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Review

Calcium signaling in B cells: Regulation of cytosolic Ca^{2+} increase and its sensor molecules, STIM1 and STIM2☆

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ABSTRACT

Calcium signals are crucial for diverse cellular functions including adhesion, differentiation, proliferation, effector functions and gene expression. After engagement of the B cell receptor, the intracellular calcium ion (Ca^{2+}) concentration is increased promoting the activation of various signaling cascades. While elevated Ca^{2+} in the cytosol initially comes from the endoplasmic reticulum (ER), a continuous influx of extracellular Ca^{2+} is required to maintain the increased level of cytosolic Ca^{2+}. Store-operated Ca^{2+} entry manages this process, which is regulated by an ER calcium sensor, stromal interaction molecule (STIM). STIM proteins sense changes in the levels of Ca^{2+} stored within the ER lumen and regulates the Ca^{2+}-release activated Ca^{2+} channel in the plasma membrane. This review focuses on the signaling pathways leading to Ca^{2+} influx and the role of Ca^{2+} signals in B cell functions.

1. Store-operated calcium entry (SOCE)

There is no denying that calcium, an essential component of bone and teeth, is also of importance as a universal second messenger in various lineages of cells including immune cells. The intracellular calcium ion concentration ($[Ca^{2+}]$) controls a diverse range of cell functions and the elevation of intracellular calcium ions (Ca^{2+}) regulates various cellular events after cells are stimulated. Ligand binding to its specific receptor, such as the B cell receptor (BCR; Baba et al., 2006; Matsumoto et al., 2011), T cell receptor (TCR; Lewis, 2001; Hogan et al., 2010), Fc receptor (Beaven and Baumgartner, 1996; Melendez and Khaw, 2002; Bryceson et al., 2006; Baba et al., 2008; Braun et al., 2009), and chemokine receptors (Partida-Sánchez et al., 2004; Hsu et al., 2001; Barbet et al., 2008), or interactions between costimulatory molecules (Nicolaou et al., 2009; Tseng et al., 2008) all initiate the upregulation of intracellular $[Ca^{2+}]$, and there is a close correlation between increased Ca^{2+} levels and the regulation of cellular functions.

In the steady state, intracellular $[Ca^{2+}]$ is $\sim 10^{-7}$ M, approximately $1/10^4$ of extracellular $[Ca^{2+}]$ ($\sim 10^{-3}$ M). However, when cells are activated, intracellular $[Ca^{2+}]$ markedly increases ($\sim 10^{-6}$ M) because of the release of Ca^{2+} from intracellular storage, the endoplasmic reticulum (ER). While Ca^{2+} release form the ER generates primary Ca^{2+} signals, a continuous influx of extracellular Ca^{2+} is required to maintain the increased levels of Ca^{2+} inside cells because of the limitations of cellular Ca^{2+} storage. The mechanism for Ca^{2+} influx, where the entry of extracellular Ca^{2+} is initiated by the exhaustion of Ca^{2+} ER storage, is termed store-operated calcium entry (SOCE) through the Ca^{2+}-release activated Ca^{2+} (CRAC) channel, a type of SOCE channel. Thus, SOCE is an essential process that ensures a continuous extracellular source of Ca^{2+}.

2. Ca^{2+} signaling in B cells

Following the activating stimulation of B cells via several functional molecules including BCR, $[Ca^{2+}]$ is increased and controls various cell functions. The binding of antigens to BCR initiates the activation of tyrosine kinase, and the phosphorylation of adaptor molecules, resulting in the activation of two main signal cascades: phospholipase-γ2 (PLC-γ2) and phosphoinositide 3-kinase (PI3K) pathways (Kurosaki et al., 2009) (Fig. 1).

Abbreviations: BCR, B cell receptor; CRAC, Ca^{2+}-release activated Ca^{2+}; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP_3, inositol-1,4,5,-triphosphate; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; PLC-γ2, phospholipase-γ2; PI3K, phosphoinositide 3-kinase; EAE, experimental allergic encephalomyelitis.

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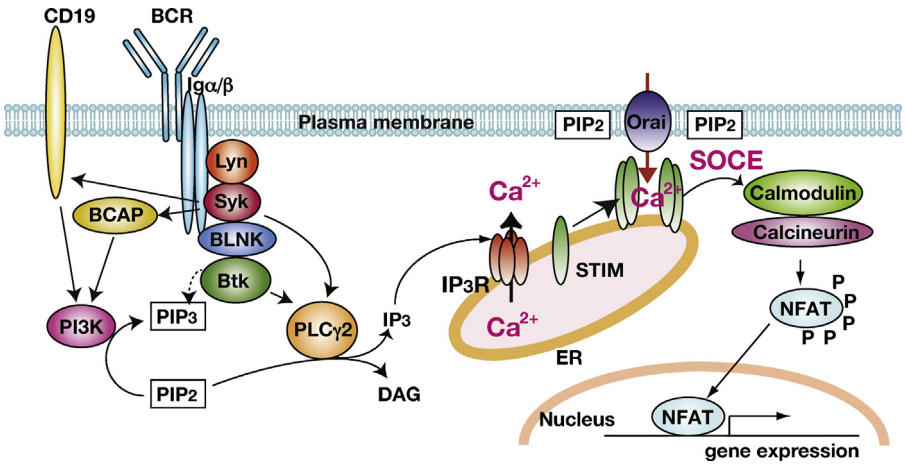

Fig. 1. Ca^{2+} signaling cascade. After BCR stimulation, Syk is recruited to phosphorylated ITAMs in Igα/Igβ, and then phosphorylates BLNK. Phosphorylated BLNK provides binding sites for Btk and PLC-γ2. Subsequently, PLC-γ2 is fully activated by Syk and Btk through phosphorylation. BCR stimulation induces phosphorylation of CD19 by Lyn and BCAP by Syk, which activates PI3 K to produce phosphatidylinositol-3,4,5-trisphosphate (PIP_3) from phosphatidylinositol-4,5-bisphosphate (PIP_2). PIP_3 is important for the membrane recruitment of Btk through its PH domain. PIP_2 serves as a substrate of PLC-γ2 to produce diacylglycerol (DAG) and inositol-1,4,5,-triphosphate (IP_3). The resultant IP_3 binds to its receptor IP_3R, which leads to Ca^{2+} release from the ER. The reduction of Ca^{2+} in the ER lumen causes STIM relocation and association of STIM and Orai into puncta at the ER-plasma membrane junction. Subsequently, the Orai channel is activated and induces a Ca^{2+} influx (store-operated Ca^{2+} entry: SOCE). Sustained Ca^{2+} increase by SOCE activates the calmodulin/calcineurin pathway. Phosphatase calcineurin dephosphorylates NFAT, which leads to its nuclear localization to regulate gene expression.

B-cell linker (BLNK), identified as a protein tyrosine-phosphorylated after BCR stimulation and as a substrate of Syk (Goitsuka et al., 1998), is a pivotal adaptor molecule in the BCR to PLC-γ2 activation signaling cascade. After BCR stimulation, Syk is recruited to phosphorylated ITAM (immunoreceptor tyrosine-based activation motif) in Igα/Igβ, which then phosphorylates BLNK allowing phosphorylated BLNK to bind to PLC-γ2 and Btk through their SH2 domains. Eventually, PLC-γ2 becomes tyrosine phosphorylated and activated by Syk and Btk. Activated PLC-γ2 hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to inositol-1,4,5,-triphosphate (IP_3) and diacylglycerol (DAG), and the resultant IP_3 binds to the receptor for IP_3 (IP_3R), an IP_3-gated intracellular Ca^{2+} channel itself, on the ER membrane. This leads to the release of Ca^{2+} stored in the lumen of the ER. Our previous study on the mutated N-terminal Src homology 2 (SH2) domain of PLC-γ2 demonstrated a central role for the SH2 domain in directing PLC-γ2 to close proximity to the BCR signaling complex by its association with BLNK (Ishiai et al., 1999). Thus, BLNK functions as a bridging molecule in the PLC-γ2 pathway to recruit Syk and Btk to PLC-γ2. It should be noted that Ca^{2+} mobilization is significantly reduced when examined in DT40 B cells deficient in Syk, Btk, BLNK, or PLC-γ2, indicating the importance of the concerted action of this molecular complex (Kurosaki and Tsukada, 2000). After the IP_3-mediated transient release of Ca^{2+} from the ER, extracellular Ca^{2+} entry is controlled by SOCE and CRAC channels.

The PI3K pathway positively regulates BCR-elicited Ca^{2+} flux (Scharenberg and Kinet, 1998; Okkenhaug and Vanhaesebroeck, 2003). The activation of PI3K is mainly mediated by CD19 or BCAP (B cell adaptor molecule for PI3 K) that contains a binding site for the SH2 domain in the p85α subunit of PI3K. After stimulation of BCR, CD19 is phosphorylated by Lyn, and BCAP is phosphorylated by Syk and Btk. Phosphorylated CD19 or BCAP (by binding to the p85 subunit of PI3K) activates PI3K to produce phosphatidylinositol-3,4,5-trisphosphate (PIP_3) from PIP_2, and PIP_3 transmits signals downstream (Okada et al., 2000). The accumulation of PIP_3 allows Btk to interact with its PH domains, thereby promoting their membrane targeting and activation. Subsequently, Btk effectively activates PLC-γ2 to induce Ca^{2+} release and influx. In particular, p110δ, a catalytic subunit of PI3K, is thought to be critical for BCR-mediated Ca^{2+} mobilization (Okkenhaug et al., 2002). Interestingly, in BCAP-deficient B cells, reduced Ca^{2+} mobilization (60–70% of the normal level) is observed suggesting insufficient PLC-γ2 activation in BCAP-deficient B cells contributes to the decreased Ca^{2+} flux. The normal tyrosine phosphorylation status of PLC-γ2 was observed in BCAP-deficient B cells, suggesting BCAP participates in PLC-γ2 activation in a phosphorylation-independent manner (Yamazaki et al., 2002).

In addition to BCR signaling, the signaling pathway through CD20 also induces cytosolic Ca^{2+} influx. Using inhibitor analyzes, fluorescence resonance energy transfer and variant Ramos human B cell lines lacking BCR, Ca^{2+} mobilization via CD20 was shown to be highly correlated with BCR expression, suggesting the direct association of CD20 with BCR "hijacks" the signaling potential downstream of BCR (Walshe et al., 2008).

In contrast to these "positive-signal" delivering molecules, CD22 is an inhibitory coreceptor of BCR exclusively expressed on B cells (Nitschke and Tsubata, 2004). Engagement of CD22 initiates its phosphorylation, subsequently activating SH2 domain-containing protein tyrosine phosphatase 1 (SHP-1). This leads to the attenuation of BCR signaling via dephosphorylation of BCR intracellular signaling pathway substrates, including Igα, Igβ, Syk, and BLNK (Tamir et al., 2000). Accordingly, reduced BCR-induced Ca^{2+} mobilization was observed through the ligation of CD22 (using anti-CD22 $F(ab)_2$ mAb); a long-term decrease in intracellular $[Ca^{2+}]$ was observed in anti-CD22 mAb pretreated B cells though an initially rapid increase in intracellular $[Ca^{2+}]$ was detected after BCR activation. This suggests Ca^{2+} mobilization regulation by CD22 is focused on the influx of extracellular Ca^{2+}. Interestingly, adding Ca^{2+} to EGTA-treated cells induced an immediate influx of Ca^{2+}, although a significantly faster reduction in intracellular Ca^{2+} was observed in B cells pretreated with anti-CD22 $F(ab)_2$ mAb. This indicates that the ligation of CD22 not only inhibits kinase phosphorylation events downstream of BCR, but also modulates Ca^{2+} efflux pump activity, and thus regulates Ca^{2+} homeostasis (Sieger et al., 2013).

3. Key molecule for SOCE: STIM

The key molecule for the operation of SOCE was recently identified as stromal interaction molecule 1 (STIM1), a ubiquitously expressed single-transmembrane protein localized in the ER that is conserved from *Drosophila* to mammals. It was reported to possess dual functions: as a sensor for $[Ca^{2+}]$ levels in the ER, and an

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