Review

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Divalent cation signaling in immune cells

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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. We review here 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.

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 several roles as a vital structural and functional cofactor through strong electrostatic interactions. By contrast, the ionized fraction (0.05–10%) 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). 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 of 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 PM, 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, respectively $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$, are maintained at approximately 10^4-fold lower levels than the physiological extracellular concentration, thus generating a large chemical gradient for their mobilization into the cytosol (Table 1). By contrast, there is a much smaller difference (<twofold) between cytosolic free Mg^{2+} ($[Mg^{2+}]_i$) and extracellular free Mg^{2+} ($[Mg^{2+}]_o$) levels, 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 (~ -70 mV), if intracellular free Mg^{2+} was at electrical equilibrium its resting concentration should be 50 mM [3,4]. Nevertheless, $[Mg^{2+}]_i$ 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 PM (Table 1 and Section 'Regulation of cytosolic free Mg^{2+} in immune cells') [3,5,6]. Indeed, the physiological function of rapid Mg^{2+} fluxes in T lymphocytes has been recently demonstrated and has 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.

We discuss here 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, the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) 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 Ca^{2+}/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 store-operated Ca^{2+} entry (SOCE), which involves mobilization across the PM

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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. In addition, the size and rapidity of movement could mean that the effective 'range' of signaling cations is fairly 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 (Figure I).

(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 2000 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 the 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 that generally have a comparatively high association constant near the concentration achieved by the mobilized free pool and above the resting free pool concentration.

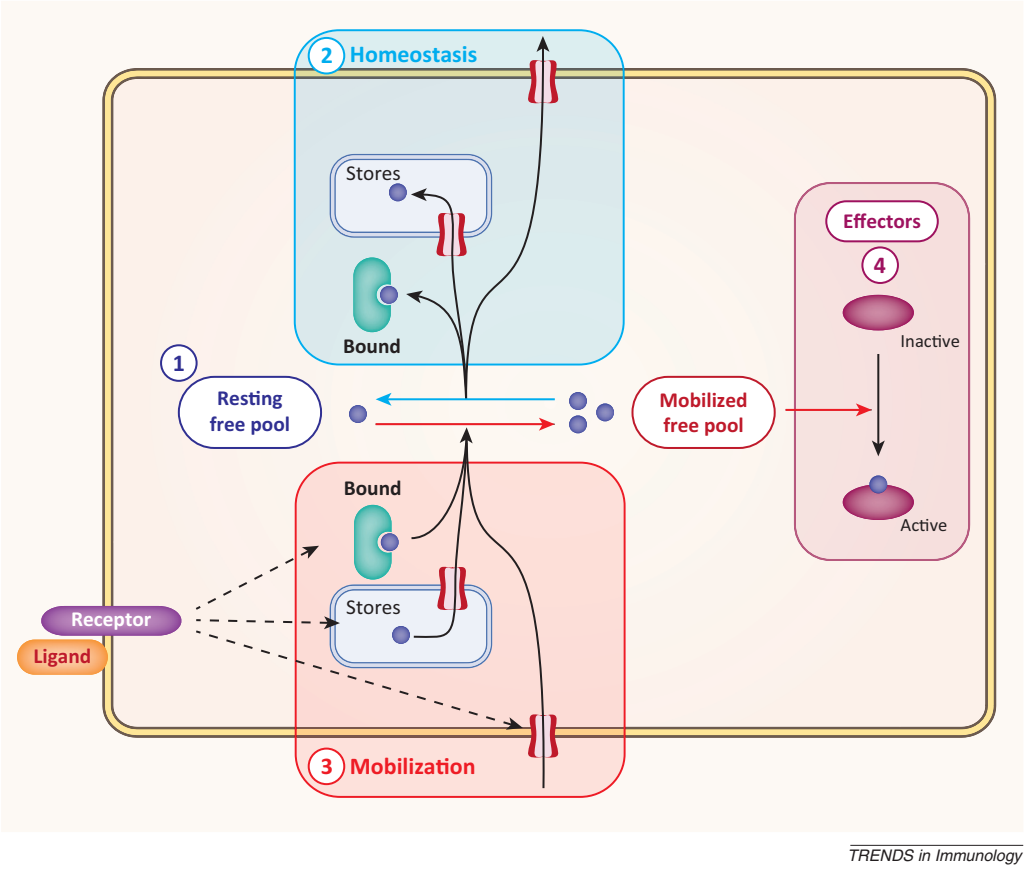

Figure I. Intracellular ion signaling.

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-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 activates Ca^{2+} release through 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