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Calcium signaling as a regulator of hematopoiesis

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1. ABSTRACT

Different extracellular signaling molecules that bind to receptors on the cell membrane use calcium ions for signal transduction. Due to the opening of receptor-operated calcium channels, some cytokine receptors and G-protein coupled receptors induce an increase of intracellular calcium concentration upon activation. Calcium ion is a versatile intracellular secondary messenger that control many different cellular functions by changing its cytoplasmic concentration. A specific and complex network of signaling proteins recognizes intracellular calcium alterations to modulate cellular processes. Some reports have previously demonstrated that calcium also regulates hematopoiesis. This review examines the participation of intracellular calcium in hematopoiesis after the stimulus of various myeloid cytokines such as interleukin-3 and granulocyte-macrophage colony-stimulating factor. In addition, the role of adenosine triphosphate and its receptors in inducing calcium increases during hematopoiesis is discussed. Lastly, the participation of this ion in myeloid proliferation and differentiation by cytokines and P2 receptors is also discussed.

2. INTRODUCTION

2.1. General aspects of Ca^{2+} signaling

Intracellular Ca^{2+} (Ca^{2+}_i) is an important secondary messenger that plays an important role in cellular processes by regulating several intracellular pathways. The large difference between Ca^{2+} concentrations of the extracellular (~1 mM) and the intracellular medium (100 nM) helps to produce rapid increases in cytoplasmic Ca^{2+} concentration through the alterations in Ca^{2+} permeability and through the activation of channels receptor on the plasma membrane (1, 2). Similarly, a difference in Ca^{2+} concentration is observed between the cytoplasm and organelles that store Ca^{2+} (3); however, the concentration within organelles has not been precisely determined. Therefore, the increase in Ca^{2+}_i levels may occur due to the opening of channels on the cellular membrane or organelles membranes (Figure 1).

Various classes of agonists can activate ligand-gated ion channels called receptor-operated calcium channels (e.g., P2X receptors) to directly increase Ca^{2+}_i concentrations by using the difference in the

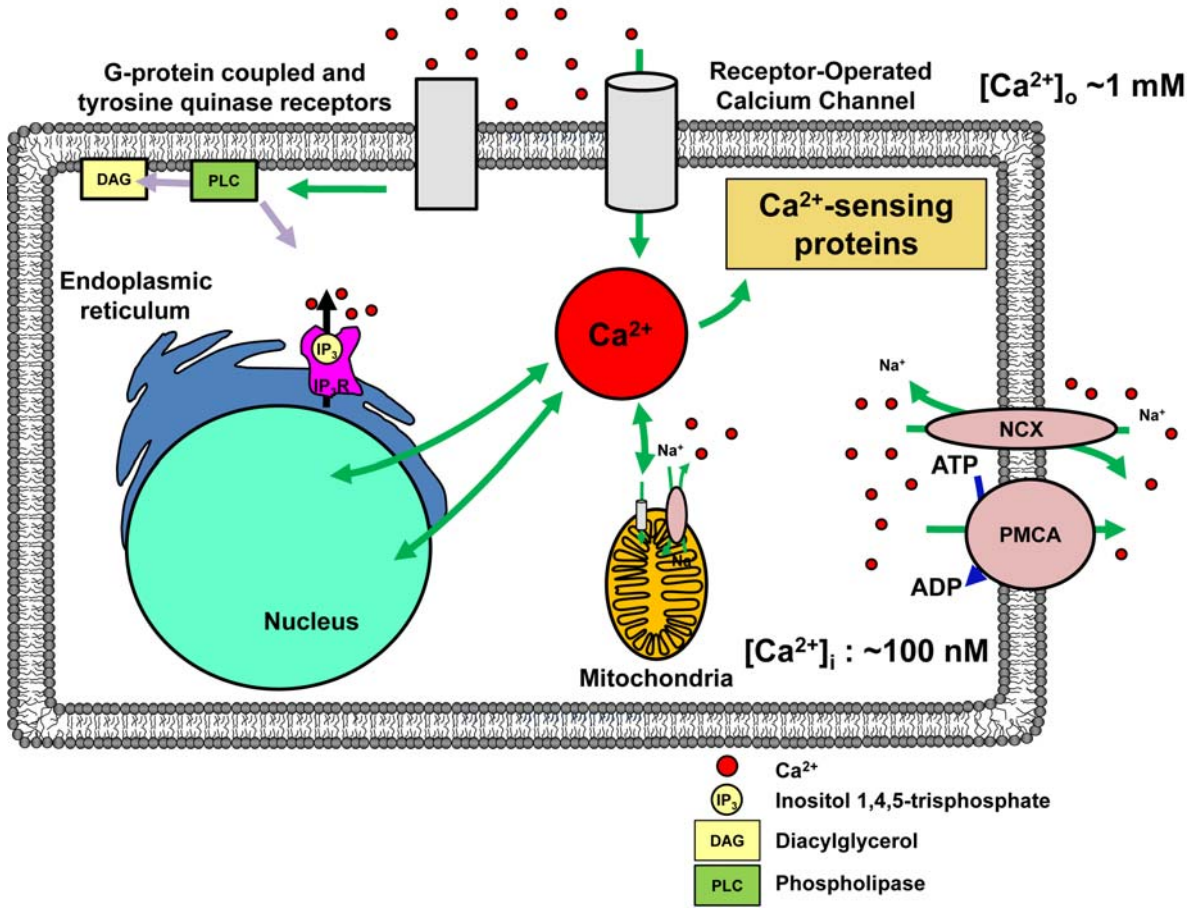

Figure 1. Different signaling molecules promote changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by either the activation of receptor-operated calcium channels, which directly induces the entry of Ca^{2+} ions, or by the activation of G-protein-coupled receptors and tyrosine kinase receptors, which active PLC, an enzyme that catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce IP_3 and DAG. IP_3 binds to the IP_3 receptor (IP_3R) to release Ca^{2+}_i from the Endoplasmic Reticula. The released Ca^{2+} acts on different Ca^{2+}-sensing proteins to regulate cellular process. Basal $[Ca^{2+}]_i$ is restored by the action of pumps, such as plasma membrane Ca^{2+} ATPase (PMCA) and antiporters, such as Na^+/Ca^{2+}- exchanger (NCX) that removes Ca^{2+} from cytoplasm.

electrochemical gradients. In addition, other classes of agonists can bind to G-protein coupled receptors that activate phospholipase Cβ (PLCβ) or can bind to tyrosine receptors that activate phospholipase Cγ (PLCγ) (1, 2). Both PLCβ and PLCγ catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (4, 5). DAG and IP_3 act as secondary messengers: DAG activates protein kinase C (PKC) on the cell membrane, whereas IP_3 spreads through the cytoplasm to release Ca^{2+}_i from the Endoplasmic Reticula (Figure 1). This Ca^{2+}_i release is required for the activation of proteins via their calcium-dependent domain, such as the classical Ca^{2+}-dependent PKCs (PKCα, βI, βII, and γ) and calmodulin (CaM), which acts as an activator of different kinases such as the CaM kinases (CaMK) family (6-8).

Basal Ca^{2+}_i concentration is restored by an action specific mechanism that recognizes high Ca^{2+} concentration, which is mainly carried out by the Na^+/Ca^{2+} exchanger (NCX) that rapidly removes Ca^{2+} from the cytoplasm. On the other hand, plasma membrane Ca^{2+} ATPase (PMCA) is also important in maintaining Ca^{2+}_i at the cell's basal level (1, 9).

Variations in cytoplasmic Ca^{2+} concentrations do not occur randomly, but they may occur in the whole cytoplasm or at specific locations (microdomains) (10). Intensity and temporal features of Ca^{2+}_i concentration alterations are also relevant in determining the effects induced by the Ca^{2+}_i signaling. Several proteins, with or without kinase activity, are responsible for decoding the Ca^{2+}_i signal into a physiological cellular process. Ca^{2+}-dependent proteins are sensitive to the location, magnitude and duration of the Ca^{2+} signal in order to properly translate Ca^{2+} changes into a cellular, physiological effect (10-13). Proteins such as PKC, CaM and CaMKs are among the most well-established proteins known to interpret these Ca^{2+} events.

The history of Ca^{2+} as an intracellular molecule that acts in cell signaling started with studies investigating muscular contraction in frogs (14). Since then, several studies have reported the participation of Ca^{2+} ions in other cellular processes such as secretion, cellular motility, proliferation, differentiation and cell death. In these last few years, studies have shown the participation of Ca^{2+}_i signaling in lympho-hematopoiesis. This review examines the participation of intracellular Ca^{2+} in hematopoiesis after the stimulus of various myeloid cytokines such as interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF). In addition, the role of ATP and its receptors in inducing Ca^{2+} increases during hematopoiesis is discussed. Lastly, the participation of this ion in myeloid proliferation and differentiation by cytokines and P2 receptors activation is also discussed.

2.2. Hematopoiesis

The formation of blood cells is called hematopoiesis. Hematopoiesis is based on the existence of hematopoietic stem cells with the capacity to self-proliferate and self-renew or to differentiate into specialized cells. Although several models have been proposed to describe hematopoiesis, the most accepted model follows a hierarchical scheme of differentiation where the hematopoietic stem cells are located at the top of this system (15, 16). The cells originating from hematopoietic stem cells can commit to two lineages: the myeloid lineage, including granulocytes, erythrocytes, megakaryocytes/platelets and monocytes; and the lymphoid lineage comprising B cells, T lymphocytes and natural killer cells. In mammals, hematopoiesis begins in the yolk sac, and hematopoietic stem cells become the region's embryonic "aorta-gonad-mesonephros" (known as the AGM region). Hematopoietic stem cells gradually progresses through the liver until permanently implanting in the bone marrow (17) due to the expression of homing proteins such as the chemokine receptor 4 (CXCR4) and extracellular calcium sensing receptor (CaSR) (18, 19).

Hematopoietic stem cells are found in specialized areas called the hematopoietic niche, and they mostly remains in a quiescent state (20-22). The hematopoietic niche and stromal cells regulate stem cell activity (23-27). The hematopoietic stroma comprises different cell types such as endothelial cells, fibroblasts, adipocytes and osteoblasts (23, 25, 28-31). Stromal cells produce specific extracellular matrix molecules that support hematopoiesis. Diverse components such as glycosaminoglycans, laminin, collagens and other molecules are present in this extracellular matrix of the bone marrow (26, 27, 32-36).

Hematopoietic and stromal cells can also produce cytokines to regulate hematopoiesis. Cytokines are a group of molecules that couple with their respective receptors to trigger diverse intracellular signaling pathways (37, 38). The binding of cytokines to their receptors induces receptor dimerization and can further induce the activation of a family of tyrosine kinases termed Janus kinases (JAK), depending on the particular cytokine receptor activated (37, 38). Cytokines such as IL-3, GM-CSF, granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) bind to cytokine receptor class I proteins that activate the JAK family to further phosphorylate tyrosine residues of other receptors. This activation allows the bind of other SH2-domain containing signaling molecules, such as signal transducers and activators of transcription (STAT), Src kinases, phosphatidylinositol 3-kinase (PI3K), PLCγ, or other adaptor signaling proteins, such as Shc and Grb2 (39-44). Downstream signal mediators, such as members of the mitogen-activated protein kinase (MAPK) families and Akt, further impart signal specificity in the hematopoietic system (44-46). On other hands, cytokines such as stem cell factor (SCF) and macrophage-colony stimulator factor (M-CSF), which bind to receptors belonging to the family of cytokine receptor class V, active tyrosine receptor with intrinsic tyrosine activity triggering similar intracellular pathway except JAK/STAT (47-49).

3. Ca^{2+} SIGNALING IN HEMATOPOIETIC PROGENITOR CELLS BY CYTOKINES

An early study showed the participation of CaM, a Ca^{2+} sensitive protein, in myeloid and erythroid colony formation when human progenitors cells were stimulated with IL-3, GM-CSF and EPO (50). Subsequently, the participation of a 68 kD CaM-binding protein in the G_1 to S phase of the cellular cycle was demonstrated when hematopoietic cell lineages were stimulated with cytokines such as IL-3, IL-6, GM-CSF and G-CSF (51). Recent report has been shown the contribution on maintenance of quiescence of hematopoietic stem cell by calmodulin kinase IV (CaMKIV), a kinase dependent of CaM (13). Mice $Camk4^{-/-}$ exhibit increased apoptosis and proliferation rates in hematopoietic progenitor cells, which may be associated with the observed decrease in hematopoietic progenitor cell numbers and the decrease in the animal's ability to reconstitute the bone marrow microenvironment (13).

In addition, the direct participation of Ca^{2+}_i signaling in hematopoietic proliferation and differentiation was demonstrated when the Ca^{2+}_i concentration increased in primitive hematopoietic cells from long term bone marrow cultures treated with several cytokines such as GM-CSF, G-CSF, IL-3, IL-6, IL-7, EPO, SCF and M-CSF. The increase in Ca^{2+}_i induces by IL-3 and GM-CSF was related to cytokine-dependent proliferation as observed by the activation of Ca^{2+}_i signaling molecules such as PLCγ, PKC and calmodulin kinase II (CaMKII) (52). Moreover, the participation of gap junctions in the proliferative and Ca^{2+}-dependent effect of cytokines was observed; this observation served as evidence that a complex Ca^{2+}_i signaling regulates this system (52). A recent study demonstrated that IL-3 and GM-CSF lead to the activation of PLCγ2 that induces a Ca^{2+}_i release. This process in turn actives PKC and CaMKII, which modulate the proliferation and activation of the Ras/Raf/ERK pathway in murine and human hematopoietic progenitor cells (53). Interestingly, variation in Ca^{2+}_i signaling was observed, as these reports demonstrated the ability of several cytokines to induce different effects in hematopoiesis. Thus, the Ca^{2+} ion can act as an intracellular coordinator of diverse cytokine-induced effects by activating different proteins such as

PLCγ, PKCs, CaMKs or by modulating different pathways that control the quiescence, proliferation, differentiation or cell death of primitive hematopoietic cells by the activation of ERK, PI3K or Wnt pathways, as observed in other cells types (54-56). Moreover, oscillatory Ca^{2+}_i elicited by cytokines in hematopoietic progenitor cells (52, 53) may be interpreted by novel Ca^{2+}-sensitive proteins, such as RASAL or PLCε, that have been linked to oscillatory Ca^{2+} increases and Ras activation. It is probable, then, that each cytokine induces a particular pattern from the different components in Ca^{2+} signaling in hematopoietic progenitors to generate a particular Ca^{2+} signal with its corresponding effect, as postulated to be the case for other cell types (10, 57, 58).

In addition, other signaling molecules may also modulate the activity of hematopoietic progenitor cells by activating G-proteins-coupled receptors. It is well established that hematopoietic stem cell homing is dependent on the expression of receptor proteins associated with the recognition of hematopoietic niches, such as CXCR4 (18, 59) and CaSR (19). CXCR4 and CaSR are known to active the intracellular cascade related to Ca^{2+}_i signaling by the recruitment of PLCβ (60-62) when activated by their ligands; however, the involvement of these receptors in the Ca^{2+}_i signaling in hematopoietic stem cells remains unknown.

The direct effect of ionomicin, a Ca^{2+} ionophore, was also tested in diverse cells of the hematopoietic system. Ionomicin was able to induce morphological and phenotypical changes in monocytes, HL-60 lineage cells, $CD34^+$ cells and $CD33^+$ cells from a chronic myeloid leukemia patient (63-65). The exposure to ionomicin induces the differentiation of these cells into dendrocytic cells (63-65).

4. Ca^{2+} SIGNALING IN HEMATOPOIETIC PROGENITOR CELLS BY P2 RECEPTORS

Extracellular ATP and its physiological analogs are molecules that may also act in the regulation of hematopoiesis through the activation of specific plasma membrane receptors. The pharmacology action of ATP as a neurotransmitter has been known since the early 1970s (66). Subsequent investigation of the cellular functions of ATP and its receptors currently place ATP as a very important signaling molecule in most tissues. Burnstock (67) first divided the receptors activated by purines into two families: P1 receptors activated by adenosine and P2 receptors activated by ATP. Classically, the P2 receptor family is further divided into ionic channel P2X receptors and G-protein-coupled P2Y receptors. (68, 69).

While ATP-gated ion channels ($P2X_{1-7}$ receptors) are permeable to cations such as Ca^{2+} (70-72), the heptahelical receptors coupled to G-proteins ($P2Y_{1,2,4,6,11,12,13,14}$ receptors) propagate a signal transduction mainly via the activation of PLCβ, and this activation leads to the formation of IP_3 and DAG, which releases stored intracellular Ca^{2+}, and PKC activation, respectively (73, 74). In addition, the positive and negative modulation of adenylate cyclase activity was also described to be dependent on some form of P2Y receptor activation (75, 76).

Several reports since the 1980s have identified the molecular and functional presence of P2 receptors in all hematopoietic cells types (77-83). However, the establishment of the physiological function of this family of receptors is not fully understood in the hematopoietic system. P2X receptors have also been shown to have an important role in the activation and cell death of hematopoietic cells, which is probably related with immune actions (75, 84-90).

On the other hand, the participation of P2 receptors in hematopoietic proliferation and differentiation is still under investigation. Initial studies of the participation of the P2 receptors in hematopoiesis were performed in the human promyelocytic leukemia cell line, HL-60. This lineage expresses several P2X and P2Y receptors, and the expression of some of these receptors is modulated during cellular differentiation into granulocytes or monocytes (91-93). Several follow-up studies have demonstrated that ATP can also act synergistically with cytokines to induce an increase in human $CD34^+$ cells (94). This small fraction of primitive hematopoietic cells expresses several P2 receptors, such as $P2Y_1$, $P2Y_2$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2X_{1-7}$ receptors, and increases Ca^{2+}_i when stimulated by ATP (94, 95). In addition, pre-incubation with UTP increased the migratory and adhesive ability of human $CD34^+$ cells to fibronectin *in vitro* and improved their bone marrow homing ability *in vivo* (96). Furthermore, during myeloid differentiation in murine bone marrow cultures, the ability of ATP and its analogs to induce a high elevation of Ca^{2+}_i concentration has been described (52). ATP and its analogs were able to induce high Ca^{2+}_i increases and promote transient proliferation of primitive hematopoietic cells. ATP also decreased this population by inducing myeloid differentiation, which shows the participation of Ca^{2+} signaling in these ATP-induced effects (52). More recently, the expression and functional participation of P2X receptors in hematopoietic stem cell proliferation was described (97). Moreover, we have investigated the direct effect of ATP on hematopoietic stem cell differentiation, which showed that ATP induces hematopoietic stem cell differentiation through the activation of P2 receptors in a Ca^{2+}_i-dependent manner (98). However, the identification of P2 receptor subtypes that participate in proliferation and differentiation of hematopoietic progenitor/stem cells are still under investigation. Since some of these effects are promoted by ADP and UTP as well, the participation of $P2Y_1$ and $P2Y_{2/4}$ is probable. On the other hand, agonists such as αβMeATP, βγMeATP and BzATP also induce a steep rise in Ca^{2+}_i in primitive hematopoietic cells, which could lead to myeloid differentiation (52).

5. CONCLUSIONS

The great versatility in the different responses to Ca^{2+} makes this ion a central signaling molecule that regulates many systems, including the hematopoietic

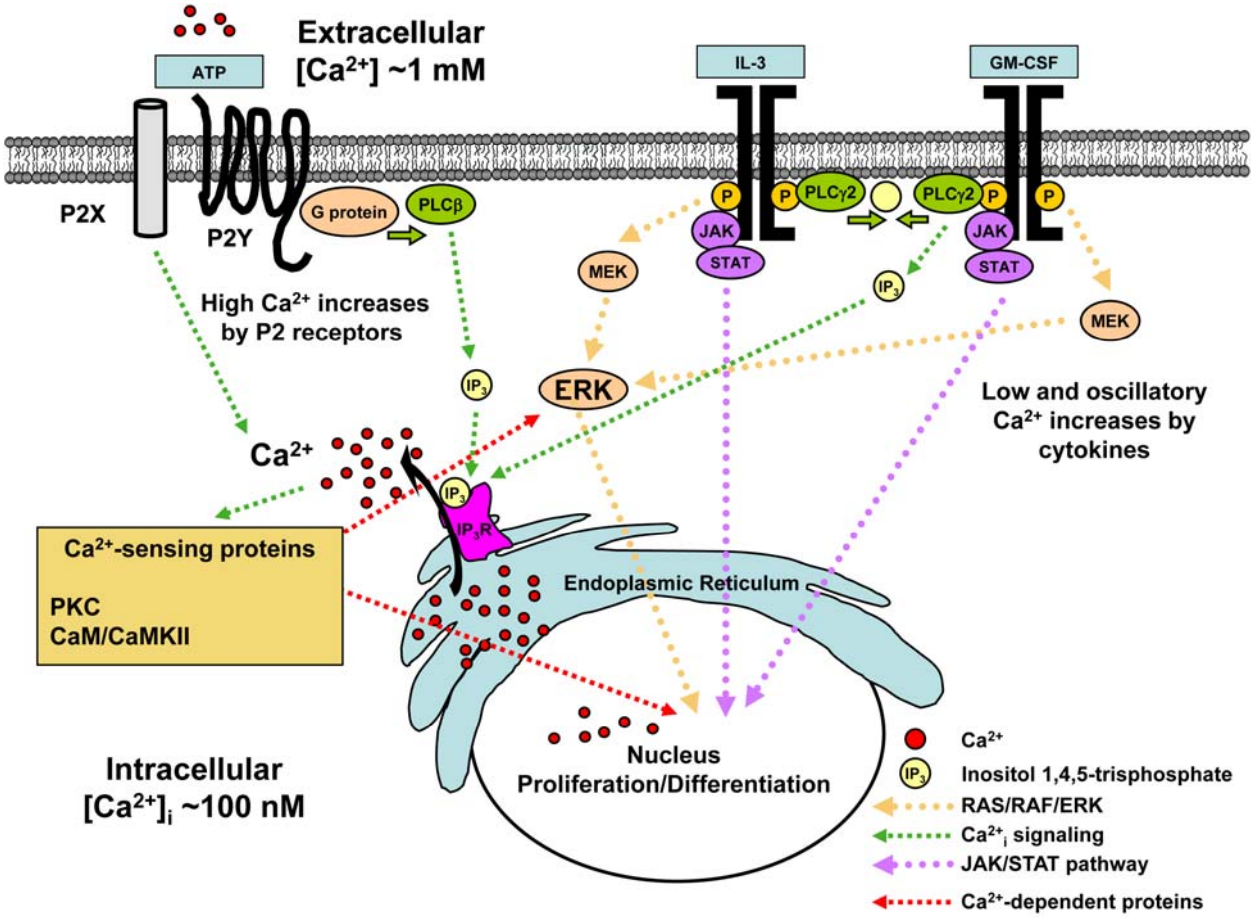

Figure 2. The participation of Ca^{2+} signaling in the activation of cytokine receptors such as IL-3 receptors and GM-CSF receptors or in the activation of nonidentified P2 receptors have been proposed in hematopoietic cells. The binding of IL-3 and GM-CSF to their cognate receptors induces the dimerization of these cytokine receptors and triggers classical JAK/STATs and RAS/RAF/ERK pathways. In addition, Ca^{2+}_i is also released through the activation of PLCγ2, which in turn produces IP_3 and DAG. The resulting low Ca^{2+}_i concentration increase activates Ca^{2+}-sensing proteins such as PKC and CaMKII that are related to the proliferation and differentiation of primitive hematopoietic cells. On the other hand, ATP actives ion channels (P2X) and G-protein-coupled receptors (P2Y) to induce a high Ca^{2+}_i increase that is strongly related with Ca^{2+}-dependent myeloid differentiation; however, other intracellular pathways involved in this process remain under investigation.

system. This versatility occurs through different components within the Ca^{2+}_i cascade that generate a particular Ca^{2+} signal translated by Ca^{2+}-sensitive proteins. Important groups of signaling mediators are modulated by Ca^{2+} variations, permitting this ion to regulate several intracellular pathways. In the hematopoietic system, the participation of Ca^{2+}_i in proliferation and differentiation though the activation of cytokine receptors and G-protein coupled receptors is also evident. Recent reports have demonstrated that cytokines promote a modest Ca^{2+}_i increase associated with the proliferation and differentiation, which is probably through the key participation of PLCγ2 (Figure 2). On the other hand, ATP that binds to purinergic receptors promotes large increases in Ca^{2+}-related differentiation (Figure 2) and may be associated to cell death of disease cells. However, the mechanisms Ca^{2+}-dependent are still under investigation. Further evaluation of Ca^{2+} participation in this complex system could identify possible role of this ion in some hematopoietic disorders opened an extensive area of research.

6. ACKNOWLEDGEMENTS

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