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Ion Channels in Innate and Adaptive Immunity

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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; $K_V1.3$; $K_{Ca}3.1$; 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.

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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 $[Ca^{2+}]_i$ 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 $K_V1.3$ and the Ca^{2+}-activated intermediate-conductance $K_{Ca}3.1$, 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).

K_V1.3 and K_{Ca}3.1—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

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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-C2β, for $K_{Ca}3.1$ (15–17). In the case of $K_{Ca}3.1$, TCR activation recruits and activates PI3K-C2β 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 Fcγ and Fcε 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 (PLCγ, PLCβ) and production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. IP_3 binding to its receptor causes the opening of IP_3R 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 $K_V1.3$ and $K_{Ca}3.1$ maintain the hyperpolarized PM potential necessary for Ca^{2+} influx, pharmacological inhibition of $K_V1.3$ 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

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(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 (β3, β4) 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 $Ca_V1.4$ (68) and moderately reduced in T cells from *Cacnb3*$^{-/-}$ mice lacking the β3

subunit (69, 70). In T helper 2 (Th2) cells, Ca^{2+} influx was reduced when $Ca_V1.2$ and $Ca_V1.3$ 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 $[K^+]$ or when $K_V1.3$ and/or $K_{Ca}3.1$ 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 $[Mg^{2+}]_i$ 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 $[Zn^{2+}]$ 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 $[Zn^{2+}]$ 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, Zir/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 $[Zn^{2+}]$ 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).

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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

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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 *$K_{2P}3.1^{-/-}$* 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 Ca^{2+} 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 Ca^{2+}, Mg^{2+}, Zn^{2+}, and Cl^- channels have been shown to regulate T cell development.

Calcium—Ca^{2+} 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 $[Ca^{2+}]_i$ (118), and Ca^{2+} 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 Ca^{2+} influx, whereas positive selection by weak peptide-MHC-TCR interactions induced only moderate Ca^{2+} influx (120, 121). However, a truly causative role for Ca^{2+} signals or specific Ca^{2+} channels in T cell development and selection has been missing.

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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 Vβ 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 TCRαβ$^+$ T cells (131). When crossed to HY TCR-transgenic mice, these mice display a moderate impairment in positive and negative selection but a normal Vβ repertoire of TCRαβ$^+$ 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 $K_V1.3$ and $K_{Ca}3.1$ (72, 133, 134). Why CRAC channels are dispensable for the development of conventional TCRαβ 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 IP_3R homologs in endothelial and hematopoietic lineage cells with Tie2-Cre resulted in an arrest of thymocyte development at the $CD4^-CD8^-$ 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 $CD4^+CD8^+$ double-positive (DP) transition is impaired.

In contrast to conventional TCRαβ 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 CD8αα$^+$ 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). CD8αα$^+$ 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

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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-2Rα (CD25, unique to the IL-2R) and IL-2Rβ (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 Ca^{2+}-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 Ca^{2+} 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 Ca^{2+} 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 Mg^{2+} is critical for T cell development. Patients with XMEN disease (X-linked immunodeficiency with Mg^{2+} defect, EBV infection, and neoplasia) due to mutations in *MAGT1* lack Mg^{2+} 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 Mg^{2+} channel TRPM7. Whereas complete deletion of TRPM7 in mice is prenatally lethal (E6.5), T cell–specific deletion in *Trpm7$^{fl/-}$ Lck-Cre* mice resulted in viable mice that showed a block of T cell development at the DN stage (Figure 6) (149). *Trpm7$^{fl/-}$ 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 Mg^{2+} influx through TRPM7 is essential for T cell development, but this

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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

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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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} flux via activation of PKCδ 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 IP_3 to inositol-1,3,4,5-tetrakisphosphate (IP_4). 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 Ca^{2+} 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 Zn^{2+} 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 Zn^{2+} 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 Zn^{2+} 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 Ca^{2+} 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 Ca^{2+} 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. Ca^{2+} 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 Ca^{2+} influx through CRAC channels is the regulation of cytokine and chemokine gene expression, primarily via the calcineurin-NFAT pathway (3, 166). Gene

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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 RORγt 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 RORγt and RORα (168), whereas mRNA and protein levels of RORγt, RORα, 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

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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.

Ca^{2+} 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 $[Ca^{2+}]_i$ 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 Ca^{2+} signals (181, 182), but it is not fully understood which channels mediate Ca^{2+} influx or release and which steps in these processes—including granule polarization toward the lytic immunological synapse, docking, or PM fusion—are Ca^{2+} 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 Ca^{2+} 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

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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 Ca^{2+} influx is largely abolished in T and B cells lacking ORAI1, STIM1, or both STIM1/2, other Ca^{2+} channels such as Ca_V channels, P2X receptors, and some TRP channels have been proposed to mediate Ca^{2+} influx and T cell functions.

Voltage-gated Ca^{2+} channels: $Ca_V1.4$-deficient murine $CD4^+$ and $CD8^+$ T cells had impaired TCR-induced Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} channel complex (70). Two other L-type Ca^{2+} channels, $Ca_V1.2$ and $Ca_V1.3$, may play a role in Th2 cells, given that their depletion by RNAi impaired TCR-induced Ca^{2+} 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 Ca^{2+} 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

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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 Mg^{2+} influx channels below, the other TRP channels implicated in lymphocyte function are Ca^{2+}-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 Ca^{2+} store depletion, although they may mediate other forms of Ca^{2+} influx (201). Whereas RNAi knockdown of TRPC3 in CD4$^+$ T cells has only marginal effects on Ca^{2+} influx and proliferation (202), TRPC5 is upregulated in activated CD4$^+$ and CD8$^+$ effector T cells and mediates Ca^{2+} influx after binding of surface GM1 ganglioside by galectin-1 on Treg cells, potentially contributing to Treg-mediated suppression (203). The Ca^{2+}-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 Ca^{2+} influx in CD4$^+$ T cells from *Trpv6*$^{-/-}$ mice is normal (S. Feske, unpublished observations). Somewhat more substantial evidence supports a role for the Ca^{2+}-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 Ca^{2+}. Besides K^+ channels, TRPM4 is therefore essential for the regulation of V_m and, thereby, indirectly Ca^{2+} influx (7, 8). Opening of the Ca^{2+}-activated TRPM4 at the peak of Ca^{2+} influx in T cells results in Na^+ influx, which then depolarizes V_m, limits Ca^{2+} influx, and generates an oscillatory pattern of Ca^{2+} 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 Ca^{2+} influx into a prolonged and sustained Ca^{2+} response, leading to increased IL-2 secretion (7). Similar findings were made in Th2 cells, where TRPM4 knockdown led to sustained Ca^{2+} influx and increased

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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 Ca^{2+}-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 Ca^{2+} 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 Ca^{2+} signaling events in activated T cells (210, 211). Pharmacological inhibition of $K_V1.3$ and $K_{Ca}3.1$ suppresses Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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

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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 Mg^{2+} 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 Mg^{2+} influx in MAGT1-deficient $CD4^+$ T cells, resulting in impaired PLCγ1 phosphorylation and impaired IP_3 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 Mg^{2+} increased NKG2D surface expression and the cytolytic function of $CD8^+$ T cells and NK cells in vitro, and XMEN patients given Mg^{2+} orally showed restored NKG2D levels on $CD8^+$ T cells and decreased the number of circulating EBV-infected B cells in vivo. The mechanisms of Mg^{2+}-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 Mg^{2+} influx channels exist in T cells. One such channel could be TRPM7, which besides Mg^{2+} conducts other divalent cations including Ca^{2+}, Zn^{2+}, and Ni^{2+} (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 Mg^{2+} signaling or other cations conducted by TRPM7, especially Ca^{2+}, remains to be investigated.

Zinc transporters—T cell function is regulated by Zn^{2+} with both activating and inhibitory effects noted (reviewed in 227–229). In some studies, the presence of Zn^{2+} enhanced in vitro T cell proliferation (82, 230) and synthesis of IL-2 and IFN-γ (231), whereas in other studies higher Zn^{2+} 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 $[Zn^{2+}]$ 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 $[Zn^{2+}]_i$ (82). Increased free $[Zn^{2+}]_i$ 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 Ca^{2+} influx (82). Dynamic changes in $[Zn^{2+}]_i$ 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 Zn^{2+} signal was not determined. ZIP8 localized in lysosomes was shown to regulate IFN-γ

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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 Zn^{2+} signaling in immune cells, we are just beginning to understand the function of Zn^{2+} transporters and Zn^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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-ΔF508* mice with the same mutation and *Cftr$^{fl/fl}$ 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

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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.

Ca^{2+} channels—Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} influx in STIM1/2-deficient mice, B cells may be overall less dependent on Ca^{2+} 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, Ca^{2+} 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 Ca^{2+} influx (242) after BCR stimulation of the DT-40 chicken B cell lymphoma cell line (241, 242). Whether TRPC channels contribute to Ca^{2+} signaling in human or murine B cells is not clear. In primary murine B cells, mechanical stress activates Ca^{2+}-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

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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 $K_V1.3$ and $K_{Ca}3.1$ to regulate V_m. Both channels are differentially expressed in different B cell subsets (246). $K_V1.3$ expression is high in IgD^-CD27^+ class-switched memory B cells and, presumably, in plasma cells, whereas $K_{Ca}3.1$ is the dominant K^+ channel in activated naive and IgD^+CD27^+ memory B cells (246). In keeping with this expression pattern, $K_V1.3$ blockers suppress anti-CD40 antibody or PMA plus ionomycin-stimulated proliferation of class-switched memory B cells, whereas $K_{Ca}3.1$ blockers inhibit the proliferation of IgD^+ B cells (246) and inhibit antimito-chondrial 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 $K_V1.3$ and $K_{Ca}3.1$, 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 [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.

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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 Fcγ receptor (FcγR) cross-linking (see Figure 8). FcγR 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 IP_3 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 finding 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 FcγR-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 FcγR

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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 PKCα and PKCβ, which phosphor-ylate 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 Ca^{2+} channels to generate localized Ca^{2+} signals that promote high-efficiency phagocytosis (264). These findings are consistent with a moderate defect in FcγR-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 Ca^{2+}-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 Ca^{2+} oscillations induced by extracellular ATP activation of P2Y receptors (268). $K_{Ca}3.1$ in macrophages can also be activated by Ca^{2+} influx through CRAC channels and seems to be tightly coupled to SOCE by prolonging Ca^{2+} 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

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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

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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-1β 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-1β, 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 Ca^{2+} 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

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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.

TRPV2 is a Ca^{2+}-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 PIP_2, which further results in actin depolymerization that is required for phagocytic receptor clustering and internalization (58). Interestingly, this function of TRPV2 is independent of Ca^{2+} 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).

TRPM4 inhibits macrophage function by mediating Na^{+} influx, depolarizing the V_m, and thereby antagonizing Ca^{2+} 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 Ca^{2+} influx (316). *Trpm4*$^{-/-}$ macrophages are also defective in phagocytosis (316). Besides regulating Ca^{2+} influx in macrophages, TRPM4 modulates Ca^{2+} influx in DCs and is required to prevent Ca^{2+} overload, as immature DCs from *Trpm4*$^{-/-}$ mice have a sustained increase in Ca^{2+} 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 Ca^{2+}-mediated innate responses.

Proton Channels

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 O_2^{-} (Figure 10) (319). However, the flux of electrons results in membrane depolarization and a decrease in intracellular pH, both of

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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 Zn^{2+} 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 O_2^- 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

Zn^{2+} deficiency is associated with many defects in innate immune functions. For example, Zn^{2+} supplementation enhances phagocytosis of *Escherichia coli* and *S. aureus* by peritoneal macrophages and killing of *Trypanosoma cruzi* (325, 326). Zn^{2+} deficiency results in reduced neutrophil and macrophage chemotaxis, phagocytosis, production of ROS, and netosis by neutrophils (327). Zn^{2+} 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 Zn^{2+} on innate immune function are not straightforward, and they vary in a concentration-dependent manner because at low concentrations Zn^{2+} is proinflammatory and at high concentrations it suppresses the same pathways (330).

Many Zn^{2+} 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 Zn^{2+} concentrations, and the regulation of innate immune responses. Expression of ZIP8 is upregulated after LPS and TNF-α stimulation of macrophages and promotes Zn^{2+} uptake across the PM, which attenuates NF-κB activation by direct Zn^{2+} 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 Zn^{2+} transporters, the net effect being a decrease in $[Zn^{2+}]_i$, which appears to be necessary for LPS-induced DC maturation (333). Zn^{2+} supplementation or overexpression of ZIP6, which mediates Zn^{2+} transport into cells, abrogates LPS-induced upregulation of MHC class II and costimulatory molecules (333). Future studies of Zn^{2+} transporter–deficient mice, innate immune cells isolated from these mice, and the downstream signaling pathways regulated by changes in $[Zn^{2+}]_i$ will be critical to fully understanding the role of Zn^{2+} 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 $[Ca^{2+}]_i$ is an important driver for the release and synthesis of a large number of allergic mediators including histamine, proteases, prostaglandins, leukotrienes, and cytokines (334).

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Ca^{2+} Channels

Cross-linking of IgE bound to the FcεRI stimulates IP_3-mediated depletion of ER Ca^{2+} 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 Ca^{2+} influx, which was associated with impaired degranulation, release of leukotriene C_4 (LTC_4), and production of cytokines such as TNF-α (129). Similarly, mast cells isolated from *Stim1*$^{-/-}$ mice had reduced Ca^{2+} influx and activation (130). Pharmacological inhibition of CRAC channels in human lung mast cells also attenuated FcεRI-stimulated Ca^{2+} influx; histamine release; release of IL-5, IL-8, IL-13, and TNF-α; and LTC_4 production (335). Interestingly, the combination of low doses of a CRAC channel inhibitor with the LTC_4 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, TNBSinduced colitis, intestinal neutrophil infiltration, and expression of inflammatory cytokines bymast 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 Ca^{2+} 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 Ca^{2+}-calmodulin binding to the C terminus of $K_{Ca}3.1$, are

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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

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cytokine and LTC_4 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 Ca^{2+} 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

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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 Ca^{2+} 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

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$K_V1.3$ 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 immunosuppres-sion. 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 sul-fonic 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 FcεRI-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 glomulonephritis, arthritis, IBD, asthma, EAE, GvHD, and compound-

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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 Ca^{2+}, Mg^{2+}, and Zn^{2+}, 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 $[Ca^{2+}]_i$ "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 Ca^{2+} signaling; it is now evident that this is the predominant pathway by which immune and chemokine receptors stimulate Ca^{2+} 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 Ca^{2+}. 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 Ca^{2+} 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

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negative PM potential for Ca^{2+} influx via CRAC and other Ca^{2+} channels, which is mediated primarily by $K_V1.3$ and $K_{Ca}3.1$ 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 $Na_V1.5$ 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, $K_V1.3$, $K_{Ca}3.1$, 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 $K_V1.3$ 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.

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Glossary

IP_3	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

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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 PLCγ1 and the production of IP_3 and SOCE following ER Ca^{2+} store depletion. How TCR stimulation activates MAGT1 and how Mg^{2+} regulates PLCγ1 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, PLCγ1 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.

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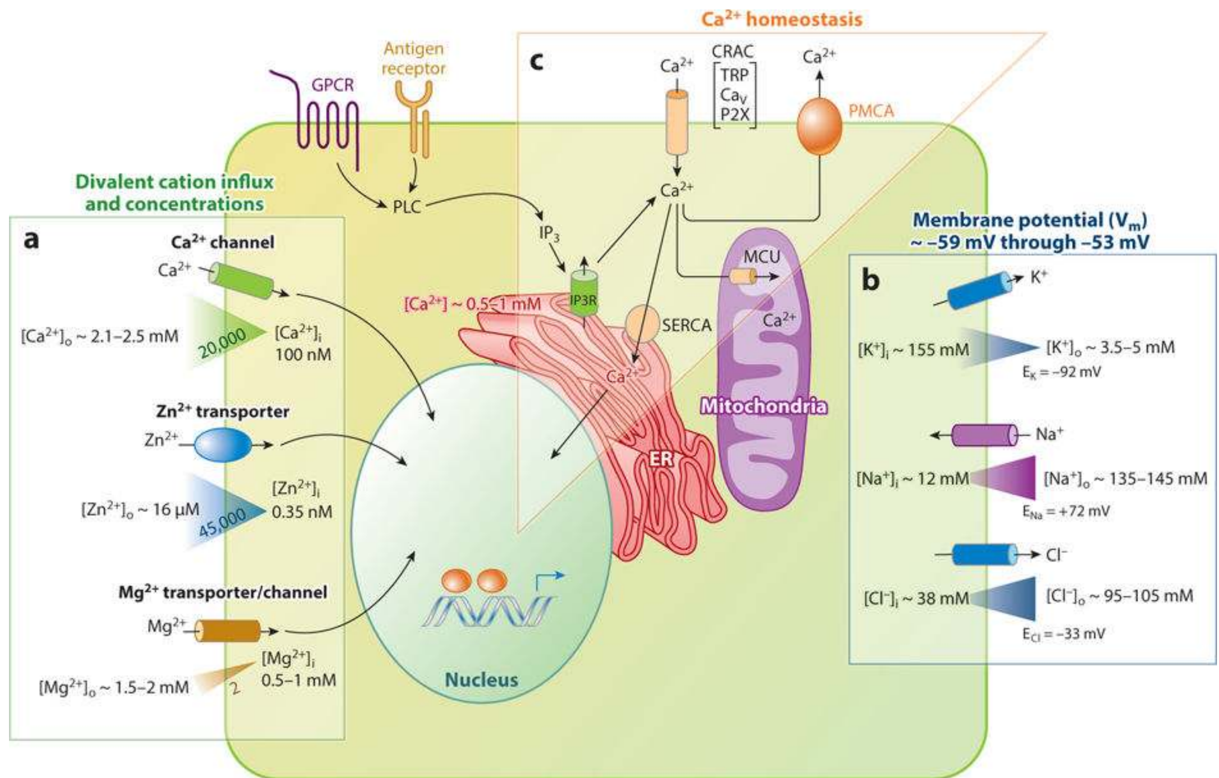

Figure 1.

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.)

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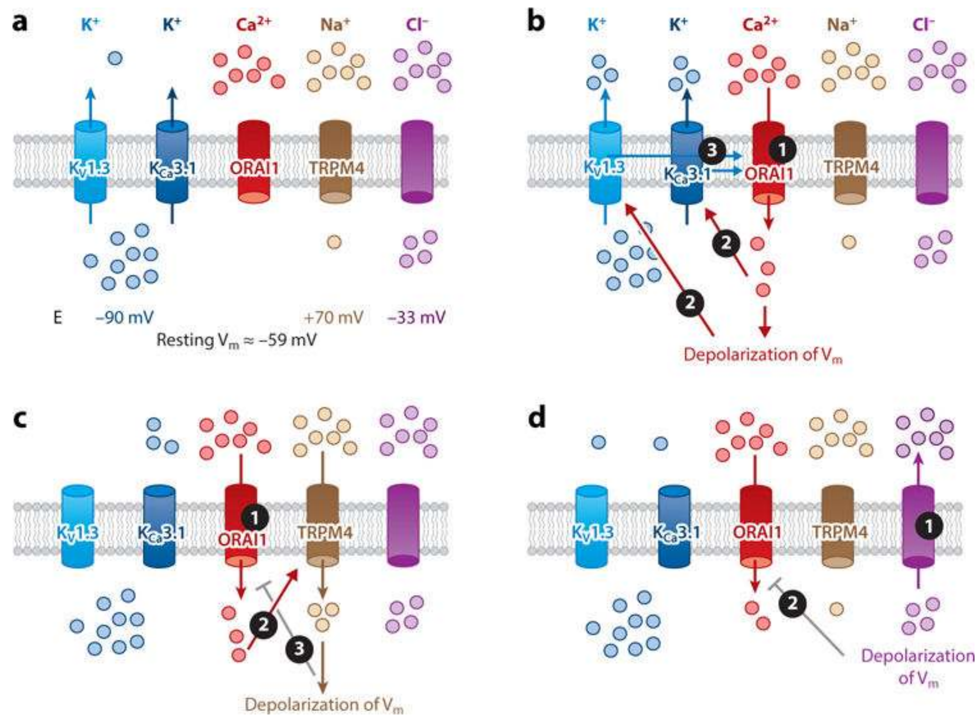

Figure 2.

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 ❶ 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. ❷ Open $K_{Ca}3.1$ and $K_V1.3$ channels mediate K^+ efflux and hyperpolarization of the PM, thereby sustaining Ca^{2+} influx ❸. (*c*) TRPM4 depolarizes the PM and inhibits Ca^{2+} influx. Ca^{2+} influx through ORAI1 channels ❶ results in the opening of Ca^{2+}-regulated TRPM4 channels ❷ that mediate Na^+ influx and depolarization of the PM, thereby inhibiting Ca^{2+} influx via ORAI1 ❸. (*d*) Opening of Cl^- channels in lymphocytes results in efflux of Cl^- ions due to the relatively high $[Cl^-]_i$ of approximately 38 mM and an equilibrium potential (approximately −33 mV) that is positive relative to V_m ❶. Cl^- efflux depolarizes V_m and inhibits Ca^{2+} influx ❷.

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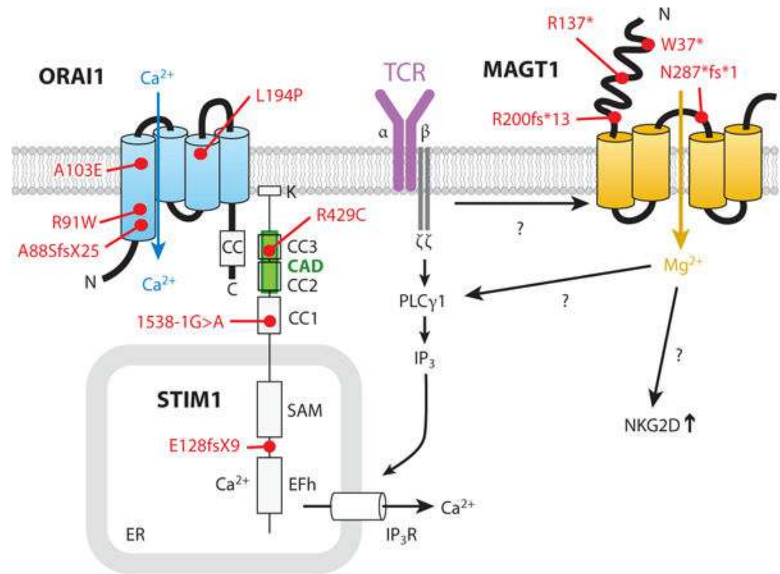

CRAC channelopathy Mutations: *ORAI1*, *STIM1*	**XMEN** Mutations: *MAGT1*
Clinical phenotype: • SCID-like immunodeficiency (recurrent and chronic viral, bacterial, and fungal infections) • Autoimmunity (autoimmune hemolytic anemia and thrombocytopenia, autoantibodies) • Muscular hypotonia and atrophy of type II muscle fibers • Ectodermal dysplasia (hypocalcified dental enamel, anhidrosis) **Immunological defect:** • Predominantly T cell activation defect • Normal lymphocyte development except Treg and NKT cells	**Clinical phenotype:** • X-linked • Magnesium defect • EBV infections (primary immunodeficiency with recurrent viral and bacterial infections) • Neoplasia (lymphoma) **Immunological defect:** • 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, IP3 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

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interaction molecule 1; TCR, T cell receptor; Treg, regulatory T cell; XMEN, X-linked immunodeficiency with Mg^{2+} defect, EBV infection, and neoplasia.)

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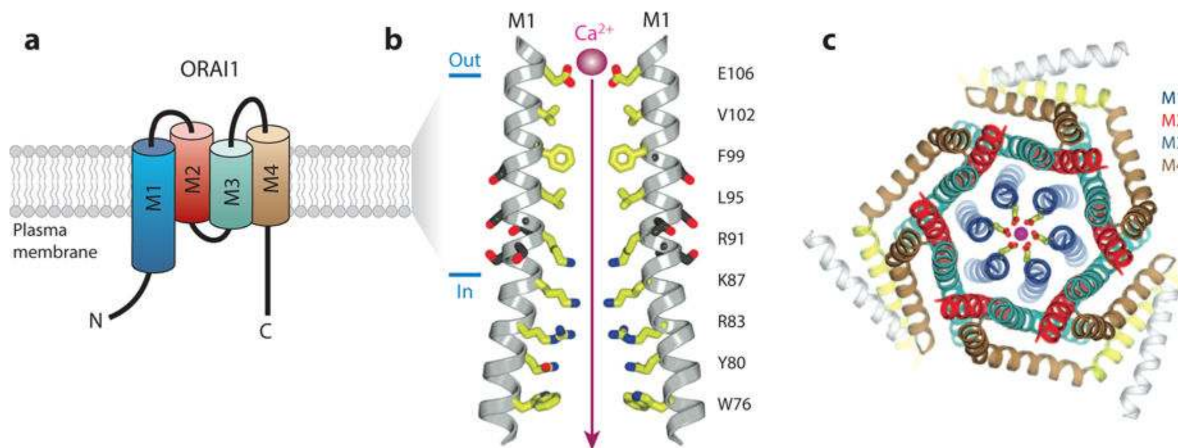

Figure 4.

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*.

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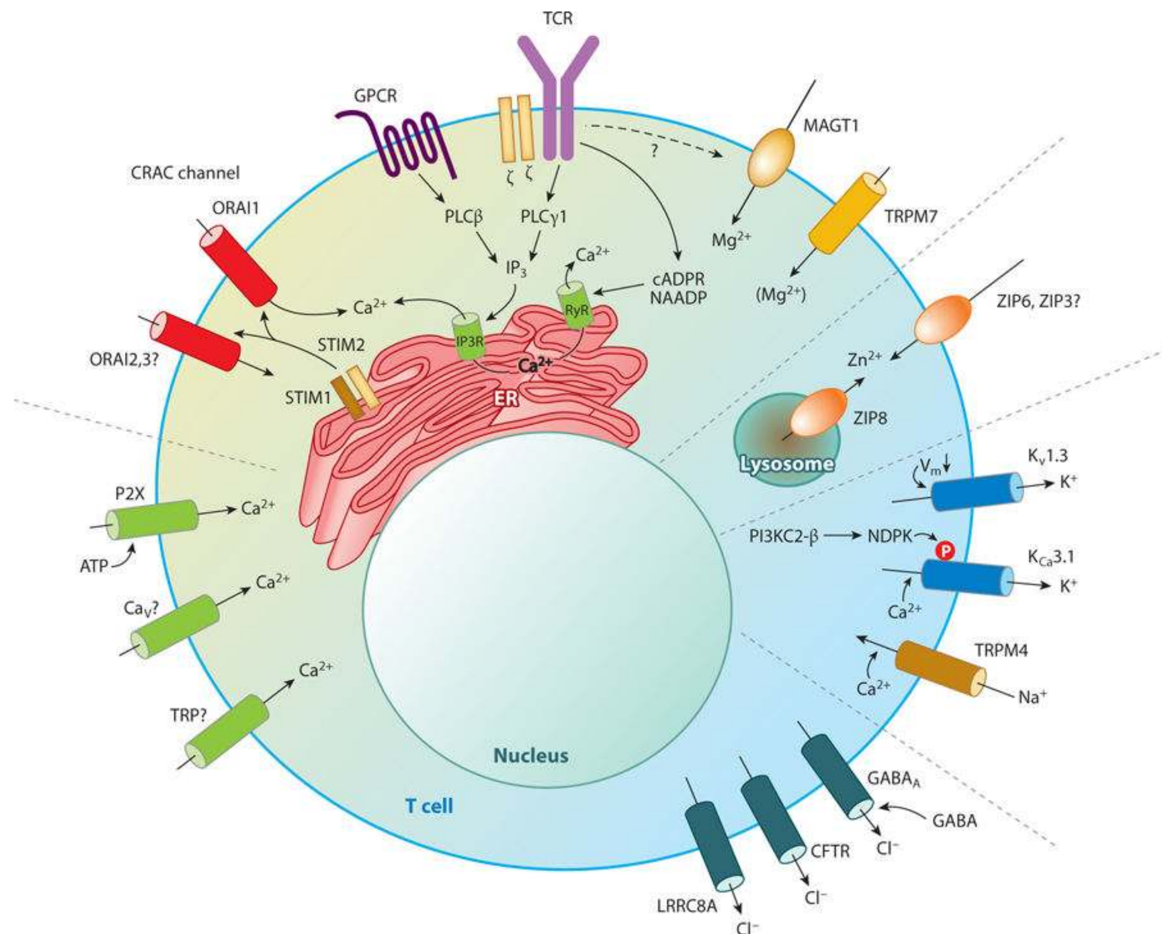

Figure 5.

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 PLCγ1, 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, $K_V1.3$ and $K_{Ca}3.1$, which are counteracted by the Ca^{2+}-activated TRPM4 channel that depolarizes V_m. $K_V1.3$ 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-C2β 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 PIP_2 and inhibited by intracellular Mg^{2+}. Several Zn^{2+} transporters, including ZIP6, ZIP3, and ZIP8, reportedly mediate Zn^{2+} 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 Ca^{2+} channel; CFTR, cystic fibrosis transmembrane conductance regulator; CRAC, Ca^{2+} release–activated Ca^{2+} channel; ER, endoplasmic reticulum; GABA, γ-aminobutyric acid; GPCR, G protein–coupled receptor; IP_3, 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 Ca^{2+} entry; STIM, stromal interaction molecule; TCR, T cell receptor; TRP, transient receptor potential; V_m, membrane potential; ZIP, Zir/Irt-like protein.)

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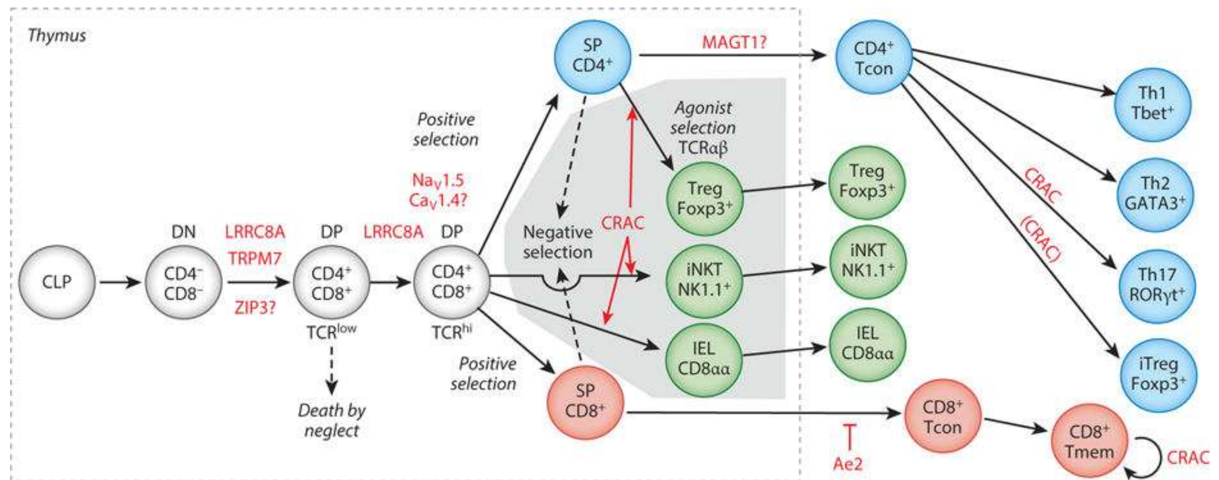

Figure 6.

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 Mg^{2+}-permeable channel TRPM7 and potentially also by the Zn^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Mg^{2+} influx through the Mg^{2+} 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^{-}/HCO_3^{-} anion exchanger Ae2, whereas the maintenance of memory CD8^{+} T cells requires Ca^{2+} influx via CRAC channels.

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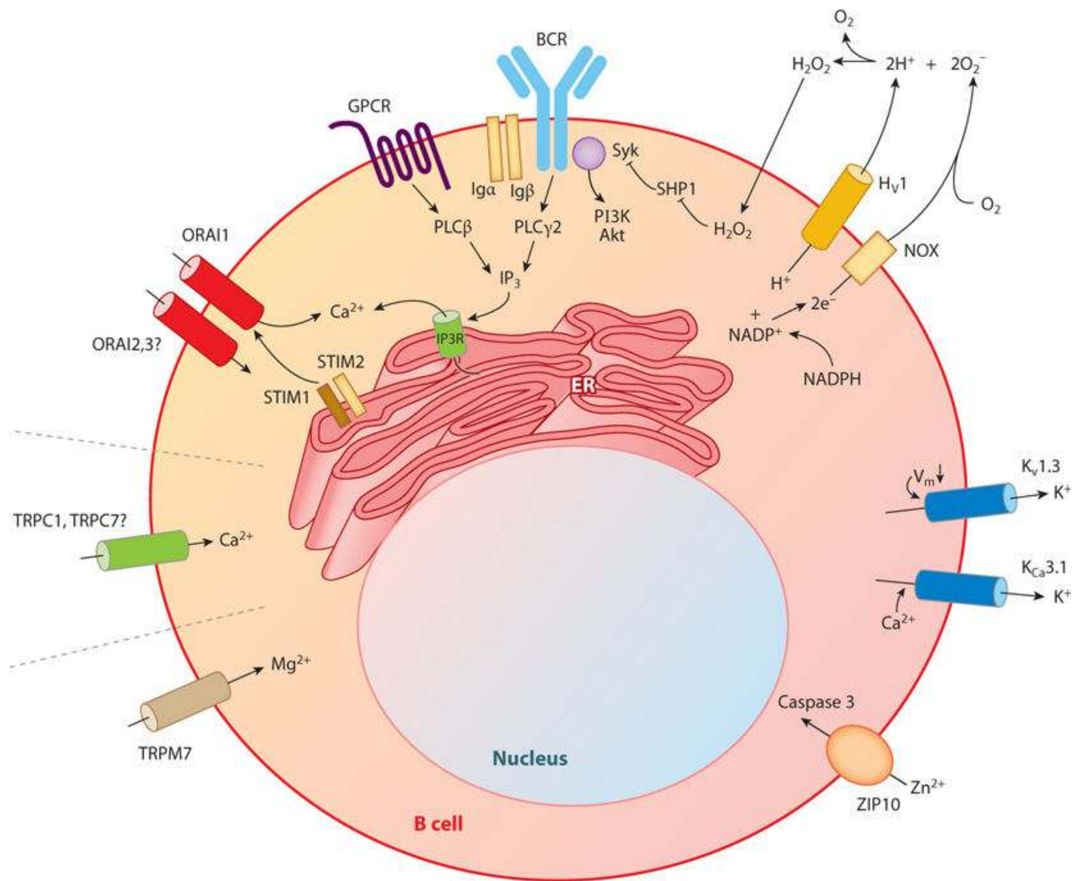

Figure 7.

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 Igα/β chains of the BCR and the scaffold protein SLP-65 (*not shown*), which helps to recruit PLCγ2 to the plasma membrane of B cells and activate it. The subsequent generation of IP_3 and the IP_3R-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 $K_V1.3$ and the Ca^{2+}-activated $K_{Ca}3.1$ channel. The proton channel H_V1 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.)

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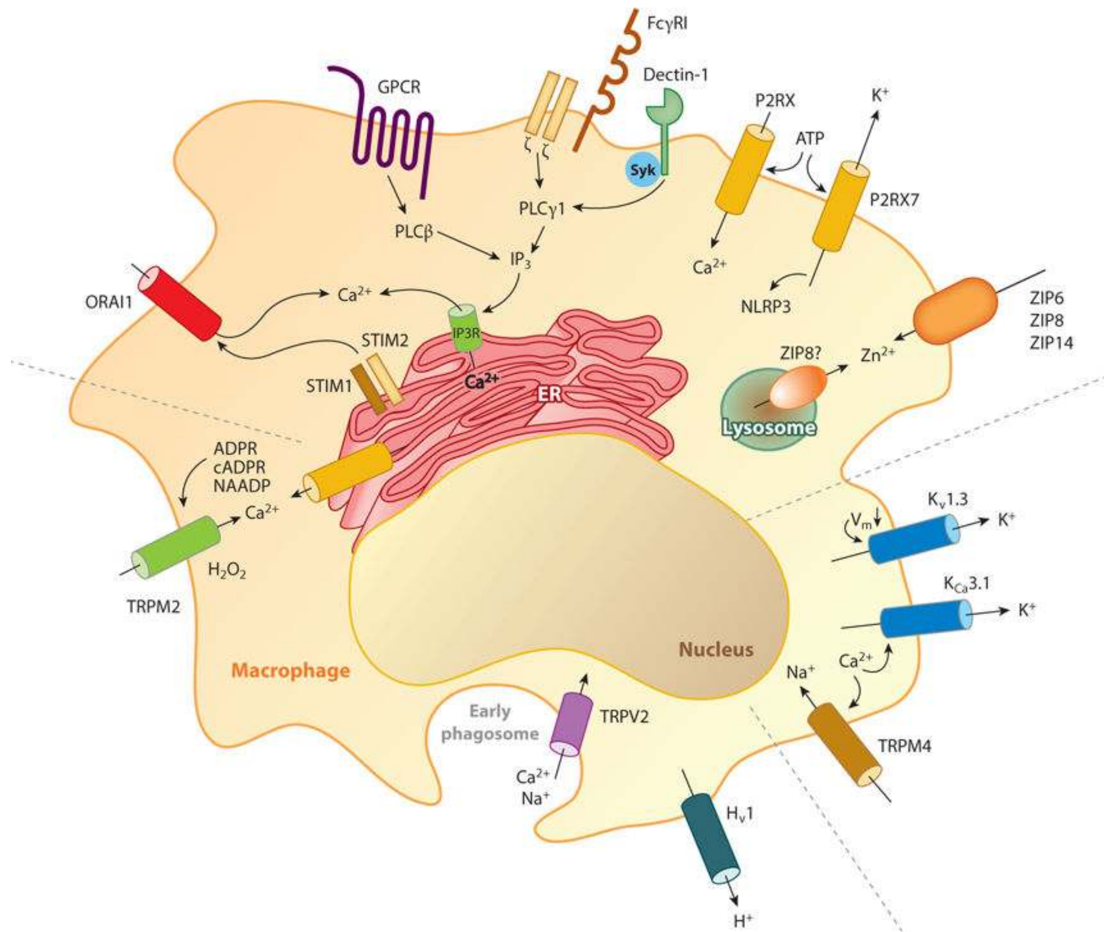

Figure 8.

Ion channels in macrophages. Macrophages express a variety of receptors that activate ion channels including FcγRI and FcγRIII, G protein–coupled chemokine receptors, and innate immunoreceptors such as Dectin-1/2 and Toll-like receptors (TLRs). FcγRI cross-linking and Dectin-1 binding result in activation of PLCγ1, and chemokine receptor engagement results in activation of PLCβ 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, $K_V1.3$ 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 FcγR 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

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reportedly regulate Zn^{2+} levels in macrophages by mediating Zn^{2+} influx after TLR stimulation, thereby inhibiting NF-κB activation. The proton channel H_V1 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, Zir/Irt-like protein.)

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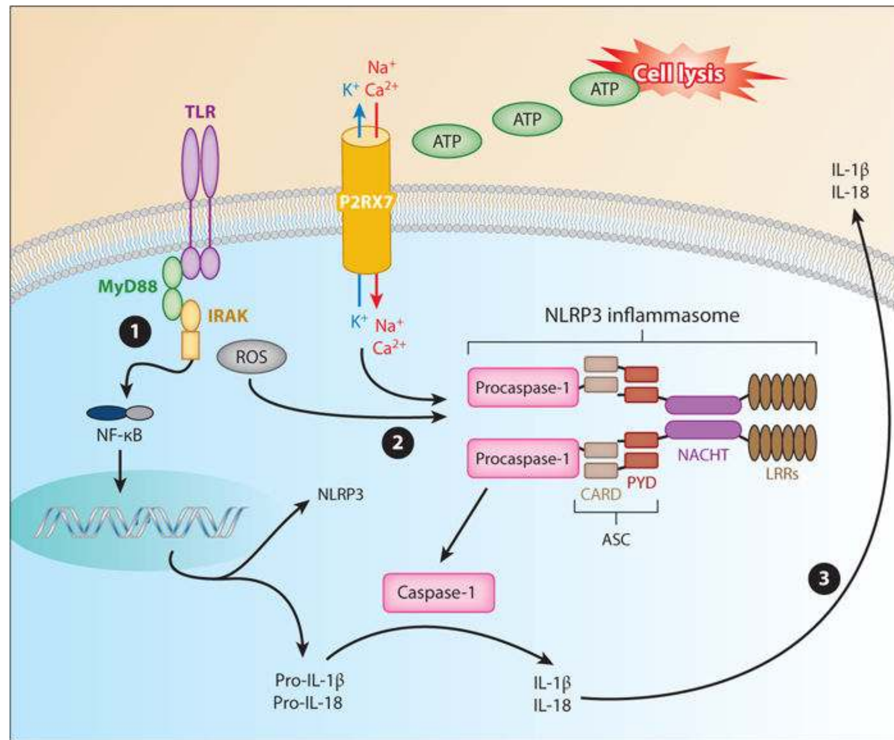

Figure 9.

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: ❶ During the priming phase, NLRP3 and pro-IL-1β are transcribed in response to signals such as TLR activation or IFN-γ, TNF-α, or other stimuli that activate NF-κB. ❷ A second signal then activates the NLRP3 inflammasome, resulting in the activation of caspase-1, cleavage of pro-IL-1β and pro-IL-18 into their mature forms, and ❸ secretion of IL-1β 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 trisphosphate; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IRAK, interleukin-1 receptor associated kinase; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor-κB; NLRP3, NLR family, pyrin domain containing 3; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α.)

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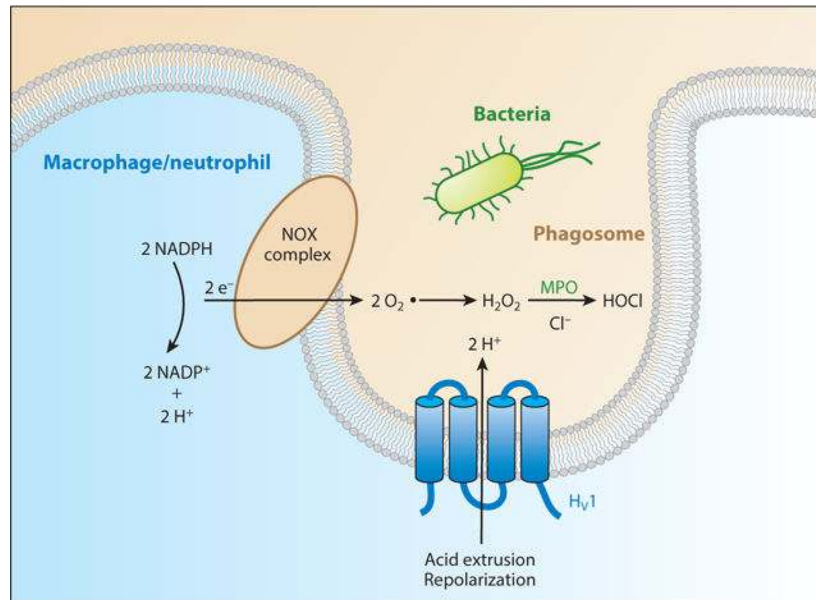

Figure 10.

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 O_2, generating O_2^-. 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 H_V1. H^+ reacts with O_2^- in the phagosome to generate H_2O_2, which undergoes further conversion to HOCl that is catalyzed by MPO. (Abbreviations: HOCl, hydrochlorus acid; MPO, myeloperoxidase; NADPH, nicotinamide adenine dinucleotide phosphate.)

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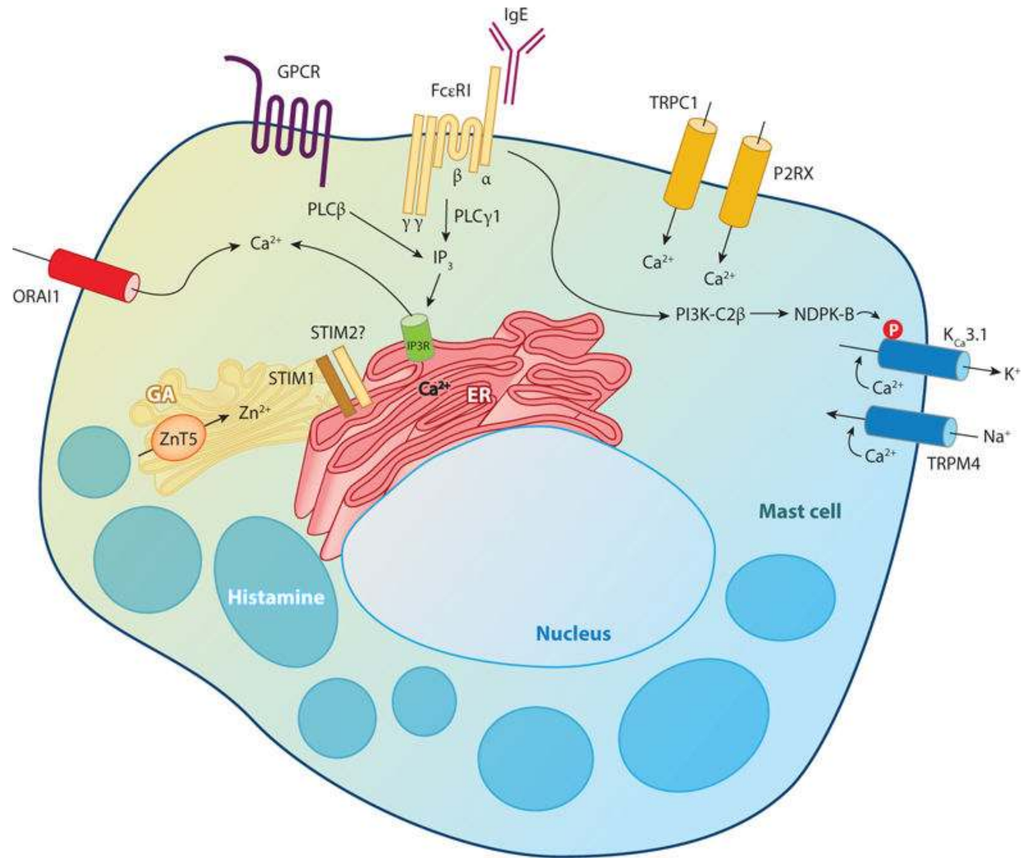

Figure 11.

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, Ca^{2+} influx, and the secretion of mediators of the allergic response. Ca^{2+} 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, IP_3 production, Ca^{2+} release from ER stores, and SOCE via STIM1 and ORAI1 activation. Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} influx, but their role in mast cell function is not well defined. The Zn^{2+} transporter ZnT5 is located in the Golgi apparatus (GA) and mediates Zn^{2+} uptake, thereby regulating FcεRI-mediated PKC recruitment to the plasma membrane and NF-κB activation. (Abbreviations: ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; NDPK-B, nucleoside diphosphate kinase B; PKC, protein kinase C; PLC, phospholipase C; SOCE, store-operated Ca^{2+} entry;

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

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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 analphylaxis Neutrophils: ROS production, adhesion (?)	CRAC channelopathy due to mutations in human *ORAI1* (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 *S. aureus* and *L. monocytogenes*? (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 *STIM1* (see *ORAI1*)
TRP					
TRPC3, C5	Ca^{2+},Na^{+}	Unknown [(GM1) 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; *Trpm2*$^{-/-}$ mice are susceptible to infection with *L. monocytogenes* and are	

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Channel	Permeability	Activation	Expression in immune cells	Immune function in vitro and in vivo	Channelopathies and immune disease in human
				resistant to obesity-induced diabetes and DSS-induced colitis	
TRPV2	Ca^{2+},Na^{+}	FcγR signaling	Macrophages	Chemotaxis and phagocytosis by macrophages; *Trpv2*$^{-/-}$ mice show increased susceptibility to *L. monocytogenes*	
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_V1.4), $CD8^+$ T cell survival, cytokine production, $CD8^+$ T cell immunity to *L. monocytogenes* infection, Th2 function in asthma (Ca_V1.2)	
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 oATP 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 *M. tuberculosis* in mice	Polymorphisms in human gene are associated with increased susceptibility to *M. tuberculosis*
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; *P2rx2/3*$^{-/-}$ 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 *ZIP4* cause acrodermatitis enteropathica with immunodeficiency (due to impaired intestinal Zn^{2+} uptake)

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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					
$K_V1.3$	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; *Kv1.3*$^{-/-}$ mice resistant to EAE and obesity-induced diabetes Macrophages: proliferation, iNOS expression DCs: expression of CD80, CD86, CD40, IL-12; chemotaxis	
$K_{Ca}3.1$	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 $K_{2P}3.1^{-/-}$ 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 *Trpm4*$^{-/-}$ mice, increases macrophage function and sepsis mortality, increases mast cell function and PCA.	
$Na_V1.5$	Na^+	Membrane depolarization (?)	Thymocytes	Sustains Ca^{2+} influx; positive selection of $CD4^+$ T cells.	
Protons, pH					
H_V1	H^+	Reduced intracellular pH	B cells, macrophages, neutrophils	Sustains activation of NAPDH oxidase and ROS production in neutrophils, macrophages, and B cells; *Hvcn1*$^{-/-}$ mice have reduced ROS production, antibody responses, and neurological damage in stroke	
Ae2	Cl^-/HCO_3^-		$CD8^+$ T cells	$CD8^+$ T cells from *Ae2*$^{-/-}$ mice have intracellular alkalization, increased IL-2 production, and increased proliferation	

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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	ΔF508 mutation impairs cytokine production by human T cells; *Cftr*$^{-/-}$ murine T cells show exacerbated IL-4, IL-13 production, and IgE response to *Aspergillus fumigatus*	
LRRC8A (alias: SWELL1), corresponding current: VRAC or Cl_{swell}	Cl^- (I^-,Br^-)	Hypotonicity-mediated cell swelling	T cells, B cells, probably ubiquitous	Volume regulation; *Lrrc8*$^{-/-}$ mice have severely impaired T cell development and moderately impaired B cell development	Heterozygous truncation mutation in *LRRC8A* 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 Ca^{2+} channel; CFTR, cystic fibrosis transmembrane conductance regulator; CIA, collagen-induced arthritis; CRAC, Ca^{2+} release–activated Ca^{2+} channel; DTH, delayed-type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; GABA, γ-aminobutyric acid; H_V1, voltage-gated proton channel; IBD, inflammatory bowel disease: GvHD, graft-versus-host disease; IEL, intraepithelial lymphocyte; K_{Ca},Ca^{2+}-activated K^+ channel; K_V, voltage-gated K^+ channel; LPS, lipopolysaccharide; MagT, Mg^{2+} 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; T_{CM}, central memory T cell; Treg, regulatory T cell; TRP, transient receptor potential; V_m, membrane potential; XMEN, X-linked immunodeficiency with Mg^{2+} defect and EBV infection and neoplasia; ZIP, Zrt-Irt-like protein; ZnT, zinc transporter.