Ca²⁺ influx in T cells: how many Ca²⁺ channels?

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Ca²⁺ signals are critical for T cell function. A number of ion channels regulate Ca²⁺ influx from the extracellular space in T cells, either by conducting Ca²⁺ ions or by modulating the membrane potential that provides the driving force for Ca²⁺ influx (Cahalan and Chandy, 2009; Feske et al., 2012). The best characterized Ca²⁺ channel in T cells is the Ca²⁺ release-activated Ca²⁺ (CRAC) channel, which mediates store-operated Ca²⁺ entry (SOCE) in response to T cell receptor (TCR) activation and is composed of ORAI and stromal interaction molecules (STIM) family proteins. Several other channels may also mediate Ca²⁺ influx directly in T cells including members of the transient receptor potential (TRP) family, P2X receptors, and voltage-gated Ca²⁺ (Ca$_v$) channels. Compared to CRAC channels, however, their contribution to TCR-induced Ca²⁺ influx and immunity is less well defined.

Ca²⁺ release-activated Ca²⁺ channels were first identified in T cells (and mast cells) over 20 years ago (Lewis and Cahalan, 1989; Hoth and Penner, 1992; Zweifach and Lewis, 1993). They mediate Ca²⁺ influx and have well defined electrophysiological properties (Parekh and Penner, 1997; Prakriya and Lewis, 2003). The long elusive molecular identity of the CRAC channel was solved with the discovery of ORAI1 by genome-wide RNAi screens and positional cloning in patients lacking CRAC channel function (Feske et al., 2006; Vig et al., 2006b; Zhang et al., 2006). ORAI1 and its two homologs, ORAI2 and ORAI3, are integral membrane proteins (**Figure 1**). Mutagenesis and structural analyses have showed that ORAI1 forms the pore of the CRAC channel through which Ca²⁺ ions enter the cell (Prakriya et al., 2006; Vig et al., 2006a; Yeromin et al., 2006; Hou et al., 2012; McNally et al., 2012). CRAC channels open after TCR-induced production of inositol 1,4,5-trisphosphate (InsP3) and release of Ca²⁺ from ER stores. Reduced Ca²⁺ levels in the ER trigger the activation of STIM 1 and 2 located in the ER membrane. After translocation to ER-plasma membrane junctions, STIM proteins bind to ORAI1 and open the CRAC channel pore, resulting in sustained Ca²⁺ influx. The molecular regulation of CRAC channel function has been described in detail elsewhere (Shaw et al., 2012).

The essential role of CRAC channels for T cell function and adaptive immunity is best illustrated by patients with loss-of-function or null mutations in *ORAI1* or *STIM1* genes, whose T cells lack CRAC channel function and SOCE (Partiseti et al., 1994; Le Deist et al., 1995; Feske et al., 1996; McCarl et al., 2009; Picard et al., 2009; Feske, 2011; Fuchs et al., 2012). CRAC channel-deficient T cells proliferate poorly *in vitro* and have a profound defect in the production of cytokines such as IFNγ, TNFα, IL-2, and IL-17. Similar defects are found in CD4⁺ and CD8⁺ T cells from *Stim1⁻/⁻*, *Orai1⁻/⁻*, and *Orai1^R91W* knock-in mice (Gwack et al., 2008; Beyersdorf et al., 2009; McCarl et al., 2010). SOCE-deficient T cells were found to be more resistant to apoptotic cell death and showed migration defects *in vitro* and *in vivo* (Ma et al., 2010; Kim et al., 2011; Greenberg et al., 2013) (and Stefan Feske unpublished data). Interestingly, SOCE is dispensable for the development and selection of conventional TCRαβ⁺ CD4⁺ and CD8⁺ T cells in SOCE-deficient patients and mice. However, their T cell function is severely compromised *in vivo*, apparent in absent delayed type hypersensitivity (DTH) responses to recall antigens in patients and mice (Le Deist et al., 1995; Feske et al., 1996; McCarl et al., 2010) and impaired skin allograft rejection in *Orai1^R93W* knock-in mice (McCarl et al., 2010). Most importantly, impaired T cell function in ORAI1 and STIM1-deficient patients results in recurrent and chronic infections with a wide spectrum of viral, bacterial and fungal pathogens (Partiseti et al., 1994; Le Deist et al., 1995; Feske et al., 1996; McCarl et al., 2009; Picard et al., 2009; Byun et al., 2010; Feske, 2010; Fuchs et al., 2012).

Besides immunity to infection, CRAC channels in T cells regulate immunological tolerance and inflammation. CD4⁺ T cells from mice lacking ORAI1 or STIM1 function showed strongly impaired expression of proinflammatory cytokines such as IFN-γ and IL-17 (Ma et al., 2010; McCarl et al., 2010). Importantly, these mice were resistant to T cell-mediated intestinal and CNS inflammation in animal models of colitis and multiple sclerosis. Complete absence of CRAC channel function in mice with T cell-specific deletion of *Stim1* and *Stim2* genes, in addition, results in impaired development and function of Foxp3⁺ regulatory T (Treg) cells (Oh-Hora et al., 2008). As a result, STIM1/2-deficient mice over time develop severe myelo-lymphoproliferative disease with lymphadenopathy, splenomegaly, and pulmonary inflammation (Oh-Hora et al., 2008). Intriguingly, these mice show an exocrine gland autoimmune disease resembling Sjogren's syndrome in humans (Cheng et al., 2012). Reduced numbers of Treg cells are also found in ORAI1- and STIM1-deficient patients (Picard et al., 2009) (and unpublished data), most of which suffer from autoimmune thrombocytopenia and hemolytic anemia due to autoantibodies against erythrocytes and platelets (Feske, 2011). The complete lack of SOCE in STIM1/2-deficient mice not only impaired the development of Treg cells but also that of natural killer T (NKT) cells and TCRαβ⁺ CD8αα⁺ intraepithelial lymphocytes (IEL) in the gut (Oh-Hora et al., 2013). These findings indicate that low to moderate SOCE is sufficient for the postselection maturation of agonist-selected T cells (Treg
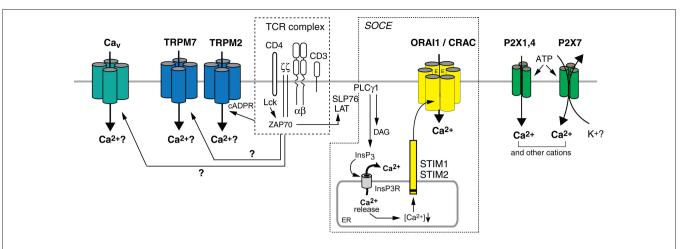
FIGURE 1 | Ca²⁺ influx pathways in T cells. Stimulation of T cells through the TCR complex results in Ca²⁺ influx, which is involved in the regulation of many T cell functions. CRAC channels mediate store-operated Ca²⁺ entry (SOCE) following activation of PLCγ1 and production of InsP₃. InsP₃ binds to and opens Ca²⁺ permeable InsP₃ receptors (InsP₃R) in the ER, resulting in the release of Ca²⁺ from ER stores (Lewis, 2001; Feske, 2007). Ca²⁺ release from the ER causes the activation of STIM 1 and 2, which oligomerize and translocate to ER-plasma membrane junctions. STIM1 and STIM2 bind to ORAI1, the pore-forming subunit of the CRAC channel, thereby mediating its opening and sustained Ca²⁺ influx. The subunit composition of the CRAC channel awaits further studies; both tetrameric and hexameric assemblies of ORAI1 subunits were proposed (Ji et al., 2008; Mignen et al., 2008; Penna et al., 2008; Maruyama et al., 2009; Hou et al., 2012). TRPM2 is a Ca²⁺ permeable cation channel that can be activated by cADPR and NAADP in human T cells (Beck et al., 2006). Increased cADPR levels after TCR stimulation (Guse et al., 1999) activate SOCE by releasing Ca²⁺ from the ER through RyR channels and potentially activate TRPM2 channels directly. TRPM7 is a non-selective cation channel implicated in Mg²⁺ homeostasis in T cells; whether its ability to conduct Ca²⁺ contributes to T cell function and how it is activated by TCR stimulation is not understood. The L-type Ca_v channels Ca_v1.2, Ca_v1.3, and Ca_v1.4, which mediate depolarization-dependent Ca²⁺ influx in excitable cells including neurons may contribute to Ca²⁺ influx in T cells but their activation mechanism is unknown and their current properties are not well defined. P2X receptors are non-selective Ca²⁺ channels activated by extracellular ATP. Several homologs, P2X1, P2X4, and P2X7, were reported to mediate Ca²⁺ influx in T cells *in vitro*.

cells, NKT cells, IEL), whereas strong SOCE is required for the proinflammatory function of Th1 and Th17 cells.

Transient receptor potential channels belong to a large family of ion channels, which conduct monovalent and divalent cations including Ca²⁺ (Nilius and Owsianik, 2011). Before the discovery of ORAI1 as the CRAC channel, several TRPC channels were proposed to mediate Ca²⁺ influx in T cells. However, a significant role of TRPC channels in Ca²⁺ influx and T cell mediated immune function has not been established. By contrast, TRPM7 is essential for T cell development as mice with T cell-specific deletion of *Trpm7* had a severe block in T cell development at the CD4⁻CD8⁻ double negative stage (Jin et al., 2008). This is the most profound effect of any ion channel on lymphocyte development demonstrated so far. TRPM7 is Mg²⁺ permeable and widely considered to regulate cellular Mg²⁺ homeostasis. However, T cells from *Trpm7⁻/⁻* mice had normal Mg²⁺ influx and total Mg²⁺ levels, raising the question whether impaired T cell development is caused by dysregulated Mg²⁺ homeostasis or rather by impaired influx of other cations including Ca²⁺ which TRPM7 is able to conduct as well. Another

TRP channel, TRPM2 is a non-selective, Ca²⁺ permeable cation channel and in human T cells, TRPM2 can be activated by a variety of intracellular agonists including adenosine diphosphate ribose (ADPR), cyclic ADPR (cADPR), and Nicotinic acid adenine dinucleotide phosphate (NAADP) (Beck et al., 2006). TCR stimulation was reported to result in increased intracellular cADPR levels and Ca²⁺ release from the ER through ryanodine receptors (RyR) (Guse et al., 1999), thereby initiating SOCE; alternatively, elevated cADPR levels could directly activate TRPM2 channels. However, the physiological function of TRPM2 channels in T cells is unknown. It is intriguing to speculate that TRPM2 may be involved in inflammatory T cell responses similar to their role in CXCL2 chemokine expression and NADPH oxidase function in monocytes (Yamamoto et al., 2008) and phagocytes (Di et al., 2011).

Voltage-gated Ca²⁺ (Ca_v) channels are highly Ca²⁺ selective channels that play an important role in Ca²⁺ influx and the function of electrically excitable cells such as neurons following cell depolarization (Tsien et al., 1987). In T cells, several members of the L-type family of Ca_v channels (Ca_v1)

were reported to be expressed but their contribution to Ca²⁺ influx has remained controversial (Hogan et al., 2010). Recent studies showed that genetic deletion of Ca_v1.4 in mouse T cells and knockdown of Cav1.2 and Cav1.3 in human T cells attenuates TCR-induced Ca²⁺ influx (Cabral et al., 2010; Omilusik et al., 2011). Similarly, mutation of the regulatory β3 and β4 subunits of Ca_v1 channels in mice results in reduced Ca²⁺ influx and impaired IL-4, IFNγ, and TNFα production in CD4⁺ and CD8⁺ T cells following TCR stimulation (Badou et al., 2006; Jha et al., 2009). CD8⁺ T cells lacking functional β3 regulatory subunits or Cav1.4 channels were more susceptible to apoptosis (Jha et al., 2009; Omilusik et al., 2011). Cav1.4-deficient mice also showed reduced cytotoxic function of CD8⁺ T cells *in vitro* and impaired CD8⁺ T cell responses to infection with *Listeria monocytogenes in vivo* (Omilusik et al., 2011). Despite these intriguing findings, the pathways by which TCR signaling activates Ca_v1 channels are unknown. In contrast to excitable cells, depolarization of T cells fails to open Ca_v1 channels and mediate Ca²⁺ influx. It has been speculated that Ca_v1 channels in T cells are activated by an alternative,

voltage-independent mechanism, but the nature of this mechanism remains to be elucidated. In addition, native Ca_V1 channel-like Ca^{2+} currents have so far been reported only once despite efforts by numerous labs and appear to be restricted to naïve CD4+ and CD8+ T cells (Omilusik et al., 2011). Intriguingly, these currents were abolished in T cells from *Cav1.4−/−* mice. Future studies will need to investigate the electrophysiological properties of Ca_V1 channels in T cells, clarify the molecular mechanisms that regulate their activation, and investigate the role of Ca_V1 channels in adaptive immunity.

P2X receptors are not, unlike the Ca^{2+} channels discussed above, activated by TCR stimulation but by extracellular ATP. Three P2X receptors, P2X1, P2X4 and P2X7 (Yip et al., 2009; Woehrle et al., 2010), were reported to mediate Ca^{2+} influx in T cells. The opening of P2X7 causes Ca^{2+} influx and activation of enzymes such as calcineurin, resulting in T cell proliferation (Baricordi et al., 1996) and IL-2 production (Adinolfi et al., 2005; Woehrle et al., 2010). Inhibition of P2X1, P2X4, and P2X7 function by RNAi or chemical antagonists attenuated Ca^{2+} influx and IL-2 production in Jurkat cells and human CD4+ T cells *in vitro* (Yip et al., 2009; Woehrle et al., 2010). A Ca^{2+} dependent role for individual P2X receptors in T cell immunity *in vivo*, however, is not well established. Using *P2X7−/−* mice, one study found that P2X7 is proinflammatory in T cells by promoting the differentiation and function of Th17 cells and inhibiting the stability of Treg cells (Schenk et al., 2011). The role of P2X7 in T cell-mediated autoimmunity, however, is ambiguous as both increased (Chen and Brosnan, 2006) and decreased (Sharp et al., 2008) CNS inflammation were observed in *P2X7−/−* mice when analyzed in animal models of multiple sclerosis. In addition, it is unclear whether the main function of P2X7 receptors in T cells is to mediate Ca^{2+} influx. At the high ATP concentrations (~1 mM) used to measure Ca^{2+} influx in most studies, P2X7 channels form a large pore (Junger, 2011) that is permeable to a variety of anorganic and organic cations (Chused et al., 1996; Adriouch et al., 2002). Similarly high ATP concentrations are used to activate inflammasomes in innate immune cells, in which P2X7 channels are thought to mediate K+ efflux and thereby production of IL-1β (Ferrari et al., 2006; Tschopp and Schroder, 2010). Future

studies need to evaluate if P2X7 receptors modulate T cell function through Ca^{2+} influx or other mechanisms. P2X1 and P2X4 conduct Ca^{2+} more selectively and open at lower (micromolar) ATP concentrations (Junger, 2011). However, P2X1 and P2X4-deficient mice have no reported immunological phenotype (Mulryan et al., 2000; Yamamoto et al., 2006) and their role in T cell immunity *in vivo* remains poorly understood.

Ca^{2+} signals have long been recognized as essential for T cell function and several channels may contribute to Ca^{2+} influx in T cells. Whereas the role of CRAC channels to T cell function and adaptive immunity is well documented by findings in ORAI1 and STIM1-deficient patients and mice, the contributions of TRP, Ca_V1, and P2X receptor channels remain to be more clearly defined. These channels could contribute to Ca^{2+} influx in specific T cell subsets, at distinct stages of T cell development or following stimuli other than TCR engagement. A better understanding of the contributions of different Ca^{2+} influx pathways in T cells will be essential to define potential drug targets for the modulation of T cell function in a variety of diseases caused by aberrant T cell function.

ACKNOWLEDGMENT

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