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Physiological and pathophysiological functions of SOCE in the immune system

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1. ABSTRACT

Calcium signals play a critical role in many cell-type specific effector functions during innate and adaptive immune responses. The predominant mechanism to raise intracellular [Ca^{2+}] used by most immune cells is store-operated Ca^{2+} entry (SOCE), whereby the depletion of endoplasmic reticulum (ER) Ca^{2+} stores triggers the influx of extracellular Ca^{2+}. SOCE in immune cells is mediated by the highly Ca^{2+} selective Ca^{2+}-release-activated Ca^{2+} (CRAC) channel, encoded by *ORAI1*, *ORAI2* and *ORAI3* genes. ORAI proteins are activated by stromal interaction molecules (STIM) 1 and 2, which act as sensors of ER Ca^{2+} store depletion. The importance of SOCE mediated by STIM and ORAI proteins for immune function is evident from the immunodeficiency and autoimmunity in patients with mutations in *STIM1* and *ORAI1* genes. These patients and studies in gene-targeted mice have revealed an essential role for ORAI/STIM proteins in the function of several immune cells. This review focuses on recent advances made towards understanding the role of SOCE in immune cells with an emphasis on the immune dysregulation that results from defects in SOCE in human patients and transgenic mice.

2. INTRODUCTION TO STORE-OPERATED CALCIUM ENTRY BY STIM AND ORAI PROTEINS

The spatio-temporal regulation of intracellular calcium (Ca^{2+}) concentrations $[Ca^{2+}]_i$ is an important, conserved mechanism of signal transduction used by virtually all cell types including cells of the immune system (1-5). The engagement of various immunoreceptors such as antigen receptors on lymphocytes, Fc receptors on mast cells and macrophages or chemokine receptors on neutrophils results in a rapid increase in $[Ca^{2+}]_i$ (2). This cytoplasmic Ca^{2+} elevation can be supplied by two sources: the release of Ca^{2+} from the endoplasmic reticulum (ER) or the influx of Ca^{2+} from the extracellular space. The emptying of ER Ca^{2+} stores is rapid but generally results in only a minor increase in $[Ca^{2+}]_i$ as the volume of the ER is small in most immune cells. However, following ER Ca^{2+} store depletion, a secondary influx of extracellular Ca^{2+} through highly selective Ca^{2+} release activated Ca^{2+} (CRAC) channels in the plasma membrane results in a more substantial and sustained increase in cytosolic Ca^{2+} levels. This secondary Ca^{2+} influx downstream of ER Ca^{2+} stores is known as store-operated calcium entry (SOCE).

SOCE was postulated in 1986 (6) and measured in pancreatic acinar cells a few years later (7). The store-operated CRAC current (I_{CRAC}) was identified soon after in mast cells and T cells (8-10). The proteins responsible for SOCE and I_{CRAC} were not identified until 2005-2006 (11-15). These discoveries led to an exponential increase in studies describing SOCE and its role in cellular functions. SOCE is initiated when the ER Ca^{2+} stores are depleted, which is sensed by stromal interaction molecules (STIM) 1 and 2 (12, 13, 16). The STIM proteins are localized to the ER membrane, with their N-terminus extending into the ER and the C-terminus into the cytoplasm. Within the N-terminal region, STIM1 and STIM2 feature a pair of low affinity EF-hand Ca^{2+} binding domains. The decrease in the $[Ca^{2+}]_{ER}$ results in the dissociation of Ca^{2+} from the EF-hand Ca^{2+} binding domains, a conformational change in the N terminus of STIM1 and STIM2 and their oligomerization (17). The kinetics of this conformational change and the resulting oligomerization are different for STIM1 and STIM2. The multimerization of STIM1 occurs significantly faster than that of STIM2, but the STIM1 aggregates are also more readily dissociated than STIM2 oligomers (18). In addition to differential expression of STIM1 and STIM2 in cell types, these differences in oligomerization dynamics likely account for the distinct roles of STIM1 and STIM2 in mediating SOCE. In T cells, STIM1 plays a dominant role in the initial, large influx of Ca^{2+} during SOCE, whereas STIM2 acts to maintain Ca^{2+} influx and mediates the homeostasis of resting Ca^{2+} levels (16, 19). The activation of the Ca^{2+} dependent family of nuclear factor of activated T cells (NFAT) transcription factors requires a prolonged increase in $[Ca^{2+}]_i$ (16, 20), and therefore, at least in T lymphocytes, STIM1 and STIM2 both play a critical role in Ca^{2+}/NFAT-dependent gene expression.

Once activated, STIM proteins translocate to sites where the ER membrane is in close proximity to the plasma membrane. There, STIM1 and STIM2 interact directly with ORAI proteins (ORAI1-3). ORAI proteins are tetraspanning membrane proteins, which are the pore-forming subunits of the CRAC channel located in the plasma membrane (21-23). The first transmembrane domain of ORAI1 contains several amino acid residues that line the ion channel pore (24, 25), of which glutamate 106 is of particular importance as it provides the binding site for Ca^{2+} ions inside the channel and imparts the high Ca^{2+} selectivity to the CRAC channel (21-23). ORAI proteins possess intracellular N- and C-termini that mediate binding to STIM1, STIM2 and potentially other proteins regulating CRAC channel function. Once activated, ORAI proteins mediate a sustained influx of extracellular Ca^{2+}. This influx is dependent on an electrochemical concentration gradient that is generated by K^+ channels and plasma membrane Ca^{2+} ATPases (PMCA) that provide a negative plasma membrane potential and maintain a low resting $[Ca^{2+}]_i$, respectively. ORAI1 and STIM1 are widely expressed in many cell types in humans and mice (26-30). ORAI1 is the best studied ORAI family member and critical for CRAC channel function in T cells, mast cells and several other types of immune cells. In addition, high levels of ORAI2 and ORAI3 mRNA are found in human $CD19^+$ B cells and $CD14^+$ monocytes, respectively (30), suggesting that these ORAI isoforms play a role in SOCE and potentially the function of these immune cells. The molecular composition of the CRAC channel, its structure and regulation by STIM proteins as well as its physiological function in many diverse tissues are an area of intense study the discussion of which is beyond the scope of this review (31, 32).

3. THE ROLE OF STIM1 AND ORAI1 IN IMMUNE CELL FUNCTION

An increase in $[Ca^{2+}]_i$ is required for a variety of cellular immune functions. This section focuses on recent studies elucidating the role of SOCE and ORAI/STIM proteins in specific immune cell functions *in vitro* and in immune responses *in vivo* (Table 1). An emphasis is placed on studies using primary immune cells from mice and human patients lacking expression of functional ORAI and STIM genes.

3.1. Effector $CD4^+$ T cells

Subsets of effector $CD4^+$ T cells such as T helper (Th) 1, Th2 and Th17 cells produce cytokines such as IFN gammą IL-4 and IL-17, respectively, that are critical in mounting immune responses against pathogens (Table 1). SOCE in both human and murine $CD4^+$ T cells is primarily mediated by STIM1 and ORAI1 following peptide-MHC binding by the TCR; in addition, binding of chemokines to their receptors can also trigger SOCE that requires STIM1 (11, 16, 33-36). While STIM1 mediates the fast activation of SOCE and the initial peak in $[Ca^{2+}]_i$, STIM2 was shown to be required for maintaining sustained Ca^{2+} influx following stimulation of $CD4^+$ T cells (16).

SOCE mediated by ORAI1, STIM1 and STIM2 is dispensable for normal T cell development as the total number of T cells and the distribution of T cell subsets were normal in mice with targeted deletion of *Orai1*, *Stim1*, *Stim2* and *Stim1/Stim2* genes as well as human patients lacking SOCE due to mutations in *ORAI1* and *STIM1* genes (16, 29, 34-39). These findings suggest that the Ca^{2+} signals observed in immature T cells in the thymus (40, 41) are either not required for T cell development or may not be due to SOCE (mediated by ORAI1, STIM1 and STIM2). The depletion of ER Ca^{2+} stores resulting in low $[Ca^{2+}]_i$ increases may be sufficient to promote T cell development. Alternatively, immature T cells may utilize non-store operated Ca^{2+} channels for Ca^{2+} signaling.

Although T cells develop normally in the absence of SOCE, their function is drastically impaired. The proliferation of T cells isolated from STIM1- and ORAI1-deficient patients was significantly reduced following *in vitro* stimulation with anti-CD3, antigens or mitogens (5, 29, 35, 42). This defect in T cell proliferation was also observed in murine lymphocytes lacking both *Stim1* and *Stim2* genes, but not in murine T cells lacking only *Stim1* or *Orai1* gene function (*Orai1*$^{-/-}$ or *Orai1* knock-in (KI) mice, the latter expressing a nonfunctional ORAI1-R93W protein unable to mediate I_{CRAC}) (16, 34, 36). Additionally, effector cytokine production by $CD4^+$ T cell subsets was almost completely absent in SOCE-deficient T cells (16, 33, 34, 36, 38). In accordance with a critical role for SOCE in T

Table 1. SOCE dependent processes in cells of the immune system and immune responses *in vivo*

Cell Type	SOCE induced by	Cellular immune functions *in vitro*	Immune responses *in vivo*	References
$CD4^+$ T cells	TCR/CD3, chemokine receptors	Proliferation: • Human T cells stimulated with anti-CD3, antigen or mitogen • Mouse $CD4^+$ T cells (impaired in STIM1/STIM2-deficient T cells only) • Mouse Th17 cells stimulated with anti-CD3 • Mouse B cells stimulated with anti-IgM (but not LPS) Cytokine production: • IFN gamma in Th1 cells • IL-4, IL-10 in Th2 cells • IL-17A, IL-17F, IL-22 in Th17 cells	Rejection of MHC class I/II mismatched skin allografts Graft vs host disease Contact hypersensitivity (Th2 cells) Autoimmune inflammation: • Inflammatory bowel disease following transfer of naive $CD4^+$ T cells (Th1, Th17 cells) • Experimental autoimmune encephalomyelitis (Th17 cells)	5, 11, 16, 29, 33-39, 42, 43, 46
$CD4^+Foxp3^+$ Regulatory T cells (Treg)	TCR/CD3	Intrathymic development of Treg cells Suppression of T cell proliferation (STIM1/2-deficient Treg only)	Myelo-lymphoproliferation and autoimmunity (in *$Stim1^{fl/fl}Stim2^{fl/fl}$ Cd4-Cre* mice)	16
$CD8^+$ T cells	TCR/CD3	Target cell lysis Degranulation IFN gamma, TNF alpha production	Rejection of MHC class I/II mismatched skin allografts	16, 34-36, 38, 39, 42, 60, 61
NK cells	CD16	Target cell lysis Degranulation IFN gamma and TNF alpha production		60-64
B cells	BCR	Anti-IgM-induced proliferation IL-10 production	Regulatory B cell function (IL-10 dependent control of EAE)	29, 35, 38, 42, 62, 63, 66
Dendritic cells	TLR4, purinergic receptors	DC maturation (CD80, CD86, MHC class II expression) TNF alpha production Chemotaxic migration (to CCL21)		73-75
Mast cells	Fc epsilon receptor I	Release of secretory granules containing serotonin, histamine, beta-hexosaminidase LTC_4 synthesis IL-6, IL-13 and TNF alpha production	Fc epsilon receptor I-dependent passive cutaneous anaphylaxis	37, 39, 79
Macrophages	Fc gamma receptor II, IIIa	Phagocytosis of opsonized mouse red blood cells, platelets MIP-2, TNF alpha production C5a production	Autoimmune hemolytic anemia, thrombocytopenia Immune complex-mediated pneumonitis	80
Neutrophils	Fc gamma receptor, fMLP receptor	Fc gamma receptor-induced phagosomal ROS production beta 2 integrin-mediated cellular arrest		29, 35, 42, 89-91, 98

Only those physiological stimuli, effector functions and immune responses are listed for which the involvement of ORAI and STIM proteins has been directly shown. Abbreviations: BCR, B cell receptor; LTC_4, leukotriene C_4; TCR, T cell receptor; Th, T helper; TLR, toll-like receptor.

cell function not just *in vitro* but also *in vivo*, fully MHC mismatched skin allografts from BALB/c mice transplanted onto C57BL/6 mice were tolerated significantly longer by ORAI1-deficient than wildtype mice (36). Since transplant rejection depends on both $CD4^+$ and $CD8^+$ T cells, this finding shows that SOCE is critical for allograft responses by $CD4^+$ T cells (43). Similar observations were made in a model of acute graft-vs-host disease (GvHD) in which $CD4^+$ T cells from *$Stim1^{-/-}$* mice were injected (together with bone marrow) into irradiated BALB/c mice (34). While all mice that had received allogenic $CD4^+$ T cells from *$Stim1^{-/-}$* mice developed GvHD, only ~ 50% of mice succumbed to the disease in contrast to 100% of mice that had received wildtype $CD4^+$ T cells.

SOCE plays a critical role in the function of all $CD4^+$ T helper cell subsets. STIM1- and ORAI1-deficient Th2 cells have reduced IL-4 and IL-10 production *in vitro* and accordingly Th2-mediated contact hypersensitivity was completely absent in *$Orai1^{KI/KI}$* mice (36). In the absence of SOCE, the production of IFN gamma and IL-2 by Th1 cells is decreased (16, 33, 34, 36, 38). Consequently, naive $CD4^+$ T cells from STIM1- and ORAI1-deficient mice failed to induce inflammatory bowel disease (IBD) when transferred into lymphocyte-deficient hosts in contrast to T cells from wildtype mice, which caused severe colitis in recipient animals (36). In this model, IBD is primarily dependent on Th1 and Th17 cells (44), demonstrating a critical role for SOCE in Th1 and Th17 effector cell function *in vivo*.

Our studies suggest that the dependence of Th17 cells on SOCE is greater than that of other $CD4^+$ T cell subsets. Murine $CD4^+$ T cells lacking expression of *Stim1* or *Stim2* genes failed to produce IL-17A, IL-17F and IL-22

when isolated directly from mice or after culture for 3 days under Th17 polarizing conditions despite normal expression of ROR gamma t, a key transcription factor responsible for the differentiation of Th17 cells (33, 45, 46). As a result of impaired Th17 function, mice lacking *Stim1* or *Stim2* gene expression in T cells or all hematopoietic cells were resistant to induction of experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis that is highly dependent on autoreactive Th17 cells (33, 46). Protection from EAE may also be due to the inability of STIM1-deficient Th17 cells to proliferate, a defect that was specific to Th17 cells and that was not observed in Th1 or Th0 cells (33). This dependence of Th17 cells on SOCE was also observed *in vivo*. When STIM1-deficient T cells were isolated from mice immunized with MOG peptide to induce EAE, restimulated and cultured under Th17 polarizing conditions *in vitro* and injected into lymphopenic host mice, they failed to expand in contrast to wildtype T cells (33). Impaired proliferation was also observed when STIM1-deficient T cells were co-injected with wildtype Th17 cells suggesting that this defect is intrinsic to SOCE-deficient Th17 cells (33). The molecular mechanisms underlying the SOCE dependence of Th17 cells is unknown, but could be due to the reduced expression of IL-23R on Th17 cells (33), which together with IL-23 is critical for the terminal differentiation and lineage stability of Th17 cells (47, 48). It was recently found that granulocyte macrophage colony stimulating factor (GM-CSF) production by Th17 cells is required to induce EAE (49, 50). Since GM-CSF production was dependent on IL-23, the decreased IL-23R expression in STIM1-deficient Th17 cells may contribute to their impaired ability to cause autoimmune CNS inflammation. Taken together, SOCE mediated by ORAI and STIM proteins is required for effector functions of Th1, Th2 and Th17 cells, in particular cytokine production, which are essential for host immune responses to pathogens and in autoimmune inflammatory processes.

3.2. Regulatory T cells

Regulatory T (Treg) cells are a suppressive subset of lymphocytes that play a critical role in maintaining immunological self-tolerance. Treg cell development and function require the transcription factor forkhead box P3 (Foxp3) (51). The importance of $Foxp3^+$ Treg cells is evident in patients and mice with mutations in Foxp3 that abolish gene expression or Foxp3 function. Immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome in humans and the scurfy phenotype in mice (52, 53) are dominated by severe autoimmunity. Mice with conditional deletion of both *Stim1* and *Stim2* genes in T cells have significantly reduced $Foxp3^+$ Treg populations in the thymus and secondary lymphoid organs indicating that Treg development requires SOCE (Table 1) (16). This requirement is intrinsic to Treg cells as mixed bone marrow chimeric mice (lymphopenic $Rag2^{-/-}$ mice which had received hematopoietic stem cells from congenically marked STIM1/STIM2-deficient and wildtype mice) also showed severely impaired Treg development (16). As a consequence, mice with T-cell specific deletion of *Stim1* and *Stim2* genes developed immunopathology in the form of lymphadenopathy, splenomegaly and inflammatory infiltration of internal organs such as lung and liver with lymphoid and myeloid cells (16). A defect in Treg development and autoimmunity was not observed in STIM1-deficient mice, suggesting that the contribution of STIM2 to SOCE in T cells is sufficient for Treg development (16, 34). ORAI1-deficient mice also had normal Treg development, presumably due to residual SOCE in naive $CD4^+$ T cells which is likely to be mediated by ORAI2 and/or ORAI3 (29). Taken together, these findings indicate that the SOCE threshold required for Treg development is low and that only a complete lack of SOCE in STIM1- and STIM2-deficient mouse T cells is sufficient to interfere with Treg development (16).

SOCE is not only required for Treg development but also their suppressive function. Residual $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells isolated from $Stim1^{fl/fl}$ $Stim2^{fl/fl}$ *Cd4-Cre* mice failed to suppress the proliferation of stimulated wildtype $CD4^+$ T cells (16). By contrast, Treg cells from STIM1- and ORAI1-deficient mice showed only a moderate reduction in their suppressive activity compared to wildtype Treg cells, indicating that Treg cells require low levels of SOCE not only for their development but also for their function (34, 36). Nevertheless, $CD4^+$ and $CD8^+$ T cells isolated from spleen and lymph nodes of ORAI1-, STIM1- and especially STIM1/STIM2-deficient mice display an activated phenotype *in vivo* that is associated with a decrease in naive $CD62L^{hi}$ T cells, an increase in $CD44^+$ antigen-experienced memory T cells as well as lymphadenopathy and splenomegaly (16, 34, 36). This phenotype is likely to be due to, at least in part, impaired Treg function *in vivo* in the absence of *Orai1*, *Stim1* or both *Stim1* and *Stim2* expression. Despite a significant reduction in the number and function of $Foxp3^+$ Tregs in mice with T-cell specific deletion of *Stim1* and *Stim2* genes, their autoimmune inflammatory phenotype is less severe than that observed in Treg-deficient scurfy mice (54), which is likely due to the significantly impaired effector T cell function in STIM1/STIM2-deficient but not scurfy mice. Taken together, SOCE emerges to be a critical regulator of Treg development and function.

3.3. Cytotoxic lymphocytes

Cytotoxic lymphocytes, such as $CD8^+$ T cells and NK cells, play a critical role in antiviral immune responses and tumor immunosurveillance through the killing of virus-infected and tumor cells, respectively. $CD8^+$ T cells recognize antigen presented to them by MHC class I molecules via their T cell receptor resulting in the polarization and release of cytotoxic granules. Natural killer (NK) cells can attack a wide spectrum of target cells. Virus-infected cells, for instance, directly activate NK cell activating receptors such as Ly49H and NKp46 (55-57), whereas antibodies bound to target cell surface structures are recognized by low affinity Fc gamma receptors for IgG (CD16) on NK cells resulting in the release of cytotoxic granules and cytokines. An important role of SOCE in $CD8^+$ T cells and NK cells can be inferred from the susceptibility of human patients with mutations in ORAI1 and STIM1 to severe viral infections (as will be discussed in more detail in chapter 4) (29, 35, 42, 58, 59).

Similar to $CD4^+$ T cells, SOCE in $CD8^+$ cells is predominantly mediated by STIM1 and ORAI1 following TCR stimulation (36, 38, 60). $CD8^+$ T cells from patients with mutations in *ORAI1* and *STIM1* or STIM1/STIM2-deficient mice lack SOCE and CRAC channel function (16, 36, 38, 61). Residual Ca^{2+} influx and I_{CRAC} are observed in naive $CD8^+$ T cells from *Orai1*$^{-/-}$ and *Orai1*$^{KI/KI}$ mice. These are no longer present when $CD8^+$ T cells are differentiated into cytotoxic T cells (CTL) *in vitro*, suggesting that the composition of the CRAC channel may change during $CD8^+$ T cell differentiation (36, 38, 39). Whether this is also the case in human $CD8^+$ T cells is unknown. Despite severely impaired SOCE in ORAI1- and STIM1-deficient mice and patients with mutations in *ORAI1* and *STIM1*, the development of $CD8^+$ T cells was unimpaired with normal numbers of $CD8^+$ T cells in the blood, thymus and secondary lymphoid organs (16, 34-36, 42, 62). SOCE-deficient $CD8^+$ T cells were, however, severely impaired in their ability to produce cytokines, especially IFN gamma, whereas expression of perforin and granzyme B, two enzymes required for cytotoxicity of CTL, was normal (Table 1) (16, 36). Human $CD8^+$ T cells from a patient homozygous for the ORAI1-R91W mutation expressed significantly reduced amounts of many cytokine and chemokine genes including TNF alpha and IFN gamma (63), which are critical for antitumor and antiviral immune responses by $CD8^+$ T cells. When treated with a pharmacologic inhibitor of SOCE, DPB162-AE (64), human $CD8^+$ T cells exhibited impaired killing of tumor target cell *in vitro* (60). The cytotoxic function of CTL requires the exocytosis of lytic granules containing perforin and granzymes. This degranulation is dependent on SOCE and was found to be impaired in human and mouse $CD8^+$ T cells lacking ORAI1 function (60). Consistent with the requirement for SOCE in $CD8^+$ T cell function, *Orai1*$^{KI/KI}$ mice exhibited an impaired ability to reject tail skin transplants from histoincompatible donor mice (36).

NK cells, like $CD8^+$ T cells, mediate antitumor and antiviral immune responses that require the production of cytokines such as IFN gamma and TNF alpha and direct, cell-mediated cytotoxicity via the release of cytolytic granules at the NK-target cell synapse. NK cells from patients with mutations in *ORAI1* and *STIM1* lacked SOCE in response to CD16 cross-linking or passive store depletion with thapsigargin indicating that SOCE in human NK cells is primarily mediated by STIM1 and ORAI1 (60). As a consequence, the production of IFN gamma and TNF alpha was severely compromised in the patients' NK cells when these were cocultured with tumor cells (60). As for CTL, the cytolytic function of NK cells requires the polarization and release of cytotoxic granules in response to target cell recognition (65). ORAI1- and STIM1-deficient human NK cells failed to degranulate as measured by CD107a surface expression and were almost completely incapable of killing tumor target cells *in vitro* (60). A similar if less pronounced defect in NK cell degranulation was observed in NK cells from *Orai1*$^{KI/KI}$ mice (SF and S. Ehl, unpublished). The significance of the functional defect observed in SOCE-deficient NK cells for immunity to viral infections in patients and mice is not well understood. In this context it is noteworthy that hematopoietic stem cell transplantation (HSCT) in an ORAI1-deficient patient resulted in partial immune reconstitution and his protection from severe infections although the patient's blood contained only 10-15% donor T cells and no donor NK cells (60). Taken together, STIM1 and ORAI1 mediate Ca^{2+} influx and the cytotoxic function of human and mouse $CD8^+$ T cells and NK cells suggesting that SOCE is essential for antiviral and antitumor immune responses.

3.4. B cells

Antigen recognition by B cells through the B cell receptor (BCR) leads to the proliferation of B cells and their differentiation into antibody-secreting plasma cells. At the molecular level, BCR engagement results in the activation of tyrosine kinases such as Syk and Btk (Bruton's tyrosine kinase), phosphorylation of the scaffold protein BLNK (B cell linker protein), activation of PLC gamma 2 and emptying of ER Ca^{2+} stores. SOCE in B cells is dependent on STIM1 and ORAI1 as B cells from human patients with mutations in ORAI1 or STIM1 genes, *Orai1*$^{-/-}$ mice or mice with B-cell specific deletion of *Stim1* and *Stim2* lacked Ca^{2+} influx when stimulated through the BCR or by passive store depletion with thapsigargin (38, 42, 63, 66). A similar defect in SOCE was observed in DT40 chicken B cells lacking STIM1, whereas siRNA mediated suppression of ORAI1 had no effect on SOCE (67). SOCE is not required for B cell development as ORAI1 and STIM1-deficient patients and mice had normal numbers and subpopulations of B lymphocytes (Table 1) (16, 29, 35, 36, 38, 42, 66). Functionally, SOCE-deficient B cells showed a moderate decrease in proliferation in response to BCR cross-linking with anti-IgM antibodies, but not following lipopolysaccharide (LPS) stimulation confirming the important role of SOCE for BCR signaling (38, 66). By contrast, the serum levels of IgM, IgG and IgA were normal in ORAI1- and STIM1-deficient patients (29, 35, 42, 62), suggesting that SOCE is not required for B cell maturation and antibody production. Nevertheless, specific antibodies against vaccination antigens such as diphteria and tetanus toxoids or pathogens that patients had encountered previously such as *C. albicans* were absent in ORAI1- and STIM1-deficient patients (29). This lack of seroconversion is likely to be due to impaired T cell help for B cells rather than a B-cell intrinsic defect. These findings are consistent with normal T-cell independent (TI) and T-cell dependent (TD) antibody responses to immunization with 4-hydroxy-3-nitrophenyl (NP)-LPS and chicken gamma globulin (CGG), respectively, in mice with B-cell specific deletion of both *Stim1* and *Stim2* (66). T cells from these *Stim1*$^{fl/fl}$ *Stim2*$^{fl/fl}$ *Mb1-Cre* mice have normal SOCE allowing them to support TD antibody responses unlike T cells in ORAI1/STIM1-deficient patients whose T cells lack SOCE.

Expression of cytokines in B cells, like that in T cells, requires SOCE. IL-10 production was drastically reduced in splenic B cells from *Stim1*$^{fl/fl}$ *Stim2*$^{fl/fl}$ *Mb1-Cre* mice in response to LPS and anti-IgM stimulation (66). This defect is of particular importance for the function of a small subset of $CD1d^{hi}CD5^+$ regulatory B cells, which lacked IL-10 production. IL-10-producing B cells were found to have a protective role during T-cell induced CNS inflammation in EAE (68) and accordingly *Stim1*$^{fl/fl}$

Stim2$^{fl/fl}$ *Mb1-Cre* mice lacking IL-10 production showed excacerbated EAE. It is noteworthy that in contrast to Foxp3$^+$ regulatory T cells, the numbers of regulatory B cells were normal. In addition, *Stim1*$^{fl/fl}$ *Stim2*$^{fl/fl}$ *Mb1-Cre* mice apparently did not spontaneously develop autoimmunity as a consequence of impaired regulatory B cell function, which is in contrast to Treg deficient *Stim1*$^{fl/fl}$ *Stim2*$^{fl/fl}$ *Cd4-Cre* mice. This is surprising insofar as STIM1 and SOCE were recently reported to regulate the Ca^{2+} dependent activation of the MAP kinase Erk in B cells undergoing negative selection during B cell development in the bone marrow (69). Overexpression of STIM1 in bone marrow stem cells had a proapoptotic effect and resulted in the loss of those developing B cells that recognized self-antigen during negative selection. If mice lacking *Stim1* and *Stim2* expression in B cells had a significant defect in negative selection, one would expect an autoimmune phenotype in these mice, which has not been reported (66). It is possible that negative selection is unaffected in *Stim1*$^{fl/fl}$ *Stim2*$^{fl/fl}$ *Mb1-Cre* mice because STIM1 and STIM2 protein levels are not fully depleted during negative selection (which takes place in immature IgM^{lo} B cells), although Mb1-driven Cre expression occurs after the ProB cell stage and therefore precedes negative selection. Alternatively, it is possible that the proapoptotic effect of STIM1 is only observed under conditions of STIM1 overexpression but does not occur at physiological STIM1 levels, explaining why STIM-deficient mice showed no apparent signs of impaired negative selection. Taken together, SOCE in B cells appears to be largely dispensable for the development of B cells as well as antibody production, but is required for IL-10 expression and the function of regulatory B cells.

3.5. Dendritic cells

Dendritic cells (DC) are a heterogenous population of immune cells, whose primary function is to present pathogen-derived antigens to T cells and to provide them with costimulatory signals. Immature DC in peripheral tissues take up antigen, process and load it onto MHC class II molecules, and present it to T cells following their migration to lymph nodes. DC mature into fully functional antigen presenting cells (APC) after having received additional activation signals in the context of infection or inflammation (e.g. viral RNA, LPS, stimulation through CD40 on the DC cell surface). Ca^{2+} signaling is required for a number of dendritic cell functions including maturation, apoptotic body engulfment and migration (70-72). Ca^{2+} influx in murine myeloid DC is predominantly mediated by SOCE in response to LPS, ATP or thapsigargin stimulation (73, 74). Ca^{2+} currents recorded in these cells had biophysical properties similar to those of I_{CRAC} and could be blocked by SKF 96365, a non-specific inhibitor of Ca^{2+} channels (73, 74). Which ORAI and STIM proteins are responsible for SOCE in DCs remains to be elucidated but one recent study suggested that SOCE in murine bone-marrow derived DCs is mediated primarily by ORAI2 and STIM2 (but not STIM1) (75). Nevertheless, SOCE is required for various DC functions (Table 1). Expression of the DC maturation markers CD80, CD86 and MHC class II was enhanced following induction of SOCE by thapsigargin or LPS treatment and inhibited by SKF-96365 (73, 74). In addition, the expression of the proinflammatory cytokines TNF alpha and IL-6 by DCs and their chemotactic migration *in vitro* in response to CCL21 were impaired in the presence of SKF-96365 (73). Since SKF-96365 is not a selective CRAC channel inhibitor, further studies are required to assess the contribution of ORAI and STIM proteins to DC function and immune responses *in vivo*.

3.6. Mast cells

Mast cells play an important role at the interface of innate and adaptive immunity and contribute to immune responses to bacterial and viral infections (76-78). However, mast cells can also be injurious and act as the central mediator of immediate hypersensitivity reactions and allergic diseases. The primary mechanism of mast cell activation is through the crosslinking of high affinity IgE receptor (Fc epsilon RI) by multivalent antigens. Mast cell activation results in degranulation and the production and release of proinflammatory lipids and cytokines. Murine mast cells lacking either *Stim1* or *Orai1* expression exhibited severely impaired SOCE in response to antigen-mediated Fc epsilon receptor I crosslinking or thapsigargin treatment (37, 39). The development of SOCE-deficient mast cells, their proliferation, morphology and Fc epsilon receptor I expression were normal compared to wild-type mast cells. However, antigen-dependent mast cell degranulation was reduced in SOCE-deficient mast cells (37, 39), similar to SOCE-deficient CTLs (Table 1). The rearrangement of microtubules, required for cellular degranulation and Ag-dependent mast cell motility, was found to be dependent on SOCE, and drastically reduced in STIM1-deficient mast cells (79). Additionally, the Fc epsilon RI-dependent production and release of proinflammatory cytokines IL-6, TNF alpha and IL-13 required STIM1 and ORAI1 (37, 39). Based on the role of SOCE in mast cell functions *in vitro*, Fc epsilon receptor-induced mast cell-mediated passive cutaneous anaphylaxis *in vivo* was abolished in ORAI1-deficient mice and strongly reduced in *Stim1*$^{+/-}$ mice (37, 39). The reduced anaphylactic response in *Stim1*$^{+/-}$ mice suggests that even a moderate reduction in SOCE significantly impairs mast cell effector functions. Future studies will show whether the SOCE dependence of mast cell function can be exploited therapeutically to alleviate atopic diseases such as allergic asthma.

3.7. Macrophages

Macrophages constitute one of the first lines of immune defense against pathogens. Their main functions include the phagocytosis of cell debris and pathogens, and the production of cytokines such as IL-1 beta, IL-6 and IL-12, which allow them to signal to other immune cells and activate immune responses. Recognition of antibody-opsonized pathogens by macrophages occurs via Fc gamma receptors on their cell surface. Fc gamma receptor crosslinking in macrophages causes SOCE that is dependent on STIM1 as Ca^{2+} influx was severely impaired in F4/80$^+$ peritoneal macrophages isolated from *Stim1*$^{-/-}$ mice that were stimulated with thapsigargin or Fc gamma receptor II/IIIa crosslinking (80). The lack of *Stim1* expression did not interfere with the development of monocytes and their differentiation into macrophages. An

increase in $[Ca^{2+}]_i$ was shown to be required for several macrophage functions, for instance the respiratory burst resulting in the generation of reactive oxygen species (ROS), nitric oxide production, secretion of microbicidal granules and synthesis of proinflammatory mediators (81-83). In addition, a rise in $[Ca^{2+}]_i$ has been implicated in phagosome maturation, a process during which phagosomes containing ingested pathogens fuse with lysosomes in order to kill the pathogen. Several studies have shown that a rise in $[Ca^{2+}]_i$ is required for phagosome-lysosome fusion (84-86), whereas other groups reported that this process is Ca^{2+} independent (87) or inhibited by an increase in intracellular calcium (88).

The most direct evidence for a role of SOCE in macrophage function comes from studies in *Stim1*$^{-/-}$ mice (Table 1). The production of cytokines such as MIP-2 and TNF alpha by STIM1-deficient macrophages in response to Fc gamma receptor crosslinking was only moderately impaired (80). By contrast, SOCE-deficient macrophages showed significantly reduced IgG2a and IgG2b mediated phagocytosis of opsonized red blood cells (80). Accordingly, STIM1-deficient mice were resistant to the induction of hemolytic anemia and thrombocytopenia in murine models of autoimmune disease following the binding of antibody-opsonized red blood cells and platelets to Fc gamma receptors on macrophages and their phagocytosis (80). *Stim1*$^{-/-}$ mice were also protected from developing immune complex-mediated pneumonitis which is mediated by tissue resident macrophages in the lung following binding of circulating immune complexes (IC) to their surface Fc gamma receptors (80). STIM1-deficient macrophages failed to infiltrate lung alveoli and to cause hemorrhage, which has been attributed to the impaired production of the vasoactive complement factor C5a. It is noteworthy that in contrast to *Stim1*$^{-/-}$ mice, STIM1 deficient patients were not protected from autoimmune hemolytic anemia and thrombocytopenia which was associated with the presence of anti-erythrocyte and anti-platelet autoantibodies (35). The cause for this discrepancy between human and mice is unknown. Further studies are required to elucidate, for instance, the role of SOCE for the function of tissue resident macrophages in the liver, CNS and other tissues and the ability of infected macrophages to promote phagosome maturation and to kill intracellular pathogens.

3.8. Neutrophils

Neutrophils, like macrophages, are first line responders to many infections. Neutrophils recognize antibody-opsonized pathogens and immune complexes (IC) containing antigens and antibodies via Fc gamma receptors on their cell surface. This results in the phagocytosis and destruction of pathogens and IC in the phagosome in a process that requires the production of reactive oxygen species (ROS) such as O_2^- and H_2O_2. Ca^{2+} influx in neutrophils is the result of crosslinking of Fc gamma receptors IIa and IIIb or binding of chemokines such as IL-8 and formyl Met-Leu-Phe (fMLP) to their receptors (89). Ca^{2+} influx in neutrophils is mediated by SOCE through ORAI1 and STIM1 as fMLP or thapsigargin induced Ca^{2+} levels were reduced in neutrophils from *Orai1*$^{+/-}$ mice and in the HL60 neutrophil cell line following the attenuation of ORAI1 and STIM1 expression by siRNA (90, 91). Furthermore, Ca^{2+} influx was absent in neutrophils from immunodeficient patients (42) that were later identified to lack ORAI1 protein expression (29). The numbers of polymorphonuclear cells including neutrophils, basophils and eosinophils in this and other ORAI1/STIM1 patients were normal suggesting that SOCE is not required for granulocyte development (29, 35, 42). Increases in $[Ca^{2+}]_i$ and SOCE in response to Fc gamma receptor and chemokine signals are, however, essential for neutrophil function (Table 1). This was recognized long before ORAI1 and STIM1 were identified as both phagocytosis of pathogens and the production of ROS, for instance, were shown to be dependent on Ca^{2+} influx (89, 92-94). ROS in neutrophils are generated by NADPH oxidase 2 (NOX2), a multiprotein enzyme complex, in response to Fc gamma receptor crosslinking or integrin-mediated neutrophil adhesion, both of which cause an increase in $[Ca^{2+}]_i$ (95). The mechanism by which Ca^{2+} regulates NOX2 activation is not well understood but has been suggested to involve PKC, PI3K and sphingosine kinase (96, 97). In the human neutrophil-like cell line HL-60 and in primary human neutrophils SOCE mediated by ORAI1 and STIM1 (but not STIM2) is necessary for phagosomal ROS production (91, 98). Furthermore, neutrophil recruitment to sites of inflammation required chemokine and beta 2-integrin signaling, both of which induced an increase in $[Ca^{2+}]_i$ (95, 99, 100). It has recently been shown that the Beta 2 integrin-mediated transition of neutrophils from a rolling state to an arrested state is dependent on SOCE mediated by ORAI1 (90). Whether SOCE is required for neutrophil migration *in vivo* remains to be studied. Non-store operated Ca^{2+} channels such as the Ca^{2+} permeable channel TRPM2 also contribute to neutrophil trafficking resulting in impaired CXCL2 chemokine ligand-dependent neutrophil migration to the colon of *Trpm2*$^{-/-}$ mice in a rodent model of IBD (101). In summary, neutrophils require Ca^{2+} signals for several of their effector functions including phagocytosis, ROS production and migration and SOCE emerges to be an important pathway mediating Ca^{2+} influx in these cells.

4. IMMUNE DEFICIENCIES ASSOCIATED WITH DEFECTS IN SOCE

SOCE in immune cells depends on a series of signaling events that include the engagement of antigen receptors (such as the TCR, BCR and FcR), the depletion of ER Ca^{2+} stores and the STIM- and ORAI-dependent activation of CRAC channels. Other ion channels such as K^+ channels indirectly control SOCE by generating a negative membrane potential and enabling Ca^{2+} influx along an electrical gradient. We here focus on defects in genes linking antigen receptor stimulation to Ca^{2+} store depletion and in the CRAC channel genes ORAI1 and STIM1.

4.1. Mutations in immunoreceptor-associated genes upstream of store-depletion

In T and B cells, mutations in the signaling molecules ZAP-70 (Zeta-chain-associated protein kinase), ITK (IL-2-inducible T cell kinase), BTK (Bruton's tyrosine kinase) and BLNK (B cell linker protein) have been described in immunodeficient patients. Mutations in these genes are associated with impaired lymphocyte signal transduction, Ca^{2+} store depletion and SOCE. ZAP-70 links phosphorylation of the TCR zeta chain to the phosphorylation

of LAT (Linker of activated T cells) and the assembly of a scaffold protein complex that is required for PLC gamma (phospholipase C) activation and IP_3 production. Mutations in ZAP-70 cause Severe Combined Immunodeficiency (SCID), which is due to a selective defect in the development of $CD8^+$ T cells and impaired activation of $CD4^+$ T cells. SOCE in ZAP-70-deficient T cells isolated from the blood of patients was found to be impaired in response to TCR stimulation (but not, as expected, ionomycin treatment) (102). A similar defect is observed in *Zap70*$^{-/-}$ mice, which have a more severe defect in T cell development and lack both $CD4^+$ and $CD8^+$ T cells (103).

Following ZAP-70 activation, tyrosine kinases of the Tec family such as ITK (in T cells) and BTK (in B cells) are recruited into signaling complexes with the scaffold proteins SLP-76 (SH2 domain containing leukocyte protein) and BLNK. ITK and BTK mediate the phosphorylation of PLC gamma 1 and PLC gamma 2 in T and B cells, respectively, and thus regulate IP_3 production and store depletion. ITK-deficient patients have normal T cell numbers but fail to control infection with Epstein Barr virus (EBV) (104). *Itk*$^{-/-}$ mice showed reduced numbers of immature T cells in the thymus (105) and $CD4^+$ T cells in the blood (106) as well as a defect in T cell activation (105). T cells from ITK-deficient patients were not analyzed for SOCE but T cells from *Itk*$^{-/-}$ mice showed reduced SOCE upon TCR crosslinking (106, 107). Ca^{2+} influx is also severely impaired in immature pre-B cells isolated from the bone marrow of patients with *BTK* gene mutations and in B cells from *Btk*$^{-/-}$ mice following BCR crosslinking (108). A similar defect in SOCE is observed in B cells from *Blnk*$^{-/-}$ mice (109). BTK- and BLNK-deficient patients suffer from Agammaglobulinemia due to severe defects in B cell maturation (110) (111). The defect in B cell development has been attributed to impaired signaling through the pre-BCR complex, which is required for survival of immature B cells.

In addition, targeted deletion of signaling molecules that link TCR stimulation to store depletion in murine T cells such as SLP-76, LAT and PLC gamma 1 severely impairs SOCE, T cell function and T cell development (112-114). Taken together, these data from human immunodeficient patients and knockout mice demonstrate that signaling pathways regulating SOCE in lymphocytes are critical for T and B cell function. They also suggested that SOCE in response to antigen receptor stimulation may be required for lymphocyte development. This idea was further supported by the observation of Ca^{2+} signals in immature lymphocytes (40, 41). As will be discussed in the next section, however, SOCE is not – at least not SOCE mediated by STIM1, STIM2 and ORAI1 proteins – required for T and B cell development (16, 33, 34, 38, 66).

4.2. Mutations in CRAC channel genes ORAI1 and STIM1

Since the discovery of STIM1 and ORAI1 as critical mediators of SOCE in 2005 and 2006, respectively, patients with defects in CRAC channel function and mutations in *ORAI1* and *STIM1* genes have been identified that provide the most direct insight into the physiological role of SOCE in humans *in vivo*. These patients lack CRAC channel function and SOCE in a variety of cell types and suffer from a unique genetic syndrome ("CRAC channelopathy") that is defined by immunodeficiency, autoimmunity, ectodermal dysplasia and myopathy (5, 27, 29, 35). The mutations and phenotype of these patients have been described in detail elsewhere (5, 27, 115) and we here provide a brief, updated overview of critical findings in these patients.

4.2.1. Mutations in ORAI1

Inherited mutations in ORAI1 are rare and only six patients with autosomal recessive mutations in the coding region of ORAI1 are known to date (Table 2). Positional cloning in two patients and healthy members of one family with CRAC channel deficiency has let to the identification of a missense mutation in ORAI1 that causes a single amino acid substitution, R91W, which is located at the cytoplasmic end of the first transmembrane (TM) domain of ORAI1 (11). Whereas ORAI1 mRNA and protein expression were normal in cells from these patients, SOCE and CRAC channel function were abolished in T cells (11, 61). The R91W mutation in ORAI1 is likely to abolish CRAC channel function by interfering with channel gating or by constricting the permeation pathway of the channel. Substitution of R91 with the hydrophobic amino acid tryptophan (W) and, alternatively, leucine (L), valine (V) or phenylalanine (F) inhibited CRAC channel function, whereas replacement of R91 with neutral or negatively charged amino acids had no effect on I_{CRAC} (116) (S.Feske, M. Prakriya unpublished). This effect was explained by an increase in the transmembrane probability of TM1 and thus presumably a more stable insertion of TM1 in the plasma membrane bilayer when R91 is replaced with a hydrophobic residue (116).

Patients from two other families, by contrast, had null mutations in ORAI1 and lacked ORAI1 protein expression (Table 2). Insertion of an adenine nucleotide in the coding sequence of ORAI1 in two siblings born to consanguineous parents resulted in a frameshift, premature termination of ORAI1 translation and abolished ORAI1 mRNA and protein expression (ORAI1-A88SfsX25) (29, 58). Two siblings from a third family were born to unrelated parents and were compound heterozygous for two missense mutations (A103E, L194P) in TM1 and TM3 that interfered with stable ORAI1 protein expression (29, 42). Lack of ORAI1 expression in cells of patients from the last two families completely abolished SOCE (29, 42, 58) suggesting that endogenously expressed ORAI2 and ORAI3, which are detectable at the mRNA level in many immune cells including T and B cells, monocytes and neutrophils (30), fail to substitute for ORAI1. In addition, ectopic expression of ORAI2 and ORAI3 in T cells or fibroblasts from ORAI1-R91W-deficient patients also failed to rescue SOCE (28). Patients with mutations in ORAI2 or ORAI3 have not been reported so far and the role of these channel isoforms in immune cells and their function remains to be determined.

It is noteworthy that heterozygosity for all reported ORAI1 mutations in relatives of the patients was not associated with clinical symptoms in general and infections in particular. At the cellular level, however, T

Table 2. Autosomal recessive mutations in *ORAI1* and *STIM1* genes that cause CRAC channelopathy

Gene	Mutation	Type of mutation	mRNA	Protein	SOCE	I_{CRAC}	Primary Immuno-deficiency	Auto-immunity	Ectodermal Dysplasia Anhidrosis	Muscular Hyptonia	Ref
ORAI1	R91W	missense	normal	normal	–	–	yes	–	yes	yes	11
ORAI1	A88SfsX25	insertion	–	–	–	–	yes	Yes	yes	yes	29
ORAI1	A103E / L194P	missense	↓↓	–	–	nt	yes	–	yes	yes	29
STIM1	E128RfsX9	insertion	–	–	–	nt	yes	Yes	yes	yes	35
STIM1	1538-1G>A (exon 8)	spice site	–	–	–	nt	(yes)	Yes	yes	yes	59

cells from individuals heterozygous for the R91W mutation had reduced SOCE at subphysiological extracellular $[Ca^{2+}]_o$ (0.2 - 0.5 mM) (11). By contrast, SOCE in T cells from individuals heterozygous for the A103E and L194P mutations was comparable to that in healthy controls under similar recording conditions (29). These findings suggest that the R91W mutation has a dominant negative effect on CRAC channel function, presumably through the assembly of the non-functional ORAI1 subunit into the tetrameric channel complex. This idea is supported by the finding that one mutant ORAI1-R91W subunit, when forced into a tetrameric configuration with wildtype ORAI1 subunits in the context of a concatenated tetramer, was sufficient to reduce I_{CRAC} by ~ 50%; two mutant subunits abolished I_{CRAC} (117). Under non-concatenated conditions, however, heterozygous expression of mutant ORAI1-R91W reduced I_{CRAC} in T cells from the patients relatives by only ~ 50-70% (36), failed to impair SOCE at physiological $[Ca^{2+}]_o$ (11) and did not cause increased susceptibility to infections. Taken together, these findings indicate that monoallelic expression of ORAI1 is sufficient to support normal SOCE and immune function (29).

4.2.2. Mutations in STIM1

Autosomal recessive mutations in STIM1 are rare and to date only four patients from two families have been reported (Table 2) (35, 59). Insertion of an adenine nucleotide in exon 3 of STIM1 resulted in a frameshift and premature termination at position 136 (E128RfsX9) in siblings of one family (35). No full-length or truncated STIM1 protein was detected in cells from these patients. A patient from a second family was homozygous for a G>A substitution in the splice acceptor site of STIM1 exon 8 (1,538–1G>A). Normally spliced, full-length STIM1 mRNA transcripts and STIM1 protein were completely absent in the patient's B cells. As a consequence, SOCE in response to Ca^{2+} store depletion with thapsigargin was undetectable in B cells from this patient and fibroblasts from patients with the E128RfsX9 mutation (35, 59). While SOCE could be restored in fibroblasts from STIM1-deficient patients by overexpression of STIM1 and, interestingly, also STIM2 (35). Endogenous STIM2 levels and function, however, are insufficient in human patients to rescue SOCE and immune function. Taken together, the mutations in ORAI1 and STIM1 in immunodeficient patients and the resulting lack of SOCE suggest that both genes are responsible for the majority of SOCE in immune cells whereas STIM2, ORAI2 and ORAI3 play a minor role.

4.3. Clinical manifestations of ORAI1 and STIM1 deficiency

4.3.1. Immunodeficiency and autoimmunity

Mutations in ORAI1 and STIM1 that abolish SOCE and CRAC channel function result in a very similar clinical phenotype suggesting that both genes act in the same pathway. The disease is characterized by a constellation of symptoms that is unique to CRAC channelopathy and is dominated by immunodeficiency with recurrent infections early in live, autoimmunity, hypohidrotic ectodermal dysplasia (HED) and muscular dysplasia. Infections are caused by a variety of bacterial, fungal and viral pathogens, especially herpes viruses such as cytomegalovirus (CMV), Epstein-Barr Virus (EBV), varizella zoster virus and human herpes virus 8 (HHV8). The latter caused disseminated Kaposi sarcoma (KS) in a patient with a STIM1 splice site mutation (1,538–1G>A) when she was two years old (59, 118). The spectrum of infections and their first occurrence early in life is similar to infections observed in patients with Severe Combined Immunodeficiency (SCID), who lack T and/or B cells, although opportunistic infections such as *P. jirovecii* pneumonia have not been observed in CRAC channel-deficient patients. As is the case for SCID patients, hematopoietic stem cell transplantation (HSCT) is the only cure for patients with CRAC channelopathy.

In addition to immunodeficiency, patients with mutations in STIM1, and to a lesser degree those with mutations in ORAI1, suffer from lymphadenopathy, hepatosplenomegaly and symptoms of autoimmune disease (29, 35). All four STIM1-deficient patients suffered from autoimmune hemolytic anemia and thrombocytopenia (35), whereas only one of six ORAI1-deficient patients showed autoimmune thrombocytopenia and neutropenia (29). Autoimmunity in STIM1-deficient patients is likely due to a reduction in the total number of $Foxp3^+$ regulatory T cells (Treg) (35), a subpopulation of $CD4^+$ T cells. One of their main functions is to restrict the activation of potentially autoreactive T cells (as discussed in more detail in Chapter 3.2). A similar phenotype of lymphoproliferation with splenomegaly and lymphadenopathy was observed in mice with T-cell specific deletion of *Stim1* and *Stim2* genes, which show a severe defect in the development and function of Treg cells (16). By contrast, mice lacking only *Stim1* expression in T cells have normal numbers of Treg cells (16, 34). It is not understood why only mice lacking both STIM1 and STIM2 have impaired Treg development and autoinflammatory disease, whereas in human patients STIM1 deficiency alone is sufficient to cause a similar phenotype. A possible explanation is that STIM2 may contribute significantly to SOCE in murine, but not human Treg cells.

The immunopathology in ORAI1- and STIM1-deficient patients is due to impaired activation of T cells, NK cells and potentially other cell types in the immune

system. By contrast, no defect in lymphocyte development (with the exception of $CD4^+$ $Foxp3^+$ regulatory T cells as described above) has been observed. All patients with mutations in ORAI1 and STIM1 had normal leukocyte numbers and subpopulations. In particular, the numbers of $CD4^+$ and $CD8^+$ T cells, B cell and NK cells were comparable to healthy controls (29, 35), which is in contrast to most forms of SCID in which T, B and/or NK cell development is impaired depending on the underlying gene defect (110). Altogether, these findings argue against an important role of ORAI1, STIM1 and most likely SOCE itself for lymphocyte development, especially since normal T, B and NK cell development has also been observed in mice lacking *Orai1*, *Stim1*, *Stim2* and *Stim1/Stim2* gene expression (16, 34, 36-39, 66). In contrast to normal lymphocyte development, the activation of T and NK cells was severely impaired in the absence of SOCE. T cells failed to proliferate in response to TCR and mitogen stimulation with anti-CD3 antibodies and PHA, ConA or PMA / ionomycin, respectively. In addition, SOCE-deficient $CD4^+$ and $CD8^+$ T cells showed severely impaired expression of multiple cytokines such as IL-2, IL4, IL-10, IFN gamma and TNF alpha (62, 63). The patients' T cell function was also compromised *in vivo* as no delayed-type hypersensitivity responses to intraepidermal challenges with recall antigens could be observed (58, 62). As discussed in chapter 3, $CD8^+$ T cells and NK cells from ORAI1- and STIM1-deficient patients showed severe defects in degranulation and cell-mediated cytotoxicity of tumor cells (60). The function of SOCE-deficient B cells appears to be unimpaired as serum concentrations of IgG, IgA and IgM were normal or elevated in ORAI1- and STIM1-deficient patients. Patient B cells stimulated with pokeweek mitogen *in vitro* produced normal amounts of IgG and IgA, although IgM levels were moderately reduced (119). Taken together, B cells appear to be able to differentiate into Ig producing plasma cells and to produce antibodies in the absence of SOCE, which is confirmed by recent findings in STIM1- and STIM2-deficient B cells in mice (66).

4.3.2. Non-immunological symptoms of SOCE deficiency

The non-immunological features of CRAC channelopathy in human patients have been described in detail elsewhere (27, 29, 35). They are dominated by a generalized muscular hypotonia and hypohidrotic ectodermal dysplasia (HED). Muscular hypotonia is present from birth in ORAI1- and STIM1-deficient patients and represents a severe clinical problem in those patients surviving after HSCT (29, 35, 60). The patients not only suffer from a generalized muscle weakness and impaired endurance; in addition they developed bronchiectases with respiratory insufficiency, potentially due to impaired respiratory muscle function. A muscle biopsy in one patient with ORAI1-R91W mutation revealed an atrophy of type II muscle fibers as the cause of the muscular hypotonia. It is of note that $Orai1^{KI/KI}$ mice (which are homozygous for the equivalent ORAI1-R93W mutation) did not display significant histological or ultrastructural abnormalities of their skeletal muscles (36). This is in contrast to STIM1-deficient mice, whose muscle fibers had reduced diameters, displayed signs of mitochondriopathy and fatigued more quickly than those of wildtype controls (120). The latter finding suggests that SOCE is required for refilling of SR calcium stores and muscle fiber contraction. Taken together, the studies in ORAI1- and STIM1-deficient patients and mice illustrate the important role of SOCE in skeletal muscle function and differentiation.

The ectodermal dysplasia in ORAI1- and STIM1-deficient patients is characterized by a severe defect in dental enamel calcification. This finding suggests that CRAC channels may be required for mediating vectorial Ca^{2+} transport in ameloblasts, which is necessary for the deposition of Ca^{2+} in the enamel extracellular space (121). ORAI1- and, to a lesser degree, STIM1- deficient patients have impaired sweat production resulting in dry skin and heat intolerance. This defect is most likely due to the impaired function of eccrine sweat glands as anion secretion in sweat glands was reported to be regulated by Ca^{2+} influx (122, 123). Further studies are required to elucidate the role of ORAI1 and STIM1 in regulating SOCE and the function of eccrine sweat glands, ameloblasts and myocytes.

5. CONCLUSIONS

Following the identification of STIM and ORAI proteins as critical mediators of SOCE, there has been an exponential growth in studies exploring the role of SOCE and STIM/ORAI proteins in many cellular processes including the function of immune cells. Mutations in *STIM1* and *ORAI1* genes are linked to primary immunodeficiency in patients, illustrating the critical role of STIM1 and ORAI1 in immune responses to infection, especially those mediated by T cells. Furthermore, an increasing number of studies have explored STIM/ORAI-mediated SOCE and its function in lymphoid and myeloid immune cells. The picture emerging from these studies is that ORAI1, STIM1 and their homologues mediate SOCE in many immune cells and that they are essential for many cell-type specific effector functions. While SOCE does not appear to be important for the development of immune cells (with the exception of regulatory T cells), it is indispensable for effector functions of immune cells such as $CD4^+$ effector and regulatory T cells, cytotoxic $CD8^+$ T cells and NK cells, mast cells and neutrophils. However, the majority of the data identifying a critical role for SOCE in cellular immune function is derived from cell lines and *in vitro* experiments, and therefore the significance of SOCE for immune responses *in vivo* remains unknown. The emerging role of ORAI and STIM proteins in SOCE and the function of immune cells in both the adaptive and innate immune system begins to explain, however, the complex immune phenotype observed in STIM1- and ORAI1-deficient patients. Future studies will have to investigate how SOCE regulates, for instance, immunity to infection with viral and bacterial pathogens as well as inflammatory and allergic immune responses. Results from these experiments will provide a mechanistic basis to assess the potential for therapeutic immunomodulation through SOCE inhibition in inflammatory and autoimmune diseases.

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Abbreviations: BCR, B cell receptor; CRAC, Ca^{2+} release activated Ca^{2+} channel; EAE, experimental autoimmune encephalomyelitis; ER, endoplasmic reticulum; IL, interleukin; IFN, interferon; SOCE, store-operated Ca^{2+} entry; STIM, stromal interaction molecule; TCR, T cell receptor; Th, T helper

Key Words: Calcium, Ca^{2+}, ORAI1, STIM1, STIM2, CRAC, SOCE, T cell, Lymphocyte, Macrophage, Neutrophil, Immunodeficiency, SCID, Review

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