and migration (1). The most-studied event in immune cells involving ion channels is probably the Ca^{2+} influx following receptor activation that regulates the function of many enzymes and transcription factors (2–6). Ca^{2+} influx is associated with oscillations in intracellular calcium concentrations $[\text{Ca}^{2+}]_i$ that are generated by an intricate interplay of multiple channels, including K^+ , Na^+ , and Cl^- channels, that regulate V_m .

Regulation of Membrane Potential

One of the consequences of the Ca^{2+} influx following receptor activation is depolarization of the membrane, which, if left unchecked, limits further Ca^{2+} influx by removing the favorable electrochemical gradient to drive Ca^{2+} influx. Thus, lymphocytes as well other immune cells require K^+ channels that, by effluxing K^+ , maintain a hyperpolarized membrane potential critical for sustaining the gradient for Ca^{2+} entry via Ca^{2+} release-activated Ca^{2+} (CRAC) channels (1). Several K^+ channels have been shown to exist in lymphocytes (Table 1; see below). Of these, the best studied are the voltage-activated $\text{K}_V1.3$ and the Ca^{2+} -activated intermediate-conductance $\text{K}_{Ca3.1}$, the K^+ channels that predominantly regulate membrane potential. Transient receptor potential cation channel, subfamily M, member 4 (TRPM4) is a Ca^{2+} -activated monovalent cation channel that is highly permeable to Na^+ and when activated depolarizes the PM and thereby limits Ca^{2+} influx (7, 8) (Figures 1 and 2).

$\text{K}_V1.3$ and $\text{K}_{Ca3.1}$ —Two seminal back-to-back papers in 1984 describing the expression of voltage-gated K^+ channels in human T lymphocytes first kindled the interest of immunologists in a possible role for ion channels in the immune system (9, 10). Following

its cloning, the K_V channel in human T cells was identified as $K_V1.3$ (11), one of the 40 voltage-gated K^+ channels in the human genome. $K_V1.3$ is a homotetramer of four α subunits, each composed of six transmembrane segments (S1–S6) and a P-loop. PM depolarization is sensed by four arginines localized in the S4 segment, which then results in a conformational change opening the channel. The second major K^+ channel in human T cells, the intermediate-conductance Ca^{2+} -activated K^+ channel $K_{Ca}3.1$, was cloned in 1997 (12). In contrast to $K_V1.3$, $K_{Ca}3.1$ is voltage independent and activated by Ca^{2+} binding to calmodulin, which is constitutively associated with the C terminus of the channel (13). Activation of $K_V1.3$ by depolarization and of $K_{Ca}3.1$ by Ca^{2+} makes physiological sense as it provides a mechanism whereby the initial influx of Ca^{2+} feeds forward, thereby preserving the negative V_m required for sustained Ca^{2+} influx.

Both $K_V1.3$ and $K_{Ca}3.1$ localize to the immunological synapse following T cell receptor (TCR) activation (14), presumably so as to be regulated by kinases that are recruited to the immunological synapse, such as Lck, protein kinase C (PKC) ζ , and PKA for $K_V1.3$ (1) and PKA and the class 2 phosphatidylinositol-3-kinase (PI3K), PI3K-C2 β , for $K_{Ca}3.1$ (15–17). In the case of $K_{Ca}3.1$, TCR activation recruits and activates PI3K-C2 β to the immunological synapse, increasing PI3P concentrations in the PM, thereby enabling the histidine kinase nucleoside diphosphate kinase B (NDPK-B) to activate $K_{Ca}3.1$ by phosphorylating histidine H358 in the C terminus of the channel (16, 18, 19) (Figure 5). Consistent with these findings, the PI3P phosphatase myotubularin-related protein 6 (MTMR6) and the histidine phosphatase phosphohistidine phosphatase-1 (PHPT-1) inhibit $K_{Ca}3.1$ function, TCR-stimulated Ca^{2+} influx, and T cell proliferation by dephosphorylating PI3P and $K_{Ca}3.1$, respectively (20, 21). Thus, regulation of $K_{Ca}3.1$ is currently the best example of reversible histidine phosphorylation in mammals.

TRPM4—TRPM4 is a cation channel that depolarizes the PM. It belongs to the large family of transient receptor potential (TRP) channels (see below) and is unique among TRP channels in that it is more highly permeable to Na^+ than it is to Ca^{2+} (7, 8). It is activated by Ca^{2+} influx following antigen receptor stimulation, resulting in Na^+ influx and PM depolarization, which then limit the magnitude of Ca^{2+} influx and account for the oscillatory pattern of Ca^{2+} transients in lymphocytes (7).

Calcium

Stimulation of antigen receptors results in a rapid increase in $[Ca^{2+}]_i$ originating from two sources: (a) a release from intracellular stores, mainly the ER; and (b) influx from the extracellular space through PM Ca^{2+} channels that is required for sustained $[Ca^{2+}]_i$ elevations (3). Several PM Ca^{2+} channels have been reported to mediate Ca^{2+} influx in immune cells (see Table 1, below).

CRAC channels—CRAC channels are present in most immune cells but were first identified in leukemic mast cells (RBL) and T cells (Jurkat) (22–24). CRAC channel currents are highly selective for Ca^{2+} compared to other cations, are inwardly rectifying, and are activated by depletion of ER Ca^{2+} stores. Because of the latter property, CRAC channels are the prototypical store-operated Ca^{2+} entry (SOCE) channel. Depletion of ER Ca^{2+} stores

results from antigen binding to immunoreceptors including the TCR and BCR, the Fc γ and Fc ϵ receptors, G protein-coupled chemokine receptors, and some innate pattern-recognition receptors such as Dectin-1. Stimulation of these receptors results in activation of phospholipase C isoforms (PLC γ , PLC β) and production of inositol 1,4,5-trisphosphate (IP $_3$) and diacylglycerol. IP $_3$ binding to its receptor causes the opening of IP $_3$ R channels and release of Ca $^{2+}$ from ER stores. Other second messengers—including cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP)—can also release Ca $^{2+}$ from intracellular stores, resulting in SOCE (25).

ORAI1 is the gene encoding the CRAC channel and was identified by forward genetic screens and linkage analysis in human patients with defects in SOCE (see sidebar, Figure 3, and Figure 4) (26–30). Deletion of *ORAI1* abolishes CRAC channel function and SOCE in human T cells and strongly attenuates it in murine T cells and most other immune cells. *ORAI1* is ubiquitously expressed and essential for the function of T cells, mast cells, and other immune cells (31–34). The term CRAC channel, firmly established in the literature before the identification of *ORAI1*, refers to the Ca $^{2+}$ channel with the functional properties described above, but the term ORAI channel is often used alternatively. *ORAI1* is a tetraspanning PM membrane protein (Figures 3 and 4) (35–37). The first transmembrane (M1) domain of *ORAI1* lines the pore and contains several amino acid residues, which define the biophysical properties of the channel, including a glutamate (E106 in human *ORAI1*) that is responsible for Ca $^{2+}$ binding and the high Ca $^{2+}$ selectivity of the CRAC channel (38, 39). *ORAI1* has two homologs, *ORAI2* and *ORAI3*, which are ubiquitously expressed in murine immune cells. Although *ORAI2* and *ORAI3* proteins can form Ca $^{2+}$ channels when ectopically expressed (40), their role in immune responses is not yet understood.

The activation of CRAC channels is mediated by stromal interaction molecules (STIM) 1 and 2, which are located in the ER membrane (see sidebar, Figure 3, and Figure 5) (27, 28). Upon depletion of ER Ca $^{2+}$ stores, Ca $^{2+}$ dissociates from a paired EF hand domain in the ER lumen, resulting in conformational changes first in the N terminus and then in the cytoplasmic C terminus of STIM (41). These structural changes expose domains that allow STIM1 and STIM2 to oligomerize and translocate to the PM, where they form large clusters, or puncta, into which *ORAI1* is recruited, resulting in localized Ca $^{2+}$ influx (reviewed in 42–44). In immune cells, STIM1 is the more important of the two proteins because its deletion strongly reduces SOCE in many immune cells, whereas deletion of STIM2 causes weaker defects and appears to interfere mainly with prolonged Ca $^{2+}$ influx (45). In keeping with the concept that K $_V$ 1.3 and K $_{Ca}$ 3.1 maintain the hyperpolarized PM potential necessary for Ca $^{2+}$ influx, pharmacological inhibition of K $_V$ 1.3 and K $_{Ca}$ 3.1 or genetic deletion of K $_{Ca}$ 3.1 also reduces SOCE (46–48).

Other Ca $^{2+}$ -permeable channels—Although the CRAC channel is the best-characterized Ca $^{2+}$ influx channel, certain TRP channels and P2X receptors contribute to Ca $^{2+}$ influx in immune cells, predominantly in those of the innate immune system.

TRP channels: The 30 mammalian TRP proteins are divided into 6 subgroups based on similar amino acid sequence and domain architecture and include TRPC (canonical), TRPM

(melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPML (mucolipin), and TRPP (polycystin). TRP channels respond to a diverse array of environmental stimuli including temperature, pH, and osmolality, making them uniquely suitable to function as environmental sensors of pain and noxious stimuli by neurons. Cells of the innate and adaptive immune systems also express several TRP channels (49–51), which for the most part are relatively nonselective channels permeable to Ca^{2+} , Na^+ , and other cations. Some are monovalent selective for Na^+ (TRPM4), and others preferentially conduct divalent cations such as Ca^{2+} (TRPM2) and Mg^{2+} (TRPM6, TRPM7) (49). TRPM2 is an example of a Ca^{2+} -permeable, nonselective TRP channel that is highly expressed in macrophages but also in T cells. It is gated by the intracellular second messengers adenosine diphosphate ribose (ADPR) (52, 53), cyclic ADPR (cADPR), and nicotinic acid adenine dinucleotide phosphate (NAADP) (54), which bind to a C-terminal domain related to Nudix family pyrophosphatases (55). TRPM2 is also modulated by high levels of intracellular Ca^{2+} and by oxidative stresses such as H_2O_2 , which activates TRPM2 at least in part through the generation of ADPR. TRPV2 is a weakly Ca^{2+} -selective cation channel that has been proposed to be an endosomal Ca^{2+} release channel and function in endosomal fusion and exocytosis (56). In macrophages, formyl Met-Leu-Phe (fMLP) stimulation and IgG binding to the FcR have been shown to cause TRPV2 translocation to the PM and developing phagosomes, and this translocation plays a role in macrophage chemotaxis and phagocytosis (57, 58).

P2X receptors and pannexin hemichannels: The ionotropic P2X receptors are ligand-gated, nonselective cation channels that are activated by extracellular ATP. Of the seven P2X receptor isoforms, P2RX1, P2RX2, P2RX4, and P2RX7 are expressed on innate and adaptive immune cells (59). P2RX7 is expressed on macrophages, lymphocytes, mast cells, and neutrophils (60, 61) and appears to play a predominant role in inflammation and autoimmune disease (62, 63). P2RX7, like other P2X receptors, is a homotrimer, with each monomer containing two transmembrane domains and an ATP-binding extracellular loop. In response to its ligand ATP, P2RX7 mediates the influx of Ca^{2+} and Na^+ and the efflux of K^+ . The low sensitivity of P2RX7 receptors to ATP limits their activation to conditions in which immune cells or dying cells release high amounts of ATP into the extracellular space (62, 63) and enables them to function as a danger sensor associated with inflammation and tissue damage (64). In addition to ATP release from dying cells, controlled release of ATP from intact cells by pannexin hemichannels is used for purinergic signaling (65). Of the three known pannexin hemichannels, Panx1–3, Panx1 is the most closely associated with immune activation. Panx1 is upregulated in human neutrophils by various stimuli including fMLP, IL-8, C5a, and leukotrienes, and it mediates ATP release from these cells (66).

Voltage-gated Ca^{2+} (Ca_v) channels: Ca_v channels are highly Ca^{2+} -selective channels that mediate Ca^{2+} influx in neurons, cardiomyocytes, and other excitable cells. They are grouped into three subfamilies (Ca_v1 , Ca_v2 , Ca_v3) that contain 10 pore-forming α subunits and many associated regulatory β , γ , and δ subunits (67). Ca_v1 (or L-type) channels and two regulatory subunits ($\beta3$, $\beta4$) have been described in immune cells. TCR-induced Ca^{2+} influx was strongly decreased in CD4^+ and CD8^+ T cells from *Cacna1f*^{-/-} mice lacking expression of $\text{Ca}_v1.4$ (68) and moderately reduced in T cells from *Cacnb3*^{-/-} mice lacking the $\beta3$

subunit (69, 70). In T helper 2 (Th2) cells, Ca^{2+} influx was reduced when $\text{Ca}_V1.2$ and $\text{Ca}_V1.3$ expression was suppressed by RNAi (71). A major gap in our understanding is how Ca_V1 channels are activated in immune cells. In excitable cells, PM depolarization is sensed by the channel's voltage sensor. Except in one study (68), de-polarization of T cells in the hands of most investigators has failed to result in Ca^{2+} currents typical of Ca_V channels and Ca^{2+} influx. In fact, most studies show that TCR-induced Ca^{2+} influx in T cells is reduced, rather than increased, under depolarization conditions, for instance in the presence of elevated extracellular $[\text{K}^+]$ or when $\text{K}_V1.3$ and/or $\text{K}_{Ca3.1}$ are inhibited or deleted, because of the reduced electrical driving force for Ca^{2+} influx through CRAC channels (47, 48, 72). An alternative molecular pathway linking TCR activation to the opening of Ca_V1 channels has not yet been established, and it therefore remains difficult to explain how TCR-induced Ca^{2+} influx via Ca_V channels controls T cell function in Ca_V knockout mice.

Magnesium Channels and Transporters

Mg^{2+} is the most abundant divalent cation in eukaryotic cells and is important for the proliferation and survival of immune cells (reviewed in 73). Although most of the cellular Mg^{2+} is bound to DNA, RNA, and ATP (74), ~5% is free and may function as a second messenger, which is consistent with the transient increases in $[\text{Mg}^{2+}]_i$ observed in T cells after TCR stimulation (75). Immune cells express several Mg^{2+} channels and transporters (see Table 1, below), including Mrs2, SLC41A1, SLC41A2, and TRPM6, but only the Mg^{2+} transporter 1 (MAGT1) and TRPM7 channels have well-documented roles in lymphocyte development and function (73). MAGT1 is widely expressed, including in the spleen, thymus, and many immune cells (76, 77). In T cells, MAGT1 is activated by TCR stimulation, although the mechanisms of activation remain unknown (75). TRPM7 is a Mg^{2+} -permeable, nonselective channel that conducts Ca^{2+} and other divalent cations including Ca^{2+} , Zn^{2+} , and Ni^{2+} (78, 79). TRPM7 is ubiquitously expressed and is thought to regulate cellular and organismal Mg^{2+} homeostasis due to its high Mg^{2+} permeability.

Zinc Transporters

Similar to Ca^{2+} , Zn^{2+} may function as a second messenger in immune cells, given that rapid increases in intracellular $[\text{Zn}^{2+}]$ were observed after stimulation of human and mouse T cells with IL-2 or coculture with dendritic cells (DCs) (80–82). The concentration of free intracellular Zn^{2+} in lymphocytes is estimated to be <1 nM (83), whereas $[\text{Zn}^{2+}]$ in the serum is 10–20 μM (84), resulting in a large Zn^{2+} gradient across the PM. Zn^{2+} homeostasis is controlled by two families of Zn^{2+} transporter proteins, SLC30A (ZnT) and SLC39A (ZIP, Zirr/Irt-like proteins) (85, 86) (see Table 1, below). The ZnT family of transporters consists of 10 members (ZnT1–10) that function to reduce intracellular Zn^{2+} levels. The 14 ZIP transporters are located either in the PM or in the membrane of intracellular organelles, where they function to increase intracellular $[\text{Zn}^{2+}]$ by mediating Zn^{2+} influx or release, respectively (87). In T cells, three Zn^{2+} transporters—ZIP3, ZIP6, and ZIP8—were reported to mediate Zn^{2+} influx (80, 82, 88). Of the 10 ZnT proteins, only ZnT5 has been described to regulate immune function in mast cells (89, 90).

Other Ion Channels

Voltage-gated Na⁺ channels—After one of the early studies investigating the existence of voltage-gated ion channels in human T cells reported small, tetrodotoxin-sensitive Na_V currents in some cells (91), many later studies concluded that human T cells did not express Na_V channels (92). However, more recent studies have reported Na_V currents in 10% of Jurkat T cells (93); in a human CD4⁺ T cell clone in response to stimulation by antigen-presenting cells (APCs) (94); and in murine thymocytes, where Na_V1.5 channels appear to regulate the positive selection of CD4⁺ T cells (95). Na_V1.5 also seems to be expressed in late endosomes of human macrophages, where it regulates endosomal acidification (96), phagocytosis of mycobacteria (97), and myelin degradation in multiple sclerosis (MS) lesions (98). Na_V1.6 has been reported to be important for the activation and phagocytosis of microglia and macrophages in MS and its animal model, experimental autoimmune encephalomyelitis (EAE) (99), whereas Na_V1.7 has been found on immature human DCs (100). As for Ca_V channels, it is currently not understood how Na_V channels are activated in electrically nonexcitable immune cells.

Chloride channels—Several electrophysiological studies in the 1990s reported at least three different, osmotically activated Cl⁻ channels in human T lymphocytes (1) (see Table 1, below). The best-characterized physiological function of these channels is volume regulation in hypotonic environments, and the channels were thus named volume-regulated anion channels (VRAC) or swelling-activated Cl⁻ channels (Cl_{swell}). The molecular identity of VRAC/Cl_{swell} had long been a mystery until two studies using genome-wide RNAi screens identified a four transmembrane domain protein with 17 leucine-rich repeats (LRRs) called LRRC8A or SWELL1 as a putative pore-forming subunit of VRAC (101, 102). Knockdown with shRNA abolished native Cl_{swell} in human CD4⁺ T cells and dramatically reduced hypotonicity-induced volume regulation in several cell types (102). LRRC8A was proposed to be a hexameric channel with a pannexin-like transmembrane topology composed of heteromultimers of LRRC8A and the related LRRC8B, -C, -D, and -E subunits (101).

Human, mouse, and rat T cells also express benzodiazepine-sensitive GABA_A receptors (103, 104), which are heteropentameric ligand-gated Cl⁻ channels. Perfusion of T cells with γ -aminobutyric acid (GABA), the main inhibitory neurotransmitter in the mature mammalian central nervous system (CNS), induces Cl⁻ inward currents and modulates T cell functions in vitro and in vivo, as is discussed in Section IV. Another Cl⁻ channel that reportedly regulates T cell function is the cystic fibrosis transmembrane conductance regulator (CFTR), an ABC transporter that functions as a cAMP-activated ATP-gated Cl⁻ channel. Best known for its multiple mutations that cause cystic fibrosis, CFTR-like Cl⁻ currents were also found in Jurkat T cells, CD4⁺ T cell clones, and Epstein-Barr virus (EBV)-transformed B cells and are defective in T and B cells from cystic fibrosis patients (105, 106).

Hydrogen voltage-gated channels—*HVCN1* codes for the voltage-gated proton channel H_V1 (Table 1, see below), which contains four transmembrane domains and shares homology with voltage-sensing domains in other voltage-gated channels (107–109) but lacks the pore-forming S5–S6 domains. H_V1 functions as a dimer and is the only proton

channel identified in mammals (109). This is consistent with the failure to detect proton currents in cells isolated from *Hvcn1*^{-/-} mice, including neutrophils, macrophages, and B cells (110–112). The primary function of H⁺ extrusion via H_V1 is to balance the intracellular pH following an acid load (107, 113) and to repolarize the PM potential following the outward electron flux during the respiratory burst of innate immune cells, as discussed in Section V under the heading Proton Channels (63). By promoting activation of NADPH oxidase (NOX) and the generation of reactive oxygen species (ROS), H_V1 also promotes signaling by the B cell receptor, as discussed in Section IV under the heading Ion Channels in B Cell Function.

K_{2P} channels—In addition to the K⁺ channels K_V1.3 and K_{Ca}3.1 discussed above, T cells also express the K_{2P} channels K_{2P}3.1 (TASK1), K_{2P}5.1 (TASK 2), and K_{2P}9.1 (TASK3) (114, 115) (Table 1, see below). These channels belong to a family of 15 so-called leak channels: voltage-independent K⁺ channels involved in setting the resting V_m, variously activated by changes in pH, lipid metabolites, stretch, and local anesthetics. K_{2P} channels are dimers of four transmembrane α subunits with two pore domains per subunit. K_{2P}3.1, K_{2P}5.1, and K_{2P}9.1 are reportedly involved in T cell functions such as proliferation and cytokine production (114, 115). K_{2P}5.1 plays a role in T cell volume regulation (116), and its expression is increased following T cell activation and in T cells from MS patients (115). It has further been shown that *K_{2P3.1}*^{-/-} mice are less susceptible to induction of EAE (117), and that K_{2P}3.1 blockers treat EAE (115). However, K_{2P} channels so far have not been demonstrated to regulate Ca²⁺ entry via CRAC channels, and it is currently unknown whether inhibiting K_{2P}3.1 protects via its role in T cells or via direct effects in the nervous system, where it is expressed at high levels (117).

III. ION CHANNELS IN LYMPHOCYTE DEVELOPMENT AND SELECTION

In recent years, data from gene-targeted mice and human patients with single gene mutations have demonstrated important roles of several ion channels and transporters in the development as well as positive and negative selection of T and B lymphocytes.

Development of T Cells

Immature T cells developing from progenitors in the thymus undergo positive and negative selection that shapes their TCR repertoire to ensure both self-MHC restriction and self-tolerance. As discussed below, several Ca²⁺, Mg²⁺, Zn²⁺, and Cl⁻ channels have been shown to regulate T cell development.

Calcium—Ca²⁺ signals are thought to be important modulators of the TCR signaling strength and have been suggested as regulators of T cell development and selection. For instance, engagement of the pre-TCR on thymocytes is associated with a rise in [Ca²⁺]_i (118), and Ca²⁺ oscillations were observed under positively selecting conditions in thymic slices (119). In vitro, negative selection of T cells induced by high-affinity peptide ligands was associated with strong Ca²⁺ influx, whereas positive selection by weak peptide-MHC-TCR interactions induced only moderate Ca²⁺ influx (120, 121). However, a truly causative role for Ca²⁺ signals or specific Ca²⁺ channels in T cell development and selection has been missing.

CRAC channels: Given that Ca^{2+} signals were observed in thymocytes and that CRAC channels play an essential role in mature T cells, it was generally expected that Ca^{2+} influx via CRAC channels would be important in the development and/or selection of thymocytes. However, this is not the case (Figure 6). Immunodeficient patients with loss-of-function or null mutations in *ORAI1* or *STIM1* that abolish TCR-induced Ca^{2+} influx in mature T cells have normal CD4^+ and CD8^+ T cell numbers with a normal TCR V β repertoire (33, 122–125). Likewise, different strains of *Orai1*^{-/-} and *Stim1*^{-/-} mice as well as *Orai1*^{R93W} knock-in mice (expressing a nonfunctional Orai1-R93W mutant that is equivalent to the loss-of-function R91W mutation in patients) all have normal thymic development of CD4^+ and CD8^+ T cells (126–130). Even *Stim1*^{fl/fl} *Stim2*^{fl/fl} *Lck-Cre* mice whose T cells completely lack TCR-induced Ca^{2+} influx show normal development of conventional TCR $\alpha\beta^+$ T cells (131). When crossed to HY TCR-transgenic mice, these mice display a moderate impairment in positive and negative selection but a normal V β repertoire of TCR $\alpha\beta^+$ T cells (132). Together these data indicate that CRAC channels do not play a significant role in the thymic development and selection of T cells. These findings are consistent with normal T cell development in mice lacking $\text{K}_V1.3$ and $\text{K}_{Ca}3.1$ (72, 133, 134). Why CRAC channels are dispensable for the development of conventional TCR $\alpha\beta$ T cells is unclear, but it is possible that still undefined PM Ca^{2+} channels or the release of Ca^{2+} from intracellular stores is sufficient for T cell development.

Consistent with a role for intracellular Ca^{2+} release in T cell development, conditional deletion of all three IP₃R homologs in endothelial and hematopoietic lineage cells with Tie2-Cre resulted in an arrest of thymocyte development at the $\text{CD4}^- \text{CD8}^-$ double-negative (DN) 4 and immature single positive stages (134a). This defect was attributed to a requirement for IP₃R-mediated Ca^{2+} release to suppress expression of Sox13, an antagonist of the Wnt transcription factor Tcf-1 that is essential for DN thymocyte differentiation. In the absence of Ca^{2+} release from ER stores, Sox13 expression is maintained beyond the DN3 stage, and thymocyte development from the DN to the $\text{CD4}^+ \text{CD8}^+$ double-positive (DP) transition is impaired.

In contrast to conventional TCR $\alpha\beta$ T cells, several agonist-selected T cell lineages require CRAC channels for development (Figure 6). Agonist-selected cells escape negative selection in the thymus despite expressing a high-affinity TCR that normally induces apoptosis in DP thymocytes and instead undergo an alternative developmental process that requires strong and sustained TCR signals. These lineages include Foxp3⁺ thymic regulatory T (Treg) cells, invariant natural killer T (iNKT) cells, and CD8 $\alpha\alpha^+$ intestinal intraepithelial lymphocytes (IELs) (135). iNKT cells express an invariant TCR that recognizes lipids derived from pathogens or self-lipids produced by APCs (136, 137). CD8 $\alpha\alpha^+$ IELs are thought to respond to danger signals in the gut and to secrete proinflammatory cytokines, mediate tissue repair, and provide protection from inflammatory bowel disease (IBD). The development of all three T cell subsets is impaired in *Stim1*^{fl/fl} *Stim2*^{fl/fl} *Vav-iCre* mice whose DN and DP thymocytes lack CRAC channel function (132). A strong reduction of thymic nTreg numbers has been observed before in *Stim1*^{fl/fl} *Stim2*^{fl/fl} *Cd4-Cre* mice, which develop a myelo- and lymphoproliferative phenotype with age (131). A similar reduction in Foxp3⁺ Treg and iNKT cell numbers is observed in patients homozygous for autosomal recessive mutations in

STIM1 and *ORAI1* (S. Feske, unpublished data) (123, 125), which is associated with splenomegaly, lymphadenopathy, and autoimmune hemolytic anemia as well as with thrombocytopenia caused by autoantibodies against red blood cells and platelets (see the sidebar above). The postselection maturation of agonist-selected cells is dependent on cytokine signals via IL-2 and IL-15 (135, 138), and the expression of IL-2R α (CD25, unique to the IL-2R) and IL-2R β (CD122, which is part of the IL-2R and IL-15R) was reduced on iNKT cells, IELs, and Treg precursor cells of *Stim1^{fl/fl} Stim2^{fl/fl} Vav-iCre* mice (132). Injection of these mice with agonist IL-2/anti-IL-2 immune complexes restored the development of Foxp3⁺CD25⁺Helios⁺ nTreg cells, suggesting that STIM1/STIM2-dependent CRAC channel function is required for their IL-2-dependent maturation. The effects of CRAC channels are mediated at least in part by the Ca²⁺-dependent transcription factor NFAT (nuclear factor of activated T cells), as *Nfat1^{-/-} Nfat2^{fl/fl} Lck-Cre* mice also show a partial block in thymic nTreg and iNKT cell development (132).

Other calcium channels: Immature T cells in the thymus also express the purinergic receptors P2RX1, P2RX2, P2RX6, and P2RX7. Stimulation of mouse thymocytes with extracellular ATP induces Ca²⁺ influx (139–141) and thymocyte apoptosis (142). These findings suggest that P2X receptors may regulate T cell development, and the pan-P2X receptor antagonist PPADS resulted in enhanced development of DP thymocytes in fetal thymic organ cultures (143). However, T cell development was normal in knockout mice lacking individual P2X receptors including P2RX1, P2RX4, and P2RX7 (144–146), whereas combined deletion of P2RX2 and P2RX3 resulted in marked atrophy or hypocellularity of bone marrow and thymus and lack of lymph follicles in spleen and mesenteric lymph nodes (147), suggesting that P2X receptors may have redundant functions in thymocytes. The L-type voltage-gated Ca²⁺ channel Ca_v1.4 may be involved in the positive selection of CD4⁺ T cells, as *Cacna1f^{-/-}* mice deficient for Ca_v1.4 showed a selective reduction of CD4⁺ single-positive (SP) thymocytes and decreased numbers of CD4⁺ T cells in secondary lymphoid organs, whereas CD8⁺ SP thymocytes were normal (Figure 6) (68).

Magnesium—Genetic evidence from human patients and mice suggests that Mg²⁺ is critical for T cell development. Patients with XMEN disease (X-linked immunodeficiency with Mg²⁺ defect, EBV infection, and neoplasia) due to mutations in *MAGT1* lack Mg²⁺ influx in T cells following TCR stimulation and exhibit mild CD4⁺ lymphocytopenia that in some patients worsens with age and renders patients susceptible to opportunistic infections (see sidebar, above; Table 1; Figure 3; Figure 6) (75, 148). In contrast to CD4⁺ T cells, the numbers of CD8⁺ T cells and B cells were normal in *MAGT1*-deficient patients (75). A much more severe defect in T cell development was observed in mice lacking the nonselective Mg²⁺ channel TRPM7. Whereas complete deletion of TRPM7 in mice is prenatally lethal (E6.5), T cell–specific deletion in *Trpm7^{fl/fl} Lck-Cre* mice resulted in viable mice that showed a block of T cell development at the DN stage (Figure 6) (149). *Trpm7^{fl/fl} Lck-Cre* mice had reduced numbers of medullary thymic epithelial cells, which was attributed to impaired expression of the thymocyte-derived growth factors FGF7, FGF13, and midkine (149). Besides LRRC8A (see below), TRPM7 has the most profound effect on T cell development of all PM ion channels or transporters known to date. It is tempting to speculate that Mg²⁺ influx through TRPM7 is essential for T cell development, but this

interpretation is complicated by the fact that Mg^{2+} influx and total cellular Mg^{2+} content were normal in thymocytes from *Trpm7^{fl/-} Lck-Cre* mice despite reduced TRPM7 currents (149). TRPM7 is also able to conduct Ca^{2+} and trace metals such as Ni^{2+} and Zn^{2+} (79, 149), and it is therefore conceivable that TRPM7 mediates its effects on T cell development through other divalent cations.

Zinc channels—Thymus atrophy and lymphopenia are part of the clinical spectrum of the inherited Zn^{2+} malabsorption syndrome acrodermatitis enteropathica (150, 151), which is caused by mutations in the Zn^{2+} transporter *ZIP4* (152, 153). Although *ZIP4* is not expressed in thymocytes, thymic atrophy is thought to be due to increased systemic glucocorticoid production and apoptosis of glucocorticoid-sensitive immature T cells (151). This interpretation is supported by the fact that the acrodermatitis enteropathica phenotype is largely recapitulated in mice with conditional deletion of *ZIP4* in intestinal epithelial cells, although lymphocyte development was not studied (154). Reduced frequencies of DP thymocytes were observed in *Zip3^{-/-}* mice that were fed a Zn^{2+} -deficient diet during pregnancy or at weaning (88). The frequencies of $CD4^+$ and $CD8^+$ SP thymocytes were increased in *Zip3^{-/-}* mice, potentially due to an accelerated maturation of T cells. It is unclear if *ZIP3* has a T cell–intrinsic role in lymphocyte development because only mice with global deletion of *ZIP3* were studied.

Sodium channels—The voltage-gated Na^+ channel $Na_v1.5$ (*SCN5A*) and its regulatory subunit $Na_v\beta4$ (*SCN4B*) were recently shown to be required for the positive selection of $CD4^+$ T cells that express a transgenic TCR specific for moth cytochrome *c* (MCC) (95). Both genes are expressed most prominently in DP thymocytes but not in peripheral $CD4^+$ or $CD8^+$ T cells. Inhibition of $Na_v1.5$ channel function reduced sustained Ca^{2+} influx induced by a weak TCR ligand and impaired the positive selection of TCR transgenic thymocytes in reaggregate thymic cultures and of $CD4^+$ SP thymocytes in recipient mice (Figure 6). Conversely, expression of $Na_v1.5$ in peripheral $CD4^+$ T cells that normally do not respond to weak peptide ligand stimulation enhanced their Ca^{2+} response, suggesting that $Na_v1.5$ amplifies Ca^{2+} signals emanating from weak TCR-peptide-MHC interactions and thereby promotes positive selection. $Na_v1.5$ currents were not investigated in this study, and it remains unclear how $Na_v1.5$ enhances Ca^{2+} influx because Na^+ influx is expected to cause PM depolarization, which decreases, rather than increases, Ca^{2+} influx through CRAC channels. This study raises the possibility that $Na_v1.5$ channel–mediated depolarization of DP thymocytes promotes Ca^{2+} influx by activating Ca_v channels, which would be consistent with the finding that *Cacna1f^{-/-}* ($Ca_v1.4$) mice exhibit selective reduction in $CD4^+$ SP and not $CD8^+$ thymocytes (68, 95). Direct electrophysiological recordings of Ca_v and Na_v currents in thymocytes, however, are required to solidify a potential role for these channels in T cell development.

Chloride channels—Recently, the Cl^- channel subunit *LRRC8A* was reported to be critical for T cell development because *Lrrc8a^{-/-}* mice displayed a ~10-fold reduction in overall thymic cellularity with decreased numbers of DN, DP, and SP thymocytes (154a). This defect was T cell intrinsic, as it could also be observed in *Lrrc8a^{-/-} → Rag2^{-/-}* bone marrow chimeras, and has been attributed to decreased proliferation and increased apoptosis

of LRRC8A-deficient thymocytes. Lack of LRRC8A was associated with reduced AKT phosphorylation in thymocytes, likely accounting for impaired thymocyte survival. In contrast to reduced numbers of conventional T cells, those of Foxp3⁺ Treg cells were increased in the thymus of *Lrrc8a*^{-/-} mice. Since the lymphocyte development defect of *Lrrc8a*^{-/-} mice was reported (154a) at the same time as the discovery of LRRC8A as the putative pore-forming subunit of VRAC (101, 102), a mechanistic understanding of how this volume-regulated Cl⁻ channel regulates T cell development remains to be investigated. Intriguingly, the induction of T cell apoptosis by cross-linking of Fas (CD95) had been shown to trigger the activation of an outwardly rectifying Cl⁻ channel (154b), suggesting that T cell apoptosis may be regulated by Cl⁻ channels.

Development of B Cells

Compared to their role in T cell development, the role of ion channels in B cell development is less well understood. The strength of BCR signaling is a critical determinant of positive selection of B cells and the elimination of potentially autoreactive B cells. Stimulation of the BCR results in the opening of CRAC channels and SOCE. Human patients with mutations in *ORAI1* or *STIM1* genes have reduced or abolished Ca²⁺ influx after direct store depletion or BCR cross-linking (124, 155). Likewise, B cells from *Orai1*^{-/-} mice and from *Stim1*^{fl/fl} *Stim2*^{fl/fl} *Mb1-Cre* mice with conditional deletion of STIM1/2 in B cells lack Ca²⁺ influx following BCR cross-linking (126, 156). Intriguingly, ORAI1, STIM1, and STIM2 are not essential for the development of B cells, given that patients with mutations in *ORAI1* and *STIM1* had normal numbers of B cells (33, 125) and that B cell populations in the bone marrow and secondary lymphoid organs were normal in *Orai1*^{-/-}, *Orai1*^{R93W} knock-in and *Stim1*^{fl/fl} *Stim2*^{fl/fl} *Mb1-Cre* mice (126, 127, 156). Although endogenous STIM1 is not required for B cell development, when STIM1 is ectopically expressed it can regulate negative selection of B cells in the bone marrow by enhancing Ca²⁺ flux via activation of PKC δ and Erk signaling (157). Altered selection of B cells was also observed in mice lacking Ins(1,4,5)P3 3-kinase B (*Itpkb*), which converts IP₃ to inositol-1,3,4,5-tetrakisphosphate (IP₄). Increased SOCE after BCR stimulation in *Itpkb*^{-/-} mice was associated with reduced numbers of all B cell subsets in the spleen, including T1 and T2 B cells, mature follicular B cells, and marginal zone B cells (158). Enhanced Ca²⁺ influx in B cells overexpressing STIM1 or lacking *Itpkb* therefore appears to alter the development of B cells in favor of B cells normally selected by strong BCR signals such as B-1 cells and CD138⁺ mature B cells (158).

Two recent discoveries have shed new light on the role of ion channels and transporters in B cell development. The first is the finding that the Zn²⁺ transporter ZIP10 regulates early B cell development. B cell-specific deletion of ZIP10 in mice resulted in spleen atrophy, reduced serum Ig levels, and decreased numbers of B cell progenitors in the bone marrow and B cells in secondary lymphoid organs (159). Decreased Zn²⁺ influx via ZIP10 was associated with increased caspase activation and apoptosis of pro-B cells. In humans, an autosomal form of congenital agammaglobulinemia with a lack of circulating B cells results from a heterozygous truncation mutation in LRRC8A, which deletes several of the C-terminal LRR domains of this Cl⁻ channel (160). Similarly, expression of the truncated protein in murine bone marrow inhibited B cell development (160). The profound effects of

the LRRC8A mutation on B cell development were reported before the more recent identification of LRRC8A as the putative pore-forming subunit of VRAC (101, 102). Complete deletion of this Cl⁻ channel in *Lrrc8a*^{-/-} mice resulted in a more modest impairment of B cell development (compared to human patients) with reduced (but not abolished) frequencies of pre-B cells, immature B cells, and recirculating B cells in the BM and decreased numbers of B220⁺ B cells in the spleen (154a). In contrast to impaired B cell development, the function of residual LRRC8A-deficient B cells appeared normal. The role of the other LRRC8 subunits (B, C, D, and E) in B cell development and function remains to be determined. These findings demonstrate the unexpectedly profound impact of Cl⁻ and Zn²⁺ fluxes on B cell development.

IV. ION CHANNELS IN ADAPTIVE IMMUNE RESPONSES

Ion Channels in T Cell–Mediated Immunity

In keeping with ion fluxes playing important roles in T cell signaling, numerous studies in recent years have shown that genetic deletion or mutation of several ion channels can have profound effects on T cell functions.

CRAC channels—CRAC channels are essential for T and B cell function, as is evident from the SCID-like syndrome in patients with null or loss-of-function mutations in *ORAI1* and *STIM1* (Figure 3). Patients with CRAC channelopathy (31) are susceptible to life-threatening infections with bacteria, fungi, and viruses, in particular herpes viruses including cytomegalovirus, EBV, and herpes simplex virus (26, 122–125, 161, 162). CD4⁺ and CD8⁺ T cells from ORAI1- and STIM1-deficient patients lack CRAC channel function and Ca²⁺ influx in response to TCR stimulation (26, 33, 123, 125, 163). Targeted deletion of *Orai1*, *Stim1*, and *Stim2* genes in mice confirms their essential role. CD4⁺ and CD8⁺ T cells isolated from STIM1- or STIM1/2-deficient mice lacked SOCE (128, 131, 164), whereas T cells from *Orai1*^{-/-} and *Orai1*^{R93W} knock-in mice had strongly impaired SOCE when isolated ex vivo and abolished SOCE when differentiated into CD4⁺ Th cells or CD8⁺ cytotoxic lymphocytes in vitro (126, 127, 131). The residual Ca²⁺ influx in ORAI1-deficient murine T cells may point to a role for ORAI2 and ORAI3 in CRAC channel function, although there is no direct evidence yet.

One of the initial observations in CRAC channel–deficient patients was the impaired ability of their T cells to proliferate in response to TCR stimulation and mitogens (33, 122, 124, 125). In mice, a proliferation defect was only apparent in STIM1/2 double-deficient T cells, whereas individual deletion of STIM1, STIM2, or ORAI1 had no effect (126–128, 131), indicating that ORAI and STIM homologs may have more redundant functions in mouse than in human T cells. Ca²⁺ influx via CRAC channels is proapoptotic, as ORAI1- and STIM1-deficient CD4⁺ T are more resistant to apoptotic cell death upon repeated TCR stimulation (S. Feske, unpublished observations) (165), which is likely to contribute to the lymphoproliferative phenotype observed in STIM1/2-deficient mice and ORAI1- and STIM1-deficient patients (33, 125, 131).

An essential function of Ca²⁺ influx through CRAC channels is the regulation of cytokine and chemokine gene expression, primarily via the calcineurin-NFAT pathway (3, 166). Gene

expression in ORAI1-deficient human T cells is severely dysregulated, with a profound defect in key T cell cytokines such as IL-2, IL-4, IL-10, IFN- γ , and TNF- α (155, 167). Similar defects in the expression of cytokines, chemokines, and apoptosis genes are found in CD4⁺ and CD8⁺ T cells from ORAI1-, STIM1-, and STIM1/2-deficient mice (126, 127, 131, 165, 168–170).

CD4⁺ T cell-specific functions of CRAC channels: Naive CD4⁺ T cells differentiate into various Th lineages that are characterized by the expression of lineage-determining transcription factors and production of signature cytokines such as T-bet and IFN- γ (Th1 cells), GATA3 and IL-4 (Th2 cells), and ROR γ t and IL-17 (Th17 cells). These lineages play important roles in immune responses to pathogens, in autoimmunity, and in allergic diseases. The role of CRAC channels in Th cell differentiation is not well understood. T cells from *Orai1*^{-/-} mice had reduced expression of the Th17 lineage-specific transcription factors ROR γ t and ROR α (168), whereas mRNA and protein levels of ROR γ t, ROR α , and IRF4 were normal in STIM1- or STIM2-deficient T cells (164). In the latter study, lack of STIM1 did, however, result in impaired expression of IL-21 and IL-23R (164), which mediate cytokine signals required for the terminal differentiation of autoreactive Th17 cells (171, 172). Irrespective of their role in Th differentiation, CRAC channels control the expression of effector cytokines by all Th lineages.

Consistent with these reports is the finding that CRAC channels contribute to T cell-mediated autoimmunity and hypersensitivity in a number of rodent models of disease. Skin contact hypersensitivity reactions to haptens that mediate a strong Th2 cell response (173) were attenuated in *Orai1*^{KI/KI} mice (127), suggesting that CRAC channels may also be important for other Th2-mediated immune diseases such as allergic asthma (see Section VII). Th1 and Th17 cells synergize to mediate acute graft-versus-host disease (GvHD) (174), and adoptive transfer of CD4⁺ T cells from *Stim1*^{-/-} mice into MHC-mismatched BALB/c mice resulted in reduced mortality of recipient mice from acute GvHD (128). More strikingly, ORAI1- and STIM1-deficient CD4⁺ T cells failed to induce disease in rodent models of Th1- and Th17-dependent autoimmune diseases such as IBD and EAE. Adoptive transfer of SOCE-deficient CD4⁺ T cells from *Orai1*^{R93W} and *Stim1*^{fl/fl} *Cd4-Cre* mice failed to induce IBD in recipient mice (127). In this model, transferred naive CD4⁺ T cells differentiate into Th1 and Th17 cells in the gut and cause intestinal inflammation (175). Although the numbers of SOCE-deficient CD4⁺ T cells were normal in the gut of host mice, they failed to produce IL-17A, IL-17F, and IFN- γ after restimulation in vitro (127; S. Feske, unpublished observations). STIM1-deficient mice were also resistant to induction of EAE, a rodent model of MS (164, 169). STIM2-deficient mice showed partial protection from EAE consistent with only partially reduced IFN- γ and IL-17 production compared to a complete defect in STIM1-deficient T cells (164).

In addition to supporting the thymic development of nTreg cells (see Section III), CRAC channels are required for the suppressive function of Treg cells. Foxp3⁺CD25⁺ nTreg cells from STIM1/2 double-deficient mice failed to inhibit the proliferation of CD4⁺ T cells in vitro (131). By contrast, nTreg cells from *Orai1*^{fl/fl} *Lck-Cre* (176), *Stim1*^{-/-} (128), and *Orai1*^{R93W} mice (127) had no or only a moderate defect. In addition, the differentiation of naive CD4⁺ T cells into induced Foxp3⁺ Treg (iTreg) cells, which develop outside the

thymus in the intestine or during chronic inflammation (177), was normal in the absence of ORAI1 (176). These findings suggest that residual SOCE in the absence of ORAI1 or STIM1 is sufficient for nTreg function and the differentiation of iTreg cells.

Ca²⁺ signals also mediate T cell motility. Chemokine receptor signaling activates PLCβ and SOCE, and the chemotaxis of ORAI1-, STIM1-, or STIM1/2-deficient CD4⁺ T cells in vitro is impaired (164, 178). By contrast, the role of CRAC channels in T cell homing to lymphoid organs in vivo remains ambiguous. Whereas homing of adoptively transferred ORAI1-deficient T cells to lymph nodes was impaired in one study (178), no such defect was observed for naive STIM1- or STIM1/2-deficient CD4⁺ T cells in another study (179). However, the spatiotemporal regulation of T cell–APC interaction in lymph nodes may be dependent on CRAC channels. An elevation of [Ca²⁺]_i induced the arrest of mature T cells and thymocytes during the formation of stable T cell–APC contacts (119, 180), and ORAI1-deficient TCR transgenic CD4⁺ T cells showed a delayed onset of arrest in lymph nodes following antigen injection (179).

CD8⁺ T cell–specific functions of CRAC channels: The release of cytolytic granules by CD8⁺ T cells and their cytotoxic function have long been known to be dependent on Ca²⁺ signals (181, 182), but it is not fully understood which channels mediate Ca²⁺ influx or release and which steps in these processes—including granule polarization toward the lytic immunological synapse, docking, or PM fusion—are Ca²⁺ regulated. Complete inhibition of SOCE in CD8⁺ T cells from STIM1/2-deficient mice impairs lytic granule exocytosis and killing of tumor cells and virus-infected cells (170, 183), whereas partial reduction of SOCE in *Orai1^{R93W}* mice has no effect on the cytolytic function of CD8⁺ T cells in vitro (S. Feske, unpublished observations). Similar quantitative requirements for SOCE in cytotoxicity were observed in human and mouse CD8⁺ T cells treated with CRAC channel inhibitors, suggesting that moderate SOCE is sufficient for cytolytic CD8⁺ T cell function (170, 184). This may explain why the cytolytic function of CD8⁺ T cells from STIM1-deficient patients was normal (123). However, STIM1-deficient natural killer (NK) cells from the same patient as well as from patients with an ORAI1 mutation had a severe defect in degranulation and killing of target cells (184). This finding indicates that the quantitative SOCE requirements for NK and CD8⁺ T cell cytotoxicity may differ or that other Ca²⁺ channels may compensate for lack of ORAI1 or STIM1 in CD8⁺ T cells.

The important role of CRAC channels for CD8⁺ T cell–mediated cytotoxicity is emphasized by the recurrent viral infections and virus-associated malignancies (including EBV-associated B cell lymphoma and human herpes virus 8–associated Kaposi sarcoma) in ORAI1- and STIM1-deficient patients (33, 161, 185) and by impaired antitumor responses in STIM1/2-deficient mice that fail to effectively control tumor cell engraftment and growth in vivo (170). Besides controlling CD8⁺ T cell effector functions, CRAC channels also control the maintenance of virus-specific memory CD8⁺ T cells and their ability to mediate recall responses to secondary infection (183). *Stim1^{fl/fl} Stim2^{fl/fl} Cd4-Cre* mice infected with lymphocytic choriomeningitis virus (LCMV) developed chronic viral infection, which was associated with defective maintenance of memory CD8⁺ T cells. The latter was due to the impaired function of STIM1/2-deficient CD4⁺ T cells and their inability to express CD40L, activate DCs, and support the production of LCMV-specific antibodies. In the

absence of STIM1 and STIM2, residual memory CD8⁺ T cells were nonfunctional and failed to mount a recall response to LCMV reinfection. CRAC channels therefore play distinct but synergistic roles in CD4⁺ and CD8⁺ T cells during antiviral immunity.

Other calcium-permeable channels—Although TCR- and BCR-induced Ca²⁺ influx is largely abolished in T and B cells lacking ORA1, STIM1, or both STIM1/2, other Ca²⁺ channels such as Ca_v channels, P2X receptors, and some TRP channels have been proposed to mediate Ca²⁺ influx and T cell functions.

Voltage-gated Ca²⁺ channels: Ca_v1.4-deficient murine CD4⁺ and CD8⁺ T cells had impaired TCR-induced Ca²⁺ influx and showed a strong survival defect after TCR and IL-7 stimulation in vitro, which was associated with increased apoptosis and a relative loss of naive CD44^{lo} T cells in vivo (68). *Cacna1f*^{-/-} mice also failed to mount an effective antigen-specific CD8⁺ T cell response to *Listeria monocytogenes* (68). TCR-induced Ca²⁺ influx, cytokine production, and total numbers of CD8⁺ T cells in the spleen and lymph node were also moderately reduced in *Cacnb3*^{-/-} mice that lack the β3 regulatory subunit of Ca_v channels (70). The absence of β3 in naive CD8⁺ T cells resulted in impaired Ca_v1.4 protein expression, suggesting that Ca_v1.4 and β3 may form a Ca²⁺ channel complex (70). Two other L-type Ca²⁺ channels, Ca_v1.2 and Ca_v1.3, may play a role in Th2 cells, given that their depletion by RNAi impaired TCR-induced Ca²⁺ influx and IL-4 production in vitro and reduced airway inflammation in a passive transfer mouse model of allergic asthma (71, 186). However, immune dysregulation is not a significant phenotype in human patients with loss-of-function mutations in Ca_v1.2 and Ca_v1.4 channels (187–189) or in patients treated with L-type Ca_v channel blockers such as nifedipine and verapamil; the importance of Ca_v channels for adaptive immune responses therefore remains unclear.

P2X receptors: Studies of T cell function in vitro using P2X receptor inhibitors and RNAi have linked Ca²⁺ influx via P2RX1, P2RX4, and P2RX7 to the TCR-induced activation of calcineurin and NFAT, to the production of IL-2, and to T cell proliferation (190–193). Besides responding to ATP released from other cells, T cells themselves release ATP through Pannexin-1 hemichannels that colocalize with P2RX7 (193, 194). This autocrine ATP signaling may reinforce weak TCR activation and T cell effector functions (63). An important role in particular for P2RX7 receptors in vivo is supported by studies using P2RX7 agonists, antagonists, and knockout mice. Injection of mice with oxidized ATP (oATP), a pan-P2X receptor inhibitor, prevented the development of IBD and diabetes in adoptive T cell transfer models (194). In both cases, oATP treatment strongly impaired production of IFN-γ and TNF-α by proinflammatory CD4⁺ T cells. In a follow-up study using the P2RX7 agonist BzATP, the same authors showed that P2RX7 promotes the differentiation of Th17 cells while inhibiting the stability of Treg cells, whereas oATP-mediated P2RX7 inhibition in vivo strongly increased Treg numbers in mesenteric lymph nodes and prevented the development of IBD (195). Consistent with a role for P2RX7 as a regulator of Th17/Treg differentiation, two allotransplant studies showed that P2RX7 inhibition with oATP promotes the long-term survival of fully mismatched cardiac allografts (196) and delayed the rejection of pancreatic islet allografts (197). The interpretation of these results is complicated, however, by the fact that oATP inhibits not only P2RX7 but also

other P2X receptors. Furthermore, the role of P2RX7 in T cells during EAE is ambiguous, given that both reduced (198) and enhanced (199) disease severity was reported in different strains of *P2rx7*^{-/-} mice. The divergent results may be due to retained P2RX7 expression in T cells in one mouse strain (198, 200). Adding further confusion, splenocytes isolated from *P2rx7*^{-/-} mice that had decreased EAE severity produced more, not less, IFN- γ and IL-17 (199). Thus, although efficacy of inhibition is seen in many of these models, it is not always clear which immune cell and P2X receptor is being inhibited.

TRP channels: Only a few TRP channels appear to contribute significantly to lymphocyte function. Besides TRPM4 and TRPM7, which are discussed with Na⁺ and Mg²⁺ influx channels below, the other TRP channels implicated in lymphocyte function are Ca²⁺-permeable nonselective cation channels. Before the identification of ORAI1 (26, 29, 30), several TRP channels, including TRPC3, were considered candidate genes for the CRAC channel, but given the solid genetic evidence linking ORAI and STIM proteins to CRAC channel function in humans and mice, TRPC channels are no longer considered part of the CRAC channel or to be activated directly by Ca²⁺ store depletion, although they may mediate other forms of Ca²⁺ influx (201). Whereas RNAi knockdown of TRPC3 in CD4⁺ T cells has only marginal effects on Ca²⁺ influx and proliferation (202), TRPC5 is upregulated in activated CD4⁺ and CD8⁺ effector T cells and mediates Ca²⁺ influx after binding of surface GM1 ganglioside by galectin-1 on Treg cells, potentially contributing to Treg-mediated suppression (203). The Ca²⁺-selective TRPV5 and TRPV6 channels appear to be expressed in human blood lymphocytes and Jurkat T cells, but *Trpv6*^{-/-} mice have no reported immunological phenotype (204), and TCR-induced Ca²⁺ influx in CD4⁺ T cells from *Trpv6*^{-/-} mice is normal (S. Feske, unpublished observations). Somewhat more substantial evidence supports a role for the Ca²⁺-permeable TRPM2 channel in T cells, which is upregulated after TCR stimulation (202). TRPM2 is activated by ADPR (52, 53), cADPR, and NAADP (54), and TCR stimulation results in increased cADPR concentrations. However, although a role for TRPM2 in innate immunity is well documented (see Section V), information about its role in lymphocyte function is more limited. A cADPR antagonist inhibited TCR-induced T cell proliferation and expression of CD25 and HLA-DR (205). In addition, the severity of EAE was moderately reduced in *Trpm2*^{-/-} mice, and this protection was associated with impaired TCR-stimulated proliferation as well as IFN- γ and IL-17 production by TRPM2-deficient CD4⁺ T cells (206). Although these findings suggest a role for TRPM2 in proinflammatory CD4⁺ T cells, attenuated EAE in *Trpm2*^{-/-} mice could also be due to deletion of TRPM2 in innate immune cells, microglia, and cells of the CNS.

Channels regulating membrane potential—Unlike other TRP channels, TRPM4 is much more permeable to Na⁺ than to Ca²⁺. Besides K⁺ channels, TRPM4 is therefore essential for the regulation of V_m and, thereby, indirectly Ca²⁺ influx (7, 8). Opening of the Ca²⁺-activated TRPM4 at the peak of Ca²⁺ influx in T cells results in Na⁺ influx, which then depolarizes V_m, limits Ca²⁺ influx, and generates an oscillatory pattern of Ca²⁺ transients in lymphocytes (7). Expression of a dominant-negative TRPM4 or knockdown of TRPM4 by RNAi in Jurkat T cells transformed the oscillatory pattern of Ca²⁺ influx into a prolonged and sustained Ca²⁺ response, leading to increased IL-2 secretion (7). Similar findings were made in Th2 cells, where TRPM4 knockdown led to sustained Ca²⁺ influx and increased

nuclear localization of NFATc1 (207). These in vitro studies point to an important role for TRPM4 in modulating T cell activation. However, in vivo validation of a role for TRPM4 in disease models has not yet been reported.

The two K⁺ channels essential for lymphocyte function are the voltage-gated K_V1.3 and the Ca²⁺-activated K_{Ca}3.1 channel. In T cells, the expression pattern of K_V1.3 and K_{Ca}3.1 changes with the activation and differentiation status. The primary role of K_V1.3 as originally described in human T cells is to maintain a negative V_m of roughly -60 mV and to support Ca²⁺ influx through CRAC channels (48, 208). Under resting conditions, naive human T cells express predominantly K_V1.3 channels and, as a result, depend on K_V1.3. Following activation, naive human T cells transcriptionally upregulate K_{Ca}3.1 (209) to sustain the more intense Ca²⁺ signaling events in activated T cells (210, 211). Pharmacological inhibition of K_V1.3 and K_{Ca}3.1 suppresses Ca²⁺ signaling, T cell proliferation, and IL-2 production in human T cells (1, 48, 208, 209). However, what was initially confusing to the field were observations that mouse T cells do not rely on K_V1.3 to set their V_m (212) and express additional K_V channels. Whereas resting human, primate, or pig T cells on average express ~300 K_V1.3 channels per cell (209, 213, 214), resting mouse T cells have virtually no K_V channels and then upregulate expression of K_V1.1, K_V1.3, and K_V1.6 in their CD4⁺ T cells (215) and K_V3.1 in their CD8⁺ T cells (216). Mice were therefore long considered to be bad models for evaluating K_V1.3 functions (217).

Later, when different T cell classification schemes such as CCR7⁺CD45RA⁺ naive, CCR7⁺CD45RA⁻ central memory (T_{CM}), and CCR7⁻CD45RA⁻ effector memory (T_{EM}) for memory T cell subsets (218) and Th1, Th2, Th17, and Treg cells for CD4⁺ T cells became popular, K_V1.3 and K_{Ca}3.1 expression in T cell subsets was reevaluated. Mouse Th1 and Th2 cells were found to predominantly express K_{Ca}3.1 and to depend on this channel for TCR-stimulated Ca²⁺ influx and cytokine production, whereas Th17 cells predominantly express K_V1.3 and depend on K_V1.3 for activation and production of IL-17 (72). Accordingly, *Kv1.3*^{-/-} mice are resistant to EAE induction, while their CD4⁺ T cells were deficient in IFN- γ and IL-17 production and exhibited an overall decreased proliferative capacity (134). In addition, inhibition of K_{Ca}3.1 results in amelioration of colitis in both an adoptive transfer and TNBS (2,4,6-trinitro benzene sulfonic acid)-induced model of colitis, at least in part by inhibiting activation of Th1 cells (72). Treg cells, in contrast, express predominantly K_{Ca}3.1 channels, yet pharmacologic inhibition or genetic deletion of K_{Ca}3.1 does not result in impaired Treg functions (72). In humans, rats, and primates, K_V1.3 expression is high in activated CD4⁺ and CD8⁺CCR7⁻ T_{EM} cells, which makes these cells highly dependent on K_V1.3 for Ca²⁺ signaling and IL-2, IFN- γ , and IL-17 production (214, 219–221). Elegant in vivo two-photon imaging experiments with GFP-expressing ovalbumin-specific T_{EM} cells in rats showed that, in the presence of K_V1.3 blockers, T_{EM} cells still make contact with APCs, but the contact is not stable and the cells fail to enlarge, to subsequently migrate through the inflamed tissue, or to proliferate and produce cytokines (222). CCR7⁺ naive and T_{CM} cells, in contrast, were not prevented from homing to lymph nodes because these T cell subsets express high levels of K_{Ca}3.1 in the activated state and rely on this channel for Ca²⁺ signaling and proliferation (219, 220, 222). Inhibition of K_V1.3 function by expression of a dominant-negative isoform in human T_{EM} cell cultures induces

reversion of T_{EM} to T_{CM} cells, suggesting a direct role for K_v1.3 in memory T cell differentiation (223).

Magnesium transporters and channels—Two PM proteins are known to mediate Mg²⁺ influx in lymphocytes and to regulate lymphocyte function: MAGT1 and TRPM7 (Figure 5) (73). Patients with mutations in *MAGT1* suffer from XMEN disease, characterized by defective T cell function and increased susceptibility to EBV infections (Figure 3) (148). TCR stimulation fails to induce Mg²⁺ influx in MAGT1-deficient CD4⁺ T cells, resulting in impaired PLCγ1 phosphorylation and impaired IP₃ production, as well as in a secondary defect in CRAC channel activation and SOCE, thereby decreasing the nuclear translocation of NFAT and NF-κB and expression of CD69 (148). A follow-up study by Lenardo and coworkers (224) showed that MAGT1-deficient CD8⁺ T cells and NK cells have impaired cytolytic function in vitro owing to decreased expression of the NK cell-activating receptor NKG2D. NKG2D is expressed on CD8⁺ T cells and NK cells and mediates antiviral and antitumor immune responses (225). Supplementation of Mg²⁺ increased NKG2D surface expression and the cytolytic function of CD8⁺ T cells and NK cells in vitro, and XMEN patients given Mg²⁺ orally showed restored NKG2D levels on CD8⁺ T cells and decreased the number of circulating EBV-infected B cells in vivo. The mechanisms of Mg²⁺-regulated NKG2D expression are not known, but these findings provide an important link between NKG2D-dependent cytotoxicity and EBV antiviral immunity. Rescue of NKG2D expression in MAGT1-deficient T cells suggests that other Mg²⁺ influx channels exist in T cells. One such channel could be TRPM7, which besides Mg²⁺ conducts other divalent cations including Ca²⁺, Zn²⁺, and Ni²⁺ (78, 79). Mice with T cell-specific deletion of TRPM7 have a severe block in intrathymic T cell development, but whether TRPM7 is also required for the function of mature T cells is not known yet. Residual T cells isolated from *Trpm7*^{-/-} *Lck-Cre* mice were resistant to TCR restimulation-induced cell death, which was dependent on Fas and activation of caspases downstream of TRPM7 channel function (226), suggesting that TRPM7 regulates T cell homeostasis. Whether the effects of TRPM7 in lymphocytes are really mediated by Mg²⁺ signaling or other cations conducted by TRPM7, especially Ca²⁺, remains to be investigated.

Zinc transporters—T cell function is regulated by Zn²⁺ with both activating and inhibitory effects noted (reviewed in 227–229). In some studies, the presence of Zn²⁺ enhanced in vitro T cell proliferation (82, 230) and synthesis of IL-2 and IFN-γ (231), whereas in other studies higher Zn²⁺ concentrations were associated with inhibition of murine T cell proliferation (232) and cytokine expression by Jurkat T cells (233) and human CD4⁺ T cells (80). TCR activation leads to rapid changes in free intracellular [Zn²⁺]_i close to the immunological synapse between T cells and APCs, and these changes are dependent on ZIP6, as RNAi knockdown of ZIP6 abrogates the increase in [Zn²⁺]_i (82). Increased free [Zn²⁺]_i was proposed to function as a second messenger to inhibit the recruitment of the tyrosine phosphatase SHP1 to the TCR, thereby augmenting the phosphorylation and activation of ZAP70, resulting in sustained TCR-stimulated Ca²⁺ influx (82). Dynamic changes in [Zn²⁺]_i within minutes of stimulation of a murine T cell line with IL-2 were associated with enhanced proliferation and cytokine production (81), but the source of the Zn²⁺ signal was not determined. ZIP8 localized in lysosomes was shown to regulate IFN-γ

and perforin production by human T cells, given that ZIP8 knockdown decreased and its overexpression increased expression of both IFN- γ and perforin (80). Although these findings provide important insights into possible roles for Zn²⁺ signaling in immune cells, we are just beginning to understand the function of Zn²⁺ transporters and Zn²⁺ signaling in lymphocytes.

Chloride channels—Several Cl⁻ channels have been reported to contribute to lymphocyte function. GABA_A receptor channels have in most studies been shown to provide inhibitory signals to immune cells as they do to neurons. Their subunits are expressed in murine and rat CD4⁺ and CD8⁺ T cells (103, 104), and functional GABA_A channels were demonstrated in MBP_{89–101}-specific CD4⁺ T cells isolated from the CNS of mice with EAE (234). Treatment with GABA inhibited T cell proliferation as well as IL-2 and IFN- γ production in vitro (103, 234, 235). In vivo administration of GABA moderately inhibited delayed-type hypersensitivity (235) and attenuated the severity of autoimmune diabetes (103) and collagen-induced arthritis (236) in mice. Collagen-specific IgG and IgG2a antibodies were reduced in GABA-treated mice, and their T cells had impaired proliferative responses to antigen in vitro (236). Similarly, IFN- γ production by T cells from GABA-treated NOD mice was impaired in response to β cell antigen stimulation (103). How GABA_A receptors inhibit T cell functions has not been investigated in detail, although a plausible mechanism may be Cl⁻ efflux and PM depolarization leading to decreased Ca²⁺ influx. In neurons, GABA_A receptors mediate Cl⁻ influx, thereby hyperpolarizing the PM and inhibiting activation of Ca_v channels. In lymphocytes, PM hyperpolarization would enhance rather than decrease Ca²⁺ influx through CRAC channels. However, unlike neurons, the intracellular [Cl⁻] in lymphocytes has been measured to be 38 mM and the Cl⁻ equilibrium potential to be -33 mV (237). Because the resting V_m in lymphocytes is between -53 mV (237) and -59 mV (208), opening of Cl⁻ channels results in Cl⁻ efflux, cell depolarization, and decreased Ca²⁺ influx, explaining the inhibitory effects of GABA on lymphocytes. This is similar to the inhibitory mechanism by GABA_A receptors in immature neurons (238, 239). Some evidence suggests that the mutations of CFTR found in cystic fibrosis patients may impair immune cell function, contributing to abnormal immune responses to pathogens and inflammation in the lung. Cl⁻ currents with properties similar to those of the CFTR were described in Jurkat T cells, CD4⁺ T cell clones, and EBV-transformed B cell lines (105, 106), but not in B lymphoblasts (106) or CD4⁺ T cells (105) from patients with cystic fibrosis. T cell clones from cystic fibrosis patients carrying the F508 mutation had impaired cytokine production in vitro following TCR stimulation (105). By contrast, *Cftr*^{F508} mice with the same mutation and *Cftr*^{F508} *Lck-Cre* mice with T cell-specific conditional deletion of the CFTR developed an exaggerated IgE response against *Aspergillus fumigatus*, with higher levels of IL-4 and IL-13 that were primarily due to CFTR deficiency in T cells (240). The causes of this discrepancy between human and mouse T cells remain unknown, as do the mechanisms of CFTR activation and function in lymphocytes. LRRC8A, recently identified as a subunit of VRAC, may also play a role in T cell function in addition to controlling T cell development. Splenic CD3⁺ T cells from *Lrrc8a*^{-/-} mice failed to proliferate in vitro after anti-CD3 or anti-CD3/CD28 stimulation, whereas proliferation after phorbol myristate acetate (PMA) plus ionomycin treatment was normal. Likewise, the number of splenic CD4⁺CD62L^{lo}CD44^{hi} T effector memory cells was decreased in

Lrrc8a^{-/-} mice, suggesting that LRRC8A is important for peripheral T cell expansion and function.

Ion Channels in B Cell Function

As we discuss here, ion channels also play important roles in B cell functions.

Ca²⁺ channels—Ca²⁺ influx in B cells following BCR stimulation occurs via SOCE and is dependent on STIM and ORAI proteins. B cells from STIM1/2-deficient mice lacked BCR-induced Ca²⁺ influx almost completely, whereas residual SOCE was present in STIM1- and ORAI1-deficient B cells (126, 156). Accordingly, B cell proliferation in vitro following BCR cross-linking was most severely impaired in STIM1/2-deficient B cells and only partially reduced in B cells lacking either STIM1 or ORAI1 (126, 156). B cell proliferation after stimulation with anti-CD40 or lipopolysaccharide (LPS) was not affected by deletion of STIM1 or ORAI1, consistent with the hypothesis that proliferation owing to these stimuli is Ca²⁺ independent. Surprisingly, serum titers of IgM, IgG1, IgG2b, IgG3, and IgA were normal in nonimmunized *Stim1*^{fl/fl} *Stim2*^{fl/fl} *Mb1-Cre* mice with B cell-specific deletion of STIM1 and STIM2 (156). In addition, T-independent and T-dependent antibody responses following immunization were also normal, as was antigen-specific antibody production after rechallenge, which is consistent with similar frequencies of germinal center B cells and normal affinity maturation in these mice. In patients with loss-of-function mutations in *ORAI1* and *STIM1*, baseline serum IgM, IgG, and IgA levels were normal or elevated, but patients lacked specific antibody responses against vaccination antigens such as diphtheria and tetanus toxoids or pathogens such as *Candida albicans* that they had encountered before (33, 122, 124, 125). The difference in antigen-dependent antibody responses between patients and mice is likely due to the B cell-specific deletion of STIM1 and STIM2 in mice but not in humans, suggesting a potentially important role for CRAC channels in T cell help, specifically by follicular helper T cells. The most prominent phenotype in mice with B cell-specific deletion of STIM1 and STIM2 was exacerbated severity of EAE, which has been attributed to the inability of CD1d^{hi}CD5⁺ regulatory B cells to produce IL-10 after BCR stimulation (156). Overall, however, CRAC channels in B cells do not appear to be required for primary and memory antibody responses. Given the almost complete lack of BCR-induced Ca²⁺ influx in STIM1/2-deficient mice, B cells may be overall less dependent on Ca²⁺ signaling than are T cells, which is consistent with the finding that B cells are less sensitive to calcineurin inhibitors such as cyclosporine A or, alternatively, that B cells utilize additional, not yet identified, Ca²⁺ channels that compensate for the absence of CRAC channels.

The TRP channels TRPC1 and TRPC7 were reported to be involved in both SOCE (241) and store-independent Ca²⁺ influx (242) after BCR stimulation of the DT-40 chicken B cell lymphoma cell line (241, 242). Whether TRPC channels contribute to Ca²⁺ signaling in human or murine B cells is not clear. In primary murine B cells, mechanical stress activates Ca²⁺-permeable non-selective cation channels whose biophysical properties are similar to those of some TRP channels (243). They may be involved in integrin activation and adhesion of B cells (243), but because the molecular nature underlying these currents is unknown, their contribution to lymphocyte function is difficult to assess. For some time, the

tetraspanin B cell surface protein CD20, the target of several monoclonal antibodies for the treatment of lymphoma, has been suggested to be part of a Ca^{2+} channel (244). So far no direct evidence has been obtained for this hypothesis, and it is more likely that CD20 regulates Ca^{2+} signaling in B cells indirectly through modulating intracellular signal transduction (245).

Channels regulating membrane potential—Similar to T cells, B cells use the K^+ channels $\text{K}_V1.3$ and $\text{K}_{Ca}3.1$ to regulate V_m . Both channels are differentially expressed in different B cell subsets (246). $\text{K}_V1.3$ expression is high in $\text{IgD}^- \text{CD}27^+$ class-switched memory B cells and, presumably, in plasma cells, whereas $\text{K}_{Ca}3.1$ is the dominant K^+ channel in activated naive and $\text{IgD}^+ \text{CD}27^+$ memory B cells (246). In keeping with this expression pattern, $\text{K}_V1.3$ blockers suppress anti-CD40 antibody or PMA plus ionomycin-stimulated proliferation of class-switched memory B cells, whereas $\text{K}_{Ca}3.1$ blockers inhibit the proliferation of IgD^+ B cells (246) and inhibit antimitochondrial antibody production by B cells from patients with primary biliary cirrhosis (247). The role of TRPM4, which in T cells opposes the hyperpolarizing effects of $\text{K}_V1.3$ and $\text{K}_{Ca}3.1$, has not been described in B cells.

Other ion channels—In contrast to T cell development, TRPM7 is not required for mouse B cell development (149). However, in one study chicken DT-40 B cells lacking TRPM7 had reduced total cellular Mg^{2+} and defects in proliferation and viability, which could be rescued by supplementation with high extracellular $[\text{Mg}^{2+}]$ (248). Whether this is true for primary human or mouse B cells has not been investigated. More recently, mouse and human B cells have also been found to express the voltage-gated proton channel H_V1 (112). H_V1 associates with the BCR complex and is internalized with the receptor after activation (Figure 7). H_V1 knockout results in reduced Syk and Akt activation, impaired mitochondrial respiration, and diminished antibody responses in vivo, suggesting that proton channel-mediated generation of ROS plays an important role in B cell signaling by inhibiting the tyrosine phosphatase SHP1 (112). The Zn^{2+} transporter ZIP10 was recently shown to modulate BCR signaling strength and B cell function (249). Deletion of ZIP10 in mature B cells of mice resulted in reduced B cell proliferation and impaired T cell-dependent and -independent antibody responses in vivo. ZIP10 was found to mediate CD45 phosphatase activation and thereby to reduce Lyn kinase function downstream of BCR cross-linking.

V. INNATE IMMUNE RESPONSES

The innate immune response is initiated by recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors such as Toll-like receptors (TLRs), C-type lectin receptors, NOD-like receptors (NLRs), or RIG1-like receptors. Activation of these receptors induces the expression of proinflammatory cytokines and results in the recruitment and activation of neutrophils, macrophages, and DCs. Essential to these responses are ion channels and transporters.

CRAC Channels in Monocytes, Macrophages, DCs, and Neutrophils

In contrast to the critical roles for CRAC channels in adaptive immune responses, less is known about their function in innate immunity. In patients with *ORAI1* or *STIM1* mutations, the numbers and phenotypes of monocytes, neutrophils, basophils, and eosinophils were normal, suggesting that CRAC channels are not required for the development of myeloid cells (33, 124, 125).

In macrophages, intracellular Ca^{2+} regulates a variety of functions such as the production of TNF- α and nitric oxide (NO), phagocytosis (250, 251), and potentially phagosome-lysosome fusion, although the reports on the Ca^{2+} dependence of the latter process are conflicting (252–254). The role of CRAC channels in macrophage function is still poorly defined. Peritoneal macrophages isolated from *Stim1*^{-/-} mice had severely impaired Ca^{2+} influx in response to Fc γ receptor (Fc γ R) cross-linking (see Figure 8). Fc γ R receptor-mediated phagocytosis by STIM1-deficient macrophages was severely impaired in vitro, and the severity of autoimmune hemolytic anemia (AIHA) and thrombocytopenia (which depend on the phagocytosis of antibody-opsonized erythrocytes and platelets) was attenuated in *Stim1*^{-/-} mice (255). Although this may indicate that STIM1 regulates macrophage phagocytosis, an alternative interpretation is that complement activation and generation of C5–C9 membrane attack complexes required for cell lysis depend on STIM1. The phenotype in mice is at odds with that of patients with loss-of-function mutations in *STIM1* who suffer from autoantibody-mediated AIHA and thrombocytopenia (123, 125) and normal function (including phagocytosis and phagosome maturation) of SOCE-deficient bone marrow-derived macrophages from mice with conditional deletion of both *Stim1* and *Stim2* genes (S. Feske, unpublished data).

In DCs, Ca^{2+} signals promote DC activation and maturation in vitro and play a role in DC responses to TLR ligands, intact bacteria, and microbial toxins (256). Using murine bone marrow-derived CD11c⁺ DCs, two studies reported that stimulation with IP₃ or LPS induced SOCE and Ca^{2+} currents resembling I_{CRAC} (257, 258). The nonselective CRAC channel inhibitor SKF-96365 as well as the K_v1.3 blocker margatoxin blocked SOCE and decreased the LPS-induced expression of TNF- α and the CCL21-dependent migration of DCs but increased the uptake of fluorescein isothiocyanate-dextran (258). A role for CRAC channels in DC function and maturation was recently proposed in human monocyte-derived DCs (259). Culturing DCs in the presence of Ca^{2+} induced upregulation of the maturation markers CD80, CD83, CD86, and HLA-DR, which was inhibited by CRAC channel blockers and siRNA-mediated knockdown of STIM1 or ORAI1 expression. Collectively, these studies suggest that in vitro differentiated murine and human DCs may require SOCE for maturation and function. However, these findings are in contrast to normal in vitro maturation and function of bone marrow-derived DCs from mice with conditional deletion of *Stim1* and *Stim2* genes, calling into question the role of CRAC channels, at least in murine DCs (S. Feske, unpublished data). Whether DCs require SOCE to mediate immune responses in vivo remains to be investigated.

Neutrophil functions such as Fc γ R-dependent phagocytosis and the generation of ROS by NOX activation depend on Ca^{2+} signals. Since the discovery of ORAI1 and STIM1, several studies have shown that Ca^{2+} influx following stimulation with fMLP, chemokines, or Fc γ R

cross-linking is impaired when ORAI1 and STIM1 are deleted in the human neutrophil-like HL-60 cell line (260) or in primary neutrophils from *Orai1*^{+/-} and *Stim1*^{-/-} mice (260, 261). Intriguingly, SOCE is reduced only moderately in neutrophils from patients with mutations in *ORAI1* or *STIM1* that abolish SOCE in lymphocytes (S. Feske, R. Elling, P. Henneke, unpublished observations; 33, 124). SOCE is required for ROS production by NOX, as demonstrated in siORAI1- or siSTIM1-treated HL-60 cells and neutrophils from *Stim1*^{-/-} mice that had reduced ROS levels (261–263). SOCE was recently shown to activate PKC α and PKC β , which phosphorylate the p40^{phox} and p47^{phox} subunits of NOX (261). In addition to its role in activating ORAI1 channels, STIM1 appears to sustain neutrophil phagocytosis by recruiting phagosomes to the ER and inducing the opening of phagosomal Ca²⁺ channels to generate localized Ca²⁺ signals that promote high-efficiency phagocytosis (264). These findings are consistent with a moderate defect in Fc γ R-induced phagocytosis by *Stim1*^{-/-} neutrophils (261). A role for ORAI1 in neutrophil adhesion and cell arrest was reported in one study (260), whereas in another *Stim1*^{-/-} neutrophils adhered normally in vitro (261). The role of CRAC channels in neutrophil migration to sites of inflammation is not known. A recent study suggests that CRAC channels may be important for neutrophil-mediated immunity to bacterial infection, given that *Stim1*^{-/-} mice showed an increased susceptibility to *Staphylococcus aureus* and *L. monocytogenes* (261), although the absence of STIM1 in other immune cells in these mice may contribute to the phenotype. Although patients with ORAI1 and STIM1 mutations are immunodeficient, their phenotype overlaps only partially with that seen in patients with leukocyte adhesion defects or chronic granulomatous disease due to mutations in NOX complex genes, suggesting that neutrophil function is at least partially retained in the absence of CRAC channels (4, 33, 162).

K⁺ Channels in Monocytes and Macrophages

After voltage-dependent conductances were first reported in mouse macrophages in 1981 (265), subsequent patch-clamp experiments revealed that human and rodent macrophages and macrophage-like cell lines express several types of K⁺ channels. Depending on the species, the source of cells, and the culture conditions, studies reported expression of the voltage-gated K_v1.3 and K_v1.5 (potentially as a heteromultimer), the Ca²⁺-activated K_{Ca}1.1 and K_{Ca}3.1, and the inward-rectifier Kir2.1 channels (266–272). A general pattern in these studies is that differentiation and activation of cells increases K⁺ channel expression. Similar to findings in T cells, K_v1.3 blockade depolarizes macrophages, demonstrating that the channel is involved in setting the resting V_m (273), reducing chemotactic migration (269), and inhibiting M-CSF-stimulated proliferation and inducible nitric oxide synthase (iNOS) expression (266).

Human macrophages further express K_{Ca}3.1, which opens when HIV engages CCR5 and CXCR4 (274) or during Ca²⁺ oscillations induced by extracellular ATP activation of P2Y receptors (268). K_{Ca}3.1 in macrophages can also be activated by Ca²⁺ influx through CRAC channels and seems to be tightly coupled to SOCE by prolonging Ca²⁺ signaling through hyperpolarization and facilitating store refilling (275). K_{Ca}3.1 expression is also commonly observed on macrophages in infiltrating atherosclerotic plaques, transplanted organs, or inflamed tissues, such as in IBD (276–278). Macrophages from *KCa3.1*^{-/-} mice as well as macrophages treated with the K_{Ca}3.1 inhibitor TRAM-34 exhibit impaired migration to

MCP-1 (CCL2) (277). Moreover, treatment of *ApoE*^{-/-} mice with TRAM-34 led to reduced atherosclerosis, which was associated with decreased infiltration of macrophages into atherosclerotic plaques (277).

While there has been little recent work on K⁺ channels in macrophages, studies on the related microglia have shown that K_V1.3 and K_{Ca}3.1 are involved in microglia-mediated neuronal killing by LPS or amyloid-β oligomer-stimulated microglia (279, 280), leading to the suggestion of K_V1.3 and K_{Ca}3.1 as therapeutic targets for the suppression of detrimental microglia functions. So far, this therapeutic hypothesis seems to translate into rodent models of CNS pathology. ShK-170, a K_V1.3-blocking peptide, protected mice from microglia-mediated radiation-induced brain injury (281), whereas K_{Ca}3.1 blockers reduced brain edema and infarct volume caused by traumatic brain injury (282) as well as infarct area, microglia activation, and neurological deficit in ischemic stroke in rats (283). K_V1.3 (probably together with K_V1.5) also seems to regulate DC function. K_V1.3 is strongly expressed on DCs in the brain of MS patients, and K_V1.3 blockade or knockdown reduced expression of various costimulatory molecules such as CD80, CD86, CD40, and IL-12 on human DCs (284). Treatment of murine bone marrow-derived CD11c⁺ DCs with the K_V1.3 blocker margatoxin interfered with LPS-induced TNF-α production and chemotaxis of DCs (258).

P2RX7 and Inflammasome Activation

ATP is a danger signal that alerts the immune system by activating the inflammasome, a multi-protein complex that integrates microbial and danger signals by activating inflammatory caspases, leading to the generation of the proinflammatory cytokines IL-1β and IL-18 (285, 286). Several inflammasome complexes have been identified and are typically named after the NLR or HIN200 protein that initiates signaling. Activation of the NLRP3 inflammasome is the best studied and is implicated in host defense to a broad range of infectious agents and perturbations including ATP (287–290).

ATP stimulation of P2RX7 plays a prominent role in controlling inflammasome activation and macrophage function (62) (see Figure 9). Studies in vitro and in vivo using *P2rx7*^{-/-} mice have shown that the ATP-dependent release of IL-1β is dependent on P2RX7 (62, 145). Activation of the NLRP3 inflammasome in macrophages and monocytes requires two signals (290). The first signal, such as IFN-γ or TLR stimulation (291, 292), is required for cellular priming and to increase transcription of NLRP3 and pro-IL-1β via an NF-κB pathway. ATP signaling via P2RX7 (along with bacterial pore-forming toxins, particulate matter, and crystals) functions as the second signal that mediates NLRP3 activation and IL-1β release (145, 287, 293, 294). ATP and P2RX7 are thought to activate NLRP3 by promoting the efflux of K⁺, which acts upstream or directly on NLRP3, leading to caspase-1 activation (61, 63, 294, 295). The importance of K⁺ efflux is supported by the ability of high extracellular K⁺ concentrations to block ATP-stimulated IL-1β release (296, 297). Under physiological conditions, [K⁺] is about 40-fold higher inside the cell, resulting in K⁺ efflux when P2RX7 opens (see Figures 1 and 2). By increasing the extracellular [K⁺], the concentration-dependent driving force for K⁺ efflux from the cell is reduced. Although P2RX7 is critical for ATP-mediated K⁺ efflux, it does not mediate K⁺ efflux in response to

other signals such as particulate matter or bacterial pore-forming toxins, which directly insert into the PM and induce K⁺ efflux by an unknown pathway.

In DCs, the roles of ATP and P2RX7 in inflammasome activation are more ambiguous. TLR signaling has been shown in some studies to be sufficient for NLRP3-mediated activation of caspase-1 and processing of pro-IL-1 β and pro-IL-18, whereas neither ATP nor P2RX7 were required (298). In another report, ATP release from dying cells activated P2RX7 on DCs, leading to activation of the NLRP3 inflammasome and release of IL-1 β , which was then critical for DC priming of antigen-specific CD8⁺ T cells and killing of chemotherapy-treated cancers in vitro and in vivo in mouse models of cancer (299). ATP-induced activation of P2RX7 on DCs has also been shown to increase expression of CD80 and CD86, thereby providing costimulation, activation, and expansion of IFN- γ -producing CD4⁺ T cells that mediate GvHD, while simultaneously inhibiting the generation of Foxp3⁺ Treg cells (300). These data suggest that inhibiting P2RX7 may provide a novel therapy to inhibit GvHD without impairing concurrent graft-versus-leukemic effects.

The innate immune response is critical for controlling infection by *Mycobacterium tuberculosis* and other intracellular bacteria such as *L. monocytogenes* and chlamydia. Protection or susceptibility to *M. tuberculosis* infection has been linked to genetic polymorphisms in the *P2RX7* gene (301, 302). P2RX7 signaling in macrophages contributes to the antibacterial response to *M. tuberculosis*, which is mediated at least partly via P2RX7-mediated inflammasome activation. ATP treatment of *M. tuberculosis*-infected macrophages induced apoptosis and death (303–305). In contrast, *P2rx7*^{-/-} mice had increased lung burdens of *M. tuberculosis* (305). Loss-of-function polymorphisms in *P2RX7* have been identified, and macrophages from individuals homozygous for the 1513A-C polymorphism fail to kill *M. tuberculosis* in response to ATP stimulation despite priming with IFN- γ (306). The 1513A-C polymorphism is associated with increased susceptibility to *M. tuberculosis* (307) and to development of extrapulmonary *M. tuberculosis* in a Southeast Asian population (308). These findings provide strong evidence of a role for P2RX7 in controlling this infection. Whether the effects of the 1513A-C polymorphism are due to altered inflammasome activation remains to be investigated.

TRP Channels

The function of TRP channels in innate immune responses is best studied in macrophages and monocytes. TRPM2 is a nonselective Ca²⁺ channel that is regulated by ADPR (55), which is generated, for instance, in response to increasing levels of ROS (309, 310). Activation of TRPM2-deficient macrophages is impaired in several immune disease models, likely accounting for the phenotypes of *Trpm2*^{-/-} mice (311–314). *Trpm2*^{-/-} mice are highly susceptible to infection with *L. monocytogenes*, which is associated with decreased expression of iNOS by macrophages as well as of IFN- γ and IL-12 (311). In a model of dextran sulfate sodium-induced colitis, *Trpm2*^{-/-} mice had a marked reduction in colonic expression of IL-12 and IFN- γ as well as decreased expression of CXCL2 (CXCL8 in humans) by peripheral monocytes and colonic macrophages, which was associated with reduced recruitment of neutrophils to the gut and amelioration of colitis (312). Transfer of wild-type macrophages into *Trpm2*^{-/-} mice reversed the phenotype, indicating that

Author Manuscript

suppression of TRPM2 function in macrophages accounted for protection (312). In agreement with the hypothesis that TRPM2 promotes inflammation, *Trpm2*^{-/-} mice fed a high-fat diet had decreased expression of IL-6, IL-1, and keratinocyte-derived cytokine in white adipose tissue and were protected from obesity-induced insulin resistance (313). Macrophages from *Trpm2*^{-/-} mice were also resistant to crystal/liposome-induced NLRP3 inflammasome activation (314). Crystal-induced generation of ROS was found to function upstream of TRPM2 and NLRP3 activation. However, these findings are in contrast to other studies in which ROS was not necessary for inflammasome activation, as discussed above (see the subsection above entitled P2RX7 and Inflammasome Activation) (294), and in contrast to a report that TRPM2 can protect against inflammation (315). When taken together, most studies indicate that TRPM2 in macrophages plays a prominent role in innate immunity to infection but can also be detrimental and promote inflammation and disease under conditions in which macrophages are aberrantly activated.

Author Manuscript

TRPV2 is a Ca²⁺-permeable but nonselective channel that is expressed in macrophages and plays a role in chemotaxis and in the early phases of phagocytosis (58). TRPV2 is recruited to the early phagosome in response to activation of PI3K, AKT, and Src following FcγR engagement by IgG. There it mediates Na⁺ influx, resulting in membrane depolarization and the synthesis of PIP₂, which further results in actin depolymerization that is required for phagocytic receptor clustering and internalization (58). Interestingly, this function of TRPV2 is independent of Ca²⁺ signaling. Consistent with a defect in phagocytosis, *Trpv2*^{-/-} mice show increased mortality and organ bacterial levels when challenged with *L. monocytogenes*, although the bacterial dose required to kill *Trpv2*^{-/-} mice is 1,000-fold higher than that required to kill *Trpm2*^{-/-} mice (58, 311).

Author Manuscript

TRPM4 inhibits macrophage function by mediating Na⁺ influx, depolarizing the V_m, and thereby antagonizing Ca²⁺ influx (316). In a sepsis model of cecal ligation, mortality of *Trpm4*^{-/-} mice is increased and associated with greater numbers of circulating monocytes in the blood that express high levels of proinflammatory cytokines, presumably due to enhanced Ca²⁺ influx (316). *Trpm4*^{-/-} macrophages are also defective in phagocytosis (316). Besides regulating Ca²⁺ influx in macrophages, TRPM4 modulates Ca²⁺ influx in DCs and is required to prevent Ca²⁺ overload, as immature DCs from *Trpm4*^{-/-} mice have a sustained increase in Ca²⁺ influx following chemokine stimulation, resulting in impaired CCR7-mediated migration (317). In summary, studies of TRPM2, TRPV2, and TRPM4 highlight a critical role for TRP channels in macrophages and DCs to regulate V_m and to coordinate the turning on and off of Ca²⁺-mediated innate responses.

Proton Channels

Author Manuscript

The voltage-gated proton channel H_V1 is closely associated with the NOX complex in innate immune cells and is crucial for the generation of ROS that mediate killing of bacteria and microbes. Genetic loss of NOX activity in humans results in chronic granulomatous disease characterized by recurrent bacterial and fungal infections (318). Phagocytosis of extracellular organisms activates NOX, which results in the flux of electrons across the PM into the forming phagosome and the generation of O₂⁻ (Figure 10) (319). However, the flux of electrons results in membrane depolarization and a decrease in intracellular pH, both of

which would impair sustained NOX activation (111, 319). The extrusion of H⁺ by H_V1 balances the outward electron efflux, thereby limiting membrane depolarization and intracellular acidosis (319). Genetic deletion of H_V1 or its inhibition by Zn²⁺ reduces the respiratory burst in neutrophils, bone marrow cells, and B lymphocytes (320–322). Although killing of *S. aureus* by H_V1-deficient bone marrow cells is impaired, *Hvcn1*^{-/-} mice are able to clear bacterial infection, likely because of the residual O₂⁻ generation and partially redundant functions of iNOS (322, 323). H_V1 is also strongly expressed in human and mouse microglia, and *Hvcn1*^{-/-} mice show reduced neuronal damage through reduced microglial ROS production following ischemic stroke, suggesting that H_V1 inhibitors are potential therapeutics for stroke-associated inflammation (324).

Zinc Channels

Zn²⁺ deficiency is associated with many defects in innate immune functions. For example, Zn²⁺ supplementation enhances phagocytosis of *Escherichia coli* and *S. aureus* by peritoneal macrophages and killing of *Trypanosoma cruzi* (325, 326). Zn²⁺ deficiency results in reduced neutrophil and macrophage chemotaxis, phagocytosis, production of ROS, and netosis by neutrophils (327). Zn²⁺ is also important for the production of proinflammatory cytokines by macrophages (328, 329) and for the recognition and killing of target cells by NK cells. However, the effects of Zn²⁺ on innate immune function are not straightforward, and they vary in a concentration-dependent manner because at low concentrations Zn²⁺ is proinflammatory and at high concentrations it suppresses the same pathways (330).

Many Zn²⁺ transporters of the ZnT and ZIP families are expressed in innate immune cells (89). However, few direct links have been established between these proteins, intracellular Zn²⁺ concentrations, and the regulation of innate immune responses. Expression of ZIP8 is upregulated after LPS and TNF-α stimulation of macrophages and promotes Zn²⁺ uptake across the PM, which attenuates NF-κB activation by direct Zn²⁺ binding and inhibition of IκB kinase β, thereby negatively regulating proinflammatory responses (331). Similarly, ZIP14 is upregulated after LPS or LPS plus IFN-γ stimulation of macrophages and attenuates IL-6 and TNF-α release, presumably by mechanisms similar to that of ZIP8 (332). In DCs, LPS stimulation induces changes in gene expression of multiple Zn²⁺ transporters, the net effect being a decrease in [Zn²⁺]_i, which appears to be necessary for LPS-induced DC maturation (333). Zn²⁺ supplementation or overexpression of ZIP6, which mediates Zn²⁺ transport into cells, abrogates LPS-induced upregulation of MHC class II and costimulatory molecules (333). Future studies of Zn²⁺ transporter-deficient mice, innate immune cells isolated from these mice, and the downstream signaling pathways regulated by changes in [Zn²⁺]_i will be critical to fully understanding the role of Zn²⁺ in innate immune responses.

VI. ION CHANNELS IN MAST CELLS AND ALLERGY

Mast cells are closely linked to allergy, atopic dermatitis, asthma, and anaphylaxis. Therefore, understanding how mast cell function is regulated by ion channels may provide new approaches to treatment (see Figure 11). The FcεRI-stimulated increase in [Ca²⁺]_i is an important driver for the release and synthesis of a large number of allergic mediators including histamine, proteases, prostaglandins, leukotrienes, and cytokines (334).

Ca²⁺ Channels

Cross-linking of IgE bound to the FcεRI stimulates IP₃-mediated depletion of ER Ca²⁺ stores, leading to activation of CRAC channels. Rodent and human mast cells express all three ORAI channels (32, 129, 335). Mast cells from *Orai1*^{-/-} mice have strongly decreased IgE-stimulated Ca²⁺ influx, which was associated with impaired degranulation, release of leukotriene C₄ (LTC₄), and production of cytokines such as TNF-α (129). Similarly, mast cells isolated from *Stim1*^{-/-} mice had reduced Ca²⁺ influx and activation (130).

Pharmacological inhibition of CRAC channels in human lung mast cells also attenuated FcεRI-stimulated Ca²⁺ influx; histamine release; release of IL-5, IL-8, IL-13, and TNF-α; and LTC₄ production (335). Interestingly, the combination of low doses of a CRAC channel inhibitor with the LTC₄ receptor inhibitor montelukast was effective in inhibiting mast cell activation, which suggests that combining CRAC channel inhibitors with other drugs may provide a novel approach to treating IgE-mediated diseases (336). The potential for treating asthma with CRAC channel inhibitors is also supported by the finding that pharmacologic inhibition of CRAC channels attenuated allergen-induced bronchoconstriction in isolated human bronchi (335).

Rodent and human mast cells also express a variety of P2X receptors which vary based on the species or origin of the mast cells (337–339). Although ATP was shown to stimulate the release of histamine, cytokines, and leukotrienes from mast cells in vitro (339, 340), the role of P2X receptors in mediating Ca²⁺ influx and mast cell functions in vivo is poorly defined. Interestingly, increased numbers of activated mast cells expressing P2RX7 were found in colons from patients with Crohn's disease and in TNBS-induced colitis in mice (339). More importantly, TNBS-induced colitis, intestinal neutrophil infiltration, and expression of inflammatory cytokines by mast cells are ameliorated following treatment with an inhibitory P2RX7 antibody or by reconstitution of mast cell-deficient mice with *P2rx7*^{-/-} mast cells, suggesting that P2RX7 inhibitors may be a novel treatment for patients with Crohn's disease. In addition, rodent and human mast cells express a variety of different TRP channels (341). However, TRP channel function in mast cells has not been validated in knockout mouse models in vivo, and it remains to be tested whether they play important roles in mast cell functions and IgE-mediated disease.

Membrane Potential Regulation by K_{Ca}3.1 and TRPM4 Channels

As in lymphocytes, in mast cells K_{Ca}3.1 and TRPM4 play opposing roles in cell activation by regulating V_m. K_{Ca}3.1 is expressed in human mast cells isolated from the lung and blood (342, 343), whereas in rodents K_{Ca}3.1 has been described in bone marrow-derived mast cells (BMDMCs) (344). Inhibiting K_{Ca}3.1 genetically or pharmacologically reduces FcεRI-mediated Ca²⁺ influx and degranulation (344). Moreover, *KCa3.1*^{-/-} mice showed reduced passive cutaneous and systemic anaphylaxis (344), and K_{Ca}3.1 inhibitors attenuated airway remodeling and eosinophilia in murine and sheep asthma models (345, 346). However, while inhibition of mast cell function probably contributed to these effects, K_{Ca}3.1 is also expressed in T cells, fibroblasts, and proliferative airway smooth muscle cells. A number of studies have addressed the mechanism(s) by which antigen receptors activate K_{Ca}3.1. Similar to CD4⁺ T cells, in mast cells both PI3K-C2β and NDPK-B function downstream of FcεRI activation and, along with Ca²⁺-calmodulin binding to the C terminus of K_{Ca}3.1, are

required for $K_{Ca}3.1$ activation (347). FcεRI stimulation of Ca^{2+} influx, de-granulation, and cytokine production were attenuated following siRNA knockdown of PI3K-C2β in BMDMCs (347) or in BMDMCs derived from *PI3KC2β*^{-/-} mice (E. Skolnik, unpublished observation). In addition, both passive systemic and cutaneous anaphylaxis were markedly attenuated in *PI3KC2β*^{-/-} mice (E. Skolnik, unpublished observation). On the flip side, FcεRI activation of mast cells was markedly enhanced in BMDMCs derived from *trim27*^{-/-} mice; TRIM27 negatively regulates PI3K-C2β kinase activity, and in contrast to *PI3KC2β*^{-/-} mice, *trim27*^{-/-} mice exhibit enhanced systemic anaphylaxis (347). The serum and glucocorticoid-inducible kinase (SGK1) is also important in FcεRI activation of $K_{Ca}3.1$ in mast cells (348, 349). BMDMCs isolated from *Sgk1*^{-/-} mice attenuates FcεRI-stimulated Ca^{2+} influx and degranulation, and *Sgk1*^{-/-} mice are protected from systemic anaphylaxis. The mechanism whereby SGK1 regulates $K_{Ca}3.1$ channel activity remains to be defined.

Counteracting the effects of $K_{Ca}3.1$, TRPM4 limits the magnitude of Ca^{2+} influx in mast cells by depolarizing V_m . BMDMCs isolated from *Trpm4*^{-/-} mice exhibit increased FcεRI-mediated Ca^{2+} influx and mast cell responses, and *Trpm4*^{-/-} mice had more severe IgE-mediated acute passive cutaneous anaphylaxis (350). Thus, as for CD4⁺ T cells, macrophages, and DCs, Ca^{2+} -mediated activation of TRPM4 plays an important role in the negative feedback regulation of Ca^{2+} influx via CRAC channels in mast cells.

Zinc Channels

Changes in intracellular Zn^{2+} may be important for mast cell function. FcεRI stimulation increases $[Zn^{2+}]_i$, and the Zn^{2+} chelator TPEN inhibits FcεRI-stimulated NF-κB activation and degranulation (351, 352). In contrast, mast cells lacking the Zn^{2+} transporter ZnT5 that is located in secretory granules had increased $[Zn^{2+}]_i$, which was associated with impaired FcεRI-mediated PKC recruitment to the PM, NF-κB activation, and cell-mediated delayed-type hypersensitivity responses in *ZnT5*^{-/-} mice, whereas FcεRI-stimulated degranulation was normal (90). Together, these studies suggest that changes in $[Zn^{2+}]_i$ modulate mast cell function; however, we do not yet know the channels that regulate FcεRI-induced changes in $[Zn^{2+}]_i$ or under which conditions altered levels of Zn^{2+} are activating or inhibiting.

VII. ION CHANNELS AS DRUG TARGETS FOR IMMUNOTHERAPY

CRAC Channels

As discussed above, genetic deletion of *Orai1*, *Stim1*, and *Stim2* genes in mice impairs T cell and mast cell functions and attenuates inflammation in animal models of autoimmunity, transplant rejection, and asthma. For instance, CRAC channel-deficient *Orai1*^{R93W} mice failed to develop skin contact hypersensitivity and showed delayed skin allotransplant rejection (127). Likewise, murine T cells lacking ORAI1 or STIM1 function failed to induce IBD (127) and EAE (164, 169) (animal models of colitis and MS, respectively), and this failure was associated with strongly reduced production of the proinflammatory cytokines IFN-γ, TNF-α, and IL-17. In addition, deletion of *Orai1* and *Stim1* genes attenuated FcεRI-dependent mast cell functions in vitro and passive cutaneous anaphylaxis in mice in vivo (129, 130), consistent with attenuated FcεRI-evoked histamine release and proinflammatory

cytokine and LTC₄ production in human mast cells treated with CRAC channel blockers (335).

In recent years, several small molecules have been developed that block CRAC channel function and SOCE in the low micromolar range when tested in RBL mast cells, Jurkat T cells, human peripheral blood mononuclear cells (PBMCs) (353–355), and murine effector T cells (168). All compounds were effective in inhibiting both the proliferation (355) and the production of a wide range of cytokines, including IL-2, IFN- γ , TNF- α , and IL-17, by primary human T cells isolated from healthy controls (355) or from diseased tissues such as colonic lamina propria of patients with IBD (353) and lung of patients with chronic obstructive pulmonary disease (356). These findings are consistent with impaired cytokine expression in T cells from ORAI1/STIM1-deficient patients and mice (33, 125–127, 155, 164, 168). In general, CRAC channel inhibitors were comparable or slightly less potent than cyclosporine A, a calcineurin inhibitor, in suppressing cytokine production in vitro. When tested in animal models, two compounds prevented the development of EAE in mice, potentially by inhibiting the differentiation of Th17 cells (168). Furthermore, the CRAC channel inhibitor BTP2 inhibited IL-4 and IL-5 production in a murine Th2 cell clone and human whole blood cells in vitro and reduced airway inflammation and bronchoconstriction in rodent models of allergic asthma (357). So far, however, none of these CRAC channel inhibitors has reached clinical trials. In addition to small molecular inhibitors, CRAC channels are potential targets for blocking antibodies. Two independently developed monoclonal antibodies selectively bound ORAI1 (but not ORAI2 and ORAI3), inhibited CRAC currents and SOCE in lymphocytes and suppressed the proliferation and cytokine production of human PBMCs and synovial fluid cells from rheumatoid arthritis patients in vitro (358, 359). In vivo treatment with anti-ORAI1 antibodies ameliorated the severity of xenogeneic T cell-mediated GvHD in mice (358) and reduced the production of IL-2, IL-4, and IL-17 by ex vivo-stimulated T cells isolated from cynomolgus monkeys (360). Collectively, these data suggest that CRAC channel inhibition may be useful for the treatment of autoimmune diseases, transplant rejection, and allergic diseases.

The anti-inflammatory effects of CRAC channel inhibitors need to be balanced against their potential suppression of protective immune responses, i.e., immunity to infection and tolerance. However, the strength of Ca²⁺ signals required for proinflammatory cytokine production and immunity to infection or tolerance appear to be very different. Whereas proinflammatory cytokine production was impaired in T cells from *Orai1*^{-/-}, *Orai1*^{R93W}, *Stim1*^{fl/fl} *CD4-Cre*, or *Stim2*^{fl/fl} *Cd4-Cre* mice with various degrees of CRAC channel dysfunction (126, 127, 164, 168, 169), memory CD8⁺ T cell responses and antibody production were only impaired in *Stim1*^{fl/fl} *Stim2*^{fl/fl} *Cd4-Cre* mice whose T cells lack CRAC channel function completely (183). Similarly, inhibitory ORAI1 antibodies partially reduced SOCE and cytokine secretion by T cells but did not impair T-dependent antibody production (360). Patients homozygous for mutations in *ORAI1* or *STIM1* that lack SOCE completely, but not their heterozygous relatives with partial impairment of SOCE, show increased susceptibility to viral and bacterial infections (26, 123). Similar observations were made for the role of SOCE in peripheral tolerance and autoimmunity. Whereas a complete loss of SOCE in T cells from ORAI1- and STIM1-deficient patients or in T cells from STIM1/2-deficient mice results in autoimmunity associated with reduced numbers of

thymus-derived Foxp3⁺ nTreg cells, a similar defect is not observed in heterozygous relatives of the patients or mice lacking only ORAI1, STIM1, or STIM2 (123, 125, 131, 132, 361). Together, these findings suggest that residual SOCE is sufficient for Treg development and immunity to infection and that partial pharmacological inhibition of CRAC channels may selectively inhibit proinflammatory T cells without affecting protective immune functions.

K_V1.3 and K_{Ca}3.1 Channels

Based on the fact that nonselective K⁺ channel blockers like 4-aminopyridine inhibit T cell proliferation and IL-2 production, K_V1.3 has been proposed as a target for immunosuppression since 1984 (266, 362). After the channel was cloned, Merck and Pfizer initiated small-molecule K_V1.3 discovery programs in the mid-1990s but largely failed to identify compounds that were suitable for development (363). Interest in K_V1.3 subsequently waned because of the aforementioned species differences between mice and humans (213, 364), but it then revived following reports that K_V1.3 blockers selectively inhibit the Ca²⁺ signaling, proliferation, and in vivo migration of CCR7⁻ T_{EM} cells while having little effect on CCR7⁺ naive and T_{CM} cells (219, 220, 222). Several studies demonstrated that myelin antigen-reactive T cells in the blood from MS patients, islet antigen-reactive T cells from new-onset type 1 diabetic children, synovial fluid T cells from patients with rheumatoid arthritis, and brain-infiltrating T cells in postmortem brain sections from MS patients were all K_V1.3^{high} CCR7⁻ T_{EM} cells (219, 220, 365). Based on these studies, K_V1.3 blockers were tested in a number of autoimmune disease models. The K_V1.3-blocking sea anemone peptide ShK and its derivatives ShK-L5 and ShK-186, all of which have picomolar affinity, treat adoptive transfer and chronic-relapsing EAE as well as pristane-induced arthritis in rats (220, 222, 366–368). Furthermore, the small molecules Psora-4 and PAP-1 [5-(4-phenoxybutoxy)psoralen]—both of which have low nanomolar potency—prevent antiglomerular basement membrane glomerulonephritis, autoimmune diabetes, and allergic contact dermatitis in rats (220, 221, 369). K_V1.3 blockers are also effective in models of psoriasis and alopecia areata (370–371a), both diseases in which T_{EM} cells are involved in the pathogenesis. In many of these studies, K_V1.3 blockers preferentially inhibited IL-2, IFN-γ, and IL-17 production by human or rat T_{EM} cells but had little effect on TNF-α or on cytokines produced by naive and T_{CM} cells (220, 221). More recently, K_V1.3^{high} CCR7⁻ T_{EM} cells have also been reported in the induced sputum of patients with asthma and in the lungs of rats with asthma. ShK-186 administration reduced IL-4 and IL-5 levels in the lavage fluid, lung infiltration, and airway hyperresponsiveness in the rat asthma model, suggesting that the K_V1.3 blocker also targets Th2-type T_{EM} cells (372). Based on all this positive animal data, Kineta Inc. completed Phase 1a and 1b studies with ShK-186 in 2014 with no major safety findings and is currently planning to start enrolling patients for trials in psoriatic arthritis and psoriasis later in 2014. Several companies, including Amgen and Janssen, are making efforts to prolong the short half-life of venom peptides like ShK by conjugating them to Fc antibody fragments, making fusion proteins (373) or producing K_V1.3-targeting nanobodies (Ablynx).

K_V1.3 blockers preferentially target CD4⁺ and CD8⁺ CCR7⁻ T_{EM} cells and have less effect on responses mediated by naive T cells and T_{CM} cells (219, 220, 222), suggesting that

$K_V1.3$ blockers should have little effect on the ability to clear acute infections or develop vaccine responses. This indeed seems to be the case. In delayed-type hypersensitivity or oxazolone-induced allergic contact dermatitis experiments, $K_V1.3$ blockers are ineffective when administered during the sensitization phase, demonstrating that they do not interfere with antigen presentation or memory T cell development but do potently suppress the infiltration and IFN- γ , IL-2, and IL-17 production of $CD4^+$ or $CD8^+$ T_{EM} cells when given following antigen rechallenge (220–222). $K_V1.3$ blockers have no effect on the ability of rats to clear influenza virus or *Chlamydia trachomatis* infections or on the ability of rhesus macaques to develop protective, flu-specific T_{CM} responses following immunization with a live influenza vaccine (214). However, rhesus macaques treated for 30 days with the $K_V1.3$ blocker PAP-1 showed reactivation of cytomegalovirus replication, suggesting that $K_V1.3$ inhibition may result in reactivation of chronic or latent viral infections.

The other lymphocyte K^+ channel, $K_{Ca}3.1$, was also suggested as a target for immunosuppression. The relative contribution of $K_V1.3$ and $K_{Ca}3.1$ to TCR-stimulated lymphocyte Ca^{2+} influx and cytokine production is determined primarily by their relative levels of expression. T_{EM} cells predominantly express $K_V1.3$ and, as a result, are responsive to $K_V1.3$ and not to $K_{Ca}3.1$ blockers. As discussed above, $K_{Ca}3.1$ is the predominant K^+ channel expressed on Th1 and Th2 cells, which suggests that $K_{Ca}3.1$ may be useful to treat diseases mediated by these cells. Utility has been demonstrated in several rodent models of colitis in which Th1 cells are important for pathogenesis. Pharmacologic and genetic inhibition of $K_{Ca}3.1$ markedly ameliorated colitis in an adoptive transfer model (72), and $K_{Ca}3.1$ blockers also ameliorated colitis induced by trinitrobenzene sulfonic acid (72, 276), raising the possibility that $K_{Ca}3.1$ may be a novel target for Crohn's disease or ulcerative colitis. $K_{Ca}3.1$ channels are also required for Fc ϵ RI-mediated Ca^{2+} influx in mast cells, their degranulation, and cytokine production (344, 374), suggesting that $K_{Ca}3.1$ blockers may also be uniquely suitable for treating IgE-mediated diseases. In addition, $K_{Ca}3.1$ also contributes to the activation of several other immune cells, including macrophages, microglia, DCs, and many nonimmune cells such as dedifferentiated vascular smooth muscle cells and fibroblasts. The ability of $K_{Ca}3.1$ blockers to simultaneously affect all these cell types probably contributes to the beneficial effects of $K_{Ca}3.1$ blockers in mouse and sheep asthma models (345, 346), prevention of atherosclerosis in *ApoE*^{-/-} mice (277), and transplant vasculopathy (278). Several $K_{Ca}3.1$ blockers have been developed by pharmaceutical companies, including Senicapoc, a small-molecule $K_{Ca}3.1$ blocker that entered Phase 3 clinical trials for sickle cell anemia (375). Although Senicapoc did not demonstrate efficacy in preventing sickling crises, it was well tolerated, which should provide additional assurance and support for pursuing $K_{Ca}3.1$ inhibitors as therapy for some of the diseases described above.

P2RX7

P2RX7 is a particularly attractive target to treat autoimmune and inflammatory diseases, given that its inhibition should suppress simultaneously the activation of multiple innate and adaptive immune cells. Consistent with this idea, inhibition or deletion of P2RX7 is effective in a number of models of autoimmunity and inflammation including lupus nephritis and other experimental glomerulonephritis, arthritis, IBD, asthma, EAE, GvHD, and compound-

induced inflammation (for excellent summaries, see reviews in 376, 377). However, some of these studies need to be interpreted with caution because of the nonspecific effects of many of the P2RX7 antagonists and inconsistent results obtained with different knockout mouse strains, as discussed in Section IV. Despite these caveats, more specific P2RX7 antagonists have been developed in the past decade (61) that have confirmed many of the original preclinical studies that used less specific inhibitors. Moreover, several rodent models of arthritis (376) and nephrogenic nephritis (378) have shown amelioration of disease using both specific P2RX7 antagonists and *P2rx7^{-/-}* mice. These results have encouraged a number of pharmaceutical companies to develop specific P2RX7 antagonists, of which several have entered Phase 1 and Phase 2 clinical trials. So far, these compounds have been well tolerated and produced minimal side effects (377). However, P2RX7 antagonists have failed to show efficacy in rheumatoid arthritis, Crohn's disease, chronic obstructive pulmonary disease, or osteoarthritis in humans (376, 377, 379, 380). Future and ongoing studies evaluating P2RX7 antagonists in other diseases and patient populations, coupled with the generation of potentially better and more specific P2RX7 antagonists, should ultimately determine whether P2RX7 is a good target for immunosuppression.

VIII. CONCLUSIONS

Although the repertoire of ion channels and transporters expressed in lymphocyte subsets and innate immune cells such as macrophages, DCs, neutrophils, and mast cells differs, some general principles arise. A general feature common to all immune cells is that they coexpress channels conducting monovalent cations such as Na⁺ and K⁺ that mainly regulate V_m together with ion channels and transporters that conduct Ca²⁺, Mg²⁺, and Zn²⁺, which are used as second messengers for signal transduction. Critical to identifying and dissecting the function of these channels and transporters in immune function has been the study of patients with genetic immune deficiency and mice with targeted deletion of ion channel and transporter genes.

Until recently, most immunology text books showed [Ca²⁺]_i “magically” rising following TCR activation with little explanation about its source. Following the discovery of ORAI and STIM proteins forming the CRAC channel and the phenotype of CRAC-deficient patients, immunologists are now much more aware that ion channels play important roles in the immune system and not just in the heart and the nervous system. The identification of CRAC/ORAI together with STIM also provided new clarity into Ca²⁺ signaling; it is now evident that this is the predominant pathway by which immune and chemokine receptors stimulate Ca²⁺ influx. As a result, studying cells from ORAI1- and STIM1-deficient patients and knockout mice has provided a powerful tool with which to dissect the cellular responses mediated specifically by Ca²⁺. These studies have led to both expected and unexpected findings and as a result have raised many new questions that are currently under investigation. For example, it was a complete surprise that Ca²⁺ influx through CRAC channels is dispensable for conventional T and B cell development. Definitive identification of ORAI/STIM proteins has also forced researchers to readdress the specific roles of other ion channels that were previously thought to contribute to SOCE, such as TRP channels, thereby bringing new clarity to the specific functions of these and other channels in immune regulation. Another general principle that has arisen is the essential role for maintaining a

negative PM potential for Ca^{2+} influx via CRAC and other Ca^{2+} channels, which is mediated primarily by $\text{K}_V1.3$ and $\text{K}_{Ca}3.1$ and antagonized by TRPM4.

Human genetics and the deletion of ion channels and transporters in knockout animals have also reinforced the importance of other divalent cations, including Mg^{2+} and Zn^{2+} , in immune function. The identification of *MAGT1* as the gene mutated in XMEN disease and of the ZIP and ZnT family proteins as Zn^{2+} transporters has demonstrated that both intracellular Mg^{2+} and Zn^{2+} concentrations rise during immune cell activation, suggesting that both cations may serve as second messengers and initiate signaling cascades similar to Ca^{2+} . However, we are clearly only at the beginning of understanding how these transporters and cations regulate immune function and can only imagine that our insights in this area will parallel the gain in knowledge that has been achieved in the previous 10 years studying Ca^{2+} signaling. Foremost among the questions that need to be addressed are which among the more than 30 Mg^{2+} and 24 Zn^{2+} transporters function in immune cells, what their mode of regulation is, and how these cations couple to downstream signaling pathways.

Besides the more established pathways such as CRAC and K^+ channels, we are faced with a plethora of other channels and transporters that have profound effects in regulating immune function, yet for many we have only limited understanding of how they mediate their effects on immune cell function or integrate with signals emanating from other channels or signaling pathways. For example, although mutations or deletion of *MAGT1*, the VRAC subunit *LRRC8A*, K_{2P} channels, or $\text{Na}_V1.5$ cause profound phenotypes, the molecular mechanism(s) whereby these channels regulate immune cells is still largely unknown. Thus, a major challenge going forward is to move from phenotypes described in mice and patients deficient in these channels and transporters and the effect of their inhibition in various disease models to understanding molecularly how these channels integrate with other signaling pathways required for activation.

Another important future challenge will be to translate the exciting in vitro and in vivo studies of ion channels and transporters into new therapies to treat a variety of autoimmune diseases, allergy, and transplant rejection. The potential to pharmacologically target ion channels, including *ORAI1*, $\text{K}_V1.3$, $\text{K}_{Ca}3.1$, P2X receptors, and TRPM2, as therapy is supported by multiple studies using knockout animals or by treatment with specific channel blockers. As discussed, there may be several advantages to targeting some of these channels. For example, inhibiting *ORAI1* or $\text{K}_V1.3$ may provide a means to dampen down inflammatory immune responses sufficiently to prevent autoimmunity, while avoiding excessive immunosuppression that predisposes to infection or malignancy. In addition, the role of a single channel and/or transporter to regulate both innate and adaptive immune responses as well as nonimmune cells may provide a means to target multiple different cell types that contribute to disease simultaneously with a single drug. Although only a handful of drugs targeting these channels has been tried in patients, it is anticipated that the number will rapidly expand with the development of better and more specific inhibitory small molecules, peptides, and antibodies.

Acknowledgments

This work was supported by grants from the National Institutes of Health to S.F. (AI097302), H.W. (GM076063), and E.S. (GM099873 and AI080583). S.F. and E.S. are also recipients of research grants from the Crohn's and Colitis Foundation.

Glossary

IP₃	inositol 1,4,5-trisphosphate
STIM1	stromal interaction molecule 1
MAGT1	Mg ²⁺ transporter 1
XMEN disease	X-linked immunodeficiency with Mg ²⁺ defect, EBV infection, and neoplasia

LITERATURE CITED

1. Cahalan MD, Chandy KG. The functional network of ion channels in T lymphocytes. *Immunol Rev.* 2009; 231:59–87. [PubMed: 19754890]
2. Clapham DE. Calcium signaling. *Cell.* 2007; 131:1047–58. [PubMed: 18083096]
3. Feske S. Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol.* 2007; 7:690–702. [PubMed: 17703229]
4. Feske S. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca²⁺ entry in the immune system and beyond. *Immunol Rev.* 2009; 231:189–209. [PubMed: 19754898]
5. Oh-hora M. Calcium signaling in the development and function of T-lineage cells. *Immunol Rev.* 2009; 231:210–24. [PubMed: 19754899]
6. Vig M, Kinet JP. Calcium signaling in immune cells. *Nat Immunol.* 2009; 10:21–27. [PubMed: 19088738]
7. Launay P, Cheng H, Srivatsan S, Penner R, Fleig A, Kinet JP. TRPM4 regulates calcium oscillations after T cell activation. *Science.* 2004; 306:1374–77. [PubMed: 15550671]
8. Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP. TRPM4 is a Ca²⁺-activated nonselective cation channel mediating cell membrane depolarization. *Cell.* 2002; 109:397–407. [PubMed: 12015988]
9. DeCoursey TE, Chandy KG, Gupta S, Cahalan MD. Voltage-dependent ion channels in T-lymphocytes. *J Neuroimmunol.* 1985; 10:71–95. [PubMed: 2414315]
10. Matteson DR, Deutsch C. K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion. *Nature.* 1984; 307:468–71. [PubMed: 6320008]
11. Grissmer S, Dethlefs B, Wasmuth JJ, Goldin AL, Gutman GA, et al. Expression and chromosomal localization of a lymphocyte K⁺ channel gene. *PNAS.* 1990; 87:9411–15. [PubMed: 2251283]
12. Joiner WJ, Wang LY, Tang MD, Kaczmarek LK. hSK4, a member of a novel subfamily of calcium-activated potassium channels. *PNAS.* 1997; 94:11013–18. [PubMed: 9380751]
13. Fanger CM, Ghanshani S, Logsdon NJ, Rauer H, Kalman K, et al. Calmodulin mediates calcium-dependent activation of the intermediate conductance KCa channel, IKCa1. *J Biol Chem.* 1999; 274:5746–54. [PubMed: 10026195]
14. Nicolaou SA, Neumeier L, Peng Y, Devor DC, Conforti L. The Ca²⁺-activated K⁺ channel KCa3.1 compartmentalizes in the immunological synapse of human T lymphocytes. *Am J Physiol Cell Physiol.* 2007; 292:C1431–39. [PubMed: 17151145]
15. Cai X, Srivastava S, Sun Y, Li Z, Wu H, et al. Tripartite motif containing protein 27 negatively regulates CD4 T cells by ubiquitinating and inhibiting the class II PI3K-C2β. *PNAS.* 2011; 108:20072–77. [PubMed: 22128329]

16. Srivastava S, Di L, Zhdanova O, Li Z, Vardhana S, et al. The class II phosphatidylinositol 3 kinase C2 β is required for the activation of the K⁺ channel KCa3.1 and CD4 T-cells. *Mol Biol Cell*. 2009; 20:3783–91. [PubMed: 19587117]
17. Gerlach AC, Syme CA, Giltinan L, Adelman JP, Devor DC. ATP-dependent activation of the intermediate conductance, Ca²⁺-activated K⁺ channel, hIK1, is conferred by a C-terminal domain. *J Biol Chem*. 2001; 276:10963–70.
18. Srivastava S, Li Z, Ko K, Choudhury P, Albaqumi M, et al. Histidine phosphorylation of the potassium channel KCa3.1 by nucleoside diphosphate kinase B is required for activation of KCa3.1 and CD4 T cells. *Mol Cell*. 2006; 24:665–75. [PubMed: 17157250]
19. Srivastava S, Choudhury P, Li Z, Liu G, Nadkarni V, et al. Phosphatidylinositol 3-phosphate indirectly activates KCa3.1 via 14 amino acids in the carboxy terminus of KCa3.1. *Mol Biol Cell*. 2006; 17:146–54. [PubMed: 16251351]
20. Srivastava S, Ko K, Choudhury P, Li Z, Johnson AK, et al. Phosphatidylinositol-3 phosphatase myotubularin-related protein 6 negatively regulates CD4 T cells. *Mol Cell Biol*. 2006; 26:5595–602. [PubMed: 16847315]
21. Srivastava S, Zhdanova O, Di L, Li Z, Albaqumi M, et al. Protein histidine phosphatase 1 negatively regulates CD4 T cells by inhibiting the K⁺ channel KCa3.1. *PNAS*. 2008; 105:14442–46. [PubMed: 18796614]
22. Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature*. 1992; 355:353–56. [PubMed: 1309940]
23. Lewis RS, Cahalan MD. Mitogen-induced oscillations of cytosolic Ca²⁺ and transmembrane Ca²⁺ current in human leukemic T cells. *Cell Regul*. 1989; 1:99–112. [PubMed: 2519622]
24. Zweifach A, Lewis RS. Mitogen-regulated Ca²⁺ current of T lymphocytes is activated by depletion of intracellular Ca²⁺ stores. *PNAS*. 1993; 90:6295–99. [PubMed: 8392195]
25. Ernst IM, Fliegert R, Guse AH. Adenine dinucleotide second messengers and T-lymphocyte calcium signaling. *Front Immunol*. 2013; 4:259. [PubMed: 24009611]
26. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 2006; 441:179–85. [PubMed: 16582901]
27. Liou J, Kim M, Heo WD, Jones JT, Myers JW, et al. STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Curr Biol*. 2005; 15:1235–41. [PubMed: 16005298]
28. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, et al. STIM1, an essential and conserved component of store-operated Ca²⁺ channel function. *J Cell Biol*. 2005; 169:435–45. [PubMed: 15866891]
29. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca²⁺ entry. *Science*. 2006; 312:1220–23. [PubMed: 16645049]
30. Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, et al. Genome-wide RNAi screen of Ca²⁺ influx identifies genes that regulate Ca²⁺ release-activated Ca²⁺ channel activity. *PNAS*. 2006; 103:9357–62. [PubMed: 16751269]
31. Feske S. CRAC channelopathies. *Pflugers Arch*. 2010; 460:417–35. [PubMed: 20111871]
32. Gwack Y, Srikanth S, Feske S, Cruz-Guilloty F, Oh-hora M, et al. Biochemical and functional characterization of Orai proteins. *J Biol Chem*. 2007; 282:16232–43. [PubMed: 17293345]
33. McCarl CA, Picard C, Khalil S, Kawasaki T, Rother J, et al. ORAI1 deficiency and lack of store-operated Ca²⁺ entry cause immunodeficiency, myopathy, and ectodermal dysplasia. *J Allergy Clin Immunol*. 2009; 124:1311–18.e7. [PubMed: 20004786]
34. Williams RT, Manji SS, Parker NJ, Hancock MS, Van Stekelenburg L, et al. Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem J*. 2001; 357:673–85. [PubMed: 11463338]
35. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature*. 2006; 443:230–33. [PubMed: 16921383]
36. Vig M, Beck A, Billingsley JM, Lis A, Parvez S, et al. CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr Biol*. 2006; 16:2073–79. [PubMed: 16978865]

37. Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature*. 2006; 443:226–29. [PubMed: 16921385]
38. McNally BA, Yamashita M, Engh A, Prakriya M. Structural determinants of ion permeation in CRAC channels. *PNAS*. 2009; 106:22516–21. [PubMed: 20018736]
39. Zhou Y, Ramachandran S, Oh-hora M, Rao A, Hogan PG. Pore architecture of the ORAI1 store-operated calcium channel. *PNAS*. 2010; 107:4896–901. [PubMed: 20194792]
40. Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, et al. CRACM1, CRACM2, and CRACM3 are store-operated Ca²⁺ channels with distinct functional properties. *Curr Biol*. 2007; 17:794–800. [PubMed: 17442569]
41. Stathopulos PB, Zheng L, Li GY, Plevin MJ, Ikura M. Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell*. 2008; 135:110–22. [PubMed: 18854159]
42. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol*. 2010; 28:491–533. [PubMed: 20307213]
43. Lewis RS. Store-operated calcium channels: new perspectives on mechanism and function. *Cold Spring Harb Perspect Biol*. 2011; 3:a003970. [PubMed: 21791698]
44. Shaw PJ, Qu B, Hoth M, Feske S. Molecular regulation of CRAC channels and their role in lymphocyte function. *Cell Mol Life Sci*. 2013; 70:2637–56. [PubMed: 23052215]
45. Shaw PJ, Feske S. Regulation of lymphocyte function by ORAI and STIM proteins in infection and autoimmunity. *J Physiol*. 2012; 590:4157–67. [PubMed: 22615435]
46. Di L, Srivastava S, Zhdanova O, Sun Y, Li Z, Skolnik EY. Nucleoside diphosphate kinase B knockout mice have impaired activation of the K⁺ channel KCa3.1, resulting in defective T cell activation. *J Biol Chem*. 2010; 285:38765–71. [PubMed: 20884616]
47. Hess SD, Oortgiesen M, Cahalan MD. Calcium oscillations in human T and natural killer cells depend upon membrane potential and calcium influx. *J Immunol*. 1993; 150:2620–33. [PubMed: 7681076]
48. Leonard RJ, Garcia ML, Slaughter RS, Reuben JP. Selective blockers of voltage-gated K⁺ channels depolarize human T lymphocytes: mechanism of the antiproliferative effect of charybdotoxin. *PNAS*. 1992; 89:10094–98. [PubMed: 1279670]
49. Wu LJ, Sweet TB, Clapham DE. International Union of Basic and Clinical Pharmacology. LXXXVI. Current progress in the mammalian TRP ion channel family. *Pharmacol Rev*. 2010; 62:381–404. [PubMed: 20716668]
50. Billeter AT, Hellmann JL, Bhatnagar A, Polk HC Jr. Transient receptor potential ion channels: powerful regulators of cell function. *Ann Surg*. 2014; 259:229–35. [PubMed: 23989052]
51. Moran MM, McAlexander MA, Biro T, Szallasi A. Transient receptor potential channels as therapeutic targets. *Nat Rev Drug Discov*. 2011; 10:601–20. [PubMed: 21804597]
52. Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, et al. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature*. 2001; 411:595–99. [PubMed: 11385575]
53. Sano Y, Inamura K, Miyake A, Mochizuki S, Yokoi H, et al. Immuncyte Ca²⁺ influx system mediated by LTRPC2. *Science*. 2001; 293:1327–30. [PubMed: 11509734]
54. Beck A, Kolisek M, Bagley LA, Fleig A, Penner R. Nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose regulate TRPM2 channels in T lymphocytes. *FASEB J*. 2006; 20:962–64. [PubMed: 16585058]
55. Perraud AL, Schmitz C, Scharenberg AM. TRPM2 Ca²⁺ permeable cation channels: from gene to biological function. *Cell Calcium*. 2003; 33:519–31. [PubMed: 12765697]
56. Saito M, Hanson PI, Schlesinger P. Luminal chloride-dependent activation of endosome calcium channels: patch clamp study of enlarged endosomes. *J Biol Chem*. 2007; 282:27327–33. [PubMed: 17609211]
57. Nagasawa M, Nakagawa Y, Tanaka S, Kojima I. Chemotactic peptide fMetLeuPhe induces translocation of the TRPV2 channel in macrophages. *J Cell Physiol*. 2007; 210:692–702. [PubMed: 17154364]

58. Link TM, Park U, Vonakis BM, Raben DM, Soloski MJ, Caterina MJ. TRPV2 has a pivotal role in macrophage particle binding and phagocytosis. *Nat Immunol.* 2010; 11:232–39. [PubMed: 20118928]
59. Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. *Nature.* 2014; 509:310–17. [PubMed: 24828189]
60. Le Feuvre RA, Brough D, Iwakura Y, Takeda K, Rothwell NJ. Priming of macrophages with lipopolysaccharide potentiates P2X7-mediated cell death via a caspase-1-dependent mechanism, independently of cytokine production. *J Biol Chem.* 2002; 277:3210–18. [PubMed: 11706016]
61. Mehta N, Kaur M, Singh M, Chand S, Vyas B, et al. Purinergic receptor P2X(7): a novel target for anti-inflammatory therapy. *Bioorg Med Chem.* 2014; 22:54–88. [PubMed: 24314880]
62. Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, et al. The P2X7 receptor: a key player in IL-1 processing and release. *J Immunol.* 2006; 176:3877–83. [PubMed: 16547218]
63. Junger WG. Immune cell regulation by autocrine purinergic signalling. *Nat Rev Immunol.* 2011; 11:201–12. [PubMed: 21331080]
64. Trautmann A. Extracellular ATP in the immune system: more than just a “danger signal”. *Sci Signal.* 2009; 2:pe6. [PubMed: 19193605]
65. Adamson SE, Leitinger N. The role of pannexin1 in the induction and resolution of inflammation. *FEBS Lett.* 2014; 588:1416–22. [PubMed: 24642372]
66. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, et al. Pannexin 1 channels mediate ‘find-me’ signal release and membrane permeability during apoptosis. *Nature.* 2010; 467:863–67. [PubMed: 20944749]
67. Catterall WA. Voltage-gated calcium channels. *Cold Spring Harb Perspect Biol.* 2011; 3:a003947. [PubMed: 21746798]
68. Omilusik K, Priatel JJ, Chen X, Wang YT, Xu H, et al. The Cav1.4 calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. *Immunity.* 2011; 5(3):349–60. [PubMed: 21835646]
69. Badou A, Jha MK, Matza D, Mehal WZ, Freichel M, et al. Critical role for the beta regulatory subunits of Cav channels in T lymphocyte function. *PNAS.* 2006; 103:15529–34. [PubMed: 17028169]
70. Jha MK, Badou A, Meissner M, McRory JE, Freichel M, et al. Defective survival of naive CD8⁺ T lymphocytes in the absence of the β3 regulatory subunit of voltage-gated calcium channels. *Nat Immunol.* 2009; 10:1275–82. [PubMed: 19838200]
71. Cabral MD, Paulet PE, Robert V, Gomes B, Renoud ML, et al. Knocking down Cav1 calcium channels implicated in Th2 cell activation prevents experimental asthma. *Am J Respir Crit Care Med.* 2010; 181:1310–17. [PubMed: 20167851]
72. Di L, Srivastava S, Zhdanova O, Ding Y, Li Z, et al. Inhibition of the K⁺ channel KCa3.1 ameliorates T cell-mediated colitis. *PNAS.* 2010; 107:1541–46. [PubMed: 20080610]
73. Brandao K, Deason-Towne F, Perraud AL, Schmitz C. The role of Mg²⁺ in immune cells. *Immunol Res.* 2013; 55:261–69. [PubMed: 22990458]
74. Scarpa A, Brinley FJ. In situ measurements of free cytosolic magnesium ions. *Fed Proc.* 1981; 40:2646–52. [PubMed: 7286245]
75. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, et al. Second messenger role for Mg²⁺ revealed by human T-cell immunodeficiency. *Nature.* 2011; 475:471–76. [PubMed: 21796205]
76. Goytain A, Quamme GA. Identification and characterization of a novel mammalian Mg²⁺ transporter with channel-like properties. *BMC Genomics.* 2005; 6:48. [PubMed: 15804357]
77. Zhou H, Clapham DE. Mammalian *MagT1* and *TUSC3* are required for cellular magnesium uptake and vertebrate embryonic development. *PNAS.* 2009; 106:15750–55. [PubMed: 19717468]
78. Bates-Withers C, Sah R, Clapham DE. TRPM7, the Mg²⁺ inhibited channel and kinase. *Adv Exp Med Biol.* 2011; 704:173–83. [PubMed: 21290295]
79. Bae CY, Sun HS. TRPM7 in cerebral ischemia and potential target for drug development in stroke. *Acta Pharmacol Sin.* 2011; 32:725–33. [PubMed: 21552293]

80. Aydemir TB, Liuzzi JP, McClellan S, Cousins RJ. Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN- γ expression in activated human T cells. *J Leukoc Biol.* 2009; 86:337–48. [PubMed: 19401385]
81. Kaltenberg J, Plum LM, Ober-Blobaum JL, Honscheid A, Rink L, Haase H. Zinc signals promote IL-2-dependent proliferation of T cells. *Eur J Immunol.* 2010; 40:1496–503. [PubMed: 20201035]
82. Yu M, Lee WW, Tomar D, Pryshchep S, Czesnikiewicz-Guzik M, et al. Regulation of T cell receptor signaling by activation-induced zinc influx. *J Exp Med.* 2011; 208:775–85. [PubMed: 21422171]
83. Haase H, Hebel S, Engelhardt G, Rink L. Flow cytometric measurement of labile zinc in peripheral blood mononuclear cells. *Anal Biochem.* 2006; 352:222–30. [PubMed: 16545333]
84. Rukgauer M, Klein J, Kruse-Jarres JD. Reference values for the trace elements copper, manganese, selenium, and zinc in the serum/plasma of children, adolescents, and adults. *J Trace Elem Med Biol.* 1997; 11:92–98. [PubMed: 9285889]
85. Huang L, Tepasorndech S. The SLC30 family of zinc transporters—a review of current understanding of their biological and pathophysiological roles. *Mol Aspects Med.* 2013; 34:548–60. [PubMed: 23506888]
86. Jeong J, Eide DJ. The SLC39 family of zinc transporters. *Mol Aspects Med.* 2013; 34:612–19. [PubMed: 23506894]
87. Lichten LA, Cousins RJ. Mammalian zinc transporters: nutritional and physiologic regulation. *Annu Rev Nutr.* 2009; 29:153–76. [PubMed: 19400752]
88. Dufner-Beattie J, Huang ZL, Geiser J, Xu W, Andrews GK. Generation and characterization of mice lacking the zinc uptake transporter ZIP3. *Mol Cell Biol.* 2005; 25:5607–15. [PubMed: 15964816]
89. Overbeck S, Uciechowski P, Ackland ML, Ford D, Rink L. Intracellular zinc homeostasis in leukocyte subsets is regulated by different expression of zinc exporters ZnT-1 to ZnT-9. *J Leukoc Biol.* 2008; 83:368–80. [PubMed: 17971500]
90. Nishida K, Hasegawa A, Nakae S, Oboki K, Saito H, et al. Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction. *J Exp Med.* 2009; 206:1351–64. [PubMed: 19451265]
91. Cahalan MD, Chandy KG, DeCoursey TE, Gupta S. A voltage-gated potassium channel in human T lymphocytes. *J Physiol.* 1985; 358:197–237. [PubMed: 2580081]
92. Verheugen JA, Oortgiesen M, Vijverberg HP. Veratridine blocks voltage-gated potassium current in human T lymphocytes and in mouse neuroblastoma cells. *J Membr Biol.* 1994; 137:205–14. [PubMed: 8182730]
93. Fraser SP, Diss JK, Lloyd LJ, Pani F, Chioni AM, et al. T-lymphocyte invasiveness: control by voltage-gated Na⁺ channel activity. *FEBS Lett.* 2004; 569:191–94. [PubMed: 15225632]
94. Lai ZF, Chen YZ, Nishimura Y, Nishi K. An amiloride-sensitive and voltage-dependent Na⁺ channel in an HLA-DR-restricted human T cell clone. *J Immunol.* 2000; 165:83–90. [PubMed: 10861038]
95. Lo WL, Donermeyer DL, Allen PM. A voltage-gated sodium channel is essential for the positive selection of CD4⁺ T cells. *Nat Immunol.* 2012; 13:880–87. [PubMed: 22842345]
96. Carrithers MD, Dib-Hajj S, Carrithers LM, Tokmoulina G, Pypaert M, et al. Expression of the voltage-gated sodium channel Nav1.5 in the macrophage late endosome regulates endosomal acidification. *J Immunol.* 2007; 178:7822–32. [PubMed: 17548620]
97. Carrithers LM, Hulseberg P, Sandor M, Carrithers MD. The human macrophage sodium channel Nav1.5 regulates mycobacteria processing through organelle polarization and localized calcium oscillations. *FEMS Immunol Med Microbiol.* 2011; 63:319–27. [PubMed: 22092558]
98. Black JA, Newcombe J, Waxman SG. Nav1.5 sodium channels in macrophages in multiple sclerosis lesions. *Mult Scler.* 2013; 19:532–42. [PubMed: 22951351]
99. Craner MJ, Damarjian TG, Liu S, Hains BC, Lo AC, et al. Sodium channels contribute to microglia/macrophage activation and function in EAE and MS. *Glia.* 2005; 49:220–29. [PubMed: 15390090]

100. Zsiros E, Kis-Toth K, Hajdu P, Gaspar R, Bielanska J, et al. Developmental switch of the expression of ion channels in human dendritic cells. *J Immunol.* 2009; 183:4483–92. [PubMed: 19748986]
101. Voss FK, Ullrich F, Munch J, Lazarow K, Lutter D, et al. Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC. *Science.* 2014; 344:634–38. [PubMed: 24790029]
102. Qiu Z, Dubin AE, Mathur J, Tu B, Reddy K, et al. SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel. *Cell.* 2014; 157:447–58. [PubMed: 24725410]
103. Tian J, Lu Y, Zhang H, Chau CH, Dang HN, Kaufman DL. Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. *J Immunol.* 2004; 173:5298–304. [PubMed: 15470076]
104. Mendu SK, Akesson L, Jin Z, Edlund A, Cilio C, et al. Increased GABA_A channel subunits expression in CD8⁺ but not in CD4⁺ T cells in BB rats developing diabetes compared to their congenic littermates. *Mol Immunol.* 2011; 48:399–407. [PubMed: 21112637]
105. Moss RB, Bocian RC, Hsu YP, Dong YJ, Kemna M, et al. Reduced IL-10 secretion by CD4⁺ T lymphocytes expressing mutant cystic fibrosis transmembrane conductance regulator (CFTR). *Clin Exp Immunol.* 1996; 106:374–88. [PubMed: 8918588]
106. Chen JH, Schulman H, Gardner P. A cAMP-regulated chloride channel in lymphocytes that is affected in cystic fibrosis. *Science.* 1989; 243:657–60. [PubMed: 2464852]
107. Decoursey TE. Voltage-gated proton channels. *Compr Physiol.* 2012; 2:1355–85. [PubMed: 23798303]
108. Demaurex N, El Chemaly A. Physiological roles of voltage-gated proton channels in leukocytes. *J Physiol.* 2010; 588:4659–65. [PubMed: 20693294]
109. DeCoursey TE. Voltage-gated proton channels: molecular biology, physiology, and pathophysiology of the H_v family. *Physiol Rev.* 2013; 93:599–652. [PubMed: 23589829]
110. El Chemaly A, Okochi Y, Sasaki M, Arnaudeau S, Okamura Y, Demaurex N. VSOP/Hv1 proton channels sustain calcium entry, neutrophil migration, and superoxide production by limiting cell depolarization and acidification. *J Exp Med.* 2010; 207:129–39. [PubMed: 20026664]
111. Morgan D, Capasso M, Musset B, Cherny VV, Rios E, et al. Voltage-gated proton channels maintain pH in human neutrophils during phagocytosis. *PNAS.* 2009; 106:18022–27. [PubMed: 19805063]
112. Capasso M, Bhamrah MK, Henley T, Boyd RS, Langlais C, et al. HVCN1 modulates BCR signal strength via regulation of BCR-dependent generation of reactive oxygen species. *Nat Immunol.* 2010; 11:265–72. [PubMed: 20139987]
113. Roos A, Boron WF. Intracellular pH. *Physiol Rev.* 1981; 61:296–434. [PubMed: 7012859]
114. Meuth SG, Bittner S, Meuth P, Simon OJ, Budde T, Wiendl H. TWIK-related acid-sensitive K⁺ channel 1 (TASK1) and TASK3 critically influence T lymphocyte effector functions. *J Biol Chem.* 2008; 283:14559–70. [PubMed: 18375952]
115. Bittner S, Bobak N, Herrmann AM, Gobel K, Meuth P, et al. Upregulation of K2P5.1 potassium channels in multiple sclerosis. *Ann Neurol.* 2010; 68:58–69. [PubMed: 20582984]
116. Bobak N, Bittner S, Andronic J, Hartmann S, Muhlpfordt F, et al. Volume regulation of murine T lymphocytes relies on voltage-dependent and two-pore domain potassium channels. *Biochim Biophys Acta.* 2011; 1808:2036–44. [PubMed: 21575593]
117. Bittner S, Meuth SG, Gobel K, Melzer N, Herrmann AM, et al. TASK1 modulates inflammation and neurodegeneration in autoimmune inflammation of the central nervous system. *Brain.* 2009; 132:2501–16. [PubMed: 19570851]
118. Aifantis I, Gounari F, Scorrano L, Borowski C, Von Boehmer H. Constitutive pre-TCR signaling promotes differentiation through Ca²⁺ mobilization and activation of NF-κB and NFAT. *Nat Immunol.* 2001; 2:403–9. [PubMed: 11323693]
119. Bhakta NR, Oh DY, Lewis RS. Calcium oscillations regulate thymocyte motility during positive selection in the three-dimensional thymic environment. *Nat Immunol.* 2005; 6:143–51. [PubMed: 15654342]

120. Daniels MA, Teixeira E, Gill J, Hausmann B, Roubaty D, et al. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature*. 2006; 444:724–29. [PubMed: 17086201]
121. Nakayama T, Ueda Y, Yamada H, Shores EW, Singer A, June CH. In vivo calcium elevations in thymocytes with T cell receptors that are specific for self ligands. *Science*. 1992; 257:96–99. [PubMed: 1621102]
122. Feske S, Muller JM, Graf D, Kroczeck RA, Drager R, et al. Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male sibs. *Eur J Immunol*. 1996; 26:2119–26. [PubMed: 8814256]
123. Fuchs S, Rensing-Ehl A, Speckmann C, Bengsch B, Schmitt-Graeff A, et al. Antiviral and regulatory T cell immunity in a patient with stromal interaction molecule 1 deficiency. *J Immunol*. 2012; 188:1523–33. [PubMed: 22190180]
124. Le Deist F, Hivroz C, Partiseti M, Thomas C, Buc HA, et al. A primary T-cell immunodeficiency associated with defective transmembrane calcium influx. *Blood*. 1995; 85:1053–62. [PubMed: 7531512]
125. Picard C, McCarl CA, Papolos A, Khalil S, Luthy K, et al. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med*. 2009; 360:1971–80. [PubMed: 19420366]
126. Gwack Y, Srikanth S, Oh-hora M, Hogan PG, Lamperti ED, et al. Hair loss and defective T- and B-cell function in mice lacking ORA1. *Mol Cell Biol*. 2008; 28:5209–22. [PubMed: 18591248]
127. McCarl CA, Khalil S, Ma J, Oh-hora M, Yamashita M, et al. Store-operated Ca^{2+} entry through ORA1 is critical for T cell-mediated autoimmunity and allograft rejection. *J Immunol*. 2010; 185:5845–58. [PubMed: 20956344]
128. Beyersdorf N, Braun A, Vogtle T, Varga-Szabo D, Galdos R, et al. STIM1-independent T cell development and effector function in vivo. *J Immunol*. 2009; 182:3390–97. [PubMed: 19265116]
129. Vig M, DeHaven WI, Bird GS, Billingsley JM, Wang H, et al. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat Immunol*. 2008; 9:89–96. [PubMed: 18059270]
130. Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nat Immunol*. 2008; 9:81–88. [PubMed: 18059272]
131. Oh-hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, et al. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol*. 2008; 9:432–43. [PubMed: 18327260]
132. Oh-hora M, Komatsu N, Pishyareh M, Feske S, Hori S, et al. Agonist-selected T cell development requires strong T cell receptor signaling and store-operated calcium entry. *Immunity*. 2013; 38:881–95. [PubMed: 23499491]
133. Grgic I, Wulff H, Eichler I, Flothmann C, Kohler R, Hoyer J. Blockade of T-lymphocyte $KCa_{3.1}$ and $Kv1.3$ channels as novel immunosuppression strategy to prevent kidney allograft rejection. *Transplant Proc*. 2009; 41:2601–6. [PubMed: 19715983]
134. Gocke AR, Lebson LA, Grishkan IV, Hu L, Nguyen HM, et al. $Kv1.3$ deletion biases T cells toward an immunoregulatory phenotype and renders mice resistant to autoimmune encephalomyelitis. *J Immunol*. 2012; 188:5877–86. [PubMed: 22581856]
- 134a. Ouyang K, Leandro Gomez-Amaro R, Stachura DL, Tang H, Peng X, et al. Loss of IP3R-dependent Ca^{2+} signalling in thymocytes leads to aberrant development and acute lymphoblastic leukemia. *Nat Commun*. 2014; 5:4814. [PubMed: 25215520]
135. Strydom GL, Jameson SC, Hogquist KA. Selection of self-reactive T cells in the thymus. *Annu Rev Immunol*. 2012; 30:95–114. [PubMed: 22149933]
136. Brigl M, Bry L, Kent SC, Gumperz JE, Brenner MB. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nat Immunol*. 2003; 4:1230–37. [PubMed: 14578883]
137. Kronenberg M, Kinjo Y. Innate-like recognition of microbes by invariant natural killer T cells. *Curr Opin Immunol*. 2009; 21:391–96. [PubMed: 19646850]

138. Godfrey DI, Berzins SP. Control points in NKT-cell development. *Nat Rev Immunol.* 2007; 7:505–18. [PubMed: 17589542]
139. Lin J, Krishnaraj R, Kemp RG. Exogenous ATP enhances calcium influx in intact thymocytes. *J Immunol.* 1985; 135:3403–10. [PubMed: 2413120]
140. Chused TM, Apasov S, Sitkovsky M. Murine T lymphocytes modulate activity of an ATP-activated P2Z-type purinoceptor during differentiation. *J Immunol.* 1996; 157:1371–80. [PubMed: 8759716]
141. Ross PE, Ehring GR, Cahalan MD. Dynamics of ATP-induced calcium signaling in single mouse thymocytes. *J Cell Biol.* 1997; 138:987–98. [PubMed: 9281578]
142. Pizzo P, Zanovello P, Bronte V, Di Virgilio F. Extracellular ATP causes lysis of mouse thymocytes and activates a plasma membrane ion channel. *Biochem J.* 1991; 274(Pt. 1):139–44. [PubMed: 1705798]
143. Freedman BD, Liu QH, Gaulton G, Kotlikoff MI, Hescheler J, Fleischmann BK. ATP-evoked Ca^{2+} transients and currents in murine thymocytes: possible role for P2X receptors in death by neglect. *Eur J Immunol.* 1999; 29:1635–46. [PubMed: 10359118]
144. Mulryan K, Gitterman DP, Lewis CJ, Vial C, Leckie BJ, et al. Reduced vas deferens contraction and male infertility in mice lacking P2X1 receptors. *Nature.* 2000; 403:86–89. [PubMed: 10638758]
145. Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, et al. Altered cytokine production in mice lacking P2X(7) receptors. *J Biol Chem.* 2001; 276:125–32. [PubMed: 11016935]
146. Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, et al. Impaired flow-dependent control of vascular tone and remodeling in P2X4-deficient mice. *Nat Med.* 2006; 12:133–37. [PubMed: 16327800]
147. Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, et al. P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. *J Physiol.* 2005; 567:621–39. [PubMed: 15961431]
148. Li FY, Chaigne-Delalande B, Su H, Uzel G, Matthews H, Lenardo MJ. XMEN disease: a new primary immunodeficiency affecting Mg^{2+} regulation of immunity against Epstein-Barr virus. *Blood.* 2014; 123:2148–52. [PubMed: 24550228]
149. Jin J, Desai BN, Navarro B, Donovan A, Andrews NC, Clapham DE. Deletion of *Trpm7* disrupts embryonic development and thymopoiesis without altering Mg^{2+} homeostasis. *Science.* 2008; 322:756–60. [PubMed: 18974357]
150. Ackland ML, Michalczyk A. Zinc deficiency and its inherited disorders—a review. *Genes Nutr.* 2006; 1:41–49. [PubMed: 18850219]
151. Fraker PJ, King LE. Reprogramming of the immune system during zinc deficiency. *Annu Rev Nutr.* 2004; 24:277–98. [PubMed: 15189122]
152. Kury S, Dreno B, Bezieau S, Giraudet S, Kharfi M, et al. Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. *Nat Genet.* 2002; 31:239–40. [PubMed: 12068297]
153. Wang K, Zhou B, Kuo YM, Zemansky J, Gitschier J. A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. *Am J Hum Genet.* 2002; 71:66–73. [PubMed: 12032886]
154. Geiser J, Venken KJ, De Lisle RC, Andrews GK. A mouse model of acrodermatitis enteropathica: loss of intestine zinc transporter ZIP4 (*Slc39a4*) disrupts the stem cell niche and intestine integrity. *PLOS Genet.* 2012; 8:e1002766. [PubMed: 22737083]
- 154a. Kumar L, Chou J, Yee CS, Borzutzky A, Vollmann EH, et al. Leucine-rich repeat containing 8A (*LRRC8A*) is essential for T lymphocyte development and function. *J Exp Med.* 2014; 211:929–42. [PubMed: 24752297]
- 154b. Szabò I, Lepple-Wienhues A, Kaba KN, Zoratti M, Gulbins E, Lang F. Tyrosine kinase-dependent activation of a chloride channel in CD95-induced apoptosis in T lymphocytes. *PNAS.* 1998; 95:6169–74. [PubMed: 9600936]
155. Feske S, Giltman J, Dolmetsch R, Staudt LM, Rao A. Gene regulation mediated by calcium signals in T lymphocytes. *Nat Immunol.* 2001; 2:316–24. [PubMed: 11276202]

156. Matsumoto M, Fujii Y, Baba A, Hikida M, Kurosaki T, Baba Y. The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. *Immunity*. 2011; 34:703–14. [PubMed: 21530328]
157. Limnander A, Depeille P, Freedman TS, Liou J, Leitges M, et al. STIM1, PKC- δ and RasGRP set a threshold for proapoptotic Erk signaling during B cell development. *Nat Immunol*. 2011; 12:425–33. [PubMed: 21441934]
158. Miller AT, Sandberg M, Huang YH, Young M, Sutton S, et al. Production of Ins(1,3,4,5)P4 mediated by the kinase Itpkb inhibits store-operated calcium channels and regulates B cell selection and activation. *Nat Immunol*. 2007; 8:514–21. [PubMed: 17417640]
159. Miyai T, Hojyo S, Ikawa T, Kawamura M, Irie T, et al. Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development. *PNAS*. 2014; 111:11780–85. [PubMed: 25074913]
160. Sawada A, Takihara Y, Kim JY, Matsuda-Hashii Y, Tokimasa S, et al. A congenital mutation of the novel gene LRRC8 causes agammaglobulinemia in humans. *J Clin Invest*. 2003; 112:1707–13. [PubMed: 14660746]
161. Byun M, Abhyankar A, Lelarge V, Plancoulaine S, Palanduz A, et al. Whole-exome sequencing-based discovery of STIM1 deficiency in a child with fatal classic Kaposi sarcoma. *J Exp Med*. 2010; 207:2307–12. [PubMed: 20876309]
162. Feske S. Immunodeficiency due to defects in store-operated calcium entry. *Ann NY Acad Sci*. 2011; 1238:74–90. [PubMed: 22129055]
163. Feske S, Prakriya M, Rao A, Lewis RS. A severe defect in CRAC Ca^{2+} channel activation and altered K^{+} channel gating in T cells from immunodeficient patients. *J Exp Med*. 2005; 202:651–62. [PubMed: 16147976]
164. Ma J, McCarl CA, Khalil S, Luthy K, Feske S. T-cell-specific deletion of STIM1 and STIM2 protects mice from EAE by impairing the effector functions of Th1 and Th17 cells. *Eur J Immunol*. 2010; 40:3028–42. [PubMed: 21061435]
165. Kim KD, Srikanth S, Yee MK, Mock DC, Lawson GW, Gwack Y. ORAI1 deficiency impairs activated T cell death and enhances T cell survival. *J Immunol*. 2011; 187:3620–30. [PubMed: 21873530]
166. Macian F. NFAT proteins: key regulators of T-cell development and function. *Nat Rev Immunol*. 2005; 5:472–84. [PubMed: 15928679]
167. Feske S, Draeger R, Peter HH, Eichmann K, Rao A. The duration of nuclear residence of NFAT determines the pattern of cytokine expression in human SCID T cells. *J Immunol*. 2000; 165:297–305. [PubMed: 10861065]
168. Kim KD, Srikanth S, Tan YV, Yee MK, Jew M, et al. Calcium signaling via Orail is essential for induction of the nuclear orphan receptor pathway to drive Th17 differentiation. *J Immunol*. 2014; 192:110–22. [PubMed: 24307733]
169. Schuhmann MK, Stegner D, Berna-Erro A, Bittner S, Braun A, et al. Stromal interaction molecules 1 and 2 are key regulators of autoreactive T cell activation in murine autoimmune central nervous system inflammation. *J Immunol*. 2010; 184:1536–42. [PubMed: 20028655]
170. Weidinger C, Shaw PJ, Feske S. STIM1 and STIM2-mediated Ca^{2+} influx regulates antitumour immunity by CD8^{+} T cells. *EMBO Mol Med*. 2013; 5:1311–21. [PubMed: 23922331]
171. Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, et al. Late developmental plasticity in the T helper 17 lineage. *Immunity*. 2009; 30:92–107. [PubMed: 19119024]
172. Zhou L, Chong MM, Littman DR. Plasticity of CD4^{+} T cell lineage differentiation. *Immunity*. 2009; 30:646–55. [PubMed: 19464987]
173. Dearman RJ, Kimber I. Role of CD4^{+} T helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. *Immunology*. 2000; 101:442–51. [PubMed: 11122447]
174. Yu Y, Wang D, Liu C, Kaosaard K, Semple K, et al. Prevention of GVHD while sparing GVL effect by targeting Th1 and Th17 transcription factor T-bet and $\text{ROR}\gamma\text{T}$ in mice. *Blood*. 2011; 118:5011–20. [PubMed: 21856864]
175. Maynard CL, Weaver CT. Intestinal effector T cells in health and disease. *Immunity*. 2009; 31:389–400. [PubMed: 19766082]

176. Jin S, Chin J, Kitson C, Woods J, Majmudar R, et al. Natural regulatory T cells are resistant to calcium release-activated calcium (CRAC/ORAI) channel inhibition. *Int Immunol*. 2013; 25:497–506. [PubMed: 23667148]
177. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive Foxp3⁺ regulatory T cells: more of the same or a division of labor? *Immunity*. 2009; 30:626–35. [PubMed: 19464985]
178. Greenberg ML, Yu Y, Leverrier S, Zhang SL, Parker I, Cahalan MD. Orai1 function is essential for T cell homing to lymph nodes. *J Immunol*. 2013; 190:3197–206. [PubMed: 23455504]
179. Waite JC, Vardhana S, Shaw PJ, Jang JE, McCarl CA, et al. Interference with Ca²⁺ release activated Ca²⁺ (CRAC) channel function delays T-cell arrest in vivo. *Eur J Immunol*. 2013; 43:3343–54. [PubMed: 23939929]
180. Negulescu PA, Krasieva TB, Khan A, Kerschbaum HH, Cahalan MD. Polarity of T cell shape, motility, and sensitivity to antigen. *Immunity*. 1996; 4:421–30. [PubMed: 8630728]
181. MacLennan IC, Gotch FM, Golstein P. Limited specific T-cell mediated cytotoxicity in the absence of extracellular Ca²⁺ Immunology. 1980; 39:109–17. [PubMed: 6769782]
182. Takayama H, Sitkovsky MV. Antigen receptor-regulated exocytosis in cytotoxic T lymphocytes. *J Exp Med*. 1987; 166:725–43. [PubMed: 2442289]
183. Shaw PJ, Weidinger C, Vaeth M, Luethy K, Kaech S, Feske S. CD4⁺ and CD8⁺ T cell-dependent antiviral immunity requires STIM1 and STIM2. *J Clin Invest*. 2014; 124:4549–63. [PubMed: 25157823]
184. Maul-Pavicic A, Chiang SC, Rensing-Ehl A, Jessen B, Fauriat C, et al. ORAI1-mediated calcium influx is required for human cytotoxic lymphocyte degranulation and target cell lysis. *PNAS*. 2011; 108:3324–29. [PubMed: 21300876]
185. Sahin G, Palanduz A, Aydogan G, Cassar O, Ertem AU, et al. Classic Kaposi sarcoma in 3 unrelated Turkish children born to consanguineous kindreds. *Pediatrics*. 2010; 125:e704–8. [PubMed: 20156905]
186. Robert V, Triffaux E, Paulet PE, Guery JC, Pelletier L, Savignac M. Protein kinase C-dependent activation of CaV1.2 channels selectively controls human TH2-lymphocyte functions. *J Allergy Clin Immunol*. 2014; 133:1175–83. [PubMed: 24365142]
187. Splawski I, Timothy K, Sharpe L, Decher N, Kumar P, et al. CaV1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell*. 2004; 119:19–31. [PubMed: 15454078]
188. Strom TM, Nyakatura G, Apfelstedt-Sylla E, Hellebrand H, Lorenz B, et al. An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nat Genet*. 1998; 19:260–63. [PubMed: 9662399]
189. Striessnig J, Bolz HJ, Koschak A. Channelopathies in Cav1.1, Cav1.3, and Cav1.4 voltage-gated L-type Ca²⁺ channels. *Pflugers Arch*. 2010; 460:361–74. [PubMed: 20213496]
190. Baricordi OR, Ferrari D, Melchiorri L, Chiozzi P, Hanau S, et al. An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. *Blood*. 1996; 87:682–90. [PubMed: 8555491]
191. Padeh S, Cohen A, Roifman CM. ATP-induced activation of human B lymphocytes via P2-purinoceptors. *J Immunol*. 1991; 146:1626–32. [PubMed: 1847168]
192. Adinolfi E, Callegari MG, Ferrari D, Bolognesi C, Minelli M, et al. Basal activation of the P2₇ ATP receptor elevates mitochondrial calcium and potential, increases cellular ATP levels, and promotes serum-independent growth. *Mol Biol Cell*. 2005; 16:3260–72. [PubMed: 15901833]
193. Woehrle T, Yip L, Elkhali A, Sumi Y, Chen Y, et al. Pannexin-1 hemichannel-mediated ATP release together with P2X1 and P2X4 receptors regulate T-cell activation at the immune synapse. *Blood*. 2010; 116:3475–84. [PubMed: 20660288]
194. Schenk U, Westendorf AM, Radaelli E, Casati A, Ferro M, et al. Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels. *Sci Signal*. 2008; 1:ra6. [PubMed: 18827222]
195. Schenk U, Frascoli M, Proietti M, Geffers R, Traggiai E, et al. ATP inhibits the generation and function of regulatory T cells through the activation of purinergic P2X receptors. *Sci Signal*. 2011; 4:ra12. [PubMed: 21364186]

196. Vergani A, Tezza S, D'Addio F, Fotino C, Liu K, et al. Long-term heart transplant survival by targeting the ionotropic purinergic receptor P2X7. *Circulation*. 2013; 127:463–75. [PubMed: 23250993]
197. Vergani A, Fotino C, D'Addio F, Tezza S, Podetta M, et al. Effect of the purinergic inhibitor oxidized ATP in a model of islet allograft rejection. *Diabetes*. 2013; 62:1665–75. [PubMed: 23315496]
198. Sharp AJ, Polak PE, Simonini V, Lin SX, Richardson JC, et al. P2×7 deficiency suppresses development of experimental autoimmune encephalomyelitis. *J Neuroinflamm*. 2008; 5:33.
199. Chen L, Brosnan CF. Exacerbation of experimental autoimmune encephalomyelitis in P2X7R^{-/-} mice: evidence for loss of apoptotic activity in lymphocytes. *J Immunol*. 2006; 176:3115–26. [PubMed: 16493071]
200. Taylor SR, Gonzalez-Begne M, Sojka DK, Richardson JC, Sheardown SA, et al. Lymphocytes from P2X7-deficient mice exhibit enhanced P2X7 responses. *J Leukoc Biol*. 2009; 85:978–86. [PubMed: 19276178]
201. Saul S, Stanisz H, Backes CS, Schwarz EC, Hoth M. How ORAI and TRP channels interfere with each other: Interaction models and examples from the immune system and the skin. *Eur J Pharmacol*. 2013; 739:49–59. [PubMed: 24291108]
202. Wenning AS, Neblung K, Strauss B, Wolfs MJ, Sappok A, et al. TRP expression pattern and the functional importance of TRPC3 in primary human T-cells. *Biochim Biophys Acta*. 2011; 1813:412–23. [PubMed: 21215279]
203. Wang J, Lu ZH, Gabius HJ, Rohowsky-Kochan C, Ledeen RW, Wu G. Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. *J Immunol*. 2009; 182:4036–45. [PubMed: 19299701]
204. Suzuki Y, Kovacs CS, Takanaga H, Peng JB, Landowski CP, Hediger MA. Calcium channel TRPV6 is involved in murine maternal-fetal calcium transport. *J Bone Miner Res*. 2008; 23:1249–56. [PubMed: 18348695]
205. Guse AH, da Silva CP, Berg I, Skapenko AL, Weber K, et al. Regulation of calcium signalling in T lymphocytes by the second messenger cyclic ADP-ribose. *Nature*. 1999; 398:70–73. [PubMed: 10078531]
206. Melzer N, Hicking G, Gobel K, Wiendl H. TRPM2 cation channels modulate T cell effector functions and contribute to autoimmune CNS inflammation. *PLOS ONE*. 2012; 7:e47617. [PubMed: 23077651]
207. Weber KS, Hildner K, Murphy KM, Allen PM. Trpm4 differentially regulates Th1 and Th2 function by altering calcium signaling and NFAT localization. *J Immunol*. 2010; 185:2836–46. [PubMed: 20656926]
208. Verheugen JA, Vijverberg HP, Oortgiesen M, Cahalan MD. Voltage-gated and Ca²⁺-activated K⁺ channels in intact human T lymphocytes. Noninvasive measurements of membrane currents, membrane potential, and intracellular calcium. *J Gen Physiol*. 1995; 105:765–94. [PubMed: 7561743]
209. Ghanshani S, Wulff H, Miller MJ, Rohm H, Neben A, et al. Up-regulation of the IKCa1 potassium channel during T-cell activation. Molecular mechanism and functional consequences. *J Biol Chem*. 2000; 275:37137–49. [PubMed: 10961988]
210. Fanger CM, Neben AL, Cahalan MD. Differential Ca²⁺ influx, KCa channel activity, and Ca²⁺ clearance distinguish Th1 and Th2 lymphocytes. *J Immunol*. 2000; 164:1153–60. [PubMed: 10640725]
211. Fanger CM, Rauer H, Neben AL, Miller MJ, Wulff H, et al. Calcium-activated potassium channels sustain calcium signaling in T lymphocytes. Selective blockers and manipulated channel expression levels. *J Biol Chem*. 2001; 276:12249–56. [PubMed: 11278890]
212. Ishida Y, Chused TM. Lack of voltage sensitive potassium channels and generation of membrane potential by sodium potassium ATPase in murine T lymphocytes. *J Immunol*. 1993; 151:610–20. [PubMed: 8393035]

213. Koo GC, Blake JT, Talento A, Nguyen M, Lin S, et al. Blockade of the voltage-gated potassium channel Kv1.3 inhibits immune responses in vivo. *J Immunol.* 1997; 158:5120–28. [PubMed: 9164927]
214. Pereira LE, Villinger F, Wulff H, Sankaranarayanan A, Raman G, Ansari AA. Pharmacokinetics, toxicity, and functional studies of the selective Kv1.3 channel blocker 5-(4-phenoxybutoxy)psoralen in rhesus macaques. *Exp Biol Med.* 2007; 232:1338–54.
215. Liu QH, Fleischmann BK, Hondowicz B, Maier CC, Turka LA, et al. Modulation of Kv channel expression and function by TCR and costimulatory signals during peripheral CD4⁺ lymphocyte differentiation. *J Exp Med.* 2002; 196:897–909. [PubMed: 12370252]
216. Grissmer S, Ghanshani S, Dethlefs B, McPherson JD, Wasmuth JJ, et al. The *Shaw*-related potassium channel gene, Kv3.1, on human chromosome 11, encodes the type *I*K⁺ channel in T cells. *J Biol Chem.* 1992; 267:20971–79. [PubMed: 1400413]
217. Beeton C, Chandy KG. Potassium channels, memory T cells, and multiple sclerosis. *Neuroscientist.* 2005; 11:550–62. [PubMed: 16282596]
218. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol.* 2004; 22:745–63. [PubMed: 15032595]
219. Wulff H, Calabresi PA, Allie R, Yun S, Pennington M, et al. The voltage-gated Kv1.3 K⁺ channel in effector memory T cells as new target for MS. *J Clin Investig.* 2003; 111:1703–13. [PubMed: 12782673]
220. Beeton C, Wulff H, Standifer NE, Azam P, Mullen KM, et al. Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. *PNAS.* 2006; 103:17414–19. [PubMed: 17088564]
221. Azam P, Sankaranarayanan A, Homerick D, Griffey S, Wulff H. Targeting effector memory T cells with the small molecule Kv1.3 blocker PAP-1 suppresses allergic contact dermatitis. *J Invest Dermatol.* 2007; 127:1419–29. [PubMed: 17273162]
222. Matheu MP, Beeton C, Garcia A, Chi V, Rangaraju S, et al. Imaging of effector memory T cells during a delayed-type hypersensitivity reaction and suppression by Kv1.3 channel block. *Immunity.* 2008; 29:602–14. [PubMed: 18835197]
223. Hu L, Gocke AR, Knapp E, Rosenzweig JM, Grishkan IV, et al. Functional blockade of the voltage-gated potassium channel Kv1.3 mediates reversion of T effector to central memory lymphocytes through SMAD3/p21cip1 signaling. *J Biol Chem.* 2012; 287:1261–68. [PubMed: 22110135]
224. Chaigne-Delalande B, Li FY, O'Connor GM, Lukacs MJ, Jiang P, et al. Mg²⁺ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. *Science.* 2013; 341:186–91. [PubMed: 23846901]
225. Rezaei N, Hedayat M, Aghamohammadi A, Nichols KE. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol.* 2011; 127:1329–41.e2. [PubMed: 21514636]
226. Desai B, Krapivinsky G, Navarro B, Krapivinsky L, Carter BC, et al. Cleavage of TRPM7 releases the kinase domain from the ion channel and regulates its participation in Fas-induced apoptosis. *Dev Cell.* 2012; 22(6):1149–62. [PubMed: 22698280]
227. Haase H, Rink L. Functional significance of zinc-related signaling pathways in immune cells. *Annu Rev Nutr.* 2009; 29:133–52. [PubMed: 19400701]
228. Hirano T, Murakami M, Fukada T, Nishida K, Yamasaki S, Suzuki T. Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule. *Adv Immunol.* 2008; 97:149–76. [PubMed: 18501770]
229. Honscheid A, Rink L, Haase H. T-lymphocytes: a target for stimulatory and inhibitory effects of zinc ions. *Endocr Metab Immune Disord Drug Targets.* 2009; 9:132–44. [PubMed: 19519463]
230. Kirchner H, Ruhl H. Stimulation of human peripheral lymphocytes by Zn²⁺ in vitro. *Exp Cell Res.* 1970; 61:229–30. [PubMed: 5431626]
231. Fraker PJ, Jardieu P, Cook J. Zinc deficiency and immune function. *Arch Dermatol.* 1987; 123:1699–701. [PubMed: 3120653]

232. Wellinghausen N, Martin M, Rink L. Zinc inhibits interleukin-1-dependent T cell stimulation. *Eur J Immunol.* 1997; 27:2529–35. [PubMed: 9368606]
233. Tanaka S, Akaishi E, Hosaka K, Okamura S, Kubohara Y. Zinc ions suppress mitogen-activated interleukin-2 production in Jurkat cells. *Biochem Biophys Res Commun.* 2005; 335:162–67. [PubMed: 16055081]
234. Bjurstom H, Wang J, Ericsson I, Bengtsson M, Liu Y, et al. GABA, a natural immunomodulator of T lymphocytes. *J Neuroimmunol.* 2008; 205:44–50. [PubMed: 18954912]
235. Tian J, Chau C, Hales TG, Kaufman DL. GABA_A receptors mediate inhibition of T cell responses. *J Neuroimmunol.* 1999; 96:21–28. [PubMed: 10227421]
236. Tian J, Yong J, Dang H, Kaufman DL. Oral GABA treatment downregulates inflammatory responses in a mouse model of rheumatoid arthritis. *Autoimmunity.* 2011; 44:465–70. [PubMed: 21604972]
237. Grinstein S, Clarke CA, Dupre A, Rothstein A. Volume-induced increase of anion permeability in human lymphocytes. *J Gen Physiol.* 1982; 80:801–23. [PubMed: 7175489]
238. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev.* 2007; 87:1215–84. [PubMed: 17928584]
239. Spitzer NC. How GABA generates depolarization. *J Physiol.* 2010; 588:757–58. [PubMed: 20194137]
240. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. Lack of cystic fibrosis transmembrane conductance regulator in CD3⁺ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Biol.* 2011; 44:922–29. [PubMed: 20724552]
241. Mori Y, Wakamori M, Miyakawa T, Hermosura M, Hara Y, et al. Transient receptor potential 1 regulates capacitative Ca²⁺ entry and Ca²⁺ release from endoplasmic reticulum in B lymphocytes. *J Exp Med.* 2002; 195:673–81. [PubMed: 11901194]
242. Lievreumont JP, Numaga T, Vazquez G, Lemonnier L, Hara Y, et al. The role of canonical transient receptor potential 7 in B-cell receptor-activated channels. *J Biol Chem.* 2005; 280:35346–51. [PubMed: 16123040]
243. Liu QH, Liu X, Wen Z, Hondowicz B, King L, et al. Distinct calcium channels regulate responses of primary B lymphocytes to B cell receptor engagement and mechanical stimuli. *J Immunol.* 2005; 174:68–79. [PubMed: 15611229]
244. Bubien JK, Zhou LJ, Bell PD, Frizzell RA, Tedder TF. Transfection of the CD20 cell surface molecule into ectopic cell types generates a Ca²⁺ conductance found constitutively in B lymphocytes. *J Cell Biol.* 1993; 121:1121–32. [PubMed: 7684739]
245. Anolik J, Looney RJ, Bottaro A, Sanz I, Young F. Down-regulation of CD20 on B cells upon CD40 activation. *Eur J Immunol.* 2003; 33:2398–409. [PubMed: 12938216]
246. Wulff H, Knaus HG, Pennington M, Chandy KG. K⁺ channel expression during B cell differentiation: implications for immunomodulation and autoimmunity. *J Immunol.* 2004; 173:776–86. [PubMed: 15240664]
247. Moritoki Y, Lian ZX, Wulff H, Yang GX, Chuang YH, et al. AMA production in primary biliary cirrhosis is promoted by the TLR9 ligand CpG and suppressed by potassium channel blockers. *Hepatology.* 2007; 45:314–22. [PubMed: 17256753]
248. Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, et al. Regulation of vertebrate cellular Mg²⁺ homeostasis by TRPM7. *Cell.* 2003; 114:191–200. [PubMed: 12887921]
249. Hojyo S, Miyai T, Fujishiro H, Kawamura M, Yasuda T, et al. Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength. *PNAS.* 2014; 111:11786–91. [PubMed: 25074919]
250. Chen BC, Chou CF, Lin WW. Pyrimidinoceptor-mediated potentiation of inducible nitric-oxide synthase induction in J774 macrophages. Role of intracellular calcium. *J Biol Chem.* 1998; 273:29754–63. [PubMed: 9792689]
251. Watanabe N, Suzuki J, Kobayashi Y. Role of calcium in tumor necrosis factor- α production by activated macrophages. *J Biochem.* 1996; 120:1190–95. [PubMed: 9010769]

252. Malik ZA, Denning GM, Kusner DJ. Inhibition of Ca²⁺ signaling by *Mycobacterium tuberculosis* is associated with reduced phagosome-lysosome fusion and increased survival within human macrophages. *J Exp Med*. 2000; 191:287–302. [PubMed: 10637273]
253. Vergne I, Chua J, Deretic V. Tuberculosis toxin blocking phagosome maturation inhibits a novel Ca²⁺/calmodulin-PI3K hVPS34 cascade. *J Exp Med*. 2003; 198:653–59. [PubMed: 12925680]
254. Zimmerli S, Majeed M, Gustavsson M, Stendahl O, Sanan DA, Ernst JD. Phagosome-lysosome fusion is a calcium-independent event in macrophages. *J Cell Biol*. 1996; 132:49–61. [PubMed: 8567729]
255. Braun A, Gessner JE, Varga-Szabo D, Syed SN, Konrad S, et al. STIM1 is essential for Fcγ receptor activation and autoimmune inflammation. *Blood*. 2009; 113:1097–104. [PubMed: 18941110]
256. Connolly SF, Kusner DJ. The regulation of dendritic cell function by calcium-signaling and its inhibition by microbial pathogens. *Immunol Res*. 2007; 39:115–27. [PubMed: 17917060]
257. Hsu S, O’Connell PJ, Klyachko VA, Badminton MN, Thomson AW, et al. Fundamental Ca²⁺ signaling mechanisms in mouse dendritic cells: CRAC is the major Ca²⁺ entry pathway. *J Immunol*. 2001; 166:6126–33. [PubMed: 11342632]
258. Matzner N, Zemtsova IM, Nguyen TX, Duszenko M, Shumilina E, Lang F. Ion channels modulating mouse dendritic cell functions. *J Immunol*. 2008; 181:6803–9. [PubMed: 18981098]
259. Felix R, Crottes D, Delalande A, Fauconnier J, Lebranchu Y, et al. The Orai-1 and STIM-1 complex controls human dendritic cell maturation. *PLOS ONE*. 2013; 8:e61595. [PubMed: 23700407]
260. Schaff UY, Dixit N, Procyk E, Yamayoshi I, Tse T, Simon SI. Orai1 regulates intracellular calcium, arrest, and shape polarization during neutrophil recruitment in shear flow. *Blood*. 2010; 115:657–66. [PubMed: 19965684]
261. Zhang H, Clemens RA, Liu F, Hu Y, Baba Y, et al. STIM1 calcium sensor is required for activation of the phagocyte oxidase during inflammation and host defense. *Blood*. 2014; 123:2238–49. [PubMed: 24493668]
262. Brechard S, Plancon S, Melchior C, Tschirhart EJ. STIM1 but not STIM2 is an essential regulator of Ca²⁺ influx-mediated NADPH oxidase activity in neutrophil-like HL-60 cells. *Biochem Pharmacol*. 2009; 78:504–13. [PubMed: 19433064]
263. Steinckwich N, Schenten V, Melchior C, Brechard S, Tschirhart EJ. An essential role of STIM1, Orai1, and S100A8-A9 proteins for Ca²⁺ signaling and FcγR-mediated phagosomal oxidative activity. *J Immunol*. 2011; 186:2182–91. [PubMed: 21239714]
264. Nunes P, Cornut D, Bochet V, Hasler U, Oh-hora M, et al. STIM1 juxtaposes ER to phagosomes, generating Ca²⁺ hotspots that boost phagocytosis. *Curr Biol*. 2012; 22:1990–97. [PubMed: 23041196]
265. Gallin EK. Voltage clamp studies in macrophages from mouse spleen cultures. *Science*. 1981; 214:458–60. [PubMed: 7291986]
266. Vicente R, Escalada A, Coma M, Fuster G, Sanchez-Tillo E, et al. Differential voltage-dependent K⁺ channel responses during proliferation and activation in macrophages. *J Biol Chem*. 2003; 278:46307–20. [PubMed: 12923194]
267. Gallin EK. Calcium- and voltage-activated potassium channels in human macrophages. *Biophys J*. 1984; 46:821–25. [PubMed: 6097318]
268. Hanley PJ, Musset B, Renigunta V, Limberg SH, Dalpke AH, et al. Extracellular ATP induces oscillations of intracellular Ca²⁺ and membrane potential and promotes transcription of IL-6 in macrophages. *PNAS*. 2004; 101:9479–84. [PubMed: 15194822]
269. Chung I, Zelyvanskaya M, Gendelman HE. Mononuclear phagocyte biophysiology influences brain transendothelial and tissue migration: implication for HIV-1-associated dementia. *J Neuroimmunol*. 2002; 122:40–54. [PubMed: 1177542]
270. Nelson DJ, Jow B, Jow F. Lipopolysaccharide induction of outward potassium current expression in human monocyte-derived macrophages: lack of correlation with secretion. *J Membr Biol*. 1992; 125:207–18. [PubMed: 1556735]
271. Seydel U, Scheel O, Muller M, Brandenburg K, Blunck R. A K⁺ channel is involved in LPS signaling. *J Endotoxin Res*. 2001; 7:243–47. [PubMed: 11581578]

272. DeCoursey TE, Kim SY, Silver MR, Quandt FN. Ion channel expression in PMA-differentiated human THP-1 macrophages. *J Membr Biol.* 1996; 152:141–57. [PubMed: 9139125]
273. Mackenzie AB, Chirakkal H, North RA. Kv1.3 potassium channels in human alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol.* 2003; 285:L862–68. [PubMed: 12909584]
274. Liu QH, Williams DA, McManus C, Baribaud F, Doms RW, et al. HIV-1 gp120 and chemokines activate ion channels in primary macrophages through CCR5 and CXCR4 stimulation. *PNAS.* 2000; 97:4832–37. [PubMed: 10758170]
275. Gao YD, Hanley PJ, Rinne S, Zuzarte M, Daut J. Calcium-activated K⁺ channel (K_{Ca}3.1) activity during Ca²⁺ store depletion and store-operated Ca²⁺ entry in human macrophages. *Cell Calcium.* 2010; 48:19–27. [PubMed: 20630587]
276. Strobaek D, Brown DT, Jenkins DP, Chen YJ, Coleman N, et al. NS6180, a new K_{Ca}3.1 channel inhibitor prevents T-cell activation and inflammation in a rat model of inflammatory bowel disease. *Br J Pharmacol.* 2013; 168:432–44. [PubMed: 22891655]
277. Toyama K, Wulff H, Chandy KG, Azam P, Raman G, et al. The intermediate-conductance calcium-activated potassium channel KCa3.1 contributes to atherogenesis in mice and humans. *J Clin Investig.* 2008; 118:3025–37. [PubMed: 18688283]
278. Chen YJ, Lam J, Gregory CR, Schrepfer S, Wulff H. The Ca²⁺-activated K⁺ channel KCa3.1 as a potential new target for the prevention of allograft vasculopathy. *PLOS ONE.* 2013; 8:e81006. [PubMed: 24312257]
279. Fordyce CB, Jagasia R, Zhu X, Schlichter LC. Microglia Kv1.3 channels contribute to their ability to kill neurons. *J Neurosci.* 2005; 25:7139–49. [PubMed: 16079396]
280. Maezawa I, Calafiore M, Wulff H, Jin LW. Does microglial dysfunction play a role in autism and Rett syndrome? *Neuron Glia Biol.* 2011; 7:85–97. [PubMed: 22717189]
281. Peng Y, Lu K, Li Z, Zhao Y, Wang Y, et al. Blockade of Kv1.3 channels ameliorates radiation-induced brain injury. *Neuro Oncol.* 2014; 16:528–39. [PubMed: 24305723]
282. Mauler F, Hinz V, Horvath E, Schuhmacher J, Hofmann HA, et al. Selective intermediate-/small-conductance calcium-activated potassium channel (KCNN4) blockers are potent and effective therapeutics in experimental brain oedema and traumatic brain injury caused by acute subdural haematoma. *Eur J Neurosci.* 2004; 20:1761–68. [PubMed: 15379997]
283. Chen YJ, Raman G, Bodendiek S, O'Donnell ME, Wulff H. The KCa3.1 blocker TRAM-34 reduces infarction and neurological deficit in a rat model of ischemia/reperfusion stroke. *J Cereb Blood Flow Metab.* 2011; 31:2363–74. [PubMed: 21750563]
284. Mullen KM, Rozycka M, Rus H, Hu L, Cudrici C, et al. Potassium channels Kv1.3 and Kv1.5 are expressed on blood-derived dendritic cells in the central nervous system. *Ann Neurol.* 2006; 60:118–27. [PubMed: 16729292]
285. Kanneganti TD. Central roles of NLRs and inflammasomes in viral infection. *Nat Rev Immunol.* 2010; 10:688–98. [PubMed: 20847744]
286. Lamkanfi M, Dixit VM. Inflammasomes and their roles in health and disease. *Annu Rev Cell Dev Biol.* 2012; 28:137–61. [PubMed: 22974247]
287. Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature.* 2006; 440:228–32. [PubMed: 16407890]
288. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature.* 2006; 440:237–41. [PubMed: 16407889]
289. Schroder K, Zhou R, Tschopp J. The NLRP3 inflammasome: a sensor for metabolic danger? *Science.* 2010; 327:296–300. [PubMed: 20075245]
290. Tschopp J, Schroder K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol.* 2010; 10:210–15. [PubMed: 20168318]
291. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature.* 2009; 458:509–13. [PubMed: 19158676]
292. Bauernfeind F, Ablasser A, Bartok E, Kim S, Schmid-Burgk J, et al. Inflammasomes: current understanding and open questions. *Cell Mol Life Sci.* 2011; 68:765–83. [PubMed: 21072676]
293. Franchi L, Kamada N, Nakamura Y, Burberry A, Kuffa P, et al. NLR4-driven production of IL-1 β discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. *Nat Immunol.* 2012; 13:449–56. [PubMed: 22484733]

294. Munoz-Planillo R, Kuffa P, Martinez-Colon G, Smith BL, Rajendiran TM, Nunez G. K⁺ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity*. 2013; 38:1142–53. [PubMed: 23809161]
295. Petrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J. Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ*. 2007; 14:1583–89. [PubMed: 17599094]
296. Kahlenberg JM, DUBYAK GR. Mechanisms of caspase-1 activation by P2X7 receptor-mediated K⁺ release. *Am J Physiol Cell Physiol*. 2004; 286:C1100–8. [PubMed: 15075209]
297. Andrei C, Margiocco P, Poggi A, Lotti LV, Torrisi MR, Rubartelli A. Phospholipases C and A2 control lysosome-mediated IL-1 β secretion: implications for inflammatory processes. *PNAS*. 2004; 101:9745–50. [PubMed: 15192144]
298. He Y, Franchi L, Nunez G. TLR agonists stimulate Nlrp3-dependent IL-1 β production independently of the purinergic P2X7 receptor in dendritic cells and in vivo. *J Immunol*. 2013; 190:334–39. [PubMed: 23225887]
299. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1 β -dependent adaptive immunity against tumors. *Nat Med*. 2009; 15:1170–78. [PubMed: 19767732]
300. Wilhelm K, Ganesan J, Muller T, Durr C, Grimm M, et al. Graft-versus-host disease is enhanced by extracellular ATP activating P2X7R. *Nat Med*. 2010; 16:1434–38. [PubMed: 21102458]
301. Azad AK, Sadee W, Schlesinger LS. Innate immune gene polymorphisms in tuberculosis. *Infect Immun*. 2012; 80:3343–59. [PubMed: 22825450]
302. Coutinho-Silva R, Ojcius DM. Role of extracellular nucleotides in the immune response against intracellular bacteria and protozoan parasites. *Microbes Infect*. 2012; 14:1271–77. [PubMed: 22634346]
303. Kusner DJ, Adams J. ATP-induced killing of virulent *Mycobacterium tuberculosis* within human macrophages requires phospholipase D. *J Immunol*. 2000; 164:379–88. [PubMed: 10605033]
304. Lammas DA, Stober C, Harvey CJ, Kendrick N, Panchalingam S, Kumararatne DS. ATP-induced killing of mycobacteria by human macrophages is mediated by purinergic P2Z(P2X₇) receptors. *Immunity*. 1997; 7:433–44. [PubMed: 9324363]
305. Santos AA Jr, Rodrigues-Junior V, Zanin RF, Borges TJ, Bonorino C, et al. Implication of purinergic P2X7 receptor in *M. tuberculosis* infection and host interaction mechanisms: a mouse model study. *Immunobiology*. 2013; 218:1104–12. [PubMed: 23583008]
306. Saunders BM, Fernando SL, Sluyter R, Britton WJ, Wiley JS. A loss-of-function polymorphism in the human P2X7 receptor abolishes ATP-mediated killing of mycobacteria. *J Immunol*. 2003; 171:5442–46. [PubMed: 14607949]
307. Areeshi MY, Mandal RK, Panda AK, Bisht SC, Haque S. CD14–159 C>T gene polymorphism with increased risk of tuberculosis: evidence from a meta-analysis. *PLOS ONE*. 2013; 8:e64747. [PubMed: 23741383]
308. Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Goldberg H, et al. A polymorphism in the P2X₇ gene increases susceptibility to extrapulmonary tuberculosis. *Am J Respir Crit Care Med*. 2007; 175:360–66. [PubMed: 17095747]
309. Perraud AL, Takanishi CL, Shen B, Kang S, Smith MK, et al. Accumulation of free ADP-ribose from mitochondria mediates oxidative stress-induced gating of TRPM2 cation channels. *J Biol Chem*. 2005; 280:6138–48. [PubMed: 15561722]
310. Knowles H, Li Y, Perraud AL. The TRPM2 ion channel, an oxidative stress and metabolic sensor regulating innate immunity and inflammation. *Immunol Res*. 2013; 55:241–48. [PubMed: 22975787]
311. Knowles H, Heizer JW, Li Y, Chapman K, Ogden CA, et al. Transient receptor potential melastatin 2 (TRPM2) ion channel is required for innate immunity against *Listeria monocytogenes*. *PNAS*. 2011; 108:11578–83. [PubMed: 21709234]
312. Yamamoto S, Shimizu S, Kiyonaka S, Takahashi N, Wajima T, et al. TRPM2-mediated Ca²⁺ influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nat Med*. 2008; 14:738–47. [PubMed: 18542050]

313. Zhang Z, Zhang W, Jung DY, Ko HJ, Lee Y, et al. TRPM2 Ca^{2+} channel regulates energy balance and glucose metabolism. *Am J Physiol Endocrinol Metab.* 2012; 302:E807–16. [PubMed: 22275755]
314. Zhong Z, Zhai Y, Liang S, Mori Y, Han R, et al. TRPM2 links oxidative stress to NLRP3 inflammasome activation. *Nat Commun.* 2013; 4:1611. [PubMed: 23511475]
315. Di A, Gao XP, Qian F, Kawamura T, Han J, et al. The redox-sensitive cation channel TRPM2 modulates phagocyte ROS production and inflammation. *Nat Immunol.* 2011; 13:29–34. [PubMed: 22101731]
316. Serafini N, Dahdah A, Barbet G, Demion M, Attout T, et al. The TRPM4 channel controls monocyte and macrophage, but not neutrophil, function for survival in sepsis. *J Immunol.* 2012; 189:3689–99. [PubMed: 22933633]
317. Barbet G, Demion M, Moura I, Serafini N, Léger T, et al. The calcium-activated nonselective cation channel TRPM4 is essential for the migration but not the maturation of dendritic cells. *Nat Immunol.* 2008; 9:1148–56. [PubMed: 18758465]
318. Holmes B, Quie PG, Windhorst DB, Good RA. Fatal granulomatous disease of childhood. An inborn abnormality of phagocytic function. *Lancet.* 1966; 1:1225–28. [PubMed: 4161205]
319. DeCoursey TE. Voltage-gated proton channels find their dream job managing the respiratory burst in phagocytes. *Physiology.* 2010; 25:27–40. [PubMed: 20134026]
320. Femling JK, Cherny VV, Morgan D, Rada B, Davis AP, et al. The antibacterial activity of human neutrophils and eosinophils requires proton channels but not BK channels. *J Gen Physiol.* 2006; 127:659–72. [PubMed: 16702353]
321. Okamura Y, Sasaki M. Phagocytosis and membrane potential. *Seikagaku.* 2007; 79:454–58. (in Japanese). [PubMed: 17571596]
322. Ramsey IS, Ruchti E, Kaczmarek JS, Clapham DE. Hv1 proton channels are required for high-level NADPH oxidase-dependent superoxide production during the phagocyte respiratory burst. *PNAS.* 2009; 106:7642–47. [PubMed: 19372380]
323. Shiloh MU, MacMicking JD, Nicholson S, Brause JE, Potter S, et al. Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase. *Immunity.* 1999; 10:29–38. [PubMed: 10023768]
324. Wu LJ, Wu G, Akhavan Sharif MR, Baker A, Jia Y, et al. The voltage-gated proton channel Hv1 enhances brain damage from ischemic stroke. *Nat Neurosci.* 2012; 15:565–73. [PubMed: 22388960]
325. Nowak JE, Harmon K, Caldwell CC, Wong HR. Prophylactic zinc supplementation reduces bacterial load and improves survival in a murine model of sepsis. *Pediatr Crit Care Med.* 2012; 13:e323–29. [PubMed: 22760431]
326. Brazao V, Caetano LC, Del Vecchio Filipin M, Paula Alonso Toldo M, Caetano LN, do Prado JC Jr. Zinc supplementation increases resistance to experimental infection by *Trypanosoma cruzi*. *Vet Parasitol.* 2008; 154:32–37. [PubMed: 18394811]
327. Hasan R, Rink L, Haase H. Zinc signals in neutrophil granulocytes are required for the formation of neutrophil extracellular traps. *Innate Immun.* 2013; 19:253–64. [PubMed: 23008348]
328. Haase H, Ober-Blobaum JL, Engelhardt G, Hebel S, Heit A, et al. Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. *J Immunol.* 2008; 181:6491–502. [PubMed: 18941240]
329. Ryu MS, Langkamp-Henken B, Chang SM, Shankar MN, Cousins RJ. Genomic analysis, cytokine expression, and microRNA profiling reveal biomarkers of human dietary zinc depletion and homeostasis. *PNAS.* 2011; 108:20970–75. [PubMed: 22171008]
330. Rink L, Haase H. Zinc homeostasis and immunity. *Trends Immunol.* 2007; 28:1–4. [PubMed: 17126599]
331. Liu MJ, Bao S, Galvez-Peralta M, Pyle CJ, Rudawsky AC, et al. ZIP8 regulates host defense through zinc-mediated inhibition of NF- κ B. *Cell Rep.* 2013; 3:386–400. [PubMed: 23403290]
332. Sayadi A, Nguyen AT, Bard FA, Bard-Chapeau EA. Zip14 expression induced by lipopolysaccharides in macrophages attenuates inflammatory response. *Inflamm Res.* 2013; 62:133–43. [PubMed: 23052185]

333. Kitamura H, Morikawa H, Kamon H, Iguchi M, Hojyo S, et al. Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. *Nat Immunol.* 2006; 7:971–77. [PubMed: 16892068]
334. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med.* 2012; 18:693–704. [PubMed: 22561833]
335. Ashmole I, Duffy SM, Leyland ML, Morrison VS, Begg M, Bradding P. CRACM/Orai ion channel expression and function in human lung mast cells. *J Allergy Clin Immunol.* 2012; 129:1628–35 e2. [PubMed: 22409987]
336. Di Capite J, Nelson C, Bates G, Parekh AB. Targeting Ca^{2+} release-activated Ca^{2+} channel channels and leukotriene receptors provides a novel combination strategy for treating nasal polyposis. *J Allergy Clin Immunol.* 2009; 124:1014–21.e3. [PubMed: 19895990]
337. Wareham K, Vial C, Wykes RC, Bradding P, Seward EP. Functional evidence for the expression of P2X1, P2X4 and P2X7 receptors in human lung mast cells. *Br J Pharmacol.* 2009; 157:1215–24. [PubMed: 19552691]
338. Bulanova E, Bulfone-Paus S. P2 receptor-mediated signaling in mast cell biology. *Purinergic Signal.* 2010; 6:3–17. [PubMed: 19921464]
339. Kurashima Y, Amiya T, Nochi T, Fujisawa K, Haraguchi T, et al. Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoceptors. *Nat Commun.* 2012; 3:1034. [PubMed: 22948816]
340. Sudo N, Tanaka K, Koga Y, Okumura Y, Kubo C, Nomoto K. Extracellular ATP activates mast cells via a mechanism that is different from the activation induced by the cross-linking of Fc receptors. *J Immunol.* 1996; 156:3970–79. [PubMed: 8621938]
341. Freichel M, Almering J, Tsvilovskyy V. The role of TRP proteins in mast cells. *Front Immunol.* 2012; 3:150. [PubMed: 22701456]
342. Duffy SM, Lawley WJ, Conley EC, Bradding P. Resting and activation-dependent ion channels in human mast cells. *J Immunol.* 2001; 167:4261–70. [PubMed: 11591748]
343. Duffy SM, Leyland ML, Conley EC, Bradding P. Voltage-dependent and calcium-activated ion channels in the human mast cell line HMC-1. *J Leukoc Biol.* 2001; 70:233–40. [PubMed: 11493615]
344. Shumilina E, Lam RS, Wolbing F, Matzner N, Zemtsova IM, et al. Blunted IgE-mediated activation of mast cells in mice lacking the Ca^{2+} -activated K^{+} channel KCa3.1 . *J Immunol.* 2008; 180:8040–47. [PubMed: 18523267]
345. Girodet PO, Ozier A, Carvalho G, Iliina O, Ousova O, et al. Ca^{2+} -activated K^{+} channel-3.1 blocker TRAM-34 attenuates airway remodeling and eosinophilia in a murine asthma model. *Am J Respir Cell Mol Biol.* 2013; 48:212–19. [PubMed: 23204391]
346. Van Der Velden J, Sum G, Barker D, Koumoundouros E, Barcham G, et al. KCa3.1 channel-blockade attenuates airway pathophysiology in a sheep model of chronic asthma. *PLOS ONE.* 2013; 8:e66886. [PubMed: 23826167]
347. Srivastava S, Cai X, Li Z, Sun Y, Skolnik EY. Phosphatidylinositol-3-kinase $\text{C2}\beta$ and TRIM27 function to positively and negatively regulate IgE receptor activation of mast cells. *Mol Cell Biol.* 2012; 32:3132–39. [PubMed: 22645315]
348. Sobiesiak M, Shumilina E, Lam RS, Wolbing F, Matzner N, et al. Impaired mast cell activation in gene-targeted mice lacking the serum- and glucocorticoid-inducible kinase SGK1. *J Immunol.* 2009; 183:4395–402. [PubMed: 19748978]
349. Wolbing F, Sobiesiak M, Shumilina E, Kaesler S, Lam RS, et al. Impaired anaphylaxis and mast cell degranulation in mice lacking serum- and glucocorticoid-inducible kinase 1. *J Investig Dermatol.* 2009; 129:S8.
350. Vennekens R, Olausson J, Meissner M, Bloch W, Mathar I, et al. Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated nonselective cation channel TRPM4. *Nat Immunol.* 2007; 8:312–20. [PubMed: 17293867]
351. Yamasaki S, Sakata-Sogawa K, Hasegawa A, Suzuki T, Kabu K, et al. Zinc is a novel intracellular second messenger. *J Cell Biol.* 2007; 177:637–45. [PubMed: 17502426]
352. Kabu K, Yamasaki S, Kamimura D, Ito Y, Hasegawa A, et al. Zinc is required for Fc epsilon RI-mediated mast cell activation. *J Immunol.* 2006; 177:1296–305. [PubMed: 16818790]

353. Di Sabatino A, Rovedatti L, Kaur R, Spencer JP, Brown JT, et al. Targeting gut T cell Ca^{2+} release-activated Ca^{2+} channels inhibits T cell cytokine production and T-box transcription factor T-bet in inflammatory bowel disease. *J Immunol.* 2009; 183:3454–62. [PubMed: 19648266]
354. Derler I, Schindl R, Fritsch R, Heftberger P, Riedl MC, et al. The action of selective CRAC channel blockers is affected by the Orai pore geometry. *Cell Calcium.* 2013; 53:139–51. [PubMed: 23218667]
355. Chen G, Panicker S, Lau KY, Apparsundaram S, Patel VA, et al. Characterization of a novel CRAC inhibitor that potently blocks human T cell activation and effector functions. *Mol Immunol.* 2013; 54:355–67. [PubMed: 23357789]
356. Grundy S, Kaur M, Plumb J, Reynolds S, Hall S, et al. CRAC channel inhibition produces greater anti-inflammatory effects than glucocorticoids in CD8 cells from COPD patients. *Clin Sci.* 2014; 126:223–32. [PubMed: 23905758]
357. Yoshino T, Ishikawa J, Ohga K, Morokata T, Takezawa R, et al. YM-58483, a selective CRAC channel inhibitor, prevents antigen-induced airway eosinophilia and late phase asthmatic responses via Th2 cytokine inhibition in animal models. *Eur J Pharmacol.* 2007; 560:225–33. [PubMed: 17307161]
358. Cox JH, Hussell S, Sondergaard H, Roepstorff K, Bui JV, et al. Antibody-mediated targeting of the orai1 calcium channel inhibits T cell function. *PLOS ONE.* 2013; 8:e82944. [PubMed: 24376610]
359. Lin FF, Elliott R, Colombero A, Gaida K, Kelley L, et al. Generation and characterization of fully human monoclonal antibodies against human Orai1 for autoimmune disease. *J Pharmacol Exp Ther.* 2013; 345:225–38. [PubMed: 23475901]
360. Gaida K, Salimi-Moosavi H, Subramanian R, Almon V, Knize A, et al. Inhibition of CRAC with a human anti-ORAI1 monoclonal antibody inhibits T-cell-derived cytokine production but fails to inhibit a T-cell-dependent antibody response in the cynomolgus monkey. *J Immunotoxicol.* 2014; 3:1–10.
361. Cheng KT, Alevizos I, Liu X, Swaim WD, Yin H, et al. STIM1 and STIM2 protein deficiency in T lymphocytes underlies development of the exocrine gland autoimmune disease, Sjogren's syndrome. *PNAS.* 2012; 109:14544–49. [PubMed: 22904194]
362. Chandy KG, DeCoursey TE, Cahalan MD, McLaughlin C, Gupta S. Voltage-gated potassium channels are required for human T lymphocyte activation. *J Exp Med.* 1984; 160:369–85. [PubMed: 6088661]
363. Wulff H, Beeton C, Chandy KG. Potassium channels as therapeutic targets for autoimmune disorders. *Curr Opin Drug Discov Devel.* 2003; 6:640–47.
364. Beeton C, Pennington MW, Wulff H, Singh S, Nugent D, et al. Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channels for therapy of autoimmune diseases. *Mol Pharmacol.* 2005; 67:1369–81. [PubMed: 15665253]
365. Rus H, Pardo CA, Hu L, Darrah E, Cudrici C, et al. The voltage-gated potassium channel Kv1.3 is highly expressed on inflammatory infiltrates in multiple sclerosis brain. *PNAS.* 2005; 102:11094–99. [PubMed: 16043714]
366. Beeton C, Barbaria J, Giraud P, Devaux J, Benoliel AM, et al. Selective blocking of voltage-gated K^{+} channels improves experimental autoimmune encephalomyelitis and inhibits T cell activation. *J Immunol.* 2001; 166:936–44. [PubMed: 11145670]
367. Beeton C, Wulff H, Barbaria J, Clot-Faybesse O, Pennington M, et al. Selective blockade of T lymphocyte K^{+} channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. *PNAS.* 2001; 98:13942–47. [PubMed: 11717451]
368. Tarcha EJ, Chi V, Munoz-Elias EJ, Bailey D, Londono LM, et al. Durable pharmacological responses from the peptide ShK-186, a specific Kv1.3 channel inhibitor that suppresses T cell mediators of autoimmune disease. *J Pharmacol Exp Ther.* 2012; 342:642–53. [PubMed: 22637724]
369. Hyodo T, Oda T, Kikuchi Y, Higashi K, Kushiyama T, et al. Voltage-gated potassium channel Kv1.3 blocker as a potential treatment for rat anti-glomerular basement membrane glomerulonephritis. *Am J Physiol Renal Physiol.* 2010; 299:F1258–69. [PubMed: 20810612]

370. Gilhar A, Bergman R, Assay B, Ullmann Y, Etzioni A. The beneficial effect of blocking Kv1.3 in the psoriasiform SCID mouse model. *J Investig Dermatol.* 2011; 131:118–24. [PubMed: 20739949]
371. Gilhar A, Keren A, Shemer A, Ullmann Y, Paus R. Blocking potassium channels (Kv1.3): a new treatment option for alopecia areata? *J Investig Dermatol.* 2013; 133:2088–91. [PubMed: 23636064]
- 371a. Kundu-Raychaudhuri S, Chen YJ, Wulff H, Raychaudhuri SP. Kv1.3 in psoriatic disease: PAP-1, a small molecule inhibitor of Kv1.3 is effective in the SCID mouse psoriasis–Xenograft model. *J Autoimmun.* 2014; 55:63–72. [PubMed: 25175978]
372. Koshy S, Huq R, Tanner MR, Atik MA, Porter PC, et al. Blocking KV1.3 channels inhibits Th2 lymphocyte function and treats a rat model of asthma. *J Biol Chem.* 2014; 289:12623–32. [PubMed: 24644290]
373. Edwards W, Fung-Leung WP, Huang C, Chi E, Wu N, et al. Targeting the ion channel Kv1.3 with scorpion venom peptides engineered for potency, selectivity, and half-life. *J Biol Chem.* 2014; 289:22704–14. [PubMed: 24939846]
374. Cruse G, Duffy SM, Brightling CE, Bradding P. Functional KCa3.1 K⁺ channels are required for human lung mast cell migration. *Thorax.* 2006; 61:880–85. [PubMed: 16809411]
375. Ataga KI, Stocker J. Senicapoc (ICA-17043): a potential therapy for the prevention and treatment of hemolysis-associated complications in sickle cell anemia. *Expert Opin Investig Drugs.* 2009; 18:231–39.
376. Bartlett R, Stokes L, Sluyter R. The P2X7 receptor channel: recent developments and the use of P2X7 antagonists in models of disease. *Pharmacol Rev.* 2014; 66:638–75. [PubMed: 24928329]
377. Arulkumaran N, Unwin RJ, Tam FW. A potential therapeutic role for P2X7 receptor (P2X7R) antagonists in the treatment of inflammatory diseases. *Expert Opin Investig Drugs.* 2011; 20:897–915.
378. Taylor SR, Turner CM, Elliott JI, McDaid J, Hewitt R, et al. P2X7 deficiency attenuates renal injury in experimental glomerulonephritis. *J Am Soc Nephrol.* 2009; 20:1275–81. [PubMed: 19389853]
379. Keystone EC, Wang MM, Layton M, Hollis S, McInnes IB. Clinical evaluation of the efficacy of the P2X7 purinergic receptor antagonist AZD9056 on the signs and symptoms of rheumatoid arthritis in patients with active disease despite treatment with methotrexate or sulphasalazine. *Ann Rheum Dis.* 2012; 71:1630–35. [PubMed: 22966146]
380. Stock TC, Bloom BJ, Wei N, Ishaq S, Park W, et al. Efficacy and safety of CE-224,535, an antagonist of P2X7 receptor, in treatment of patients with rheumatoid arthritis inadequately controlled by methotrexate. *J Rheumatol.* 2012; 39:720–27. [PubMed: 22382341]
381. Hou X, Pedi L, Diver MM, Long SB. Crystal structure of the calcium release-activated calcium channel Orai. *Science.* 2012; 338:1308–13. [PubMed: 23180775]

IMMUNODEFICIENCY DUE TO MUTATIONS IN *ORAI1*, *STIM1*, AND *MAGT1* GENES

ORAI1, *STIM1*, and *MAGT1* mediate Ca^{2+} and Mg^{2+} influx, respectively. Mutations in these three genes cause primary immunodeficiency by impairing the function of T cells and other immune cells (see Figure 3).

Homozygous mutations in *ORAI1* and *STIM1* cause CRAC channelopathy. *ORAI1* is the pore-forming subunit of the CRAC channel located in the PM. Autosomal recessive mutations in *ORAI1* (indicated by red circles in Figure 3) abolish either channel expression or function (R91W) by blocking Ca^{2+} permeation through the channel pore (26). *STIM1* is a single-pass transmembrane protein located in the ER membrane. Changes in the Ca^{2+} concentration in the ER are sensed by a paired EF hand domain in the N terminus of *STIM1*, which undergoes conformational changes upon ER store depletion and Ca^{2+} dissociation from the EF hand domain. The cytoplasmic C terminus contains three coiled-coil (CC) domains and a lysine-rich (K) domain, which mediate *STIM1* interaction with *ORAI1* and PM phospholipids, respectively, thereby enabling the translocation of *STIM1* from the bulk ER to ER-PM junctions after cell stimulation and Ca^{2+} depletion from ER stores. *STIM1* binding to *ORAI1* is mediated by the CRAC activation domain (CAD, in Figure 3) that encompasses CC2 and CC3. Autosomal recessive mutations in *STIM1* (indicated by red circles in Figure 3) abolish either protein expression or function (R429C) by inhibiting *STIM1* activation (S. Feske, unpublished data; 123). CRAC channelopathy due to mutations in either *ORAI1* or *STIM1* is characterized by severe combined immunodeficiency (SCID)-like disease, autoimmunity, muscular hypotonia, and ectodermal dysplasia. The clinical spectrum in *ORAI1*- and *STIM1*-deficient patients is very similar (see Figure 3), suggesting that both genes are essential for CRAC channel function and that defects cannot be compensated by their homologs *ORAI2*, *ORAI3*, and *STIM2*. Viral and bacterial infections in all known *ORAI1*- and most *STIM1*-deficient patients are life threatening and require hematopoietic stem cell transplantation in the first year of life. Autoimmunity appears more pronounced in *STIM1*-deficient patients, although numbers of Foxp3⁺ T regulatory cells are reduced in patients with mutations in *ORAI1* and *STIM1*.

X-linked mutations in *MAGT1* cause XMEN disease. *MAGT1* is a highly selective Mg^{2+} transporter located in the PM. It is activated after TCR stimulation and mediates Mg^{2+} influx, resulting in the activation of PLC γ 1 and the production of IP₃ and SOCE following ER Ca^{2+} store depletion. How TCR stimulation activates *MAGT1* and how Mg^{2+} regulates PLC γ 1 function are unknown. Two isoforms of *MAGT1* have been reported: a longer isoform (367 amino acids) and a shorter isoform (335 amino acids), which may differ in the number of transmembrane domains. Most data indicate, however, that the N and C terminus of *MAGT1* are both extracellular. Mutations in *MAGT1* (indicated by red circles in Figure 3) abolish TCR-induced Mg^{2+} influx, PLC γ 1 activation, and SOCE, resulting in XMEN disease. This X-linked immunodeficiency is characterized by a mild CD4 lymphopenia with decreased CD4:CD8 ratio; impaired T and natural killer cell function due to decreased NKG2D expression; Epstein-Barr virus (EBV) infections; and EBV-positive lymphoproliferative disease, splenomegaly, and

dysglobulinemia (see Figure 3). Some patients have additional viral and bacterial infections, consistent with primary immunodeficiency.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

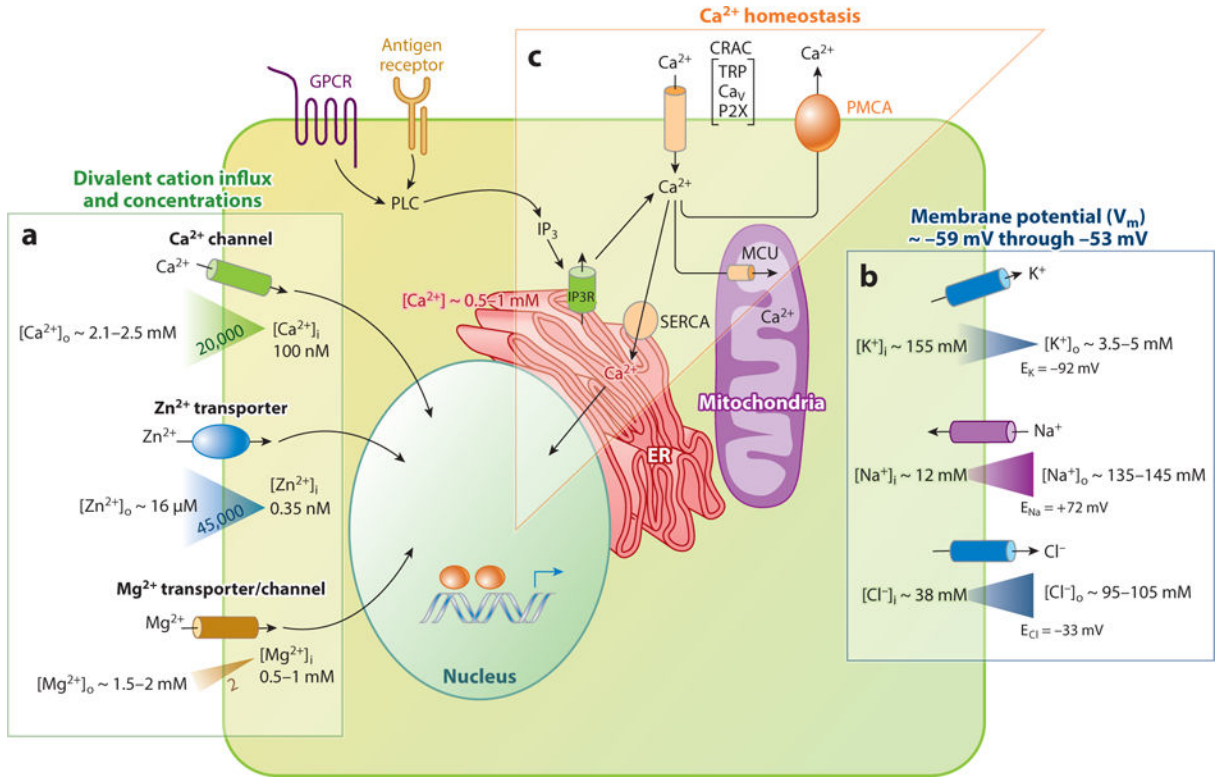
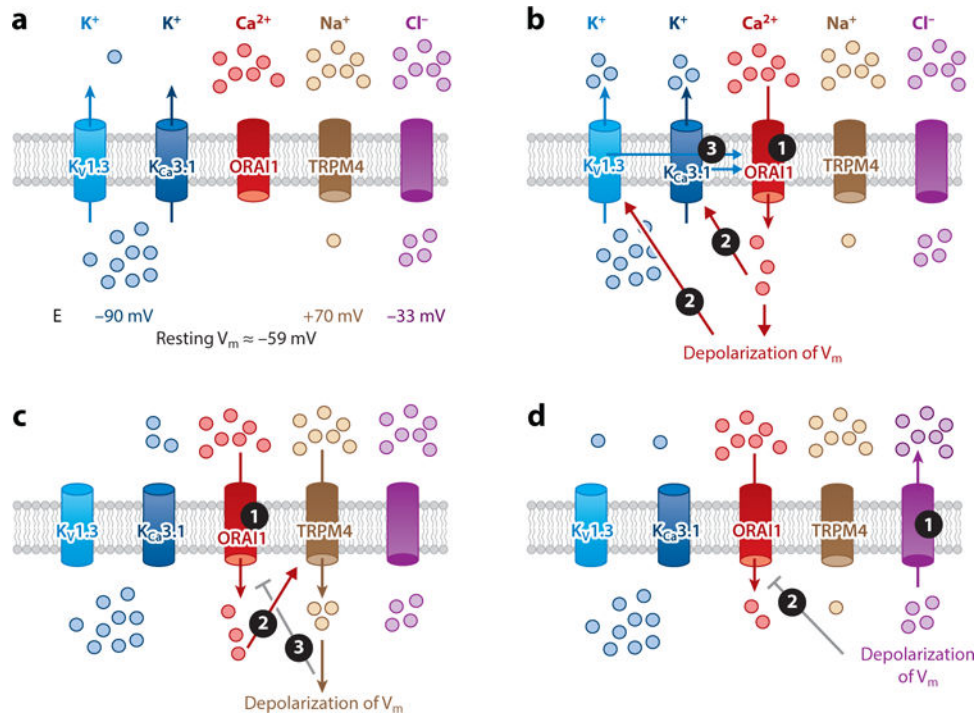
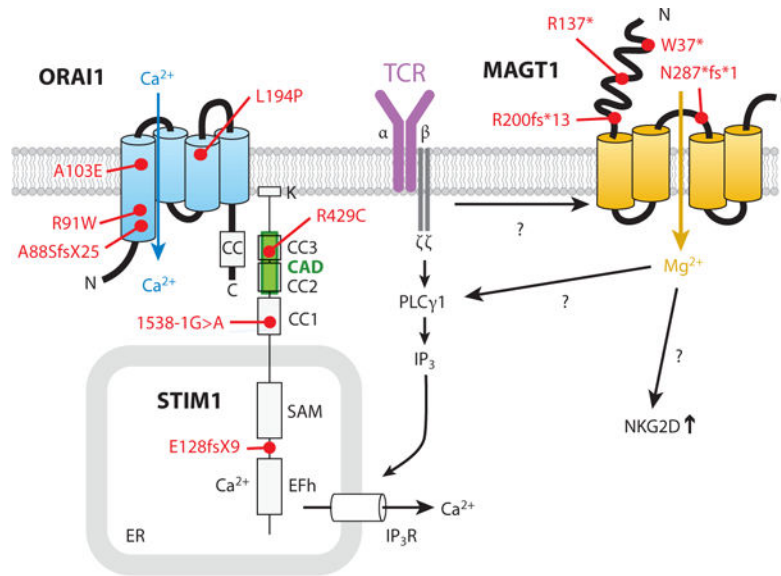


Figure 1. Ion channels and regulation of divalent cation signaling in immune cells. A variety of ion channels and transporters regulate the function of immune cells. Rather than depicting a specific immune cell type or specific ion channels/transporters, this figure presents an overview of the classes of ion channels and transporters in the plasma membrane and the pathways and compartments involved in regulating intracellular ion concentrations. (a) Divalent cation channels and transporters and the concentrations of extra- and intracellular Ca^{2+} , Mg^{2+} , and Zn^{2+} ions (concentration gradients indicated by *triangles*). (b) Monovalent cation (K^+ , Na^+) and anion (Cl^-) channels that control the V_m in immune cells and the concentrations of extra- and intracellular K^+ , Na^+ , and Cl^- ions. (See Figure 2 for details on V_m regulation.) (c) Mechanisms regulating Ca^{2+} homeostasis in immune cells. Antigen receptor or GPCR binding results in PLC activation, production of IP_3 and Ca^{2+} release from ER Ca^{2+} stores via IP_3R channels resulting in (1) a transient increase in $[Ca^{2+}]_i$ and (2) activation of store-operated Ca^{2+} entry (SOCE; for details see Figures 3, 4, 5 and the sidebar, below). The resulting increase in $[Ca^{2+}]_i$ is balanced by Ca^{2+} reuptake into the ER via SERCA pumps, Ca^{2+} export via PMCA pumps, and uptake into the mitochondrial matrix via the MCU. (Abbreviations: $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; GPCR, G protein-coupled receptor; IP_3R , inositol 1,4,5-trisphosphate receptor; MCU, mitochondrial Ca^{2+} uniporter; PLC, phospholipase C; PMCA, plasma membrane Ca^{2+} pump; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; V_m , membrane potential. For additional abbreviations, see Table 1, below.)

**Figure 2.**

Regulation of membrane potential (V_m) and Ca^{2+} influx. (a) In nonactivated lymphocytes, the resting V_m is approximately -59 mV, as determined by the equilibrium potentials of K^+ ($K_V1.3$, $K_{Ca}3.1$), Na^+ (TRPM4), and Cl^- channels in the plasma membrane (PM). The equilibrium potentials (E) and V_m are shown; the intra- and extracellular ion concentrations are as indicated in Figure 1. (b) K^+ channels hyperpolarize the PM and enhance Ca^{2+} influx. The opening of ORAI1 Ca^{2+} channels following T cell activation results in Ca^{2+} influx ① and depolarization of V_m , causing the subsequent opening of Ca^{2+} -activated $K_{Ca}3.1$ channels and voltage-gated $K_V1.3$ channels, respectively. ② Open $K_{Ca}3.1$ and $K_V1.3$ channels mediate K^+ efflux and hyperpolarization of the PM, thereby sustaining Ca^{2+} influx ③. (c) TRPM4 depolarizes the PM and inhibits Ca^{2+} influx. Ca^{2+} influx through ORAI1 channels ① results in the opening of Ca^{2+} -regulated TRPM4 channels ② that mediate Na^+ influx and depolarization of the PM, thereby inhibiting Ca^{2+} influx via ORAI1 ③. (d) Opening of Cl^- channels in lymphocytes results in efflux of Cl^- ions due to the relatively high $[Cl^-]$; of approximately 38 mM and an equilibrium potential (approximately -33 mV) that is positive relative to V_m ①. Cl^- efflux depolarizes V_m and inhibits Ca^{2+} influx ②.



CRAC channelopathy Mutations: <i>Orai1</i> , <i>Stim1</i>	XMEN Mutations: <i>Magt1</i>
Clinical phenotype: <ul style="list-style-type: none"> • SCID-like immunodeficiency (recurrent and chronic viral, bacterial, and fungal infections) • Autoimmunity (autoimmune hemolytic anemia and thrombocytopenia, autoantibodies) • Muscular hypotonia and atrophy of type II muscle fibers • Ectodermal dysplasia (hypocalcified dental enamel, anhidrosis) Immunological defect: <ul style="list-style-type: none"> • Predominantly T cell activation defect • Normal lymphocyte development except Treg and NKT cells 	Clinical phenotype: <ul style="list-style-type: none"> • X-linked • Magnesium defect • EBV infections (primary immunodeficiency with recurrent viral and bacterial infections) • Neoplasia (lymphoma) Immunological defect: <ul style="list-style-type: none"> • CD4 lymphopenia (CD4:CD8 ratio ↓) • T cell activation defect • NKG2D expression ↓

Figure 3.

Immunodeficiency due to mutations in *Orai1*, *Stim1*, and *Magt1* genes. *Orai1* is the pore-forming subunit of the CRAC channel in the PM and mediates Ca^{2+} influx following TCR stimulation. It is activated by the Ca^{2+} -sensing protein *Stim1* localized in the ER. Mutations in *Orai1* and *Stim1* genes (indicated by red circles) cause CRAC channelopathy, a primary immunodeficiency syndrome characterized by severe, life-threatening infections, autoimmunity, and nonimmunological symptoms. *Magt1* is a selective Mg^{2+} transporter in the PM activated by TCR stimulation. Mutations in the human *Magt1* gene (indicated by red circles) cause XMEN disease. For details, see sidebar. (Abbreviations: CAD, CRAC activation domain; CC, coiled-coil domain; CRAC, Ca^{2+} release-activated Ca^{2+} channel; EBV, Epstein-Barr virus; EFh, EF hand; ER, endoplasmic reticulum; IP_3 , inositol 1,4,5-trisphosphate; IP_3R , IP_3 receptor; *Magt1*, Mg^{2+} transporter; NKG2D, natural killer group 2, member D; PLC, phospholipase C; PM, plasma membrane; SAM, sterile alpha motif; SCID, severe combined immunodeficiency; *Stim1*, stromal

interaction molecule 1; TCR, T cell receptor; Treg, regulatory T cell; XMEN, X-linked immunodeficiency with Mg²⁺ defect, EBV infection, and neoplasia.)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

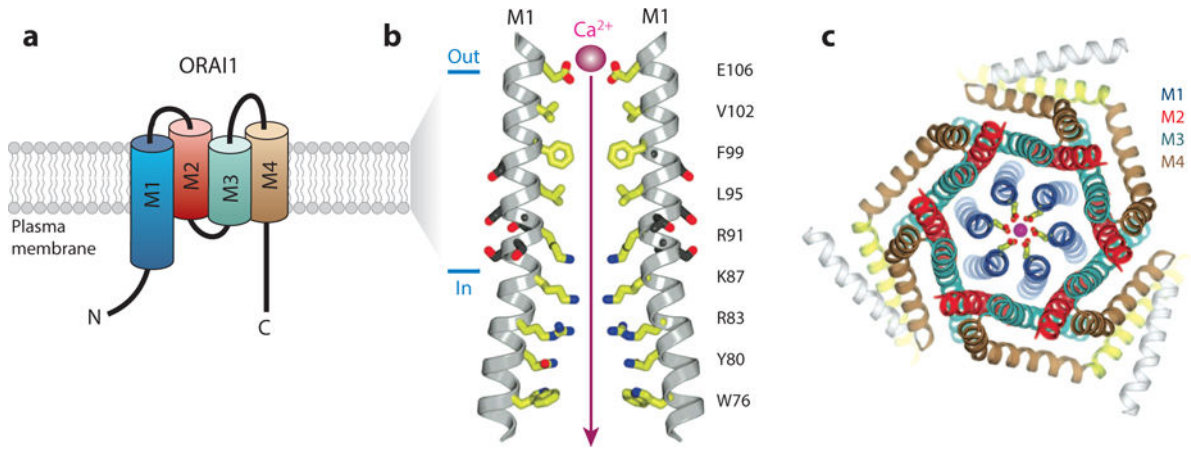


Figure 4.

CRAC channel structure. The CRAC channel is a multimer of ORAI1 subunits that form the pore of the channel. (a) Membrane topology of one ORAI1 subunit. Each subunit has four transmembrane domains (M1–M4), intracellular N and C termini, and two extracellular loops. (b) Ion pore of the *Drosophila* Orai channel, whose transmembrane domains are conserved in the human ORAI1 channel. M1 lines the conduction pathway for Ca^{2+} . Two M1 alpha-helices from two separate ORAI1 subunits are shown, with amino acid side chains protruding into the pore indicated in yellow. Amino acid residue numbers refer to human ORAI1. Glutamate (E) 106 at the outer end of the pore is the Ca^{2+} -binding site in the selectivity filter of the CRAC channel that determines its preference for conducting Ca^{2+} over other divalent or monovalent cations. Arginine (R) 91, together with lysine (K) 87 and R83, is part of a basic region of residues that form the narrowest part of the pore; R91 is mutated in patients with abolished CRAC channel function (see sidebar and Figure 3). (c) Crystal structure of the *Drosophila* Orai channel. The hexameric assembly of Orai subunits is shown in an orthogonal view from the extracellular side. The colors for each transmembrane helix in this panel are the same as in panel a (e.g., M1 helices are shown in blue, E106 is depicted in yellow, and a Ca^{2+} ion in magenta). Panels b and c have been reproduced with permission from Reference 381. A Ca^{2+} ion bound to E106 and the path of Ca^{2+} through the pore have been added to panel b.

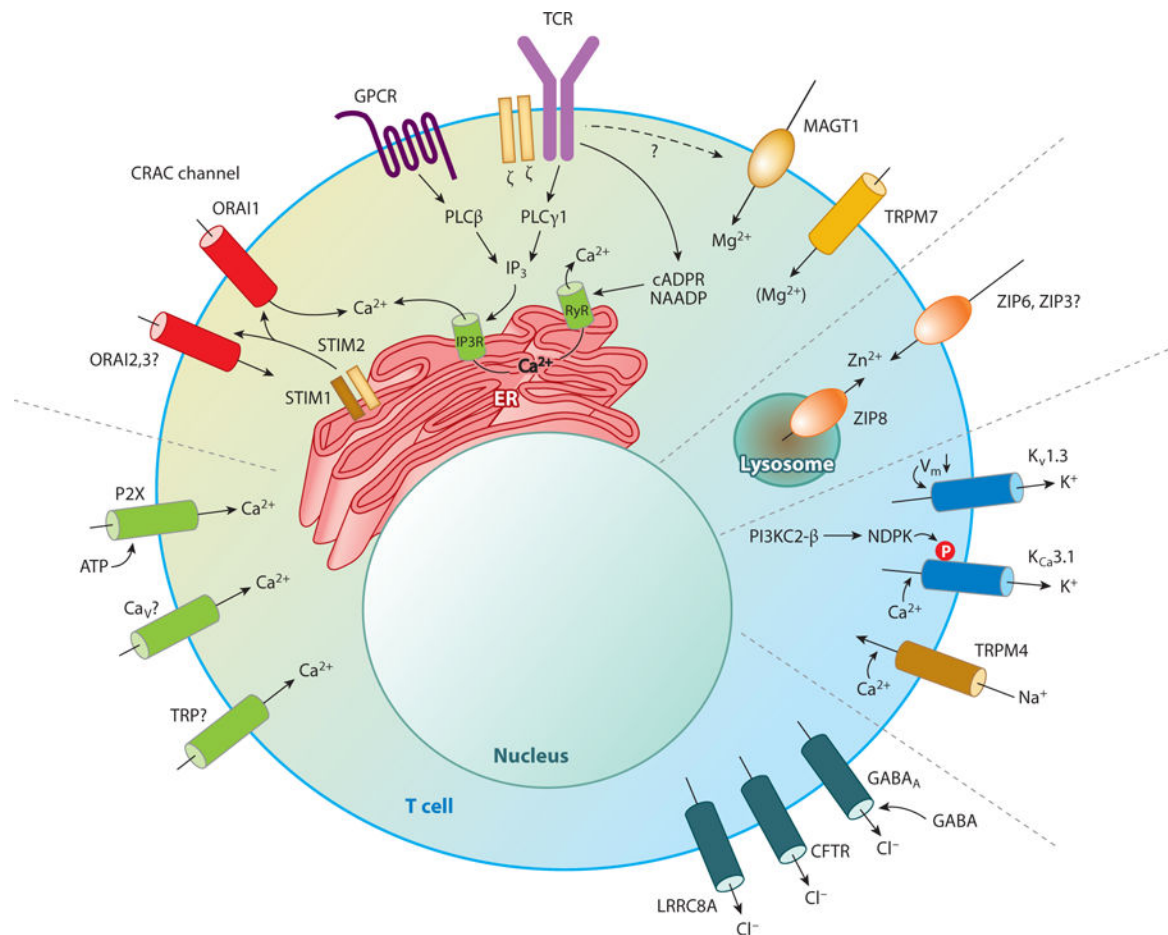


Figure 5.

Ion channels in T cells. Antigen binding to the TCR results in activation of tyrosine kinases and phosphorylation of ITAM motifs of the TCR and phosphorylation of the scaffold proteins SLP-76 and LAT (*not shown*) that facilitate recruitment and activation of PLC γ 1, production of IP $_3$, and release of Ca $^{2+}$ from ER stores. Other second messengers such as NAADP and cADPR have also been reported to cause ER store depletion via RyR after TCR activation. Reduction of [Ca $^{2+}$] $_{ER}$ activates STIM1 and STIM2, which then translocate to ER-PM junctions, where they bind to ORAI1. ORAI1 encodes the CRAC channel and mediates SOCE; the role of ORAI2 and ORAI3 isoforms for SOCE in T cells and T cell function is unknown. Other Ca $^{2+}$ channels reported in T cells include P2X1, P2X4, and P2X7 receptors; TRPM2; and several L-type voltage-gated Ca $^{2+}$ channels (Ca $_V$), although their roles in T cell function are less well defined. Ca $^{2+}$ influx depends on the negative membrane potential (V_m) established by two K $^+$ channels, K $_V$ 1.3 and K $_{Ca}$ 3.1, which are counteracted by the Ca $^{2+}$ -activated TRPM4 channel that depolarizes V_m . K $_V$ 1.3 activation is mediated by membrane depolarization, whereas K $_{Ca}$ 3.1 activation is mediated by two signals that include Ca $^{2+}$ binding to the calmodulin-bound C terminus of K $_{Ca}$ 3.1 and the recruitment of PI3K-C2 β to the immunological synapse, which results in histidine phosphorylation of K $_{Ca}$ 3.1 by NDPK-B. The Mg $^{2+}$ transporter MAGT1 and the divalent cation channel TRPM7 mediate Mg $^{2+}$ influx; their activation mechanisms following TCR

stimulation are not understood, but that for TRPM7 is regulated by PIP₂ and inhibited by intracellular Mg²⁺. Several Zn²⁺ transporters, including ZIP6, ZIP3, and ZIP8, reportedly mediate Zn²⁺ influx from the extracellular space and release from intracellular compartments, respectively. The Cl⁻ channels LRRC8A, CFTR, and GABA_A regulate T cell function, likely by mediating Cl⁻ efflux and depolarization of V_m. (Abbreviations: cADPR, cyclic ADP ribose; Ca_v, voltage-gated Ca²⁺ channel; CFTR, cystic fibrosis transmembrane conductance regulator; CRAC, Ca²⁺ release-activated Ca²⁺ channel; ER, endoplasmic reticulum; GABA, γ-aminobutyric acid; GPCR, G protein-coupled receptor; IP₃, inositol 1,4,5-trisphosphate; ITAM, immunoreceptor tyrosine-based activation motif; LAT, linker for activation of T cells; LRRC8A, leucine-rich repeat containing 8 family member A; NAADP, nicotinic acid adenine dinucleotide phosphate; NDPK-B, nucleoside diphosphate kinase B; PLC, phospholipase C; PM, plasma membrane; RyR, ryanodine receptor; SOCE, store-operated Ca²⁺ entry; STIM, stromal interaction molecule; TCR, T cell receptor; TRP, transient receptor potential; V_m, membrane potential; ZIP, Zlr/Irt-like protein.)

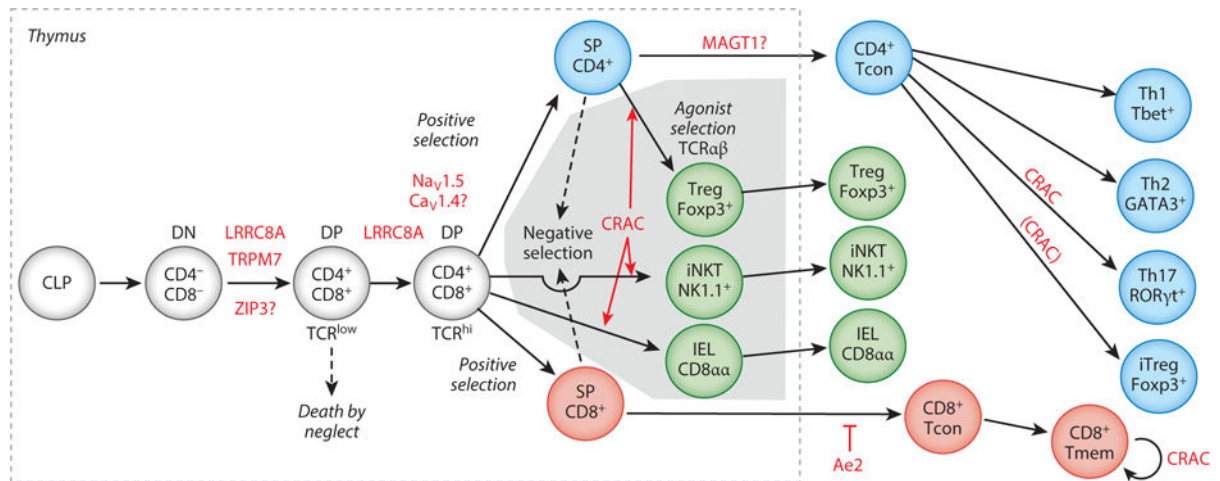


Figure 6.

Ion channels in T cell development and lineage differentiation. Studies in knockout mice and human patients have implicated a number of ion channels and transporters in T cell development. During T cell development, common lymphoid progenitors (CLPs) enter the thymus and differentiate into CD2⁺, CD4⁻CD8⁻ (double-negative, DN) T cells that have not yet rearranged their T cell receptor (TCR) genes. These DN cells further develop into CD4⁺CD8⁺ (double-positive, DP) thymocytes that express low levels of a rearranged TCRαβ. This developmental step is regulated by the Mg²⁺-permeable channel TRPM7 and potentially also by the Zn²⁺ transporter ZIP3. The volume-regulated Cl⁻ channel LRRC8A also controls thymocyte development, and *Lrrc8a*^{-/-} mice have strongly reduced numbers of DN, DP, and SP thymocytes. DP thymocytes next undergo positive selection, during which cells whose TCR interacts weakly with self-peptide-MHC on thymic epithelial cells are rescued from death by neglect. The voltage-gated Na⁺ channel Na_v1.5 and potentially also the voltage-gated Ca²⁺ channel Ca_v1.4 regulate positive selection. During negative selection, DP thymocytes with high-affinity TCR-self-peptide-MHC interactions and strong TCR signals are eliminated to delete potentially self-reactive T cells. A subset of DP thymocytes escapes negative selection despite expressing a high-affinity TCR, and instead they become agonist-selected T cells whose development requires strong and sustained TCR signals. These include Foxp3⁺ natural regulatory T (nTreg) cells, invariant natural killer T cells (iNKT), and CD8αα⁺ intestinal intraepithelial lymphocytes (IELs). The thymic development of nTreg, iNKT, and IEL cells, in contrast to the development of conventional αβ T cells (denoted in figure as Tcon), requires Ca²⁺ influx via CRAC channels, likely by controlling the expression of cytokines needed for their homeostasis. The development or survival of CD4⁺ T cells partially depends on Mg²⁺ influx through the Mg²⁺ transporter MAGT1. Once conventional CD4⁺ T cells leave the thymus, they further differentiate into distinct T helper lineages of which Th17 cells, but not Th1 or Th2 cells, have been suggested to depend on CRAC channels for differentiation. The proliferation and differentiation of CD8⁺ T cells into effector and memory cells (in figure as Tmem) is regulated by the Cl⁻/HCO₃⁻ anion exchanger Ae2, whereas the maintenance of memory CD8⁺ T cells requires Ca²⁺ influx via CRAC channels.

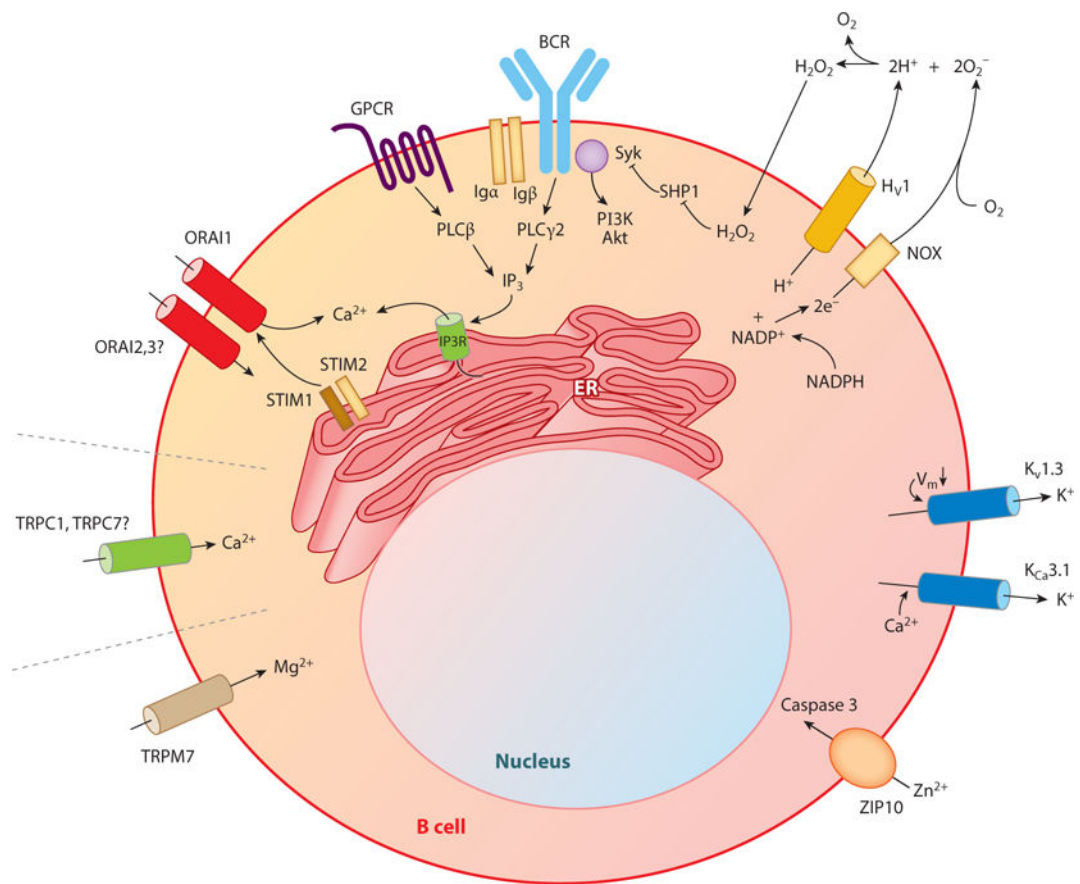


Figure 7.

Ion channels in B cells. Similar to TCR activation in T cells, cross-linking of the BCR results in tyrosine phosphorylation of ITAM motifs in the Ig α / β chains of the BCR and the scaffold protein SLP-65 (*not shown*), which helps to recruit PLC γ 2 to the plasma membrane of B cells and activate it. The subsequent generation of IP $_3$ and the IP $_3$ R-mediated Ca $^{2+}$ release from the ER result in the activation of STIM1 and STIM2 and in the opening of ORAI1/CRAC channels. The nonselective cation channels TRPC1 and TRPC7 have been implicated in store-dependent and -independent Ca $^{2+}$ influx in chicken DT-40 B cells. Likewise, the nonselective cation channel TRPM7 regulates total cellular Mg $^{2+}$ levels and, thereby, the proliferation and viability of DT-40 B cells. The V_m in B cells is established by the voltage-gated K $^+$ channel K $_V$ 1.3 and the Ca $^{2+}$ -activated K $_C$ a3.1 channel. The proton channel H $_V$ 1 associates with the BCR complex and is required for activation of NOX and the generation of reactive oxygen species, and these events promote BCR signaling by inhibiting the tyrosine phosphatase SHP1, leading to enhanced activation of signaling pathways such as Syk, PI3-kinase, and Akt. (Abbreviations: BCR, B cell receptor; CRAC, Ca $^{2+}$ release-activated Ca $^{2+}$ channel; ER, endoplasmic reticulum; GPCR, G protein-coupled receptor; IP $_3$, inositol 1,4,5-trisphosphate; ITAM, immunoreceptor tyrosine-based activation motif; NOX, NADPH oxidase; PLC, phospholipase C; PM, plasma membrane; STIM, stromal interaction molecule; TRP, transient receptor potential; V_m , membrane potential.)

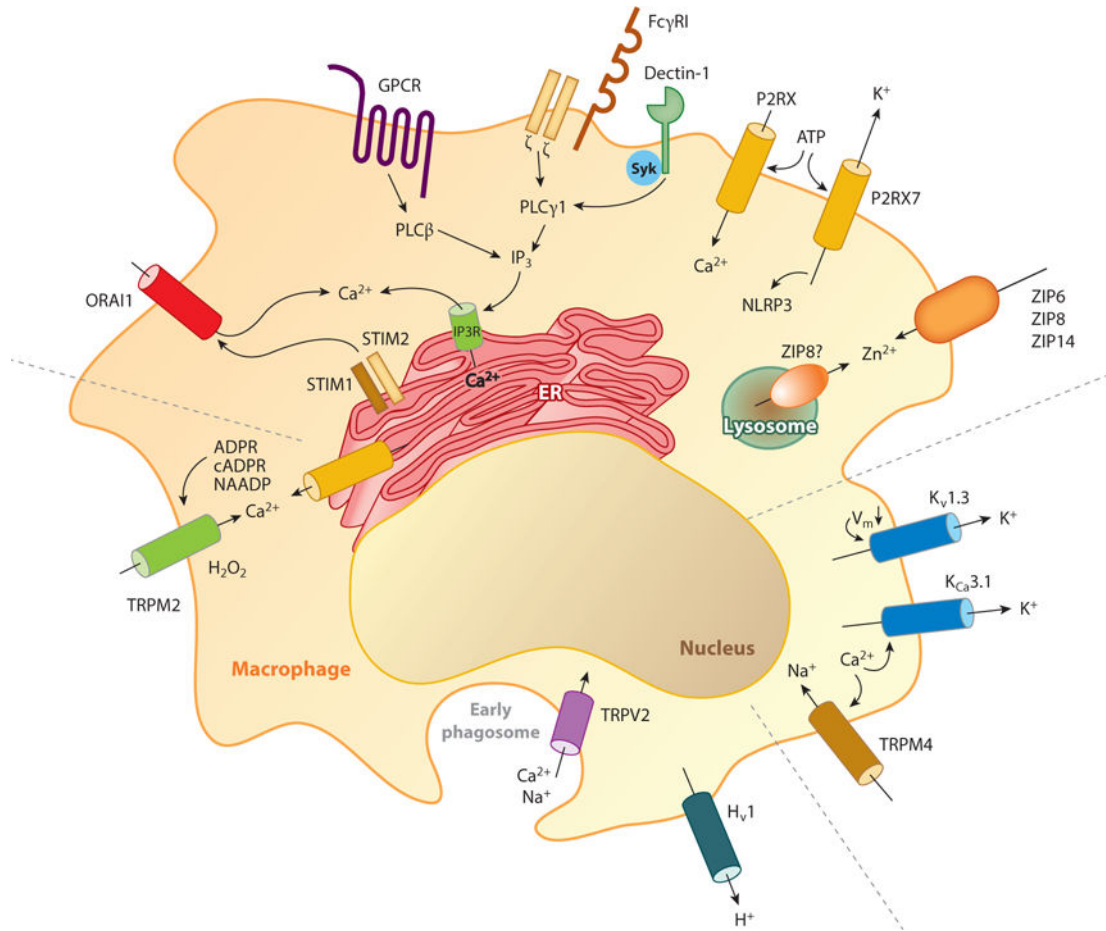


Figure 8.

Ion channels in macrophages. Macrophages express a variety of receptors that activate ion channels including $Fc\gamma RI$ and $Fc\gamma RIII$, G protein-coupled chemokine receptors, and innate immunoreceptors such as Dectin-1/2 and Toll-like receptors (TLRs). $Fc\gamma RI$ cross-linking and Dectin-1 binding result in activation of $PLC\gamma 1$, and chemokine receptor engagement results in activation of $PLC\beta$ and in the opening of CRAC channels, as described in Figures 5 and 7 for T and B cells. The membrane potential (V_m) required for SOCE in macrophages is established by two K^+ channels, $K_v 1.3$ and $K_{Ca} 3.1$, and by the Na^+ channel TRPM4 channel, as described in Figure 2. Other Ca^{2+} channels in macrophages are TRPM2, TRPV2, and P2RX7. TRPM2 is a nonselective Ca^{2+} channel located in both the plasma and ER membranes. TRPM2 is activated by the second messengers ADPR, cADPR, and NAAPD, resulting in Ca^{2+} influx and release from the ER. TRPM2 function in macrophages is further regulated by ROS levels, which increase after TLR stimulation. TRPV2 is a nonselective, Ca^{2+} -permeable channel that is recruited to the early phagosome after $Fc\gamma R$ or zymosan stimulation and is required for the early steps of phagocytosis, presumably by mediating Na^+ but not Ca^{2+} influx, leading to membrane depolarization resulting in the generation of PIP_2 and actin depolymerization. P2RX7 appears to play a dual role in macrophages by mediating the ATP-induced influx of Ca^{2+} and the efflux of K^+ that is required for inflammasome activation (see Figure 9). The Zn^{2+} transporters ZIP6 and ZIP8

reportedly regulate Zn^{2+} levels in macrophages by mediating Zn^{2+} influx after TLR stimulation, thereby inhibiting NF- κ B activation. The proton channel H ν 1 is closely associated with the NADPH oxidase complex (*not shown*) and is essential for the generation of ROS in macrophages (see Figure 10). (Abbreviations: CRAC, Ca $^{2+}$ release-activated Ca $^{2+}$ channel; ER, endoplasmic reticulum; GPCR, G protein-coupled receptor; NLRP3, NLR family, pyrin domain containing 3; PIP $_2$, phosphatidylinositol 4,5-bisphosphate; ROS, reactive oxygen species; SOCE, store-operated Ca $^{2+}$ entry; TLR, Toll-like receptor; TRP, transient receptor potential; ZIP, Zlr/Irt-like protein.)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

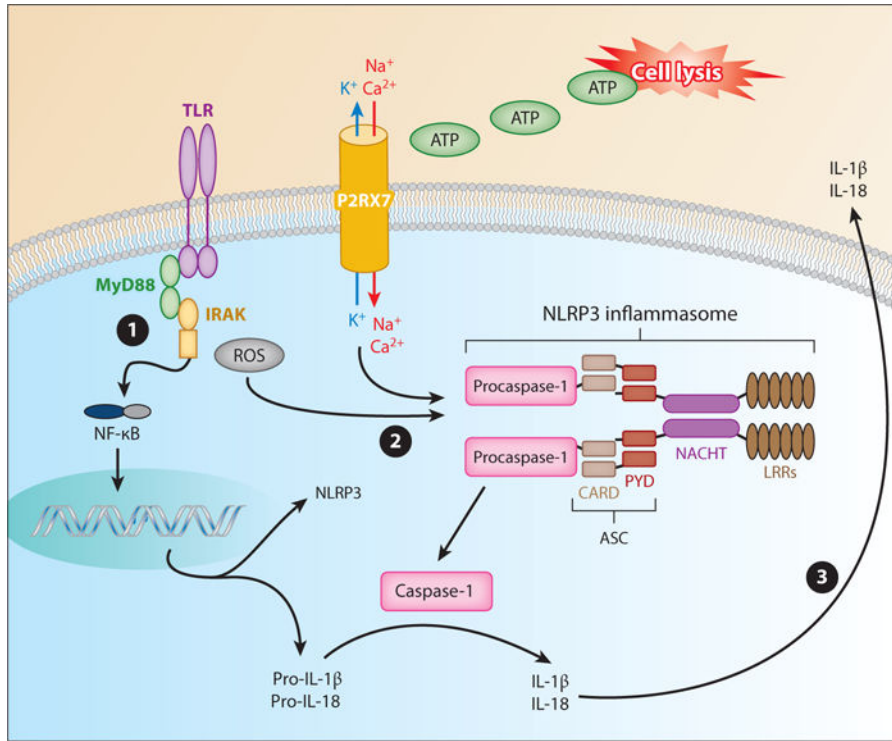


Figure 9.

P2RX7 activation of the NLRP3 inflammasome in macrophages. High concentrations of extracellular ATP released from dying cells function as danger signals to initiate NLRP3 inflammasome activation, which requires two signals: **1** During the priming phase, NLRP3 and pro-IL-1 β are transcribed in response to signals such as TLR activation or IFN- γ , TNF- α , or other stimuli that activate NF- κ B. **2** A second signal then activates the NLRP3 inflammasome, resulting in the activation of caspase-1, cleavage of pro-IL-1 β and pro-IL-18 into their mature forms, and **3** secretion of IL-1 β and IL-18. Central to NLRP3 activation during the second phase is K⁺ efflux from the cytoplasm of macrophages, which mediates assembly and activation of the NLRP3 inflammasome by an unknown mechanism(s). P2RX7 mediates only ATP-stimulated K⁺ efflux, whereas K⁺ efflux by other second signals can be mediated by plasma membrane insertion of bacterial pore-forming toxins or by yet-to-be-defined mechanisms, as is the case for particulate matter. (Abbreviations: ASC, apoptosis associated speck-like protein containing a CARD; ATP, adenosine triphosphate; IFN- γ , interferon- γ ; IL-1 β , interleukin-1 β ; IRAK, interleukin-1 receptor associated kinase; MyD88, myeloid differentiation primary response gene 88; NF- κ B, nuclear factor- κ B; NLRP3, NLR family, pyrin domain containing 3; TLR, Toll-like receptor; TNF- α , tumor necrosis factor α .)

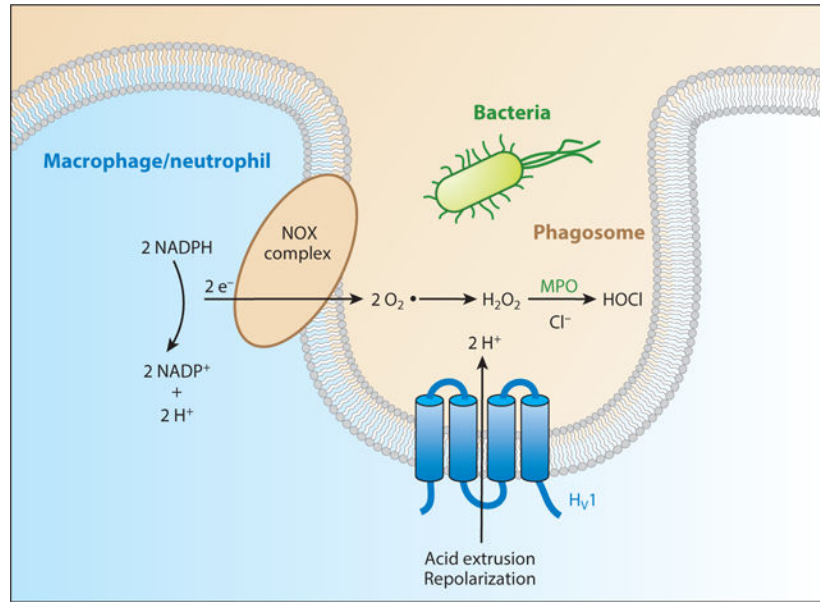


Figure 10.

Ion channels in NADPH oxidase (NOX) activation and phagocytic respiratory burst. The multiprotein NOX complex is critical for the killing of microorganisms by macrophages and neutrophils by mediating the generation of reactive oxygen species (ROS). The NOX2 subunit (also known as gp91^{phox}, *not shown*) located in the nascent phagosome membrane accepts electrons from cytosolic NADPH, which are then transported across the plasma membrane where they are transferred to O₂, generating O₂⁻. The transfer of electrons results in the depolarization and acidification of the cytosol, either of which would inhibit NOX activity. This is prevented by the transport of H⁺ from the cytosol into the phagosome by the proton channel Hv1. H⁺ reacts with O₂⁻ in the phagosome to generate H₂O₂, which undergoes further conversion to HOCl that is catalyzed by MPO. (Abbreviations: HOCl, hydrochlorus acid; MPO, myeloperoxidase; NADPH, nicotinamide adenine dinucleotide phosphate.)

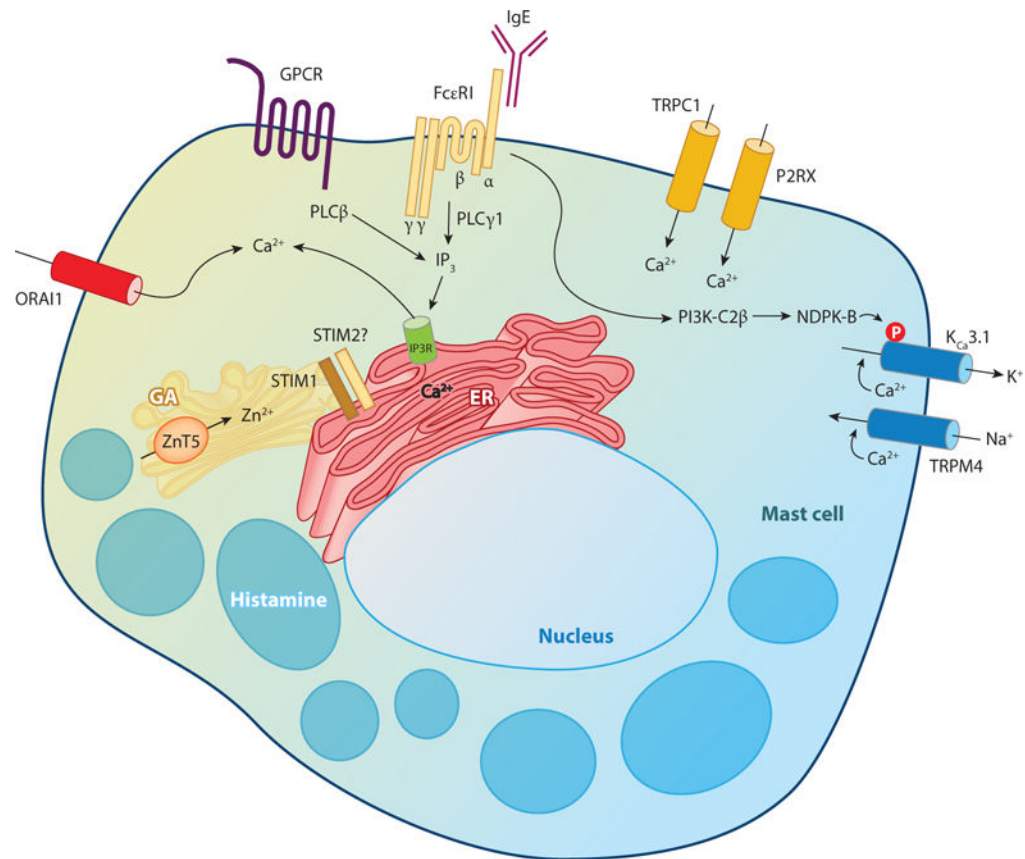


Figure 11.

Ion channels in mast cells. Antigen binding and cross-linking of IgE antibodies bound to the high-affinity FcεRI are central to mast cell activation, Ca²⁺ influx, and the secretion of mediators of the allergic response. Ca²⁺ mediates both acute mast cell degranulation and the delayed transcriptional production of cytokines, leukotrienes, and other secondary mediators by mast cells. IgE cross-linking leads to phosphorylation of the β and γ FcεRI subunits and to the activation of several signaling molecules including PLCγ, IP₃ production, Ca²⁺ release from ER stores, and SOCE via STIM1 and ORAI1 activation. Ca²⁺ influx results in the activation of K_{Ca}3.1, which by mediating the efflux of K⁺ maintains a hyperpolarized membrane potential (V_m) and thereby enables Ca²⁺ influx via ORAI1. The function of K_{Ca}3.1 is counteracted by TRPM4, which mediates Na⁺ influx and V_m depolarization, thereby limiting the magnitude of Ca²⁺ influx in mast cells. FcεRI activation also results in the activation of the class II PI3K-C2β, which is critical for K_{Ca}3.1 activation by stimulating the histidine phosphorylation at the C terminus of K_{Ca}3.1 by NDPK-B. Mast cells express several TRP channels and P2X receptors that potentially mediate Ca²⁺ influx, but their role in mast cell function is not well defined. The Zn²⁺ transporter ZnT5 is located in the Golgi apparatus (GA) and mediates Zn²⁺ uptake, thereby regulating FcεRI-mediated PKC recruitment to the plasma membrane and NF-κB activation. (Abbreviations: ER, endoplasmic reticulum; IP₃, inositol 1,4,5-trisphosphate; NDPK-B, nucleoside diphosphate kinase B; PKC, protein kinase C; PLC, phospholipase C; SOCE, store-operated Ca²⁺ entry;

STIM, stromal interaction molecule; TRP, transient receptor potential; V_m , membrane potential.)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Ion channels in immune cells

Channel	Permeability	Activation	Expression in immune cells	Immune function in vitro and in vivo	Channelopathies and immune disease in human
Calcium					
CRAC					
ORAI1	Ca ²⁺	TCR, BCR, FcR, dectin, chemokine receptors → Ca ²⁺ depletion from ER stores → STIM1, STIM2 activation	T cells, B cells, NK cells, macrophages, DCs, neutrophils, ubiquitous	T cells in vitro: proliferation, cytokine production, cytotoxicity, apoptosis, chemotaxis T cells in vivo: immunity to infection (human), Th1/Th17 cell-mediated autoimmunity (EAE, IBD); DTH; skin allograft rejection; motility and homing (?) NK cells: degranulation, cytotoxicity (human) DCs (in vitro cultured): maturation (?), cytokine production (?) Mast cells: degranulation, histamine release, cytokine and LTC ₄ production, passive cutaneous anaphylaxis Neutrophils: ROS production, adhesion (?)	CRAC channelopathy due to mutations in human <i>ORAI1</i> (immunodeficiency, autoimmunity, muscular hypotonia, ectodermal dysplasia)
ORAI2	Ca ²⁺	Presumably similar to ORAI1	B cells (human); ubiquitous (mouse)	t.b.d.	
ORAI3	Ca ²⁺	Presumably similar to ORAI1	Ubiquitous (lowest in T cells)	t.b.d.	
STIM1, STIM2	NA	ER store depletion	Ubiquitous	Similar to ORAI1, STIM1-deficient mice have attenuated EAE, IBD, GvHD (T cells), reduced cytokine production, FcγR-dependent phagocytosis of antibody-opsonized RBC and platelets (macrophage), reduced ROS and phagocytosis, increased susceptibility to <i>S. aureus</i> and <i>L. monocytogenes</i> ? (neutrophils) STIM1/2-deficient mice have impaired development of Foxp3 ⁺ Treg, NKT, and IEL cells, myelo-lymphoproliferation, inflammation (lung, intestine, salivary glands), impaired antitumor and antiviral immunity, impaired CD8 ⁺ T cell memory	CRAC channelopathy due to mutations in <i>STIM1</i> (see <i>ORAI1</i>)
TRP					
TRPC3, C5	Ca ²⁺ , Na ⁺	Unknown [(GMI) ganglioside activates TRPC5 (?)]		Unknown	
TRPM2	Ca ²⁺ , Na ⁺	ADPR, cADPR, NAADP	T cells, macrophages	Activation and cytokine production by macrophages (and T cells); inflammasome activation: <i>Trpm2</i> ^{-/-} mice are susceptible to infection with <i>L. monocytogenes</i> and are	

Channel	Permeability	Activation	Expression in immune cells	Immune function in vitro and in vivo	Channelopathies and immune disease in human
TRPV2	Ca ²⁺ , Na ⁺	FcγR signaling	Macrophages	Chemotaxis and phagocytosis by macrophages; <i>Trpv2</i> ^{-/-} mice show increased susceptibility to <i>L. monocytogenes</i>	
Ca_v					
Ca 1.2, 1.3, V 1.4 (and β3, β4 regulatory subunits)	Ca ²⁺	Activation mechanism unknown, inhibition by STIM1 (in Jurkat T cells)	T cells	Positive selection of CD4 ⁺ T cells (?) (Ca _v 1.4), CD8 ⁺ T cell survival, cytokine production, CD8 ⁺ T cell immunity to <i>L. monocytogenes</i> infection, Th2 function in asthma (Ca _v 1.2)	
P2X					
P2RX7	Ca ²⁺ , Na ⁺ , K ⁺ , other	Extracellular ATP	Ubiquitous	T cell proliferation, cytokine production; promotes Th17 and inhibits Treg differentiation; the pan-P2RX inhibitor αATP prevents IBD, cardiac and pancreatic islet allograft rejection Innate immune cells: NLRP3 inflammasome activation (K ⁺ efflux); activation/priming of DCs in CD8 ⁺ T cell-mediated antitumor immunity and CD4 ⁺ T cell-mediated GvHD; immunity to <i>M. tuberculosis</i> in mice	Polymorphisms in human gene are associated with increased susceptibility to <i>M. tuberculosis</i>
P2RX1, 2, 3, 4, 5	Ca ²⁺ , Na ⁺	Extracellular ATP	T cells, thymocytes, innate immune cells	T cells: T cell proliferation, cytokine production, thymocyte apoptosis; <i>P2rx2/3</i> ^{-/-} mice have hypocellularity of bone marrow and thymus.	
Magnesium					
TRPM7	Ni ²⁺ > Zn ²⁺ > Mg ²⁺ , Ca ²⁺	Unknown; regulators include intracellular Mg ²⁺ , PIP ₂ , extracellular pH	T, B, NK cells	Thymocyte development, production of thymocyte growth factors, proliferation and survival of DT-40 B cells	
MAGT1	Mg ²⁺	TCR (activation mechanism unknown)	T, NK cells	CD4 ⁺ T cell homeostasis, NKG2D expression on CD8 ⁺ T and NK cells, cytotoxicity, immunity to viral infection (EBV)	XMEN disease (immunodeficiency, CD4 lymphopenia, EBV infections)
Zinc					
ZIP3, ZIP6, ZIP8, ZIP10, ZIP14	Zn ²⁺	Unknown; TCR stimulation (ZIP6)	T cells, B cells, macrophages, DCs	T cells: activation (ZIP6); IFN-γ and perforin expression (ZIP8); T cell development (ZIP3) (?) B cells: early B cell development, apoptosis, cytokine signaling (ZIP10) Macrophages: inhibition of proinflammatory cytokine production (ZIP8, ZIP14) DCs: inhibition of LPS induced expression of MHC class II and costimulatory molecules (ZIP6)	Mutations in <i>ZIP4</i> cause acrodermatitis enteropathica with immunodeficiency (due to impaired intestinal Zn ²⁺ uptake)

Channel	Permeability	Activation	Expression in immune cells	Immune function in vitro and in vivo	Channelopathies and immune disease in human
ZnT5	Zn ²⁺	Unknown; FcεRI stimulation (?)	Mast cells	Zn ²⁺ efflux; promotes NF-κB activation in mast cells and FcεRI-dependent DTH	
Potassium					
Kv1.3	K ⁺	Depolarization of V _m	Th17 and T _{EM} , IgD ⁺ CD27 ⁺ class switched memory B and plasma cells, macrophages, DCs	Function: hyperpolarization of V _m ; volume regulation. T cells: activation of Th17 and T _{EM} cells, proliferation, cytokine production, T cell-mediated autoimmunity; inhibition in mice attenuates EAE; autoimmune diabetes, RA, and ACD; <i>Kv1.3</i> ^{-/-} mice resistant to EAE and obesity-induced diabetes Macrophages: proliferation, iNOS expression DCs: expression of CD80, CD86, CD40, IL-12; chemotaxis	
KCa3.1	K ⁺	Intracellular Ca ²⁺	Th1, Th2, and T _{CM} , naive and IgD ⁺ CD27 ⁺ memory B cells, macrophages	Function: hyperpolarization of V _m T cells: activation of Th1, Th2, T _{CM} cells; cytokine production; IBD. Inhibition attenuates IBD (mice), asthma (rodents, sheep), and allograft vasculopathy Macrophages: chemotaxis, infiltration of atherosclerotic plaques	
K _{2p} 3.1, 5.1, 9.1	K ⁺	K ⁺ leak channels, activated by pH changes, lipids, stretch	T cells	T cell volume regulation, proliferation, cytokine production; attenuation of EAE in <i>K_{2p3.1}</i> ^{-/-} mice	
Sodium					
TRPM4	Na ⁺	Intracellular Ca ²⁺	T cells, DCs, macrophages	Depolarization of V _m ; inhibits Ca ²⁺ influx and cytokine production in macrophages and mast cells; promotes phagocytosis. In <i>Tipin4</i> ^{-/-} mice, increases macrophage function and sepsis mortality, increases mast cell function and PCA.	
Nav1.5	Na ⁺	Membrane depolarization (?)	Thymocytes	Sustains Ca ²⁺ influx; positive selection of CD4 ⁺ T cells.	
Protons, pH					
Hv1	H ⁺	Reduced intracellular pH	B cells, macrophages, neutrophils	Sustains activation of NADPH oxidase and ROS production in neutrophils, macrophages, and B cells; <i>Hv1</i> ^{-/-} mice have reduced ROS production, antibody responses, and neurological damage in stroke	
Ae2	Cl ⁻ /HCO ₃ ⁻		CD8 ⁺ T cells	CD8 ⁺ T cells from <i>Ae2</i> ^{-/-} mice have intracellular alkalinization, increased IL-2 production, and increased proliferation	

Channel	Permeability	Activation	Expression in immune cells	Immune function in vitro and in vivo	Channelopathies and immune disease in human
Chloride					
GABA _A	Cl ⁻	Extracellular GABA	T cells	Inhibition of T cell proliferation, cytokine production, cytotoxicity, T cell-mediated autoimmunity; inhibition attenuates EAE, CIA, diabetes in rodent models	
CFTR	Cl ⁻	cAMP	T and B cells	F508 mutation impairs cytokine production by human T cells; <i>Cftr</i> ^{-/-} murine T cells show exacerbated IL-4, IL-13 production, and IgE response to <i>Aspergillus fumigatus</i>	
LRRc8A (alias: SWELL1), corresponding current: VRAC or Cl _{swell}	Cl ⁻ (I ⁻ , Br ⁻)	Hypotonicity-mediated cell swelling	T cells, B cells, probably ubiquitous	Volume regulation: <i>Lrrc8</i> ^{-/-} mice have severely impaired T cell development and moderately impaired B cell development	Heterozygous truncation mutation in <i>LRRc8A</i> causes congenital agammaglobulinemia with no circulating B cells

This table summarizes ion channels and transporters whose function in immune cells in vitro and in vivo is supported by genetic evidence (mutations in human patients, knockout mice, RNAi in mammalian cells) or by selective channel inhibitors. Nevertheless, the degree of evidence for a role of the listed channels and transporters in immune function varies greatly, as discussed in the text. Question marks (?) indicate greater level of uncertainty or ambiguous results in different studies. > indicates the selectivity of ion channels/transporters for specific cations. Arrows (→) indicate activating signals.

Abbreviations: ACD, allergic contact dermatitis; ADPR, ADP ribose; Ae2, anion exchanger 2; cADPR, cyclic ADP ribose; cAMP, cyclic adenosine monophosphate; Ca_v, voltage-gated Ca²⁺ channel; CFTR, cystic fibrosis transmembrane conductance regulator; CIA, collagen-induced arthritis; CRAC, Ca²⁺ release-activated Ca²⁺ channel; DTH, delayed-type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; GABA, γ-aminobutyric acid; HV1, voltage-gated proton channel; IBD, inflammatory bowel disease; GvHD, graft-versus-host disease; IEL, intraepithelial lymphocyte; K_{Ca}Ca²⁺-activated K⁺ channel; KV, voltage-gated K⁺ channel; LPS, lipopolysaccharide; MagT, Mg²⁺ transporter; MHC, major histocompatibility complex; NAADP, nicotinic acid adenine dinucleotide phosphate; NKT, natural killer T cell; PCA, passive cutaneous anaphylaxis; RA, rheumatoid arthritis; ROS, reactive oxygen species; STIM, stromal interaction molecule; t.b.d., to be determined; TCM, central memory T cell; Treg, regulatory T cell; TRP, transient receptor potential; V_m, membrane potential; XMEN, X-linked immunodeficiency with Mg²⁺ defect and EBV infection and neoplasia; ZIP, Zrt-Irt-like protein; ZnT, zinc transporter.