The role of voltage-dependent calcium (Ca\(^{2+}\)) channels is prominent in excitable cells while store-operated calcium channels (SOC\(C\)) were considered as characteristic of non-excitable cells. Ca\(^{2+}\) channels are implicated in excitation transcription. Store-operated calcium channels (SOC\(C\)) activity is increased during cardiac stress and would contribute to Ca\(^{2+}\) influx and expression of genes responsible for cardiac hypertrophy and heart failure (Luo et al., 2012). Several lines of evidence now show the importance of Ca\(^{2+}\) channels in non-excitable cells including lymphocytes (reviewed in Robert et al., 2011, 2013). Ca\(^{2+}\) channels are defined by their voltage sensitivity and their sensitivity to drugs as dihydropyridines, phenylalkylamines, benzothiazepines, known to alter T-cell functions. However the drug concentrations needed were higher compared to excitable cells. The absence of cell membrane depolarization upon activation and possible non-specific effects of the drugs questioned the putative role of Ca\(^{2+}\) channels in T-cells.

Ca\(^{2+}\) channels are formed by the ion forming pore \(\alpha_1\) subunit encoded by four genes conferring some tissue-specific expression pattern in excitatory cells. Ca\(\alpha_1\) is characteristic of skeletal muscle cells. Ca\(\alpha_{1,2}\) is found in neurons, heart, and smooth muscle cells while Ca\(\alpha_{1,3}\) is detected in neuroendocrine cells. Ca\(\alpha_{1,2}\) and Ca\(\alpha_{1,3}\) can be found in the same tissues even if their role is not redundant as shown by the differential phenotypes of Ca\(\alpha_{1,2}\) and Ca\(\alpha_{1,3}\) null mice. Ca\(\alpha_{1,4}\) is the retinal form. Ca\(\alpha_1\) channel isoforms differ by their sensitivity to depolarization and to antagonizing drugs such as dihydropyridines (DHP) as well as by their inactivation properties (Lipscombe et al., 2004). For example, Ca\(\alpha_{1,4}\) channels activate at more negative potentials than Ca\(\alpha_{1,3}\) and Ca\(\alpha_{1,2}\), which highlights the potential involvement of Ca\(\alpha_{1,4}\) in non-excitable cells as mast cells (McRory et al., 2004) and more recently in mouse T-lymphocytes (Omilusik et al., 2011).

**CALCIN IN T-LYMPHO CYTES: PROMINENT ROLE OF THE STIM-Orai PATHWAY**

In T-lymphocytes, Ca\(^{2+}\) ions are important for the activation of many enzymes including phospholipase C gamma (PLC\(\gamma\)), classical protein kinases C, for proper protein folding, for the accessibility of key enzymes in T-cell transduction, and as a second messenger (Vig and Kinet, 2009). Variations in the intracellular calcium concentration ([Ca\(^{2+}\)]\(_{\text{free}}\)) are responsible for modulating the transcription of more than 75% of genes induced or down-regulated by T-cell receptor engagement in T-lymphocytes (Feske et al., 2001). The intracellular [Ca\(^{2+}\)] that decides the cellular fate is tightly regulated in both resting and activated conditions. The calcium concentration in the external medium is about 1–2 mM whereas the [Ca\(^{2+}\)] is about 50–100 nM and depends on the calcium channels expressed at both the cell and endoplasmic reticulum (ER) membranes, on exchangers, pumps, ... Activation of potassium channels that extrude the potassium from the cell is needed for supporting the electrochemical driving force allowing the calcium influx. In T-lymphocytes, TCR engagement results in a cascade of tyrosine kinase activation, the constitution of a platform transducing the signal with the recruitment of adapters and enzymes such as PLC\(\gamma\) that generates inositol trisphosphate (IP3) and diacylglycerol. IP3 binds to its receptors on the ER membrane leading to the release of Ca\(^{2+}\) stores, which induces a conformational change of STIM1, an ER Ca\(^{2+}\) sensor. STIM1 then localizes near the cell membrane, and activates the SOCC ORAI1 at the cell membrane (Barr et al., 2009; Oh-hora, 2009; Vig and Kinet, 2009; Zhou et al., 2010). The sustained entry of Ca\(^{2+}\) into the cell through ORAI1 channels is responsible for the activation of calcineurin, resulting in the nuclear translocation of the transcription factor NFAT as well as the activation of calmodulin kinase-dependent pathways. The severe immunodeficiency observed in mice or Humans with defective STIM1 (Picard et al., 2009) and ORAI1 testifies the importance of these molecules in T-cell biology (Partiseti et al., 1994; Feske et al., 2006, 2012).

However, this scheme accounts neither for the heterogeneity of calcium responses induced by TCR stimulation depending upon the state of activation and differentiation of T-lymphocytes nor for the possible implication of other calcium channels at the T-cell membrane.

**Ca\(\alpha_1\) CHANNELS IN T-CELLS**

An increasing line of evidence pleads for the involvement of Ca\(\alpha_1\) channels in T-lymphocyte biology (Kotturi et al., 2003, 2006; Stokes et al., 2004; Kotturi and Jeffreries, 2005; Badou et al., 2006; Matza et al., 2008, 2009; Jha et al., 2009). Thus, the analysis of mice with ablation of the auxiliary subunits Ca\(\beta_3\) (Jha et al., 2009) and Ca\(\beta_4\) (Badou et al., 2006) and more recently of mice deleted for Ca\(\alpha_{1,4}\) (Omilusik et al., 2011) reveals the role of Ca\(\alpha_1\) channels in T-lymphocyte survival and activation. Ca\(\alpha_{1,4}\) was recently described as interacting with Vav and Ick src kinase (Jha et al., 2009), which could result in Ca\(^{2+}\) entry required for maintaining [Ca\(^{2+}\)] and the ER Ca\(^{2+}\) stores (Figure 1A). As a consequence, Ca\(\alpha_{1,4}\) defective T-cells are more prone to apoptosis and have a reduced homeostatic proliferation capacity. Naïve Ca\(\alpha_{1,4}\) null T-cells also harbor defective calcium
Ca1 Channels in Th2-Cells

Depending upon the strength of TCR stimulation, the chronicity of antigenic exposure, the route of antigen administration, and the cytokines present during T-cell differentiation, CD4+ T-cells can differentiate into Th1, Th2, and Th17-cells that produce distinct sets of cytokines and exert different functions. In addition, these subpopulations express lineage specific and common transmembrane receptors. Th1-cells produce gamma interferon (IFN-γ) and are implicated in the eradication of intracellular pathogens, viruses; Th2-cells produce interleukin (IL)-4, IL-5, and IL-13, contribute to the elimination of parasites and Th17, producing IL-17 and IL-22, participate in the elimination of extracellular pathogens as fungi. These subsets may also be pathogenic. Th1 and Th17 can promote autoimmune diseases, whereas Th2-cells can cause allergic diseases. Especially, Th2-cells can induce all the cardinal features of allergic asthma through all the cytokines they produce.

The calcium signature differs between Th1, Th2, and Th17-cells suggesting that components regulating calcium entry may differ between each T-cell subset. The resting [Ca]i is the lowest in Th1, the highest in Th2, and intermediate in Th17. Conversely the TCR-dependent increase in [Ca]i is the highest in Th1, intermediate in Th17, and less important and sustained in Th2-cells, which could be related to the differential dependence of calcium-regulated transcription factors as NFAT, NFκB, and CREB (Dolmetsch et al., 1997) in the different T-cell subsets. It was suggested that these differences could result from lower equipment in pumps or in potassium channels required for maintaining the electrochemical driving force that supports calcium entry in Th2-cells, compared with the other T-cell subsets (Fanger et al., 2000). Our group identified voltage-dependent calcium Cav1.2 and Cav1.3 channels as selectively overexpressed in Th2-cells (Badou et al., 1997; Savignac et al., 2001, 2004; Gomes et al., 2006; Djata Cabral et al., 2010). Knocking down Cav1.2 and/or Cav1.3 α1 subunits by transfection with specific antisense oligodeoxynucleotides (Cav1AS) did not affect the proliferative response of Th2-cells but strongly impaired the TCR-dependent increase in [Ca]i and Th2 cytokine production without any effect on Th1-cells. We have then injected OVA-specific DO11.10 transgenic Th2-cells transfected or not with Cav1.2 plus Cav1.3 AS into BALB/c mice that were given intranasal OVA. Th2 Cav1AS localized into the lungs and proliferated as well as control Th2-cells. However they were unable to support a sustained inflammation characteristic of asthma. On the contrary, Th1 Cav1AS were as effective as control Th1-cells in the induction of inflammation. Antisense oligodeoxynucleotides were shown to remain localized into the airways when given by inhalation (Tanaka and Nyce, 2001). A mixture of Cav1.2 and Cav1.3 AS given by this route protected mice against the development of asthma (Djata Cabral et al., 2010), suggesting that these channels may represent an interesting new approach in the treatment of allergic diseases. Interestingly TCR stimulation is associated with polarized signaling as shown by an enrichment of Cav1AS (Lioudyno et al., 2008) and other ionic channels near the immune synapse, an area where the T-cell membrane contacts the antigen-presenting cell (Cahalan et al., 2011).
and Chandy, 2009). It will be important to assess whether Ca_1 channels traffic at the immune synapse upon TCR activation, in which areas and to identify the partners with which they associate.

Another important feature of the regulation of [Ca], will be the understanding of ionic channels as potassium and non-specific cationic channels in Ca_1 channel opening. Indeed K- channels comprising voltage and Ca^{2+}-activated channels maintain the electrochemical gradient of Ca^{2+} required for Ca^{2+} entry and tend to hyperpolarize the cell membrane favoring Ca_1 channel inactivation. Conversely, TCR-dependent TRPM4 activation induces Na^+ entry as limiting the Ca^{2+} entry and permitting Ca^{2+} oscillations (Launay et al., 2004). It would be interesting to determine if TRPM4 can favor Ca_1 channel opening.

**REGULATION OF Ca_1 CHANNELS IN LYMPHOCYTES**

All the authors showing the presence of Ca_1 channels in T-lymphocytes agree that these channels are not voltage-operated in physiological conditions. Therefore, how they are regulated in T-lymphocytes must be explained. Differences in the sequence/structure of Ca_1 channels in T-cells relative to the canonical forms in excitable cells have been reported and could provide an account for the absence of voltage sensitivity in T-cells (Stokes et al., 2004; Kotturi and Jefferies, 2005). However, the authors must demonstrate that the truncated Ca_1 channels are true Ca^{2+} channels. The analysis of T-lymphocytes from Ca_1.4 null and sufficient mice reveals the presence of voltage-gated currents in control cells, which were undetectable in null T-cells (Omilusik et al., 2011). Noticeably, the authors used peculiar conditions for their patch clamp experiments and mentioned that normal T-cells were pre-activated before recordings. This suggests that TCR activation could induce or enhance the number of Ca_1 channels at the cell membrane. The TCR-induced opening of Ca_1 channels may alternatively be explained by the existence of partners able to drive channel recruitment via two non-exclusive pathways: (i) post-translational modifications regulating Ca_1.2 channel trafficking, targeting, recycling, or degradation induced by TCR stimulation. We demonstrate that the sequence of Ca_1 channels in Th2-lymphocytes is similar to neuronal forms of the channel. However, Ca_1 channels do not seem to be voltage-operated in Th2-lymphocytes. We have already demonstrated that TCR-induced L-type dependent calcium influx is at least sensitive to Src kinases and the PKC in an IL-4 producing T-cell hybridoma (Savignac et al., 2001). In fact, the application of PP2, an inhibitor of Src kinases or an inhibitor of PKCα on Th2-cells suppresses the Ca_1 channel-dependent Ca^{2+} influx. In addition, we showed that PKC activator induced an entry of Ca^{2+}, suppressed by an antagonist of Ca_1 channels (Savignac et al., 2001). These data mean that kinase activation is implicated in Ca_1 dependent currents (Figure 1B). PKCα is a good candidate since Ca_1.2 channels can be constitutively activated at the resting potential of smooth arteriolar cells due to their interaction with PKCζ (Navedo et al., 2005; Santana and Navedo, 2010). Ryanodine receptors (RyR) are channels releasing Ca^{2+} from the ER into the cytosol. They are activated directly or not by Ca_1 channels. It is not known if Ca_1 channels interact with RyR in T-lymphocytes, inducing ER Ca^{2+} depletion and the activation of the STIM-ORAI pathway (Figure 1B).

The pending questions deal with how Ca_1 channels work in lymphocytes and their integration with other channels to generate a specific calcium signature. The relationships between STIM, ORAI, and Ca_1 are puzzling. STIM was shown as a negative regulator of Ca_1 signaling (Park et al., 2010). The possibility of a checkpoint controlling ORAI versus Ca_1 channel-dependent calcium responses merits to be explored.

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**REFERENCES**

Badou, A., Jha, M. K., Matza, D., Mehal, W. Z., Freichel, M., Flockerzi, V., et al. (2006). Critical role for the beta regulatory subunits of Cav channels in T lymphocyte function. Proc. Natl. Acad. Sci. U.S.A. 103, 15329–15334. doi: 10.1073/pnas.0607262103

Badou, A., Savignac, M., Moreau, M., Leclerc, C., Pasquier, R., Druet, P. et al. (1997). HgCl₂-induced IL-4 gene expression in T cells involves protein kinase C-dependent calcium influx through L-type calcium channels. J. Biol. Chem. 272, 32411–32418. doi: 10.1074/jbc.272.51.32411

Barr, V. A., Bernot, K. M., Shaffer, M. H., Burkhardt, J. K., and Samelson, L. E. (2009). Formation of STIM and ORAI complexes: puncta and distal caps. Immunol. Rev. 231, 148–159. doi: 10.1111/j.1600-065X.2009.00812.x

Cahalan, M. D., and Chandy, K. G. (2009). The functional network of ion channels in T lymphocytes. Immunol. Rev. 231, 59–87. doi: 10.1111/j.1600-065X.2009.00816.x

Djata Cabral, M., Paulet, P. E., Robert, B., Gomes, M. L., Renoud, M., and Savignac, C. (2010). Knocking-down Cav1 calcium channels implicated in Th2-cell activation prevents experimental asthma. Am. J. Respir. Crit. Care Med. 181, 1310–1317. doi: 10.1164/rccm.200907-1166OC

Dolmetsch, R. E., Lewis, R. S., Goodnow, C. C., and Healy, J. I. (1997). Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. Nature 386, 855–858. doi: 10.1038/386855a0

Fanger, C. M., Neben, A. L., and Cahalan, M. D. (2000). Differential Ca^{2+} influx, KCa channel activity, and Ca^{2+} clearance distinguish Th1 and Th2 lymphocytes. J. Immunol. 164, 1153–1160.

Feske, S., Giltnane, J., Dolmetsch, R., Staudt, L. M., and Rao, A. (2001). Gene regulation mediated by calcium signals in T lymphocytes. Nat. Immunol. 2, 316–324. doi: 10.1038/86318

Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S. H., Tanasa, B., et al. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 441, 179–185. doi: 10.1038/nature04702

Feske, S., Skolnik, E. Y., and Prakriya, M. (2012). Ion channels and transporters in lymphocyte function and immunity. Nat. Rev. Immunol. 12, 532–547. doi: 10.1038/nri3233

Gomes, B., Savignac, M., Cabral, M. D., Paulet, P., Moreau, M., Leclerc, C., et al. (2006). The cGMP/protein kinase G pathway contributes to dihydropyridine-sensitive calcium response and cytokine production in TH2 lymphocytes. J. Biol. Chem. 281, 12421–12427. doi: 10.1074/jbc.M50633200

Jha, M. K., Badou, A., Meissner, M., McRory, J. E., Freichel, M., Flockerzi, V., et al. (2009). Defective survival of naive CD8+ T lymphocytes in the absence of the beta3 regulatory subunit of voltage-gated calcium channels. Nat. Immunol. 10, 1275–1282. doi: 10.1038/ni.1793

Kotturi, M. F., Carlow, D. A., Lee, J. C., Ziltener, H. J., and Jefferies, W. A. (2003). Identification and functional characterization of voltage-dependent calcium channels in T lymphocytes. J. Biol. Chem. 278, 46949–46960. doi: 10.1074/jbc.M30928200

Kotturi, M. F., Hunt, S. V., and Jefferies, W. A. (2006). Roles of CRAC and Ca(V)-like channels in T cells: more than one gatekeeper? Trends Pharmacol. Sci. 27, 360–367. doi: 10.1016/j.tips.2006.05.007

Kotturi, M. F., and Jefferies, W. A. (2005). Molecular characterization of L-type calcium channel splice variants expressed in human T lymphocytes. Mol. Immunol. 42, 1461–1474. doi: 10.1016/j.molimm.2005.01.014

Launay, P., Cheng, H., Sivatsan, S., Penner, R., Fleig, A., and Kinet, J. P. (2004). TRPM4 regulates calcium oscillations after T cell activation. Science 306, 1374–1377. doi: 10.1126/science.1098845

Liao, P., and Soong, T. W. (2010). CaV1.2 channelopathies: from arrhythmias to autism, bipolar disorder, and immunodeficiency. Pflugers Arch. 460, 353–359. doi: 10.1007/s00424-009-0753-0

Lioydono, M. I., Kozak, J. A., Penna, A., Safrina, O., Zhang, S. L., Sen, D., et al. (2008). Orai1 and STIM1 move...
Ca_{1} channels in T-cells

Dihydropyridine receptors are selective markers of Th2 cells and can be targeted to prevent Th2-dependent immunopathological disorders. J. Immunol. 172, 5206–5212.

Stokes, L., Gordon, J., and Grafton, G. (2004). Non-voltage-gated L-type Ca^{2+} channels in human T-cells: pharmacology and molecular characterization of the major alpha pore-forming and auxiliary beta-subunits. J. Biol. Chem. 279, 19566–19573. doi: 10.1074/jbc.M401481200

Tanaka, M., and Nyce, J. W. (2001). Respirable antisense oligonucleotides: a new drug class for respiratory disease. Respir. Res. 2, 5–9. doi: 10.1186/rr153

Vig, M., and Kinet, J. P. (2009). Calcium signaling in immune cells. Nat. Immunol. 10, 21–27. doi: 10.1038/ni.f.220

Zhou, Y., Meraner, P., Kwon, H. T., Machnes, D., Masatsugu, O. H., Zimmer, J., et al. (2010). STIM1 gates the store-operated calcium channel ORAI1 in vitro. Nat. Struct. Mol. Biol. 17, 112–116. doi: 10.1038/nsmb.1724

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