Published in final edited form as:

Int J Biochem Cell Biol. 2013 November ; 45(11): 2491–2495. doi:10.1016/j.biocel.2013.07.014.

The Critical Role of STIM1-Dependent Ca²⁺ Signalling during T-cell Development and Activation

Elsie Samakai, Robert Hooper, and Jonathan Soboloff

Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Abstract

T lymphocytes are key cellular effectors of adaptive immunity able to recognize a virtually limitless number of antigenic peptides and mount an immune response. Ca²⁺ signals are crucial to the development and activation of T cells and Stromal Interaction Molecule 1 (STIM1) has been identified as a critical modulator of intracellular Ca²⁺ levels in T cells. Although the role of STIM1 in T cell activation has been extensively investigated, the role of STIM1 in T cell development has been somewhat controversial. Indeed, deficiencies in STIM1 expression and function lead to both developmental defects associated with the development of autoimmunity yet also interfere with T cell activation leading to severe combined immunodeficiency signifying a multifaceted role of STIM1 in T cell physiology and pathophysiology.

1. Introduction

The effective development of an immune response depends on activation and mobilization of multiple cell types through a complex integration of various signals. This immune response is divided into 2 main branches broadly categorized as the “innate” and “adaptive” response. Whereas innate immunity represents the first line of defense through cellular effectors that recognize a broad, but limited range of pathogens, adaptive immunity serves as the latent, but more specialized response. Adaptive immunity is able to handle an unlimited number of antigenic structures through the generation of receptors from a process called somatic recombination which eventually leads to the formation of a fully functional T cell Receptor (TCR) (Danilova, 2012). T lymphocytes or T cells are key effectors of adaptive immunity derived from common lymphoid progenitors (CLP) along with B cells and Natural Killer cells (Bhandoola and Sambandam, 2006). CLPs that will differentiate into T cells first must migrate to and settle in the thymus (Fig. 1). Within the thymus, multiple naïve T cell subsets are generated which subsequently migrate into the periphery where, upon antigen exposure, they develop into effector T cells capable of mounting a potent immune response directed against highly specific targets.

Engagement of the TCR leads to sustained increases in intracellular Ca²⁺ levels which are critical for T cell activation (Fig. 2). Indeed, the amplitude, duration and dynamics of Ca²⁺ signals dictate the specificity and efficiency of downstream pathways in activated mature T

© 2013 Elsevier Ltd. All rights reserved.

To whom correspondence should be addressed to: Jonathan Soboloff, Department of Biochemistry, Temple University School of Medicine, 3440 North Broad Street, Philadelphia, PA 19140. Office: (215) 707-6567; Fax: (215) 707-7536 soboloff@temple.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

cells (Lewis, 2001). A central mediator of elevated cytosolic Ca^{2+} levels is the endoplasmic reticulum (ER) transmembrane protein, STIM1. Downstream of TCR engagement, STIM1 is activated and translocates within the ER towards the PM where it interacts with and activates Orai1 (commonly referred to as the Ca^{2+} Release Activated Ca^{2+} (CRAC) channel (Soboloff et al., 2012)). It is noteworthy that homologs of both STIM1 (STIM2) and Orai1 (Orai2 and Orai3) are expressed in T cells, although their contribution to both Ca^{2+} entry and T cell activation is less well defined. Genetic knockout of STIM2 had a minimal effect on peak Ca^{2+} entry, but Ca^{2+} elevations were not sustained in the absence of STIM2, leading to loss of NFAT nuclear localization and cytokine production (Oh-Hora et al., 2008). Unfortunately, Orai2 and Orai3 knockout models have not been generated, so the impact of these homologues has not been similarly assessed. However, both Orai2 and Orai3 function as CRAC channels when overexpressed with STIM1 or STIM2 (DeHaven et al., 2007; Lis et al., 2007). Further, residual (<5%) Ca^{2+} entry can be detected in Orai1-deficient murine T cells (Gwack et al., 2008), but not human T cells (McCarl et al., 2010) which could be restored by overexpressing Orai2 only. As such, STIM1 and Orai1 are considered the primary mediators of CRAC in T cells. The current review addresses the role of CRAC in T cell effector function as well as its somewhat controversial role in T cell development.

STIM1 and T cell development

Early stages of thymic development—CLPs generated within bone marrow travel through the circulatory system into the thymus (Ma et al., 2013), where they go through successive stages of T cell development, with each stage distinguishable by a temporally coordinated repertoire of cell surface receptors (Fig. 1). Initially, T cell precursors lack both CD4 and CD8 expression and are referred to as Double Negative (DN) thymocytes. DN thymocytes proceed through 4 stages of differentiation within the cortex where extensive interactions with non-hematopoietic stromal cells dictate their progress towards maturity (Anderson and Jenkinson, 2001). Rearrangement of the TCR (α , β , and δ loci) and lineage commitment towards either the α/β or γ/δ lineages cannot be detected until the DN3 (CD25+CD44⁻) stage, at which point the TCR becomes critical for survival and differentiation. Following lineage commitment, α/β T cells progress through the DN4 stage, progressively gaining a fully functional α chain and eventually migrate to the medulla. Here they begin to express CD4 and CD8 receptors (now referred to as Double Positive (DP) thymocytes) (Starr et al., 2003).

Very little is known about the role of CRAC during early thymic development, however, there remains indirect evidence implicating the involvement of STIM1 at multiple developmental stages. For example, early thymic development is regulated by local levels of chemokines and cytokines (Bunting et al., 2011), many of which signal via Phospholipase C, thereby leading to STIM1-mediated Ca^{2+} entry (see Fig. 2). In addition, it was shown that strong TCR signals drive differentiation towards the α/β fate via the transcription factor Early Growth Response 1 (EGR1) (Hayes et al., 2005; Lauritsen et al., 2009). We have shown that EGR1 positively regulates STIM1 expression and that increases in STIM1 expression lead to more sustained Ca^{2+} signals in T cells (Ritchie et al., 2012; Ritchie et al., 2010). Hence, TCR signal strength (and by extension, differentiation towards the α/β fate) could be determined, at least in part, by STIM1 expression. Interestingly, a recent paper concluded that STIM1 was dispensable for early thymic development based on relatively small differences in the numbers of DN, DP and SP cells (Oh-Hora et al., 2013). This conclusion contrasts with the severe developmental defects observed in thymocytes lacking PLC- γ (Fu et al., 2010), which requires STIM1 to maintain Ca^{2+} signals. Whether this difference reflects knockout-specific compensatory mechanisms or the involvement of alternative Ca^{2+} signaling mechanisms during early thymic development remains an important and unresolved question.

Positive Selection—During positive selection, highly motile DP cells within the cortex encounter self-peptide/major histocompatibility (pMHC) complexes on the surface of stromal cells. Low to moderate avidity interactions between pMHC complexes and the TCR lead to Ca^{2+} entry (Daniels et al., 2006) which promotes survival, maturation and commitment to either CD4 or CD8 lineage (Fig. 1) (Romagnani, 2006) via calcineurin/NFAT4 activation (Starr et al., 2003). Interestingly, using three dimensional imaging of thymic splices, it was shown that pauses of migrating thymocytes during positive selection coincide with persistent and substantial Ca^{2+} oscillations (Bhakta et al., 2005) that promote productive interactions between thymocytes and stromal cells. Interestingly, in the absence of PLC- γ , progression beyond the DP stage was severely compromised (Fu et al., 2010), yet STIM knockout thymocytes progressed to the semi-mature SP thymocyte stage (CD24+) before exhibiting a developmental delay (Oh-Hora et al., 2013). Similar to DN thymocytes then, progression through the DP stage involves PLC-dependent Ca^{2+} signals, yet the dependence upon STIM is not entirely clear. Whether or not this indicates the involvement of alternative Ca^{2+} signaling pathways is not known, although examination of Ca^{2+} signals in thymic slices (as in (Bhakta et al., 2005)) from STIM-KO mice could address this question.

Negative Selection—Post-positive selection, surviving T cells then migrate to the medulla where high avidity interactions between pMHC complexes on medullary epithelial cells and the TCR result in death by apoptosis, also known as clonal deletion. This process leads to elimination of self-reactive T cells (Romagnani, 2006). Intriguingly, STIM1-deficient mice exhibited a moderate increase in total CD8+ T cells after negative selection (Oh-Hora et al., 2013). If, in fact, this reflects defective negative selection, then this increase in CD8+ T cells could reflect subpopulations of self-reactive T cells which could lead to autoimmunity, which is observed in CRAC-deficient patients (Feske, 2010), although the link to negative selection is not entirely clear due to the additional loss of suppressive T cell subtypes discussed further below.

Non-Conventional T cell development—While the major developmental pathways were described above, there are a number of rare T cell subsets that are also generated within the thymus such as regulatory T cells (Tregs), invariant natural killer T cells (iNKT) and CD8⁺ intestinal intraepithelial lymphocytes (CD8⁺-IELs) which function as negative regulators of T cell activation (Ronchi and Falcone, 2008; Corthay, 2009; van Wijk and Cheroutre, 2009). Intriguingly, decreased precursor numbers of all three of these rare T cells is observed in the absence of STIM (Oh-Hora et al., 2013). Development of these rare T cell subsets is thought to arise from exposure to alternate cytokines that rescue DP thymocytes from strong antigen receptor signals that would normally result in clonal deletion (Wirnsberger et al., 2011). Therefore, the fact that STIM-KO thymocytes exhibited decreased expression of CD122, the common γ chain of IL-2 and IL-15 receptors provides a potential explanation for the unique dependence of these cell types on STIM expression. As such, during development, STIM-mediated Ca^{2+} signals serve a prominent multi-faceted role in the negative regulation of immune function, in sharp contrast to their function in mature T cells as discussed further below.

2. STIM1 and T cell Activation

T cell activation is the process whereby naïve T cells are converted into effector T cells upon exposure to antigens. The first key step is the processing of antigens by Antigen Presenting Cells (APCs), leading to the expression of antigen-MHC complexes on the surface of these cells. Binding of these complexes to the TCR initiates a multi-step process resulting in the conversion of naïve T cells into a cohort of activated T cells targeting specific antigens. It is now clear that STIM1 is a critical early effector of T cell activation.

Hence, STIM1-dependent elevations in cytosolic Ca^{2+} are required for calcineurin-dependent NFAT activation, critical for virtually all aspects of this process including proliferation, expression of activation-associated genes and effector functions such as the production of cytokines and chemokines (Feske, 2007). However, recent investigations in our lab have revealed that STIM1 is required not only for Ca^{2+} entry, but also modulates Ca^{2+} clearance in activated T cells. Here we discuss the coordination of these events during T cell activation as well as their implications to this process.

Novel STIM1-mediated control of T cell activation

Engagement of the TCR by APCs leads to the formation of the immunological synapse (IS), within which extensive intercellular interactions occur. Formation of the mature IS occurs within approximately 10 minutes of the TCR coming in contact with a ligand, however, the IS is maintained for several hours, during which time the T cell actin cytoskeleton is rearranged to facilitate the redistribution of both signaling proteins and organelles into the immediate environment of the fully engaged TCR and co-receptors (Dustin et al., 2010). Perhaps not surprisingly, a number of key Ca^{2+} signaling proteins are also redistributed to the IS in this process including STIM1, Plasma Membrane Ca^{2+} ATPase (PMCA) and Orai1 (Fig. 3) (Ritchie et al., 2012; Quintana et al., 2011; Lioudyno et al., 2008; Barr et al., 2008). This leads to the creation of novel Ca^{2+} signaling microdomains localized to the IS under the control of STIM1 as discussed further below.

There is extensive evidence that both STIM1 and Orai1 are critical for T cell activation. Hence, either genetic knockout (Gwack et al., 2008; Oh-Hora et al., 2008) or loss-of-function mutations (Feske et al., 2006; Picard et al., 2009) of either protein leads to severe combined immunodeficiency. This reflects the strength of the link between cytosolic Ca^{2+} concentration and NFAT activation as well as the dependence of T cell activation on NFAT. However, recent investigations have revealed that the mechanisms regulating Ca^{2+} signaling elevation in activated T cells are more complex than initially imagined. Hence, PMCA4, previously identified as the major PMCA isoform expressed in Jurkat cells (Caride et al., 2001) has recently been shown to be inhibited at the IS by 2 independent laboratories (Quintana et al., 2011; Ritchie et al., 2012), although disparate mechanisms were proposed for this phenomenon. Based on a comparison of mitochondrial Ca^{2+} loading with Ca^{2+} clearance under both normal and supranormal extracellular Ca^{2+} concentration, it was proposed that mitochondrial Ca^{2+} loading at the IS sequesters Ca^{2+} from IS-localized PMCA, thereby leading to an effective decrease in PMCA pumping activity (Quintana et al., 2011). While our findings were in agreement with the many of those made in Quintana et al (2011), our data ultimately led us to a different conclusion (Ritchie et al., 2012). Hence, we found that STIM1 and PMCA4 not only co-localized, but were also physically associated. While we did observe enhanced mitochondrial Ca^{2+} loading in activated T cells and blocking mitochondrial Ca^{2+} loading altered the rate of Ca^{2+} clearance there were still substantial differences between PMCA function in resting and activated T cells. However this difference could be eliminated by STIM1 knockdown. Further, expression of STIM1_{WT} led to PMCA inhibition whereas a STIM1 mutant lacking its proline-rich domain (aa 66 – 629) which was fully effective as an activator of Orai1 failed to physically associate with PMCA4 or inhibit its function. Intriguingly, examination of the dynamics of Ca^{2+} entry and release in activated T cells revealed an unexpected directionality to both. Hence, when extracellular Ca^{2+} was increased from 0 to 1 mM in store-depleted activated T cells, Ca^{2+} elevation occurred at the ‘cap’ side of the cell prior to the IS side of the cell (Fig 3). Further, when extracellular Ca^{2+} was again removed, cytosolic Ca^{2+} concentration rapidly decreased at the cap side of the cell, but this recovery was delayed at the IS. This combined with the apparent accumulation of STIM1, PMCA4 and Orai1 at BOTH the IS and the cap (Barr et al., 2008; Ritchie et al., 2012) has led us to propose that both the entry and exit Ca^{2+} occur

predominantly at the cap in activated T cells, while accumulation of STIM1 at the IS may primarily function as an inhibitor of Ca^{2+} clearance. Given the approximately 10^5 -fold difference in Ca^{2+} concentration between the cytosol and the extracellular milieu, this mechanism would provide a remarkably energy efficient and simple strategy for locally elevating cytosolic Ca^{2+} concentration while still maintaining the capacity to remove Ca^{2+} in other parts of the cell to protect against Ca^{2+} overload.

Based upon our ability to co-immunoprecipitate STIM1 and PMCA in a manner dependent on the presence of the STIM1 proline-rich domain, we were tempted to speculate that STIM1 and PMCA associate via a conformational coupling model similar to the manner in which STIM1 regulates Orai1. However, attempts to show close interactions in situ using Förster Resonance Energy Transfer (FRET) were not successful (unpublished observations). Further, at approximately the same time period, a new protein termed Partner of STIM1 (POST) was identified which physically interacted with both PMCA and STIM1 (Krapivinsky et al., 2011) along with several other Ca^{2+} signaling proteins including SERCA and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX). Using a knockdown strategy, it was shown that POST inhibits PMCA function in a manner independent of mitochondrial Ca^{2+} loading. While the specific role of POST in control of PMCA function at the IS was not investigated in that study, it is tempting to speculate that POST maybe a critical adaptor of STIM1-mediated PMCA inhibition during T cell activation. Further, if indeed, POST does co-localize with STIM1 at the IS, it may also bring its numerous additional partners with it. The extent to which the function of other Ca^{2+} pumps and exchangers is also altered at the IS is not currently clear, but will undoubtedly be the subject of future investigations.

3. Associated Pathologies and Conclusions

As discussed in the preceding sections, STIM1 exhibits multi-faceted control over T cell development and activation. Given the tremendous impact of STIM1 as a modulator of immune function, it presents a highly tempting target for therapeutic strategies. However, the paradox that loss of STIM1 function leads to both autoimmune features and severe combined immunodeficiency presents a significant problem for predicting the potential impact of this strategy. Would blocking the expression and/or function of STIM1 inhibit T cell activation and alleviate autoimmune symptoms? Or would it lead to loss of regulatory T cell subtypes and/or increased numbers of self-reactive T cells? Currently, the prediction is that all of these events would occur unless peripheral T cells could be specifically targeted, leaving T cell development to proceed unimpeded. Future investigations focused on addressing this issue could ultimately lead to potent new therapeutic approaches towards the treatment of numerous autoimmune diseases.

References

- Anderson G, Jenkinson EJ. Lymphostromal interactions in thymic development and function. *Nat Rev Immunol.* 2001; 1:31–40. [PubMed: 11905812]
- Barr VA, Bernot KM, Srikanth S, Gwack Y, Balagopalan L, Regan CK, Helman DJ, Sommers CL, Oh-Hora M, Rao A, Samelson LE. Dynamic movement of the calcium sensor STIM1 and the calcium channel Orai1 in activated T-cells: puncta and distal caps. *Mol Biol Cell.* 2008; 19:2802–2817. [PubMed: 18448669]
- Bhakta NR, Oh DY, Lewis RS. Calcium oscillations regulate thymocyte motility during positive selection in the three-dimensional thymic environment. *Nat Immunol.* 2005; 6:143–151. [PubMed: 15654342]
- Bhandoola A, Sambandam A. From stem cell to T cell: one route or many? *Nat Rev Immunol.* 2006; 6:117–126. [PubMed: 16491136]
- Bunting MD, Comerford I, McColl SR. Finding their niche: chemokines directing cell migration in the thymus. *Immunol Cell Biol.* 2011; 89:185–196. [PubMed: 21135866]

- Caride AJ, Filoteo AG, Penheiter AR, Paszty K, Enyedi A, Penniston JT. Delayed activation of the plasma membrane calcium pump by a sudden increase in Ca^{2+} : fast pumps reside in fast cells. *Cell Calcium*. 2001; 30:49–57. [PubMed: 11396987]
- Corthay A. How do regulatory T cells work? *Scand J Immunol*. 2009; 70:326–336. [PubMed: 19751267]
- Daniels MA, Teixeira E, Gill J, Hausmann B, Roubaty D, Holmberg K, Werlen G, Hollander GA, Gascoigne NR, Palmer E. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature*. 2006; 444:724–729. [PubMed: 17086201]
- Danilova N. The evolution of adaptive immunity. *Adv Exp Med Biol*. 2012; 738:218–235. [PubMed: 22399382]
- DeHaven WI, Smyth JT, Boyles RR, Putney JW Jr. Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem*. 2007; 282:17548–17556. [PubMed: 17452328]
- Dustin ML, Chakraborty AK, Shaw AS. Understanding the structure and function of the immunological synapse. *Cold Spring Harb Perspect Biol*. 2010; 2:a002311. [PubMed: 20843980]
- Feske S. Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol*. 2007; 7:690–702. [PubMed: 17703229]
- Feske S. CRAC channelopathies. *Pflugers Arch*. 2010; 460:417–435. [PubMed: 20111871]
- Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 2006; 441:179–185. [PubMed: 16582901]
- Fu G, Chen Y, Yu M, Podd A, Schuman J, He Y, Di L, Yassai M, Haribhai D, North PE, Gorski J, Williams CB, Wang D, Wen R. Phospholipase C $\{\gamma\}$ 1 is essential for T cell development, activation, and tolerance. *J Exp Med*. 2010; 207:309–318. [PubMed: 20123962]
- Gwack Y, Srikanth S, Oh-Hora M, Hogan PG, Lamperti ED, Yamashita M, Gelinis C, Neems DS, Sasaki Y, Feske S, Prakriya M, Rajewsky K, Rao A. Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol Cell Biol*. 2008; 28:5209–5222. [PubMed: 18591248]
- Hayes SM, Li L, Love PE. TCR signal strength influences alphabeta/gammadelta lineage fate. *Immunity*. 2005; 22:583–593. [PubMed: 15894276]
- Krapivinsky G, Krapivinsky L, Stotz SC, Manasian Y, Clapham DE. POST, partner of stromal interaction molecule 1 (STIM1), targets STIM1 to multiple transporters. *Proc Natl Acad Sci U S A*. 2011; 108:19234–19239. [PubMed: 22084111]
- Lauritsen JP, Wong GW, Lee SY, Lefebvre JM, Ciofani M, Rhodes M, Kappes DJ, Zuniga-Pflucker JC, Wiest DL. Marked induction of the helix-loop-helix protein Id3 promotes the gammadelta T cell fate and renders their functional maturation Notch independent. *Immunity*. 2009; 31:565–575. [PubMed: 19833086]
- Lewis RS. Calcium signaling mechanisms in T lymphocytes. *Annu Rev Immunol*. 2001; 19:497–521. [PubMed: 11244045]
- Lioudyno MI, Kozak JA, Penna A, Safrina O, Zhang SL, Sen D, Roos J, Stauderman KA, Cahalan MD. Orai1 and STIM1 move to the immunological synapse and are up-regulated during T cell activation. *Proc Natl Acad Sci U S A*. 2008; 105:2011–2016. [PubMed: 18250319]
- Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, Fleig A, Penner R. CRACM1, CRACM2, and CRACM3 are store-operated Ca^{2+} channels with distinct functional properties. *Curr Biol*. 2007; 17:794–800. [PubMed: 17442569]
- Ma D, Wei Y, Liu F. Regulatory mechanisms of thymus and T cell development. *Dev Comp Immunol*. 2013; 39:91–102. [PubMed: 22227346]
- McCarl CA, Khalil S, Ma J, Oh-hora M, Yamashita M, Roether J, Kawasaki T, Jairaman A, Sasaki Y, Prakriya M, Feske S. Store-operated Ca^{2+} entry through ORAI1 is critical for T cell-mediated autoimmunity and allograft rejection. *J Immunol*. 2010; 185:5845–5858. [PubMed: 20956344]
- Oh-Hora M, Komatsu N, Pishyareh M, Feske S, Hori S, Taniguchi M, Rao A, Takayanagi H. Agonist-Selected T Cell Development Requires Strong T Cell Receptor Signaling and Store-Operated Calcium Entry. *Immunity*. 2013

- Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, Prakriya M, Feske S, Rao A. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol.* 2008; 9:432–443. [PubMed: 18327260]
- Picard C, McCarl CA, Papolos A, Khalil S, Luthy K, Hivroz C, LeDeist F, Rieux-Laucat F, Rechavi G, Rao A, Fischer A, Feske S. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med.* 2009; 360:1971–1980. [PubMed: 19420366]
- Quintana A, Pasche M, Junker C, Al-Ansary D, Rieger H, Kummerow C, Nunez L, Villalobos C, Meraner P, Becherer U, Rettig J, Niemeyer BA, Hoth M. Calcium microdomains at the immunological synapse: how ORAI channels, mitochondria and calcium pumps generate local calcium signals for efficient T-cell activation. *Embo J.* 2011; 30:3895–3912. [PubMed: 21847095]
- Ritchie MF, Samakai E, Soboloff J. STIM1 is required for attenuation of PMCA-mediated Ca^{2+} clearance during T-cell activation. *Embo J.* 2012; 31:1123–1133. [PubMed: 22246182]
- Ritchie MF, Yue C, Zhou Y, Houghton PJ, Soboloff J. Wilms tumor suppressor 1 (WT1) and early growth response 1 (EGR1) are regulators of STIM1 expression. *J Biol Chem.* 2010; 285:10591–10596. [PubMed: 20123987]
- Romagnani S. Regulation of the T cell response. *Clin Exp Allergy.* 2006; 36:1357–1366. [PubMed: 17083345]
- Ronchi F, Falcone M. Immune regulation by invariant NKT cells in autoimmunity. *Front Biosci.* 2008; 13:4827–4837. [PubMed: 18508548]
- Soboloff J, Rothberg BS, Madesh M, Gill DL. STIM proteins: dynamic calcium signal transducers. *Nat Rev Mol Cell Biol.* 2012; 13:549–565. [PubMed: 22914293]
- Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. *Annu Rev Immunol.* 2003; 21:139–176. [PubMed: 12414722]
- van Wijk F, Cheroutre H. Intestinal T cells: facing the mucosal immune dilemma with synergy and diversity. *Semin Immunol.* 2009; 21:130–138. [PubMed: 19386513]
- Wirnsberger G, Hinterberger M, Klein L. Regulatory T-cell differentiation versus clonal deletion of autoreactive thymocytes. *Immunol Cell Biol.* 2011; 89:45–53. [PubMed: 21042335]

Cell Facts

- T cells, which are key players in adaptive immune responses, can mount specialized immune responses against foreign peptides.
- Intracellular Ca^{2+} signals are crucial to both the development and activation of T cells.
- Defective Ca^{2+} signals can result in both severe combined immunodeficiency and a paradoxical development of autoimmunity.

Figure 1. Progression of T cell differentiation

Common Lymphoid progenitors (CLP) that arise from hematopoietic stem cells in the bone marrow migrate to the thymus near the cortico-medullary junction. Upon entry into the thymus, these precursors lack expression of CD4 and CD8 and are termed double negative (DN). DN cells move through the cortex (orange) as they progress through DN subsets, DN1 (CD44⁺/CD25⁻) and DN2 (CD44⁺/CD25⁺). As these cells progress to the DN3 (CD44⁻/CD25⁺) stage, rearrangement of the TCR α and β loci is evident and two distinct lineages of T cells (γ/δ and α/β) form. Following lineage commitment after the DN4 stage (CD44⁻/CD25⁻), α/β lineage cells migrate into the medulla (pink), expand and begin to express CD4 and CD8, a stage referred to as double positive (DP). As discussed in the text, DP cells undergo positive and negative selection before downregulating one of their CD4/CD8 receptors to become single positive (SP) cells which exit the thymus and enter the circulatory system. Within the periphery, these naïve SP cells can be activated upon exposure to antigens.

Figure 2. TCR-mediated control of store-operated Ca^{2+} entry during T cell activation
 The binding of antigens to the T cell Receptor (TCR) leads to the activation of the protein tyrosine kinase (PTK) Lck. Lck phosphorylates the CD3 chain of the TCR, leading to recruitment and activation of the PTK Zeta-chain-associated protein kinase 70 (ZAP70). ZAP70 phosphorylates Linker for Activation of T-cell (LAT) and LAT in turn ligates phospholipase C- (PLC-), leading to its activation. Activated PLC- hydrolyzes phosphatidylinositol 4,5-bisphosphate into inositol-1,4,5-trisphosphate (InsP_3) and diacylglycerol (DAG). Binding of InsP_3 to its endoplasmic reticulum (ER)-localized receptors leads to the transient release of Ca^{2+} from the ER into the cytosol. The Ca^{2+} pump, plasma membrane Ca^{2+} /ATPase (PMCA), is responsible for extracellular extrusion of Ca^{2+} . Decreases in ER Ca^{2+} content are 'sensed' by STIM1, causing it to translocate within the ER towards the PM where it interacts with Orail resulting in its activation and subsequent Ca^{2+} entry. The sarco/endoplasmic calcium ATPase (SERCA) pump replenishes ER Ca^{2+} stores.

Figure 3. Proposed model for co-ordination of Ca^{2+} signals in activated T cells

Engagement of the T Cell Receptor (TCR) by an antigen leads to the formation of the immunological synapse (IS). Within the IS, STIM1, PMCA and POST accumulate, leading to inhibition of Ca^{2+} extrusion and local, sustained Ca^{2+} elevation. At the opposite end of the cell, a 'distal cap' forms, within which STIM1 and Orai1 accumulate. At this site, Orai1 activation leads to transient elevation of cytosolic Ca^{2+} levels from which Ca^{2+} can freely diffuse throughout the cell.