
ROLE OF INTRACELLULAR CALCIUM RELEASE CHANNELS IN CALCIUM SIGNALING IN T LYMPHOCYTES

A.F. FOMINA

University of California, Davis, CA, 95616, USA
affomina@ucdavis.edu



Dr. Alla Fomina received her undergraduate degree in Biophysics from Kharkiv State University in 1981. From 1981 to 1984 she performed her graduate studies in Biophysics under Prof. P.G. Kostyuk at the Bohomolets Institute of Physiology, Kyiv, Ukraine. After completing her Ph.D. in Biophysics in 1985, Dr. Fomina continued her research at the Department of general physiology of the nervous system at the Bohomolets Institute of Physiology until 1992. At the present time, Dr. Fomina is an Associate Professor at the Department of Physiology and Membrane Biology School of Medicine at the University of California, Davis, USA, where she is teaching courses in human physiology and conducting research on different aspects of calcium signaling in cells of immune system.

Elevation in intracellular Ca^{2+} concentration is crucial for T_h lymphocyte activation and effector functions

CD4^+ helper T (T_h) lymphocytes play a pivotal role in directing the immune responses against foreign pathogens, damaged or transformed cells. Naïve T_h cells express T cell receptors (TCR) that recognize a specific antigen in association with a homologous class II major histocompatibility complex on the antigen-presenting cell. The initial response of T_h cells triggered by TCR crosslinking with an antigen is commonly referred to as activation and is tightly regulated (Favero and Lafont, 1998, Germain and Stefanova, 1999). During the activation phase, which lasts at least one day, T cells begin to express cytokine interleukin 2 (IL-2) and several surface receptors. Engagement of IL-2 with its receptor expressed on T_h cells initiates cell cycle progression, which may continue with many further rounds of divisions (Favero and Lafont, 1998, Killeen et al., 1998, Swain, 1999, Germain, 2002). The continued antigenic stimulation leads to a differentiation of activated T_h cells into specific subsets of effector T_h cells characterized by divergence of cytokine production. Effector T_h cells coordinate a variety of immune responses including inflammatory and humoral responses (Swain, 1999, Szabo et al., 2003, Steinman, 2007). To maintain homeostasis after clonal expansion, activated T_h cells are removed by apoptosis once the invading antigen has been elim-

inated (Lenardo et al., 1999, Krammer et al., 2007). The balance between T_h cell expansion and elimination is critical for maximizing the ability of the immune response to defend the host while maintaining immunologic tolerance and preventing autoimmunity.

Antigenic stimulation of TCR and/or accessory molecules triggers spatio/temporally heterogeneous cytosolic Ca^{2+} signals (Lewis, 2001). A sustained or oscillatory elevation in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) which lasts for hours and days is required to drive many events of lymphocyte activation, including the activation of transcription factors, modulation of cytoskeletal elements and motility, production and release of cytokines, and cell proliferation. Diminished $[Ca^{2+}]_i$ signaling results in impaired T_h cell activation and, consequently, in the development of severe immunodeficiency (Feske, 2007, Gwack et al., 2007). Furthermore, excessive elevation in $[Ca^{2+}]_i$ following strong TCR stimulation induces activation-dependent apoptosis (Orrenius et al., 2003, Hanson et al., 2004). Therefore, understanding the complex molecular interactions that mediate Ca^{2+} signaling in T_h cells may lead to the development of strategies for manipulation of Ca^{2+} -dependent T_h cell responses, which may allow for the elimination of unwanted T_h cell subsets, for prevention of autoimmunity, or re-sensitization of apoptosis-resistant malignant T_h cells.

Molecular mechanisms for initiating of $[Ca^{2+}]_i$ signaling following TCR engagement

Understanding of the molecular mechanisms responsible for the heterogeneous Ca^{2+} signaling in T_h cells began from the discovery that stimulation of TCR leads to production of inositol 1,4,5-inositol trisphosphate (IP_3), bound to the IP_3 receptor (IP_3R) located on internal membranes and evokes Ca^{2+} release from the intracellular organelles, predominantly endoplasmic reticulum (Berridge, 1997). Two IP_3R isoforms (IP_3R -2 and IP_3R -3) have been found in lymphocytes, thymocytes, and splenocytes, whereas Jurkat T cells, a human lymphoblastic leukemia T_h cell line, express all three known isoforms (IP_3R -1, IP_3R -2, and IP_3R -3) (Sugiyama et al., 1994, Harnick et al., 1995). Depletion of intracellular Ca^{2+} store triggers activation of plasmalemmal Ca^{2+} release-activated Ca^{2+} (CRAC) channels to allow extracellular Ca^{2+} entry (Lewis, 2001).

It was also found that TCR activation stimulates production of cyclic adenosine 5'-diphosphate-ribose (cADPR) and/or nicotinic acid adenine dinucleotide phosphate (NAADP), the endogenous agonists of ryanodine receptors (RyR) (Guse et al., 1999, Gasser et al., 2006). Peripheral blood human T lymphocytes express RyR type 1 and 2 (Hosoi et al., 2001), whereas Jurkat T lymphocytes express RyR type 3 (Hakamata et al., 1994, Guse et al., 1999). It was shown that downregulation of cADPR levels or RyR type 3 expression reduced the sustained component of $[Ca^{2+}]_i$ elevation following TCR stimulation in Jurkat T cells (Guse et al., 1999,

Schwarzmann et al., 2002). Although these findings imply that RyR participate in later sustained phase of $[Ca^{2+}]_i$ elevation, the sequence of the events leading to the RyR activation in T cells remains poorly understood.

Due to the limited capacity of intracellular Ca^{2+} store (estimated volume of the endoplasmic reticulum is only ~1% of the volume of the T cell cytoplasm) and short lifetime of IP_3 , for a long time CRAC channels were considered to be the only mechanism controlling the sustained $[Ca^{2+}]_i$ elevation required for T cell activation (Lewis, 2001, Feske, 2007, Gwack et al., 2007, Hogan and Rao, 2007). The role of IP_3R was believed to be limited to store depletion leading to CRAC channels activation. Furthermore, because downregulation of IP_3R alone was sufficient to abolish sustained $[Ca^{2+}]_i$ elevation following TCR stimulation (Jayaraman et al., 1995), the role of RyR in Ca^{2+} signaling in T_h cells was not recognized as essential. However, recent work performed in our laboratory has demonstrated that both IP_3R and RyR play a crucial role in maintaining the sustained $[Ca^{2+}]_i$ elevation necessary for T cell activation.

Activation of intracellular Ca^{2+} channels during $[Ca^{2+}]_i$ elevation

Our experiments performed in Jurkat T cells revealed that in the presence of sarco-endoplasmic Ca^{2+} -ATPase (SERCA) activity, the robust elevation in $[Ca^{2+}]_i$ due to activation of CRAC channels did not result in the refilling of the intracellular Ca^{2+} store (Dadsetan et al., 2008). This can happen if Ca^{2+} influx through CRAC channels activates intracellular Ca^{2+} release channels, IP_3R and/or RyR, via Ca^{2+} -induced Ca^{2+} release (CICR) mechanism (Galione and Churchill, 2002), as it was shown in parotid acinar cells (Yao et al., 2004). To confirm that Ca^{2+} influx via CRAC channels activates CICR in T cells, CRAC channels were activated following store depletion with cyclopiazonic acid, a reversible SERCA blocker, which does not stimulate IP_3 or cADPR production. We found that IP_3R and RyR blockers of xestospongin C and ryanodine, respectively, significantly reduced $[Ca^{2+}]_i$ transients evoked upon activation of Ca^{2+} influx via CRAC channels but increased Ca^{2+} accumulated within the intracellular store (Dadsetan et al., 2008). In the absence of extracellular Ca^{2+} , neither IP_3R nor RyR inhibitors affected the $[Ca^{2+}]_i$ transients evoked by the cyclopiazonic acid-induced store depletion, indicating that in the absence of intracellular second messengers (IP_3 or cADPR) or extracellular Ca^{2+} influx, the Ca^{2+} efflux from the store was not sufficient to activate IP_3R or RyR. Furthermore, in the absence of extracellular Ca^{2+} , the RyR blockers did not affect the $[Ca^{2+}]_i$ transients induced by the store depletion with the TCR agonist, indicating that in the absence of Ca^{2+} influx, generation of second messengers following TCR stimulation and Ca^{2+} release from the store were not sufficient to activate RyR. Thus, in contrast to IP_3R activation at the resting $[Ca^{2+}]_i$ levels in the presence of IP_3 , the RyR activation in T_h cells absolutely requires Ca^{2+}

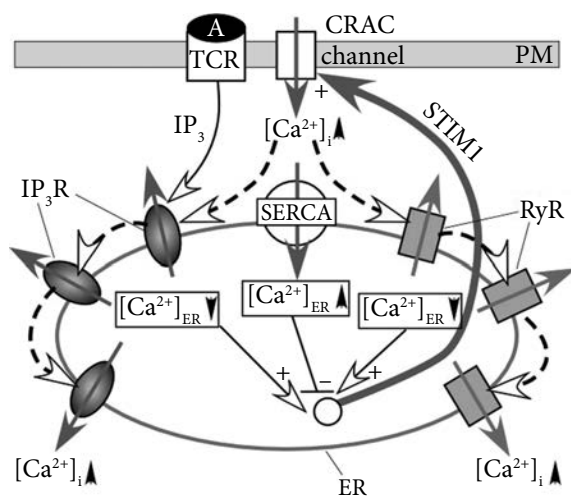


Fig. 1. Simplified diagram of intracellular Ca^{2+} dynamics in T cells following T cell receptor activation. Abbreviations: TCR (T cell receptor); A (antigen); IP_3 (inositol 1,4,5-trisphosphate); IP_3R (inositol 1,4,5-trisphosphate receptor); RyR (ryanodine receptor); CRAC (Ca^{2+} release-activated Ca^{2+} channel); ER (endoplasmic reticulum); PM (plasma membrane); STIM1 (putative ER Ca^{2+} sensor). Dashed arrows indicate activation via CICR mechanism

entry via CRAC channels. Taken together, these observations allowed us to conclude that in T_h cells the IP_3R and RyR are activated upon Ca^{2+} entry via CRAC channels, presumably via CICR mechanism, and control Ca^{2+} dynamics in T cells by controlling Ca^{2+} retention within the store.

Fig. 1 presents a simplified diagram of molecular mechanisms controlling $[\text{Ca}^{2+}]_i$ dynamics in T_h cells. The IP_3 -dependent activation of IP_3R is an earliest event following TCR engagement with an antigen, which leads to Ca^{2+} release from the store and consequent store depletion. Decrease in luminal $[\text{Ca}^{2+}]_{\text{ER}}$ activates putative Ca^{2+} sensor STIM1 (Cahalan, 2009), which consequently activates plasmalemmal CRAC channels and allows for Ca^{2+} influx. Elevation in $[\text{Ca}^{2+}]_i$ activates IP_3R and RyR via CICR mechanism. Ca^{2+} -dependent activation of IP_3R and/or RyR compensates for the SERCA activity preventing store from being refilled even at elevated $[\text{Ca}^{2+}]_i$ and CRAC channels from being inactivated by negative feedback inhibition from the refilled store. Thus, positive feedback relationships between plasmalemmal CRAC channels and intracellular IP_3R and RyR sustain $[\text{Ca}^{2+}]_i$ elevation following TCR stimulation. In addition, store may serve as an intermediate compartment into which Ca^{2+} may be pumped from the points of Ca^{2+} entry via CRAC channels. Sequential activation of IP_3R and/or RyR via CICR may allow for the generation of spatially heterogeneous Ca^{2+} signaling in T_h cells.

Targeting of T cell IP_3R and RyR in treatment of human diseases

Given the major role of IP_3R and RyR in regulating $[\text{Ca}^{2+}]_i$ dynamics in T cells, one can predict that manipulation of the these channels activity will affect Ca^{2+} -dependent functions of T_h lymphocytes. Accordingly, we have shown that pharmacological blockers of IP_3R or RyR hampered proliferation and IL-2 production

in Jurkat lymphocytes *in vitro* (Dadsetan et al., 2008). In addition, the RyR antagonists dantrolene and ruthenium red have been shown to inhibit murine T cell proliferation and IL-2 production following TCR stimulation (Conrad et al., 2004). Thus, both IP₃R and RyR may be used as potential targets for immunosuppression. Although there are no clinically relevant IP₃R inhibitors yet available, the RyR inhibitor dantrolene is clinically approved for treatment of muscular skeletal disorders in humans. We speculate that dantrolene could potentially be used for the treatment of human disorders associated with T cell dysfunctions such as autoimmune diseases or graft-versus host disease.

The future challenge in establishing the utility of IP₃R and/or RyR as a therapeutic target for immunosuppression will be to unravel the relationships between IP₃R and RyR type-specific genetic and functional expression in the specific T cell subsets implicated in pathogenesis of particular types of human disorders and in testing the efficacy of IP₃R and/or RyR blockers in alleviating the severity and progression of these disorders in relevant animal models.

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