Published in final edited form as:

Trends Immunol. 2014 July ; 35(7): 332–344. doi:10.1016/j.it.2014.05.001.

Divalent cation signaling in immune cells

Benjamin Chaigne-Delalande and Michael J. Lenardo

Molecular Development of the Immune System Section, Lymphocyte Molecular Genetics Unit, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Michael J. Lenardo: lenardo@NIH.gov

Abstract

Divalent cations of two alkaline earth metals Ca^{2+} and Mg^{2+} and the transition metal Zn^{2+} play vital roles in the immune system, and several immune disorders are associated with disturbances of their function. Until recently, only Ca^{2+} was considered to serve as a second messenger. However, signaling roles for Mg^{2+} and Zn^{2+} have been recently described, leading to a reevaluation of their role as potential second messengers. Here we review the roles of these cations as second messengers in light of recent advances in Ca^{2+} , Mg^{2+} and Zn^{2+} signaling in the immune system. Developing a better understanding of these signaling cations may lead to new therapeutic strategies for immune disorders.

Keywords

Calcium; Magnesium; Zinc; Signal transduction; Immune disorders

Cytosolic *free* ionic pool and cellular homeostasis

In eukaryotic cells, divalent cations exist in two principal states; one tightly bound to proteins or other negatively-charged macromolecules such as mono- or polyphosphates, and a second ionized state involved in dynamic chemical equilibria. The “*bound*” pool represents the majority of the intracellular cation and plays a number of roles as a vital structural and functional co-factor through strong electrostatic interactions. On the other hand, the ionized fraction (0.05% to 10%) of each cation remains “*free*” in the cytosol or sequestered into organelles, such as the endoplasmic reticulum (ER) or mitochondria. The signaling ability of an ion relies on the high chemical activity of the intracellular “*free*” pool, and its ability to be rapidly modulated without affecting the total cellular amount of the ion (Box 1: Notion of ion signaling). This modulation results from mobilization of the cation across the plasma membrane (PM) or from intracellular stores to increase the cytosolic concentration to generate transient binding complexes between the cation and proteins or other macromolecules. There are numerous technical considerations that affect the accurate measurement of intracellular concentrations divalent cations. Whereas the total amount

(*bound* and *free*) can be quantified by destructive biophysical analysis, the assessment of the *free* pool is achieved mainly by the use of chemical indicators [1].

The ability for a cation to be mobilized in the cell, especially across the plasma membrane, depends on two driving forces, the chemical and the electric gradient [2]. The chemical gradient corresponds to the net difference of concentrations between the extracellular (or reservoir) environment and the cytosol. For example, Ca^{2+} and Zn^{2+} intracellular *free* concentrations, $[\text{Ca}^{2+}]_i$ and $[\text{Zn}^{2+}]_i$, respectively, are maintained at approximately 10^4 -fold lower than the physiological extracellular concentration, thus generating a large chemical gradient for their mobilization into the cytosol (Table 1). On the other hand, there is a much smaller difference (< 2 -fold) between cytosolic *free* Mg^{2+} ($[\text{Mg}^{2+}]_i$) and extracellular *free* Mg^{2+} ($[\text{Mg}^{2+}]_o$) level leading to the conventional wisdom that it is a poor candidate for a second messenger (Table 1) [1, 2]. However, because non-excitable cells, such as immune cells, harbor a negative membrane potential $\approx -70\text{mV}$, if intracellular *free* Mg^{2+} was at electrical equilibrium, its resting concentration should be 50 mM [3, 4]. Nevertheless, $[\text{Mg}^{2+}]_i$ ranges from 0.2 to 0.5 mM, showing that intracellular *free* Mg^{2+} is regulated and maintained at a lower concentration. This creates an electrochemical gradient of 100 to 250-fold for Mg^{2+} , which is sufficient to allow rapid mobilization across the plasma membrane (PM) (Table 1, see *Regulation of cytosolic free Mg^{2+}* section) [3, 5, 6]. Indeed, the physiological function of rapid Mg^{2+} fluxes in T lymphocytes has been recently demonstrated and led to interesting insights into novel immunoregulatory mechanisms [7]. Table 1 summarizes extracellular and intracellular *free* concentrations of Ca^{2+} , Mg^{2+} and Zn^{2+} as well as their physicochemical properties.

Here, we discuss the criteria for ion signaling and report recent advances on Ca^{2+} , Zn^{2+} and Mg^{2+} mobilization and signaling in immune cells as well as their importance in human disease pathophysiology and treatment.

Homeostasis and mobilization of divalent cations in immune cells

Mobilizing Ca^{2+}

In the basal state, cytosolic *free* Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) is maintained at 100 nM by active extrusion of the Ca^{2+} from the cytosol. This occurs either through the PM by the Ca^{2+} -ATPase (PMCA) and $\text{Ca}^{2+}/\text{Na}^{+}$ exchangers (NCX), or by deposition in ER or mitochondrial stores by the sarcoplasmic/ER Ca^{2+} -ATPase (SERCA) or the mitochondrial Ca^{2+} uniporter (MCU), respectively (Figure 1a, Table 2) [8–10].

Many immune functions are triggered by receptor-mediated acute elevations of cytosolic *free* Ca^{2+} . The main mechanism leading to this elevation is the store-operated Ca^{2+} entry (SOCE), which involves mobilization across the PM triggered by the release of Ca^{2+} stored in the ER. SOCE is induced by a wide range of immune receptors including the T cell receptor (TCR), the B cell receptor (BCR), Fc receptors (FcR), Toll-like receptors (TLR) and chemokine receptors, among others. Receptor engagement activates various isoforms of phospholipase C (PLC), leading to the generation of the second messengers inositol 1, 4, 5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 activates Ca^{2+} release through the IP_3 receptor channels (IP_3R) on the ER. The depletion of Ca^{2+} from the ER stores is sensed by

the N-terminal region of the stromal interaction molecule (STIM). This results in STIM oligomerization and translocation to a region of the ER proximal to the PM. The cytosolic C-terminal region of STIM then recruits and activates the prototypical Ca^{2+} -release activated Ca^{2+} (CRAC) channel, ORAI, which promotes Ca^{2+} influx through the PM [11]. Our understanding of SOCE has increased greatly since the discovery of STIM and ORAI and recent updates are reviewed in [11].

Genetic deficiencies of ORAI1 and STIM1 in mice and humans lead to a drastic reductions of SOCE in immune cells including T cells, B cells, NK cells and mast cells and impaired T cell and mast cell activation (see *Ca²⁺ and SOCE deficiency* section [12–14]. In addition, there are two STIM isoforms, STIM1 and STIM2, that differ in their relative abundance and activation thresholds [15]. STIM1 activation requires higher Ca^{2+} store depletion than STIM2 [15]. In the mouse, deletion of both STIM isoforms in T cells profoundly decreases SOCE, greater than either one alone, and causes a lymphoproliferative disorder associated with a selective reduction of regulatory T cells (T_{regs}) [16]. Similarly, in B cells, the loss of both STIMs diminishes interleukin 10 (IL-10) production by regulatory B cells [17]. In both cell types, STIM2 deficiency alone leads to a milder SOCE decrease than STIM1 deficiency [15, 16]. However, STIM2 deficient T cells and B cells exhibit lower sustained Ca^{2+} mobilization and reduced nuclear translocation of the transcription factor, nuclear factor of activated T-cells (NFAT) [15, 16]. Additionally, recent studies suggest that both isoforms are required for optimal cytotoxic function of CD8^{+} T cells [15, 18]. The specific role of STIM2 in immune cells remains to be elucidated. Similarly, the three isoforms of ORAI are ubiquitously although differentially expressed in distinct cell types [15]. The ORAI1 knockdown (KD) in mouse T cells completely abrogates SOCE, whereas an ORAI2 KD has no effect implying that the latter may have no role in T cells [15]. However, other studies detected residual SOCE in ORAI1 deficient murine mast cells and T cells, suggesting ORAI2 participates in SOCE in these cells [13]. ORAI3 is induced after T cell activation and, unlike ORAI1 and ORAI2, is not inhibited by reactive oxygen species (ROS) [19]. Given that differentiation of naïve T cells into effector cells is associated with desensitization to ROS, ORAI3 might mediate SOCE desensitization following T cell activation [11, 19]. In addition to SOCE, store-independent or non-SOCE are also potentially important, although less established, mechanisms, for Ca^{2+} mobilization in immune cells and are discussed in Box 2.

Regulation of cytosolic free Mg^{2+} in immune cells

The homeostatic regulation of cytosolic *free* Mg^{2+} is less well understood than Ca^{2+} . It involves Mg^{2+} transporters, channels and exchangers, such as the $\text{Mg}^{2+}/\text{Na}^{+}$ exchanger (Figure 1b, Table 2). Another major participant is the melastatin transient receptor potential 7 (TRPM7) channel [3]. TRPM7 is a ubiquitously expressed, non-selective Mg^{2+} channel that also conducts Ca^{2+} , Zn^{2+} and even Na^{+} in absence of divalent cations [3, 20, 21]. TRPM7 has a serine/threonine kinase domain in its cytosolic tail [22]. Whereas the kinase domain is not essential, its activity can modulate the gating of TRPM7 [22]. The critical role of TRPM7 for Mg^{2+} homeostasis in immune cells is illustrated by decreased *free* Mg^{2+} and cell cycle arrest in TRPM7 deficient B cell lines and impaired T cell development in T cell-specific TRPM7 conditional knockout (KO) mice [22–24]. Other regulators of *free* Mg^{2+}

homeostasis in immune cells include members of the solute carrier (SLC) family SLC41A1 and SLC41A2. These transporters were identified by homology to the bacterial Mg^{2+} transporter, MgtE, and are expressed relatively ubiquitously [20, 25]. The role of SLC41A1/2 in *free* Mg^{2+} homeostasis was uncovered by ectopic expression in TRPM7-deficient B cell line that can rescue the decreased intracellular Mg^{2+} levels and defective proliferation [26, 27].

Finally, MAGT1 is a key Mg^{2+} transporter in immune signaling [30, 31]. Unlike TRPM7 and SLC41A1/2, MAGT1 is a uniquely Mg^{2+} -selective channel that is ubiquitously expressed but with higher levels in immune and epithelial cells [28, 29]. Since the early 90s, elevation of intracellular *free* Mg^{2+} has been observed after stimulation of T cells with lectins in some studies, but not others [5, 30, 31]. The functional importance of Mg^{2+} mobilization remained unknown for twenty years until the discovery of a new primary immunodeficiency named "X-linked immunodeficiency with Mg^{2+} defect, Epstein Barr Virus (EBV) infection, and neoplasia" (XMEN) disease [7, 32, 33]. This disease is due to a genetic deficiency of MAGT1, which we found to be essential for a TCR-gated Mg^{2+} flux (see *divalent cations in pathophysiology* section) [7]. The MAGT1-dependent Mg^{2+} flux is required for the optimal activation of PLC- γ 1, IP₃ generation, protein kinase C theta (PKC θ) phosphorylation, and Ca^{2+} mobilization via SOCE (Figure 2b) [7]. The molecular mechanism of TCR gating of MAGT1 is currently unknown. MAGT1 deficiency also leads to decreased cytosolic *free* Mg^{2+} and decreased Mg^{2+} uptake in T cells and B cells [7, 32, 34]. Moreover, oral Mg^{2+} supplementation restores the level of intracellular *free* Mg^{2+} in XMEN patients [32]. The channel or transporter that restores Mg^{2+} homeostasis in immune cells after supplementation remains under investigation. Conversely, the Mg^{2+} flux cannot be restored in XMEN T cells by high external Mg^{2+} and apparently requires the presence of the MAGT1 protein [32]. Recently, a study using drosophila MAGT1 expressed in human neuroblastoma SH-SY5Y cell line showed MAGT1-dependent currents induced by the protein kinase C (PKC) inducer phorbol-12-myristate-13-acetate (PMA) via phosphorylation by PKC [35]. The role of this interesting observation in immunity has yet to be established. In B cells, the phosphorylation of serine 1164 of PLC- γ 2 by the kinase domain of TRPM7 is dependent on extracellular Mg^{2+} and participates in Ca^{2+} mobilization (via SOCE) in response to BCR stimulation [36]. However, in this study, the authors did not assess Mg^{2+} mobilization in response to BCR stimulation and there is no evidence of Mg^{2+} mobilization by B cell activation (Figure 2c) [36].

Different waves of Zn^{2+} mobilization

Cytosolic *free* Zn^{2+} is maintained at low levels by tight regulation of the expression and subcellular localization of two families of Zn^{2+} transporters controlling cytosolic import and export. The zinc transporter (ZnT, SLC30) family consists of 10 transporters that reduce cytosolic Zn^{2+} levels, whereas the fourteen importers of the Zrt1-, Irt1-like Protein (ZIP, SLC39) family increase it (Figure 1c) [37, 38]. Zn^{2+} homeostasis is also regulated by intracellular Zn^{2+} -binding proteins, including metallothioneins (MT) that can sequester up to 15% of the cellular Zn^{2+} and release it during oxidative stress [39]. The role of MT in controlling Zn^{2+} homeostasis appears to be important in innate immune cells. In

macrophages, MT deficiency impairs cytokine production and bactericidal activity after lipopolysaccharide (LPS) stimulation due to Zn^{2+} -deficiency [40, 41].

Two types of Zn^{2+} mobilization have been described with different timing. First, in T cells, monocytes, neutrophils and mast cells, fast transcription-independent Zn^{2+} mobilization has been observed within minutes after the stimulation of activating receptors using fluorescent Zn^{2+} -sensitive probes [42]. How these Zn^{2+} fluxes are triggered is mostly unknown. In human T cells, co-incubation with IL-2 or antigen-loaded dendritic cells (DCs) induces a rapid Zn^{2+} elevation that requires ZIP6 on the PM and ZIP8 on the lysosomal membrane, respectively [43–45]. In monocytes/macrophages, LPS stimulation induces quick release of Zn^{2+} from intracellular stores [46, 47]. The intracellular stores and the role of MT involved in Zn^{2+} release remain to be determined. In mast cells, FcεRI stimulation induces Zn^{2+} release from the ER termed “ Zn^{2+} waves”. The mechanisms governing these “ Zn^{2+} waves” are not well understood and their biological function remains unclear [48]. The second type of Zn^{2+} mobilization occurs several hours after various stimuli and requires changes in channel expression. For example, activation of T and B cells leads to a sustained cytosolic *free* Zn^{2+} elevation due to the down-regulation of several Zn^{2+} exporters (ZnT1, ZnT4, ZnT5, ZnT6 and ZnT7) and the up-regulation of several importers (ZIP6, ZIP10 and ZIP8) over long time course [43, 49]. Silencing ZIP6 and ZIP8 in human T cells prevents the elevation of cytosolic *free* Zn^{2+} and impairs proliferation and cytokine production during T cell activation [43–45]. The physiological role of these transporters in B cells is unknown. On the other hand, stimulation of DCs with LPS decreases intracellular Zn^{2+} over time via the down-regulation of ZIP6 and ZIP10 and the up-regulation of ZnT1, ZnT4 and ZnT6 [50]. Importantly, reduced cytosolic *free* Zn^{2+} is essential for major histocompatibility complex class II (MHC II) up-regulation as well as DC maturation and phagocytic function [50]. The precise molecular mechanism of these effects is unknown and it is not clear whether this is modulation of a cofactor or a signaling function *per se*.

Over the past few years, investigation of Ca^{2+} , Mg^{2+} and Zn^{2+} signaling has provided many new insights in their regulatory roles in immune cells. The identification of ORAI as the CRAC channel provided the long-sought molecular validation of SOCE. However, there are still unanswered questions about the respective roles of STIM and ORAI isoforms in immune cells. Additionally, non-SOCE mechanisms may be important in immune functions even if we don't fully understand how they work at this time (Box 2). Recently, the discovery of inducible Mg^{2+} and Zn^{2+} fluxes in immune cells has revealed the signaling function of these cations but has raised many new questions about their regulation. For example, in T cells, the gating mechanisms and compartmentalization of MAGT1 (Mg^{2+}) and ZIP6 (Zn^{2+}) requires further investigation in immune cells. Furthermore, new specific chelators and fluorescent probes must be developed to study Mg^{2+} and Zn^{2+} mobilization with the clarity and elegance achieved in the Ca^{2+} field. Mobilizing divalent cations is only the first step for ion signaling, in the next section we discuss how changes in the concentration of cytosolic *free* Ca^{2+} , Mg^{2+} and Zn^{2+} control immune signaling pathways and effector functions.

Divalent cations functions in immune cells

Ca²⁺, a central second messenger

Ca²⁺ signaling triggers cell activation and differentiation principally through gene expression and the rapid deployment of effector functions (Figure 2a). Ca²⁺ controls the activity of several transcription factors. It stimulates NFAT via the Ca²⁺-sensitive protein calmodulin (CaM)-dependent activation of the phosphatase calcineurin (CN) (Figure 2a). Ca²⁺ also activates the cAMP response element-binding protein (CREB) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) through CaM kinases (CaMK) and PKC (Figure 2a) [51]. For cytolytic effector functions, Ca²⁺ plays an important role in immune exocytosis. In mast cells, NK cells and cytotoxic T cells (CTL), increased *free* Ca²⁺ is required for degranulation of following FcεRI and FcγRIII (CD16) and TCR stimulation, respectively [13, 52, 53]. Similarly, the up-regulation of MHC II molecules and synaptotagmin VII, a Ca²⁺ sensor involved in vesicle trafficking, during DC maturation depends on Ca²⁺ mobilization [54, 55]. Ca²⁺ chelation studies in mouse and human phagocytes have established that phagocytosis is mostly Ca²⁺-*independent* [56]. However, the Ca²⁺ requirement for the fusion of the phagosome with the lysosome is still debated [56, 57]. Moreover, in neutrophils, ROS production associated with antimicrobial functions relies on the Ca²⁺-dependent activation of NADPH oxidase 2 (NOX2). The mechanism of activation of NOX2 is not well defined, but apparently involves PKC, phosphatidylinositide 3-kinases (PI3K) and sphingosine kinase (SK) and the Ca²⁺/Zn²⁺-binding protein calprotectin (S100A8/A9) [56, 58, 59]. Thus, a broad range of immune functions can be rapidly deployed through the extraordinary speed of free Ca²⁺ moving inside and throughout the cell.

Emerging role for Mg²⁺ in immune cells

Beyond the essential role of Mg²⁺ as a cofactor for ATP, enzymes and other processes in all cells, special signaling functions have been recently documented in immune cells. Early studies documented that Mg²⁺ is important for T and B cell proliferation, but it was unclear whether this was due to a cofactor role, a signaling role, or both [7, 27, 60, 61]. As mentioned earlier, TRPM7-deficient B cell lines exhibit reduced cytosolic *free* Mg²⁺ and defective proliferation, which is restored by increasing extracellular Mg²⁺ and/or overexpressing other Mg²⁺ transporters [27, 61]. Whether TRPM7 deficiency affects total, mainly bound, Mg²⁺ in B cell lines was not determined [22]. Both TRPM7 and MAGT1 appear to play a role in T cell differentiation [3, 7]. Germline TRPM7 KO mice are embryonic lethal, whereas T cell-specific TRPM7 KO mice are viable but exhibit a blockade of thymic T cell development at the double negative stage [24]. XMEN patients (MAGT1-deficient), have reduced numbers of CD4 T cells especially the naïve CD4⁺/CD45RO⁻/CD27⁺/CD31⁺ cells, suggesting diminished thymic output possibly due to impaired positive selection [7, 33]. As mentioned previously, in mature T cells, TCR stimulation induces a MAGT1-dependent Mg²⁺ flux required for optimal activation of PLC-γ1 and Ca²⁺ mobilization via SOCE [7]. There appears to be no such requirement for B cell activation through BCR signaling [7].

Interestingly, we found that decreased cytosolic *free* Mg²⁺ in MAGT1 deficiency causes the loss of expression of NKG2D, an essential cytotoxicity activating receptor on NK and CD8+

T cells [32]. NKG2D recognizes specific nonclassical MHC class I-like ligands that are upregulated in virally-infected and transformed cells, including EBV-infected B cells [32]. Biochemical analysis in lymphocytes from XMEN patients revealed incompletely glycosylated NKG2D proteins that are retained intracellularly in reduced abundance [32]. It is not clear whether the failure of N-glycosylation is the cause of NKG2D deficiency. However, MAGT1 shares 60% amino acid identity with TUSC3, an ortholog of a subunit of the yeast N-glycosylation enzyme complex, raising the question of whether MAGT1 may participate in these processes [20, 29]. Strikingly, exogenous Mg^{2+} supplementation restores the level of cytosolic *free* Mg^{2+} and the expression of fully glycosylated NKG2D, independently of MAGT1, indicating that the full maturation and glycosylation of NKG2D is strictly dependent on the level of cellular *free* Mg^{2+} [32].

Zn^{2+} more than a trace element in immune cells

Dietary Zn^{2+} deficiency decreases T cell activation, shifts T_H1 responses toward T_H2 responses, reduces the cytotoxic function of NK and NKT cells, and impairs cytokine production by mast cells and NK cells [62]. Interestingly, Zn^{2+} supplementation restores those functions, but also induces chronic inflammatory responses and suppresses T_H17 development in the elderly [63–65]. T_H17 cell suppression appears to be caused by the direct Zn^{2+} -dependent inhibition of IL-6-induced phosphorylation of signal transducer and activator of transcription 3 (STAT3) by janus kinases (JAKs) [64]. At the cellular level, several studies using the metal chelator tetrakis-(2-Pyridylmethyl)ethylenediamine (TPEN) and the Zn^{2+} ionophore pyrithione established that Zn^{2+} mobilization regulates specific signaling pathways [66, 67]. Those pathways involve tyrosine kinases, PKCs, mitogen-activated protein kinases (MAPK), cAMP and JAK/STAT. In T cells, the role of Zn^{2+} in leukocyte C-terminal Src kinase (LCK) activation is multiple: it facilitates recruitment to the cytoplasmic tail of CD4 and CD8, stabilizes homodimerization of the enzyme, and enhances its activation by reducing the recruitment of the inhibitory phosphatase Src homology region 2 domain-containing phosphatase-1 (SHP-1) to the TCR complex [66, 67]. Reduced SHP-1 recruitment has been directly tied to Zn^{2+} mobilization in T cells, but whether Zn^{2+} plays a structural or signaling role in LCK activation is unclear [44]. In mast cells, PKC recruitment to the PM induced by Fc ϵ RI stimulation depends on Zn^{2+} mobilization and ZnT5 expression, but the mechanism has not been defined [68]. Zn^{2+} enhances mitogen-activated protein kinases (MAPK) used by phagocytes to eliminate pathogens by inhibiting MAPK phosphatases (MAPKP) [45, 46]. Also, cAMP and cyclic guanosine monophosphate (cGMP) are important second messengers produced by adenylate cyclase (AC) and guanylate cyclase (GC), respectively; and degraded by phosphodiesterases (PDE). In monocytes, the levels of cytosolic free Zn^{2+} modulates the cAMP/cGMP pathway by two different mechanisms. First, Zn^{2+} promotes cGMP accumulation by limiting the production of cAMP via direct inhibition of AC [69, 70]. This blocks monocyte differentiation without a requisite decrease of intracellular Zn^{2+} [69, 70]. Second, high *free* Zn^{2+} concentrations (in the low nM range) promote cAMP and cGMP accumulation by inhibiting PDEs [69, 70]. During T cell activation, inhibition of CN by Zn^{2+} released from the lysosomes promotes the activation of NF- κ B and IFN γ production [43]. In IL-27-induced T_{reg} cells, IL-10 production requires Zn^{2+} and is inhibited by MT [71]. Lastly, Zn^{2+} sequestration by calprotectin (S100A8/A9), expressed in monocytes and neutrophils is important to suppress

Candida albicans growth [72]. Thus, Zn^{2+} fluctuations affect many immunological functions.

Whereas many immune functions controlled by Ca^{2+} are well established, our knowledge of the effectors of Zn^{2+} and Mg^{2+} is not as well developed. Given their essential roles as structural supports and co-factors in many cell functions, it is important to further characterize the effectors involved in Mg^{2+} and Zn^{2+} signaling. Additionally, Ca^{2+} , Mg^{2+} and Zn^{2+} signaling pathways are intertwined and each cation can potentially modulate the signaling of the others.

Agonist and antagonist interactions in divalent cations signaling

Not just acting in parallel, Ca^{2+} , Mg^{2+} and Zn^{2+} signaling pathways can intersect by direct competition for the same effectors or by cross-modulation. Classically, Mg^{2+} is considered to be a Ca^{2+} antagonist. This concept stems from their physico-chemical properties as alkali metals carrying divalent positive charges. Ca^{2+} has a larger atomic radius, but Mg^{2+} has a higher affinity for electronegative oxygen, which leads to a greater hydrated radius (Table 1). These properties allow Mg^{2+} to compete with Ca^{2+} for certain binding sites. However, the propensity of Mg^{2+} to be hydrated prevents the conformational changes associated with Ca^{2+} binding which antagonizes Ca^{2+} signaling [73]. For example, Mg^{2+} competes with Ca^{2+} for the binding to the EF-hand sites of CaM and causes the protein to remain in a closed, inactivated conformation [74]. Mg^{2+} can also antagonize Ca^{2+} signaling by blocking Ca^{2+} mobilization. For example, *in vitro* studies using HEK cells engineered to express various P2X channels, show that extracellular Mg^{2+} inhibits Ca^{2+} currents induced by ATP [75]. Similar observations were made on endogenous P2X7 channels using the monocyte-like cell line THP1 [76]. Other Ca^{2+} channels were shown to be inhibited by extracellular Mg^{2+} , such as TRPV3 in mouse keratinocytes, $\text{Ca}_v1.2$ in mouse myocytes [77, 78].

In T cells, Mg^{2+} -dependent activation of PLC- γ 1 following TCR stimulation participates in Ca^{2+} mobilization, which defines a new regulatory role for Mg^{2+} signaling in the upstream control of Ca^{2+} signaling [7, 32]. Unlike the direct competition mentioned earlier, this “pro- Ca^{2+} signaling” role of Mg^{2+} does not involve common binding sites, but a convergence of two independent signaling pathways, in which Mg^{2+} increases PLC- γ 1 activation leading to a more robust generation of IP_3 . This example of coordinated Mg^{2+} and Ca^{2+} signaling opens the door to further investigations and identification of the Mg^{2+} effector(s) that control PLC- γ 1 activation [7]. In B cells, the phosphorylation of PLC- γ 2 in a Mg^{2+} -dependent manner by the kinase domain of TRPM7 might illustrate another convergence of Mg^{2+} and Ca^{2+} signaling [36].

Similar to Mg^{2+} , Zn^{2+} can inhibit Ca^{2+} signaling via inhibition of CN or S100 proteins [43, 79, 80]. However, unlike Mg^{2+} , Zn^{2+} does not compete with Ca^{2+} for the same binding site, but rather binds different sites on the same protein. For example, the inhibitory effect of Zn^{2+} on CaM does involve sites other than the Ca^{2+} -binding EF-hand sites [74]. Zn^{2+} can also promote Ca^{2+} signaling by enhancing the recruitment of Ca^{2+} -sensitive effectors, such as the recruitment of PKC to the membrane where the localized Ca^{2+} mobilization can induce activation [66, 68]. Lastly, some proteins have both Ca^{2+} -dependent and Zn^{2+} -

dependent functions involving different binding sites, such as calprotectin (S100A8/A9 dimers) which is a Ca^{2+} effector for the phagocytosis as well as a Zn^{2+} -binding protein involved in anti-bacterial function of monocytes [79, 80]. Conversely, Ca^{2+} signaling can promote Zn^{2+} mobilization in mast cells, wherein Zn^{2+} waves elicited in response to $\text{Fc}\epsilon\text{RI}$ stimulation are dependent on Ca^{2+} mobilization [48]. Moreover, Ca^{2+} and Zn^{2+} signaling coordinately regulate complex cellular processes in ways that are not completely understood. For example, during DC maturation, both Ca^{2+} elevation and Zn^{2+} diminution are required for up-regulation of MHC II and cytokine production [50, 54, 55].

Divalent cations in physiopathology

The study of human primary immunodeficiencies (PIDs) often yield novel insights into how the immune system is regulated. The understanding of the signaling roles of divalent cations is no exception. For example, the CRAC channel was identified by screening patients with severe combined immunodeficiency (SCID) associated with loss of SOCE [81]. The case is even more striking for Mg^{2+} , because XMEN disease reveals its unexpected signaling functions [7, 32].

Ca^{2+} and SOCE deficiency

In humans, deleterious mutations in *ORAI1* and *STIM1* cause a loss of SOCE in immune cells, SCID and muscle abnormalities [81]. Both ORAI1- and STIM1-deficient patients exhibit recurrent life-threatening viral, bacterial and fungal infections, and require hematopoietic stem cell transplantation (HSCT) early in life. ORAI1 and STIM1 deficiency do not alter the numbers of innate, B or T cells, except for a reduction in the T_{reg} population. However, defective SOCE severely compromises mature T cell activation, proliferation, and cytokine production, although it has a much milder effect on B cell function. Other features of SOCE-related immunodeficiency are lymphoproliferative disorders and autoimmunity. More comprehensive description of human SOCE deficiency is provided in [14].

Mg^{2+} deficiency in immunodeficiency, autoimmunity and inflammation

In XMEN disease, “loss of function” mutations in *MAGT1* result in essentially complete loss of the MAGT1 mRNA and protein [7, 33]. For unknown reasons, XMEN patients have modestly decreased numbers of CD4^{+} T cells and inversion of the CD4/CD ratio [7, 33]. As mentioned above, MAGT1 affects Mg^{2+} in two key respects : (1) the loss of a T cell receptor (TCR)-induced transient Mg^{2+} influx required for optimal T cell activation and (2) a chronic decrease of cytosolic *free* Mg^{2+} [7, 32]. The latter defect is almost entirely responsible for the deficient expression of the NKG2D receptor on NK and CD8^{+} T cells which is an important cytotoxic activating receptor against virally-infected or transformed cells (see *Emerging role for Mg^{2+} in immune cells* section) [7, 32]. The most severe and life-threatening complication of XMEN disease is uncontrolled EBV infection with increased susceptibility to EBV-driven lymphoproliferative disorders [7, 32, 33]. The fact that EBV induces the specific ligands for NKG2D might account for the severity of EBV infection when NKG2D is deficient [32]. Since increasing the cytosolic *free* Mg^{2+} to normal levels rescues the cell surface expression of NKG2D, Mg^{2+} supplementation may be an

effective therapeutic strategy for XMEN patients and could be important for immune suppression of EBV and avoiding EBV-induced lymphoma [32].

In rodents, Mg^{2+} deficiency induced by low Mg^{2+} diet leads to decreased antibody production, thymic involution and chronic inflammation [82, 83]. Similarly, in humans, Mg^{2+} deficiency promotes a chronic inflammatory state that is associated with metabolic syndrome and type 2 diabetes mellitus (T2DM) [84]. Mg^{2+} supplementation inhibits IL-1 β , TNF α and IL-6 secretion by mouse macrophages *in vitro* and *in vivo* [85, 86]. This latter observation fits with the concept of Mg^{2+} - Ca^{2+} antagonism, because reduced extracellular Mg^{2+} sensitizes immune cells to Ca^{2+} signaling leading to chronic inflammation. The precise relationship between the blood level (magnesemia) and the cytosolic level of Mg^{2+} is not well understood however free basal Mg^{2+} equilibrates more effectively with external Mg^{2+} than with the large amounts of intracellular bound Mg^{2+} [87, 88]. Thus, the full immunological impact of hypomagnesemia may be greater than we currently understand. Importantly, more than 60% of the US population does not meet the estimated daily requirement for Mg^{2+} . The impact of the Mg^{2+} dietary deficiency on cytosolic *free* Mg^{2+} , susceptibility to viral infections, and long-term health requires further investigation [89, 90].

Zn²⁺ deficiency and aging

A vital role for Zn^{2+} in immunity has been known for over 50 years, mostly through studies of dietary Zn^{2+} deficiency. The loss of expression of ZIP4 in acrodermatitis enteropathica (AE), a Zn^{2+} malabsorption syndrome, leads to immunodeficiency associated with lymphopenia and thymus atrophy [91]. Additionally, leukocytes from asthmatic children and pulmonary tuberculosis patients over-express ZIP2, and monocytes from septic patients up-regulate ZIP8 suggesting there is abnormal cytosolic *free* Zn^{2+} in these pathological settings [92–94].

As mentioned earlier, the study of PID has clarified signaling roles for Ca^{2+} , Mg^{2+} and Zn^{2+} . Nonetheless, these conditions also raise numerous questions about the cellular homeostasis of these ions. Mg^{2+} and Zn^{2+} deficiency can be compensated to a certain extent by external supplementation, but the mechanisms remains unknown. Unlike Zn^{2+} deficiency, Mg^{2+} deficiency can be masked by the lack of correlation between serum and cellular levels of Mg^{2+} , which could mask the potential role of Mg^{2+} in pathological settings.

Concluding remarks: ion signaling as therapeutic target

Recent advances in understanding the role of divalent cations in immune cells have uncovered new signaling functions for Mg^{2+} and Zn^{2+} and have unveiled new vistas for future investigation. Many questions about Mg^{2+} and Zn^{2+} signaling are still unanswered. In fact, there are a plethora of apparent Mg^{2+} transport and channel proteins whose role in human physiology has not yet been determined [20]. For example, amongst the twenty-two Mg^{2+} channels known, sixteen are expressed in T cells, but except the few mentioned in this review, little is known about their potential role in immunity. Moreover, Mg^{2+} and Zn^{2+} mobilization has been observed in a variety of cell types, however the molecular mechanisms regulating these cation changes and the molecular consequences remain elusive

[95]. Technical limitations such as probe sensitivity, low electrogenicity, and other considerations prevent the precise study of the regulated mobilization of these cations. Another aspect that begs additional experimentation is the understanding of the various effectors of divalent cations signaling and the network(s) that they generate. Beyond enhancing our fundamental scientific understanding, answering these questions has important medical implications especially, but not only, for immune disorders associated with divalent cations signaling deficiency, such as XMEN disease, SOCE deficiency or even dietary Zn^{2+} deficiency. Thirty years ago, the discovery of the immunosuppressant abilities of the Ca^{2+} signaling inhibitors such as the CN inhibitor cyclosporine A, revolutionized organ transplantation [96]. Thus, uncovering new Mg^{2+} and Zn^{2+} sensitive targets or functions could identify new therapeutic targets for immunomodulation or affecting the pathophysiology of other diseases. Moreover, the promising results of Mg^{2+} and Zn^{2+} supplementation in various pathological settings should encourage the development of new ion supplementation strategies [32, 86, 97–99].

Acknowledgments

This work was supported by the Division of Intramural Research, NIAID, NIH. We thank past and present members of the Lenardo laboratory for their intellectual and experimental contributions to this review. We thank our colleagues at Merck for generous collaboration support. We apologize to the many investigators whose work could not be cited due to space limitations.

References

1. Tsien RY. Intracellular measurements of ion activities. Annual review of biophysics and bioengineering. 1983; 12:91–116.
2. Wolf FI, et al. Cell physiology of magnesium. Molecular aspects of medicine. 2003; 24:11–26. [PubMed: 12537986]
3. Romani AM. Magnesium homeostasis in Mammalian cells. Metal ions in life sciences. 2013; 12:69–118. [PubMed: 23595671]
4. Flatman PW. Mechanisms of magnesium transport. Annu Rev Physiol. 1991; 53:259–271. [PubMed: 2042962]
5. Ng LL, et al. Intracellular free magnesium in human lymphocytes and the response to lectins. Clinical science. 1991; 80:539–547. [PubMed: 1647916]
6. Takaya J, et al. Can magnesium act as a second messenger? Current data on translocation induced by various biologically active substances. Magnesium research : official organ of the International Society for the Development of Research on Magnesium. 2000; 13:139–146. [PubMed: 10907231]
7. Li FY, et al. Second messenger role for Mg^{2+} revealed by human T-cell immunodeficiency. Nature. 2011; 475:471–476. [PubMed: 21796205]
8. Baughman JM. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature. 2011; 476:341–345. [PubMed: 21685886]
9. De Stefani D. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature. 2011; 476:336–340. [PubMed: 21685888]
10. Zampese E, Pizzo P. Intracellular organelles in the saga of Ca^{2+} homeostasis: different molecules for different purposes? Cellular and molecular life sciences : CMLS. 2012; 69:1077–1104. [PubMed: 21968921]
11. Shaw PJ, et al. Molecular regulation of CRAC channels and their role in lymphocyte function. Cellular and molecular life sciences : CMLS. 2013; 70:2637–2656. [PubMed: 23052215]
12. Baba Y, et al. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. Nat Immunol. 2008; 9:81–88. [PubMed: 18059272]

13. Vig M, et al. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat Immunol.* 2008; 9:89–96. [PubMed: 18059270]
14. Feske S. CRAC channelopathies. *Pflügers Archiv : European journal of physiology.* 2010; 460:417–435. [PubMed: 20111871]
15. Hoth M, Niemeyer BA. The neglected CRAC proteins: Orai2, Orai3, and STIM2. *Current topics in membranes.* 2013; 71:237–271. [PubMed: 23890118]
16. Oh-Hora M, et al. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol.* 2008; 9:432–443. [PubMed: 18327260]
17. Matsumoto M. The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. *Immunity.* 2011; 34:703–714. [PubMed: 21530328]
18. Weidinger C, et al. STIM1 and STIM2-mediated Ca(2+) influx regulates antitumour immunity by CD8(+) T cells. *EMBO molecular medicine.* 2013; 5:1311–1321. [PubMed: 23922331]
19. Bogeski I, et al. Differential redox regulation of ORAI ion channels: a mechanism to tune cellular calcium signaling. *Sci Signal.* 2010; 3:ra24. [PubMed: 20354224]
20. Quamme GA. Molecular identification of ancient and modern mammalian magnesium transporters. *Am J Physiol Cell Physiol.* 2010; 298:C407–C429. [PubMed: 19940067]
21. Wenning AS, et al. TRP expression pattern and the functional importance of TRPC3 in primary human T-cells. *Biochimica et biophysica acta.* 2011; 1813:412–423. [PubMed: 21215279]
22. Schmitz C, et al. Regulation of vertebrate cellular Mg2+ homeostasis by TRPM7. *Cell.* 2003; 114:191–200. [PubMed: 12887921]
23. Ryazanova LV, et al. TRPM7 is essential for Mg(2+) homeostasis in mammals. *Nature communications.* 2010; 1:109.
24. Jin J. Deletion of Trpm7 disrupts embryonic development and thymopoiesis without altering Mg2+ homeostasis. *Science.* 2008; 322:756–760. [PubMed: 18974357]
25. Brandao K, et al. The role of Mg2+ in immune cells. *Immunol Res.* 2013; 55:261–269. [PubMed: 22990458]
26. Sahni J, et al. SLC41A2 encodes a plasma-membrane Mg2+ transporter. *The Biochemical journal.* 2007; 401:505–513. [PubMed: 16984228]
27. Mandt T, et al. SLC41A1 Mg(2+) transport is regulated via Mg(2+)-dependent endosomal recycling through its N-terminal cytoplasmic domain. *The Biochemical journal.* 2011; 439:129–139. [PubMed: 21696366]
28. Goytain A, Quamme GA. Identification and characterization of a novel mammalian Mg2+ transporter with channel-like properties. *BMC Genomics.* 2005; 6:48. [PubMed: 15804357]
29. Zhou H, Clapham DE. Mammalian MagT1 and TUSC3 are required for cellular magnesium uptake and vertebrate embryonic development. *Proceedings of the National Academy of Sciences of the United States of America.* 2009; 106:15750–15755. [PubMed: 19717468]
30. Rink TJ, et al. Cytoplasmic pH and free Mg2+ in lymphocytes. *The Journal of cell biology.* 1982; 95:189–196. [PubMed: 6815204]
31. Rijkers GT, Griffioen AW. Changes in free cytoplasmic magnesium following activation of human lymphocytes. *The Biochemical journal.* 1993; 289(Pt 2):373–377. [PubMed: 8424782]
32. Chaigne-Delalande B, et al. Mg2+ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. *Science.* 2013; 341:186–191. [PubMed: 23846901]
33. Li FY, et al. XMEN disease: a new primary immunodeficiency affecting Mg2+ regulation of immunity against Epstein-Barr virus. *Blood.* 2014; 123:2148–2152. [PubMed: 24550228]
34. Qiao Y, et al. Variant ATRX syndrome with dysfunction of ATRX and MAGT1 genes. *Human mutation.* 2014; 35:58–62. [PubMed: 24130152]
35. Hu Y. Drosophila MagT1 is upregulated by PKC activation. *Biochem Biophys Res Commun.* 2013; 436:140–144. [PubMed: 23727583]
36. Deason-Towne F, et al. Identification of Ser/Thr phosphorylation sites in the C2-domain of phospholipase C gamma2 (PLCgamma2) using TRPM7-kinase. *Cell Signal.* 2012; 24:2070–2075. [PubMed: 22759789]

37. Huang L, Tepasamorndech S. The SLC30 family of zinc transporters - a review of current understanding of their biological and pathophysiological roles. *Molecular aspects of medicine*. 2013; 34:548–560. [PubMed: 23506888]
38. Jeong J, Eide DJ. The SLC39 family of zinc transporters. *Molecular aspects of medicine*. 2013; 34:612–619. [PubMed: 23506894]
39. Maret W. Redox biochemistry of mammalian metallothioneins. *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry*. 2011; 16:1079–1086. [PubMed: 21647775]
40. Sugiura T, et al. Dysfunction of macrophages in metallothionein-knock out mice. *Journal of UOEH*. 2004; 26:193–205. [PubMed: 15244072]
41. Itoh N, et al. Reduced bactericidal activity and nitric oxide production in metallothionein-deficient macrophages in response to lipopolysaccharide stimulation. *Toxicology*. 2005; 216:188–196. [PubMed: 16157437]
42. Haase H, Rink L. Zinc signals and immune function. *Biofactors*. 2014; 40:27–40. [PubMed: 23804522]
43. Aydemir TB, et al. Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-gamma expression in activated human T cells. *J Leukoc Biol*. 2009; 86:337–348. [PubMed: 19401385]
44. Yu M. Regulation of T cell receptor signaling by activation-induced zinc influx. *The Journal of experimental medicine*. 2011; 208:775–785. [PubMed: 21422171]
45. Kaltenberg J, et al. Zinc signals promote IL-2-dependent proliferation of T cells. *Eur J Immunol*. 2010; 40:1496–1503. [PubMed: 20201035]
46. Haase H, et al. Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. *J Immunol*. 2008; 181:6491–6502. [PubMed: 18941240]
47. Sayadi A, et al. Zip14 expression induced by lipopolysaccharides in macrophages attenuates inflammatory response. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]*. 2013; 62:133–143.
48. Hirano T. Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule. *Adv. Immunol*. 2008; 97:149–176. [PubMed: 18501770]
49. Overbeck S, et al. Intracellular zinc homeostasis in leukocyte subsets is regulated by different expression of zinc exporters ZnT-1 to ZnT-9. *J. Leukoc. Biol*. 2008; 83:368–380. [PubMed: 17971500]
50. Kitamura H, et al. Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. *Nat Immunol*. 2006; 7:971–977. [PubMed: 16892068]
51. Bootman MD. Calcium signaling. *Cold Spring Harbor perspectives in biology*. 2012; 4:a011171. [PubMed: 22751152]
52. Maul-Pavicic A. ORAI1-mediated calcium influx is required for human cytotoxic lymphocyte degranulation and target cell lysis. *Proc. Natl Acad. Sci. USA*. 2011; 108:3324–3329. [PubMed: 21300876]
53. Baba Y, et al. Calcium signaling in B cells: Regulation of cytosolic Ca increase and its sensor molecules, STIM1 and STIM2. *Mol Immunol*. 2013
54. Vukcevic M, et al. Ryanodine receptor activation by Ca v 1.2 is involved in dendritic cell major histocompatibility complex class II surface expression. *J Biol Chem*. 2008; 283:34913–34922. [PubMed: 18927079]
55. Becker SM, et al. Differential role of the Ca(2+) sensor synaptotagmin VII in macrophages and dendritic cells. *Immunobiology*. 2009; 214:495–505. [PubMed: 19157638]
56. Nunes P, Demaux N. The role of calcium signaling in phagocytosis. *J Leukoc Biol*. 2010; 88:57–68. [PubMed: 20400677]
57. Braun A, et al. STIM1 is essential for Fcγ receptor activation and autoimmune inflammation. *Blood*. 2009; 113:1097–1104. [PubMed: 18941110]
58. Brechard S, et al. STIM1 but not STIM2 is an essential regulator of Ca²⁺ influx-mediated NADPH oxidase activity in neutrophil-like HL-60 cells. *Biochem Pharmacol*. 2009; 78:504–513. [PubMed: 19433064]

59. Schenten V, et al. Sphingosine kinases regulate NOX2 activity via p38 MAPK-dependent translocation of S100A8/A9. *J Leukoc Biol.* 2011; 89:587–596. [PubMed: 21233411]
60. Abboud CN, et al. The requirements for ionized calcium and magnesium in lymphocyte proliferation. *J Cell Physiol.* 1985; 122:64–72. [PubMed: 3965485]
61. Deason-Towne F, et al. The Mg²⁺ transporter MagT1 partially rescues cell growth and Mg²⁺ uptake in cells lacking the channel-kinase TRPM7. *FEBS Lett.* 2011; 585:2275–2278. [PubMed: 21627970]
62. Ibs KH, Rink L. Zinc-altered immune function. *The Journal of nutrition.* 2003; 133:1452S–1456S. [PubMed: 12730441]
63. Haase H, Rink L. Functional significance of zinc-related signaling pathways in immune cells. *Annu Rev Nutr.* 2009; 29:133–152. [PubMed: 19400701]
64. Kitabayashi C, et al. Zinc suppresses Th17 development via inhibition of STAT3 activation. *International immunology.* 2010; 22:375–386. [PubMed: 20215335]
65. Wong CP, Ho E. Zinc and its role in age-related inflammation and immune dysfunction. *Molecular nutrition & food research.* 2012; 56:77–87. [PubMed: 22076675]
66. Lin RS, et al. Zinc is essential for binding of p56(lck) to CD4 and CD8alpha. *J. Biol. Chem.* 1998; 273:32878. [PubMed: 9830036]
67. Romir J, et al. Crystal structure analysis and solution studies of human Lck-SH3; zinc-induced homodimerization competes with the binding of proline-rich motifs. *Journal of molecular biology.* 2007; 365:1417–1428. [PubMed: 17118402]
68. Nishida K, et al. Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction. *The Journal of experimental medicine.* 2009; 206:1351–1364. [PubMed: 19451265]
69. von Bulow V, et al. Zinc-dependent suppression of TNF-alpha production is mediated by protein kinase A-induced inhibition of Raf-1, I kappa B kinase beta, and NF-kappa B. *J Immunol.* 2007; 179:4180–4186. [PubMed: 17785857]
70. Dubben S, et al. Cellular zinc homeostasis is a regulator in monocyte differentiation of HL-60 cells by 1 alpha, 25-dihydroxyvitamin D3. *J Leukoc Biol.* 2010; 87:833–844. [PubMed: 20089671]
71. Wu C. Metallothioneins negatively regulate IL-27-induced type 1 regulatory T-cell differentiation. *Proceedings of the National Academy of Sciences of the United States of America.* 2013; 110:7802–7807. [PubMed: 23630250]
72. Striz I, Trebichavsky I. Calprotectin - a pleiotropic molecule in acute and chronic inflammation. *Physiological research / Academia Scientiarum Bohemoslovaca.* 2004; 53:245–253. [PubMed: 15209531]
73. Gifford JL, et al. Structures and metal-ion-binding properties of the Ca²⁺ -binding helix-loop-helix EF-hand motifs. *The Biochemical journal.* 2007; 405:199–221. [PubMed: 17590154]
74. Senguen FT, Grabarek Z. X-ray structures of magnesium and manganese complexes with the N-terminal domain of calmodulin: insights into the mechanism and specificity of metal ion binding to an EF-hand. *Biochemistry.* 2012; 51:6182–6194. [PubMed: 22803592]
75. Li M. Subtype-specific control of P2X receptor channel signaling by ATP and Mg²⁺ Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:E3455–E3463. [PubMed: 23959888]
76. Lee M, et al. Mg²⁺ ions reduce microglial and THP-1 cell neurotoxicity by inhibiting Ca²⁺ entry through purinergic channels. *Brain Res.* 2011; 1369:21–35. [PubMed: 21040713]
77. Luo J, et al. Tonic inhibition of TRPV3 by Mg²⁺ in mouse epidermal keratinocytes. *The Journal of investigative dermatology.* 2012; 132:2158–2165. [PubMed: 22622423]
78. Zhang J, et al. Role of Cav1.2 L-type Ca²⁺ channels in vascular tone: effects of nifedipine and Mg²⁺ American journal of physiology. Heart and circulatory physiology. 2007; 292:H415–H425. [PubMed: 16980345]
79. Moroz OV, et al. The role of zinc in the S100 proteins: insights from the X-ray structures. *Amino acids.* 2011; 41:761–772. [PubMed: 20306096]
80. Donato R, et al. Functions of S100 proteins. *Current molecular medicine.* 2013; 13:24–57. [PubMed: 22834835]

81. Feske S, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 2006; 441:179–185. [PubMed: 16582901]
82. Malpuech-Brugere C, et al. Accelerated thymus involution in magnesium-deficient rats is related to enhanced apoptosis and sensitivity to oxidative stress. *The British journal of nutrition*. 1999; 81:405–411. [PubMed: 10615213]
83. Zimowska W, et al. Morphological and immune response alterations in the intestinal mucosa of the mouse after short periods on a low-magnesium diet. *The British journal of nutrition*. 2002; 88:515–522. [PubMed: 12425732]
84. Rayssiguier Y, et al. Magnesium deficiency and metabolic syndrome: stress and inflammation may reflect calcium activation. *Magnesium research : official organ of the International Society for the Development of Research on Magnesium*. 2010; 23:73–80. [PubMed: 20513641]
85. Lin CY, et al. L-type calcium channels are involved in mediating the anti-inflammatory effects of magnesium sulphate. *Br J Anaesth*. 2010; 104:44–51. [PubMed: 19933511]
86. Tam Tam HB, et al. Magnesium sulfate ameliorates maternal and fetal inflammation in a rat model of maternal infection. *Am J Obstet Gynecol*. 2011; 204:364, e361–e368. [PubMed: 21272846]
87. Barbagallo M, et al. Magnesium homeostasis and aging. *Magnesium research : official organ of the International Society for the Development of Research on Magnesium*. 2009; 22:235–246. [PubMed: 20228001]
88. Ulger Z, et al. Intra-erythrocyte magnesium levels and their clinical implications in geriatric outpatients. *The journal of nutrition, health & aging*. 2010; 14:810–814.
89. Castiglioni S, Maier JA. Magnesium and cancer: a dangerous liason. *Magnesium research : official organ of the International Society for the Development of Research on Magnesium*. 2011; 24:S92–S100. [PubMed: 21933757]
90. Rosanoff A, et al. Suboptimal magnesium status in the United States: are the health consequences underestimated? *Nutr Rev*. 2012; 70:153–164. [PubMed: 22364157]
91. Ackland ML, Michalczyk A. Zinc deficiency and its inherited disorders -a review. *Genes & nutrition*. 2006; 1:41–49. [PubMed: 18850219]
92. Xu TF, et al. Overexpression of Zip-2 mRNA in the leukocytes of asthmatic infants. *Pediatric pulmonology*. 2009; 44:763–767. [PubMed: 19598279]
93. Besecker BY, et al. A comparison of zinc metabolism, inflammation, and disease severity in critically ill infected and noninfected adults early after intensive care unit admission. *Am J Clin Nutr*. 2011; 93:1356–1364. [PubMed: 21525204]
94. Tao YT, et al. Up-regulation of Slc39A2(Zip2) mRNA in peripheral blood mononuclear cells from patients with pulmonary tuberculosis. *Molecular biology reports*. 2013; 40:4979–4984. [PubMed: 23686108]
95. Feske S, et al. Ion channels and transporters in lymphocyte function and immunity. *Nat Rev Immunol*. 2012; 12:532–547. [PubMed: 22699833]
96. Starzl TE, et al. Liver transplantation with use of cyclosporin a and prednisone. *The New England journal of medicine*. 1981; 305:266–269. [PubMed: 7017414]
97. Kahmann L, et al. Zinc supplementation in the elderly reduces spontaneous inflammatory cytokine release and restores T cell functions. *Rejuvenation research*. 2008; 11:227–237. [PubMed: 18279033]
98. Sugimoto J, et al. Magnesium decreases inflammatory cytokine production: a novel innate immunomodulatory mechanism. *J Immunol*. 2012; 188:6338–6346. [PubMed: 22611240]
99. Consolo LZ, et al. Zinc supplementation in children and adolescents with acute leukemia. *Eur J Clin Nutr*. 2013; 67:1056–1059. [PubMed: 23963274]
100. Gees M, et al. The role of transient receptor potential cation channels in Ca²⁺ signaling. *Cold Spring Harbor perspectives in biology*. 2010; 2:a003962. [PubMed: 20861159]
101. Knowles H, et al. The TRPM2 ion channel, an oxidative stress and metabolic sensor regulating innate immunity and inflammation. *Immunol Res*. 2013; 55:241–248. [PubMed: 22975787]
102. Sumoza-Toledo A, et al. Dendritic cell maturation and chemotaxis is regulated by TRPM2-mediated lysosomal Ca²⁺ release. *FASEB J*. 2011; 25:3529–3542. [PubMed: 21753080]

103. Di A. The redox-sensitive cation channel TRPM2 modulates phagocyte ROS production and inflammation. *Nature Immunol.* 2012; 13:29–34. [PubMed: 22101731]
104. Yamamoto S. TRPM2-mediated Ca^{2+} influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nature Med.* 2008; 14:738–747. [PubMed: 18542050]
105. Peralvarez-Marín A, et al. What do we know about the transient receptor potential vanilloid 2 (TRPV2) ion channel? *FEBS J.* 2013; 280:5471–5487. [PubMed: 23615321]
106. Saunders CI, et al. Expression of transient receptor potential vanilloid 1 (TRPV1) and 2 (TRPV2) in human peripheral blood. *Mol Immunol.* 2007; 44:1429–1435. [PubMed: 16777226]
107. Fernandes ES, et al. TRPV1 deletion enhances local inflammation and accelerates the onset of systemic inflammatory response syndrome. *J Immunol.* 2012; 188:5741–5751. [PubMed: 22547700]
108. Zhang D, et al. Mast-cell degranulation induced by physical stimuli involves the activation of transient-receptor-potential channel TRPV2. *Physiological research / Academia Scientiarum Bohemoslovaca.* 2012; 61:113–124. [PubMed: 21574765]
109. Link TM, et al. TRPV2 has a pivotal role in macrophage particle binding and phagocytosis. *Nat Immunol.* 2010; 11:232–239. [PubMed: 20118928]
110. Niemeyer BA, Hoth M. Excitable T cells: Ca(v) 1.4 channel contributions and controversies. *Immunity.* 2011; 35:315–317. [PubMed: 21943485]
111. Kotturi MF, Jefferies WA. Molecular characterization of L-type calcium channel splice variants expressed in human T lymphocytes. *Mol Immunol.* 2005; 42:1461–1474. [PubMed: 15899519]
112. Badou A, et al. Critical role for the beta regulatory subunits of Cav channels in T lymphocyte function. *Proceedings of the National Academy of Sciences of the United States of America.* 2006; 103:15529–15534. [PubMed: 17028169]
113. Grafton G, et al. A non-voltage-gated calcium channel with L-type characteristics activated by B cell receptor ligation. *Biochem Pharmacol.* 2003; 66:2001–2009. [PubMed: 14599558]
114. Omilusik K, et al. The Ca(v) 1.4 calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. *Immunity.* 2011; 35:349–360. [PubMed: 21835646]
115. Park CY, et al. The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science.* 2010; 330:101–105. [PubMed: 20929812]
116. Wang Y, et al. The calcium store sensor, STIM1, reciprocally controls Orai and $\text{CaV}1.2$ channels. *Science.* 2010; 330:105–109. [PubMed: 20929813]
117. Kawano A, et al. Regulation of P2X7-dependent inflammatory functions by P2X4 receptor in mouse macrophages. *Biochem Biophys Res Commun.* 2012; 420:102–107. [PubMed: 22405772]
118. Sakaki H, et al. P2X4 receptor regulates P2X7 receptor-dependent IL-1 β and IL-18 release in mouse bone marrow-derived dendritic cells. *Biochem Biophys Res Commun.* 2013; 432:406–411. [PubMed: 23428419]
119. Junger WG. Immune cell regulation by autocrine purinergic signalling. *Nat Rev Immunol.* 2011; 11:201–212. [PubMed: 21331080]
120. Yip L, et al. Autocrine regulation of T-cell activation by ATP release and P2X7 receptors. *FASEB J.* 2009; 23:1685–1693. [PubMed: 19211924]
121. Chen Y, et al. Purinergic signaling: a fundamental mechanism in neutrophil activation. *Sci Signal.* 2010; 3:ra45. [PubMed: 20530802]
122. Rukgauer M, et al. Reference values for the trace elements copper, manganese, selenium, and zinc in the serum/plasma of children, adolescents, and adults. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements.* 1997; 11:92–98.
123. Haase H, et al. Flow cytometric measurement of labile zinc in peripheral blood mononuclear cells. *Analytical biochemistry.* 2006; 352:222–230. [PubMed: 16545333]
124. Shaw PJ, Feske S. Physiological and pathophysiological functions of SOCE in the immune system. *Front Biosci (Elite Ed).* 2012; 4:2253–2268. [PubMed: 22202035]
125. Suzuki R, et al. Loss of TRPC1-mediated Ca^{2+} influx contributes to impaired degranulation in Fyn-deficient mouse bone marrow-derived mast cells. *J Leukoc Biol.* 2010; 88:863–875. [PubMed: 20571036]

126. Cheng KT, et al. Contribution and regulation of TRPC channels in store-operated Ca^{2+} entry. *Current topics in membranes*. 2013; 71:149–179. [PubMed: 23890115]
127. Omilusik K. The $\text{CaV}1.4$ calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. *Immunity*. 2011; 35:349–360. [PubMed: 21835646]
128. Di L. Inhibition of the K^+ channel $\text{KCa}3.1$ ameliorates T cell-mediated colitis. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:1541–1546. [PubMed: 20080610]
129. Vennekens R, Nilius B. Insights into TRPM4 function, regulation and physiological role. *Handb. Exp. Pharmacol.* 2007; 179:269–285. [PubMed: 17217063]
130. Numaga T, et al. Ca^{2+} influx and protein scaffolding via TRPC3 sustain PKC β and ERK activation in B cells. *J Cell Sci*. 2010; 123:927–938. [PubMed: 20179100]
131. Carrillo C, et al. Diacylglycerol-containing oleic acid induces increases in $[\text{Ca}^{2+}]_i$ via TRPC3/6 channels in human T-cells. *Biochimica et biophysica acta*. 2012; 1821:618–626. [PubMed: 22306362]
132. M M. Zinc improves the development of human CD34+ cell progenitors towards natural killer cells and induces the expression of GATA-3 transcription factor. *Int. J. Biochem. Cell Biol.* 2007; 39:955. [PubMed: 17306601]

Highlights

- Ca^{2+} , Mg^{2+} and Zn^{2+} are signaling cations with interconnected signal networks
- Cation signaling is essential for immunity and defects underlie immune diseases
- Mechanisms of Mg^{2+} and Zn^{2+} mobilization and effectors require further definition
- Modulation of ion signaling may lead to novel therapies for immune diseases

Box 1**Notion of ion signaling**

The second messenger concept is well defined, however its application to ion signaling requires some adjustments. Indeed, ions signal through variation of their intracellular concentrations via different transport mechanisms, which is different from the “*de novo*” production of a second messenger by an enzyme. Also, the size and rapidity of movement could mean that the effective “range” of signaling cations is quite broad in the cell. The concept of ion signaling relies on several fundamental features: (1) a cytosolic resting *free* pool present in unstimulated cells that increases in response to an extracellular stimulus, such as the engagement of a cell surface receptor to become a mobilized *free* pool through mechanisms supporting its (2) homeostasis and (3) mobilization of the ion from the extracellular milieu, internal stores, or a bound depot; and (4) the mobilized *free* pool needs to alter one or more cellular processes at physiological level.

1. **Cytosolic *free* pool.** In eukaryotic cells, divalent cations are mostly bound with protein or other bioactive molecules and play essential structural and functional roles. For example Zn^{2+} is associated with up to 10% of all cellular proteins including over 300 enzymes and more than 2,000 transcription factors. Similarly, Mg^{2+} is associated with more than 300 enzymes as well as nucleotides, nucleic acids, and other negatively charged macromolecules. Given the essential structural and functional roles of these ions, signaling functions require the existence of a pool that can be modulated without affecting those functions. This pool is the cytosolic *free* pool which is considered *free* because it is in ionized form and able to bind to potential effectors. The difference between the bound and free forms is that the association constant for the divalent cation is much lower for the former.
2. **Cellular homeostasis.** To fulfill its purpose without affecting the total amount of the cation, the cytosolic *free* pool generally represents a small fraction of the total intracellular amount. To support the signaling functions of the cytosolic *free* pool, homeostatic regulation maintains the cytosolic *free* pool low at resting state by extrusion to the outside of the cell, sequestration in intracellular pools (stores) or immobilization by binding to cytosolic binding partners (bound).
3. **Mobilization.** Extracellular, or intracellular (not represented in the figure), stimuli trigger the mobilization of the cytosolic *free* pool via release from intracellular stores or bound pool, or by transport from the extracellular environment.
4. **Effectors.** Finally, the mobilized pool modulates cellular functions via tipping the equilibrium to the cation-bound form of specific effector molecules generally with the comparatively high association constant that is near the concentration achieved by the mobilized *free* pool and above the resting *free* pool concentration.

Box 2**Non-SOCE Ca²⁺ mobilization: new directions and controversies**

In addition to SOCE, immune cells several non-SOCE, or store-*independent*, Ca²⁺ mobilization mechanisms that are triggered by specific extracellular or intracellular ligands in immune cells (summarized in Figure 3 and Table 2) [95]. However, the physiological importance of some of these mechanisms remain to be established.

The superfamily of the transient receptor potential (TRP) channels encompass 28 members divided in six subfamilies based on their sequence homology [100]. The role of certain TRPs for Ca²⁺ mobilization in immune cells is relatively well established. For example, TRP melastatin 2 (TRPM2) is regulated by various intracellular ligands related to oxidative stress, such as cyclic ADP-ribose (cADPR), hydrogen peroxide (H₂O₂), nicotinic acid adenine dinucleotide phosphate (NAADP), and adenosine monophosphate (AMP) [101]. *In vitro* stimulation of T cell and B cell lines by ADPR show that TRPM2 triggers Ca²⁺ mobilization [101]. In neutrophils and macrophages, TRPM2 is expressed in the PM and promotes the production of various inflammatory cytokines in response to ROS, LPS and cellular acidification. Whereas, in DC, TRPM2 is expressed in the lysosomes and seems to play a role in the chemotaxis and maturation [102–104]. Another interesting example is the members of the vanilloid TRP family, TRPV1 and TRPV2 that are both expressed in immune cells [105, 106]. These channels associated with nociception are activated by noxious heat (>43°C), low pH, lipidic ligands such as prostaglandins, and mechanical stress suggesting an interesting intersection between nociception and inflammation [107, 108]. TRPV2 deficiency in macrophages is linked to impaired phagocytosis, whereas TRPV1 may have a role in sepsis but this remains unclear [107, 109]. TRPV2 has also been associated with mast cell degranulation, however, in these studies the stimulus used were not physiological and the relevance of these findings requires further investigation [108].

In excitable cells, such as muscle cells and neurons, L-type voltage-*dependent* Ca²⁺ channels (Ca_v1) and ryanodine receptor channels (RyR) are responsible for SOCE after depolarization. These are also expressed in immune cells, including T cells, B cells and DC. In macrophages and DCs, Ca_v activation is voltage-*dependent* and causes SOCE by promoting Ca²⁺ release from the ER through a direct interaction with the channel RyR₁ after LPS stimulation [54]. On the other hand, the role of Ca_v in T cells is more controversial [110]. Whereas, T cells expressed all Ca_v1 channel isoforms (Ca_v1.1, Ca_v1.2, Ca_v1.3 and Ca_v1.4) along with their regulatory subunits β3 and β4, their activation in T cells is not induced by depolarization [54, 111–113]. Recent studies established roles for Ca_v1 in thymic development and naïve T cell homeostasis in mouse [114]. However, incomplete characterization of the biophysical properties of Ca_v1 in T cells, causes their role in T cell function to be debated. In addition, two recent studies report inhibition of Ca_v channels by STIM in T cells, suggesting a potential reciprocal regulation of the ORAI1 and Ca_v channels by STIM that needs to be further investigated [115, 116].

Purinergic P2X receptors are non-selective Ca^{2+} channels activated by extracellular ATP/UTP. During immune responses, the release of ATP by damaged or dying cells is considered to be an important inflammatory “danger” signal. P2X4 and P2X7 strongly influence chemotaxis and pro-inflammatory cytokine production in DCs and macrophages [117, 118]. In T cells and B cells, P2X7-dependent Ca^{2+} influx induces proliferation and cytokine production [119]. Additionally, activation of T cells and neutrophils stimulates P2X7 via autocrine release of ATP [120, 121]. Although, all these findings are supported by *in vivo* studies, the biophysical characteristics of P2X in immune cells is only partly understood.

Figure Box 2: SOCE and non-SOCE Ca^{2+} mobilization mechanisms in immune cells. In addition to the SOCE induced by released of Ca^{2+} from the ER by the IP_3R , ryanodine receptors (RyR) channels can also release Ca^{2+} from the ER. STIM senses the store depletion and triggers the opening of the Ca^{2+} CRAC channel, ORAI and potentially TRPC1. L-type “voltage-dependent” Ca^{2+} (Ca_v) channels induce voltage-dependent (+) and independent Ca^{2+} mobilization via direct interaction with RyR and are potentially inhibited by STIM. Non-SOCE mechanisms include, purinergic receptor channels (P2X), transient receptor potential vanilloid 1 and 2 (TRPV1/2) channel, TRPC3/6 and TRPM2. ADPR, ADP-ribose; NAADP, nicotinic acid adenine dinucleotide phosphate; PIP_2 , phosphoinositides diphosphate; ROS, radical oxygen species; Temp, temperature.

Figure 1. Divalent cations transporters

(a) Ca^{2+} transport mechanisms in immune cells. Ca^{2+} homeostasis regulation involves the PMCA, SERCA ATPases as well as the MCU and $\text{Na}^+/\text{Ca}^{2+}$ exchangers (NCX). SOCE is induced by released of Ca^{2+} from the ER by the IP_3R . STIM senses the store depletion and triggers the opening of the Ca^{2+} CRAC channel, ORAI. SOCE is modulated by two K^+ permeable channels, $\text{K}_{\text{Ca}} 1.3$ and $\text{K}_{\text{v}} 3.1$ regulated by intracellular Ca^{2+} and depolarization respectively. These two channels help sustain SOCE generation by inducing hyperpolarization of the cell. Additionally, the Na^+ permeable channel TRPM4 is gated by intracellular Ca^{2+} and reduces the Ca^{2+} mobilization. (b) Mg^{2+} transport mechanisms in immune cells. Mg^{2+} transporters include TRPM7, which have an intracellular Ser/Thr kinase domain, MAGT1, and SLC41A1/2 expressed on the PM and Mrs2 on the inner membrane of the mitochondria. (c) Zn^{2+} transport mechanisms in immune cells. Zn^{2+} transporters of the ZnT and ZIP families are expressed in various cellular compartments. Zn^{2+} homeostasis is completed by MT. Abbreviations: ChR, Chemokine receptor; DAG, Diacylglycerol; FcR, Fc receptor; TPC, Two pore channel.

Figure 2. Divalent cations effectors and functions in immune cells

(a) A broad range of functions can be rapidly deployed through Ca²⁺ signals. One major Ca²⁺ function is the regulation of gene expression involved in cell activation, differentiation and cytokine production. Ca²⁺ controls the activation of NFAT via CaM-CN, CREB and myocyte enhancer factor 2 (MEF2) via CaMK and CaM and NF-κB via PKC and CaMKII. Ca²⁺ controls exocytosis through Ca²⁺-sensitive proteins such as synaptotagmins (Syt) and Munc13. S100 proteins are Ca²⁺-binding proteins involved in the cytoskeleton organization during migration, lysosome-phagosome fusion during phagocytosis. In neutrophils, Ca²⁺ controls ROS production by NOX2 via PKC and S100 proteins (S100A8/A9). (b) In T and B cells, Mg²⁺ is regulating Ca²⁺ mobilization via SOCE, by modulating PLC-γ activation. In T cells, the TCR-induced MAGT1-dependent Mg²⁺ flux is required to activate PLC-γ1. In B cell, TRPM7 kinase domain participates in PLC-γ2 activation together with Bruton's tyrosine kinase (BTK) in a Mg²⁺-dependent manner. (c) Zn²⁺ is involved in the recruitment of kinases such as PKC or LCK. Zn²⁺ enhances MAPK activation through inhibition of the MAPKP. Zn²⁺ controls gene expression through NF-κB and through inhibition of CN (which promotes CREB activation). In innate immune cells, Zn²⁺ binding-proteins such as

MT and S100 proteins (S100A8/A9) are playing anti-microbial roles by chelating Zn^{2+} . Zn^{2+} inhibits IL-6 signaling by inhibiting the activation of STAT3. Finally, Zn^{2+} regulates the cAMP pathway through the inhibition of AC or PDE.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Ca²⁺, Mg²⁺ and Zn²⁺ characteristics

Ion	[x] _e	% Free	Free [x] _i	E/I gradient ^{a,b}	Ion radius (pm)	H/I ^c	References
Ca ²⁺	1–2 mM	0.05%	100 nM	10,000	100	70	[11]
Mg ²⁺	1 mM	5–10%	0.2–0.5 mM	2–5 ^b	72	210	[3, 5, 6]
Zn ²⁺	15 μM	5–10%	0.4 nM	20,000	74	196	[122, 123]

^b Extracellular (E)/intracellular (I) concentration gradient,

^b The electrochemical E/I gradient for Mg²⁺ is 100–250,

^c H/I, hydrated to ionized volume ratio

Table 2

Ca²⁺, Mg²⁺ and Zn²⁺ channels

Channel	Selectivity	Loc.	Stimuli	Regulation	Expression	Functions	References
Calcium							
SOCE and related							
ORAI1	Ca ²⁺	PM	TCR, BCR, TLR, FcR, Chemokine Rc	SOCE, inhibited by Ca ²⁺ and ROS	Ubiquitous	Cell proliferation, Cytokines production, Degranulation, Phagocytosis, Chemotaxis, T _{reg} development	[124]
ORAI3	Ca ²⁺	PM	TCR	SOCE, inhibited by Ca ²⁺	Upregulated in activated T cells	Cell proliferation, Cytokines production	[19]
TRPC1/6	Ca ²⁺ /Na ⁺	PM	TLR, FcR, chemokine Rc	SOCE, ARC	Mast cells, Neuro. (B, T cells ?)	Cytokine production, Degranulation	[125, 126]
Ca_v (1.2/1.3/1.4)	Ca ²⁺	PM	Voltage-independent	Inhibited by STIM	T cells	SOCE modulation ?, T cell homeostasis	[110, 127]
			Voltage-dependent	SOCE (STIM independent)	DC, Macro.	Cytokine production, Chemotaxis, MHC II upregulation	[54]
SOCE modulators							
K_{Ca}3.1	K ⁺	PM	TCR, BCR	Increased [Ca ²⁺] _i	T _H 1, T _H 2, T _{CM} , B cells	Hyperpolarization (sustains SOCE)	[128]
K_v1.3	K ⁺	PM	TCR, BCR	Depolarization	Naïve T cells, T _{EM} , T _H 17	Hyperpolarization (sustains SOCE)	[128]
TRPM4	Na ⁺	PM	TCR, BCR	Increased [Ca ²⁺] _i	Ubiquitous	Depolarization (inhibits SOCE)	[129]
Non SOCE							
Ca_v (1.2/1.3/1.4)	Ca ²⁺	PM	Voltage-independent	Inhibited by STIM	T cells	SOCE modulation ?, T cell homeostasis	[110, 127]
			Voltage-dependent	SOCE (STIM independent)	DC, Macro.	Cytokine production, Chemotaxis, MHC II upregulation	[54]
P2X (1/4/7)	Ca ²⁺ /Na ⁺	PM	Extracellular ATP/UTP	Inhibited by Mg ²⁺	T, B, NK cells, Neuro.	Pro-inflammatory Cytokines production, Chemotaxis, Proliferation	[117, 118, 120, 121]
TRPV1/2	Ca ²⁺ /Na ⁺	PM	Heat (fever?), low pH, mechanical stress		Mono., Macro.	Degranulation, Phagocytosis, Cytokines production	[107, 108]
TRPC3/6	Ca ²⁺ /Na ⁺	PM	PLC activation (DAG), PIP ₂		T, B, NK cells, Neuro.	Chemotaxis, Degranulation	[130, 131]
TRPM2	Ca ²⁺ /Na ⁺	PM Lys.	H ₂ O ₂ , NAADP, cADPR		T, B, Neuro., Mast cells, DC	Cytokine production, Degranulation	[101]

Channel	Selectivity	Loc.	Stimuli	Regulation	Expression	Functions	References
Magnesium							
TRPM6	Mg ²⁺ >Ca ²⁺	PM		Inhibited by [Mg ²⁺] _i	Gut, Kidney, Hematopoietic (not T cells)	Unknown in immune cells	[3]
TRPM7	Mg ²⁺ >Ca ²⁺	PM	Unknown (BCR, TCR?) PIP ₂ (?)	Inhibited by [Mg ²⁺] _i	Ubiquitous	T cell development, T and B cells proliferation, Cytokine production	[36]
MAGT1	Mg ²⁺	PM	TCR	Unknown	Ubiquitous	CD4 T cells development, SOCE induction in T cells, NKG2D expression	[7, 32]
Zinc							
Importers							
ZIP1	Zn ²⁺	PM		Unknown			[38]
ZIP2	Zn ²⁺	PM	Unknown		Upregulated in asthma and sepsis	Unknown	[38, 92, 94]
ZIP3	Zn ²⁺ >others	PM		Unknown	CD34 ⁺ progenitors	T cell development	[38, 132]
ZIP4	Zn ²⁺	PM		Unknown	Intestine	AE	[38]
ZIP6	Zn ²⁺	PM		TCR (?)	Ubiquitous, T cells	INF γ production in T cells	[43–45]
ZIP8	Zn ²⁺	Lys.		TCR (?)	Ubiquitous, T cells, Mono.	INF γ production in T cells	[43–45]
ZIP14	Zn ²⁺	PM		LPS			[46, 47]
Exporters							
ZnT1	Zn ²⁺	PM		Unknown	Upregulated in T cells	Unknown	[37]
ZnT4	Zn ²⁺	PM		Unknown	Upregulated in T cells	Unknown	[37]
ZnT5	Zn ²⁺	Endo., PM (?)	Fc ϵ R stimulation	Unknown	Upregulated in activated mast cells	Recruitment of PKC to the PM (?)	[37, 68]

Abbreviations: ARC, arachidonate-regulated Ca²⁺ channel, Endo., endosome; Loc., localization; Lys., lysosome; Mito., mitochondria; PA, Prader-Willi/Angelman syndrome; Yes., vesicles