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The immunological synapse controls local and global calcium signals in T lymphocytes

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Summary: Cell polarization is a key feature of T-cell function. The immunological synapse (IS) between T cells and antigen-presenting cells is a beautiful example of how polarization of cells is used to guide cell function. Receptors, signal transducers, the cytoskeleton, and organelles are enriched at or depleted from the IS after its formation, and in many cases these re-localizations have already been linked with certain T-cell functions. One key step for T-cell activation is a rise in the cytoplasmic calcium concentration. Whereas it is undisputed that the IS initiates and controls calcium signals in T cells, very little is known about the role of T-cell polarization for calcium signals and calcium-dependent signal transduction. We briefly summarize the basic commonly agreed principles of IS-dependent calcium signal generation but then focus on the less well understood influence of polarization on calcium signals. The discussion of the role of polarization for calcium signals leads to a model how the IS controls local and global calcium signals and calcium-dependent T-cell functions. We develop a theoretical formalism based on existing spatiotemporal calcium dynamic simulations to better understand the model in the future and allow further predictions which can be tested by fast, high resolution live-cell microscopy.

Keywords: *signal transduction, signaling proteins, T-cell receptors, T cells, calcium, immunological synapse*

Introduction: facts about Ca^{2+} entry in T cells

Ca^{2+} entry across the plasma membrane is the most important Ca^{2+} source for T-cell activation. The only Ca^{2+}-selective ion channels present in T cells are ORAI channels (1–3). ORAI channels are activated through the depletion of internal Ca^{2+} stores in a stromal interacting molecule 1 (STIM1)-dependent manner (4–6). The ORAI proteins and most prominently ORAI1 are the molecular basis for Ca^{2+} release-activated Ca^{2+} (CRAC) channels. CRAC channels were discovered more than 15 years ago in immune cells and are the prototype of store-operated Ca^{2+} selective ion channels (7–9). CRAC/ORAI1 channels are well defined through their biophysical and pharmacological properties: they are highly Ca^{2+} selective and permeate divalent cations better than monovalent cations but become permeable to monovalent cations in the absence of all

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divalent cations (9–14). They are inactivated by Ca^{2+} in several different ways (8, 9, 15–17). Part of the inactivation may be mediated in a STIM1-dependent manner (18). Ca^{2+} entry through CRAC/ORAI1 channels is responsible for most if not all Ca^{2+} entry, shaping cytosolic Ca^{2+} signals which are required for many T-cell functions including transcription, proliferation, and cytokine release (19–25). CRAC/ORAI1 channels thus play the central role for Ca^{2+} entry and Ca^{2+}-dependent T-cell activation.

Analyzing the studies about CRAC/ORAI1 channels published so far in T cells, almost all of them (we estimated around 99%) were carried out with unphysiological stimuli to induce store depletion and subsequent CRAC/ORAI1 activation. Reagents used to activate CRAC/ORAI1 channels include the SERCA [sarco-endoplasmic Ca^{2+} adenosine triphosphatase (ATPase)] inhibitor thapsigargin, the Ca^{2+} ionophores ionomycin or A23187 (often used in combination with phorbol esthers), inositol-1,4,5-trisphosphate and/or Ca^{2+} buffers like EGTA (ethylene glycol tetraacetic acid) or BAPTA [1,2-bis (o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid], and receptor stimulation by lectins (like PHA) or antibodies against T-cell receptors (TCRs) (26–31). While these stimuli are well suited to study properties of CRAC/ORAI1 channels in quantitative ways, all of these manipulations neglect the important point that T cells are highly polarized during activation.

The immunological synapse is central for T-cell activation

T cells and matching antigen-presenting cells (APCs) can form very intimate contacts resulting in the formation of a specialized junction between the two cells, the immunological synapse (IS) (32, 33). Formation of the IS is paralleled by dramatic morphological changes and cell polarization that facilitate a stable physical contact of the two cells and subsequent T-cell activation.

Cytoskeleton-dependent molecular rearrangements after antigen recognition lead to the formation of an organized IS between T cells and APCs, which was initially described to consist of a central cluster of TCR [central supramolecular activation cluster (cSMAC)] surrounded by a ring of adhesion molecules [peripheral SMAC (pSMAC)] (34). Subsequently, a third SMAC was defined, because the tyrosine phosphatase CD45, which is initially enriched in the cSMAC, was found to be enriched in a distal (d) ring outside the pSMAC at a later stage of IS formation, the dSMAC (35). Even further away from the IS, on the very opposite side of the cell, the formation of the distal pole complex (DPC) is thought to remove negative regulators of T-cell activation from the IS and might also serve as an independent signaling domain (36, 37). The concept of SMACs and the DPC highlights a very important feature of T-cell activation: how the quantification (number or concentration) of receptors and other signaling molecules in different cellular compartments is linked to qualitatively different T-cell responses. In addition, changes of the numbers or concentration of receptors and molecules in SMACs during and after IS formation point to another important kinetic parameter to modulate T-cell activation. The duration of an IS should therefore not only have a quantitative but may also have a qualitative impact on T-cell activation, resulting from dynamic changes of SMAC composition.

It has been observed that the duration of an IS can range from seconds/minutes up to several hours (38, 39). Naive $CD4^+$ or $CD8^+$ T cells have to scan their environment to find matching APCs. Very short IS durations are beneficial under these circumstances to allow as many contacts as possible to find a potentially rare match with an antigen-presenting dendritic cell. T cells still show net migration during the formation of such a short IS, which led Dustin (33) to term it ‘kinapse’ (from ‘kinetic synapse’). Kinapses have also been implicated to be important in the induction of tolerance in case naive T cells only contact low potency major histocompatibility complex (MHC)-bound antigens. T cells did not change migration pattern under these conditions, meaning that they were never arrested to form a stable long-lasting IS, but they became finally tolerant after forming several kinapses (39). Somewhat longer durations of an IS between cytotoxic T lymphocytes (CTLs) and target cells are probably necessary for CTL-dependent cell killing (40). Here, durations of 20–60 min have been proposed. Of course, shorter times would be very beneficial also in this case, because CTL efficiency could be drastically improved by shortening the IS duration required for cell killing. Considering this, it is interesting that a mature IS is not required for CTL killing (41), which could potentially speed up target cell killing. It would certainly make sense to test the hypothesis that CTLs can kill within a couple of minutes, making use of short-lived kinapses instead of stable synapses to contact target cells under physiological conditions. Longer lasting stable synapses, however, are certainly required for the maturation and proliferation of naive T cells (38). Determining the duration of an IS appears to be a crucial problem in immunology. Different signals have been implicated in synapse stability (reviewed in 33), but the general principles are not yet understood.

Theoretical models of the IS have also provided insights into our current understanding of IS formation and stability (42–

45). They highlight the importance of localization, polarization, and pattern formation to generate an IS. Key players involve of course the cytoskeleton, TCR recycling, and receptor polarization.

Over the last years it has become clear that there is not just one type of an IS, but that different ISs differ qualitatively and quantitatively. IS kinetics are one important parameter to determine the outcome and the efficiency of an immune response. One of the most important questions at the moment therefore is how qualitative and quantitative differences of the IS are linked to specific cell functions. Cytosolic Ca^{2+} signals are extremely important for T-cell activation and are well suited to mediate localized cellular functions on different time scales following IS formation.

The IS controls Ca^{2+} signals: the basic principle

The general aspects of Ca^{2+} signaling following TCR stimulation are well understood and are very similar to many other cell types that use store-operated Ca^{2+} entry as a major Ca^{2+} source (26, 28, 30, 31, 46–52). Upon TCR activation, the Src kinases Fyn and Lck phosphorylate tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAMs), which allow activation of the ζ-chain-associated protein of 70 kDa (ZAP-70). ZAP-70 phosphorylates the adapter proteins linker for activation of T cells (LAT) and Src homology 2 domain-containing leukocyte phosphoprotein of 76 kDa (SLP76), which activate phospholipase Cγ (PLCγ) through the Src-like tyrosine kinase Tec. PLCγ cleaves phosphatidylinositol-4,5-bisphosphate and generates the second messengers inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to the IP_3 receptor in the membrane of the endoplasmic reticulum (ER), which is the main Ca^{2+} store, and initiates release of its stored Ca^{2+}. The depletion of Ca^{2+} from the ER activates, in a STIM1-dependent way (4–6), store-operated ORAI1 channels in the plasma membrane (1–3). Ca^{2+} influx through these channels elevates the intracellular Ca^{2+} concentration for minutes through hours (potentially days), which among other Ca^{2+}-dependent signaling molecules activates the phosphatase calcineurin. Calcineurin dephosphorylates the nuclear factor of activated T cells (NFAT) in the cytosol inducing its activation and translocation to the nucleus (51, 53). DAG and Ca^{2+}, among many other stimuli, can activate the nuclear factor-κB (NF-κB) in a protein kinase C (PKC)-dependent way (54). Ca^{2+}, through Ca^{2+}-dependent kinases, also modulates activity of the transcription factor activator protein-1 (AP-1) (55). AP-1, NFAT, and NF-κB induce specific transcription of inducible genes which control T-cell proliferation, maturation, and differentiation. It is remarkable that 75% of all activation-regulated genes show a dependence on Ca^{2+} influx through the plasma membrane by CRAC channels (22). With decreasing Ca^{2+} signals, no or reduced T-cell activation and proliferation is observed (19–25), highlighting the great influence of Ca^{2+} signals on the T-cell-dependent immune responses.

Most of our basic insight about Ca^{2+} signaling in T cells presented in the preceding paragraph is the result of studies that did not involve formation of an IS but made use of artificial stimuli like thapsigargin or ionomycin and of course antibodies against the TCR in solution or lectins that crosslink surface receptors. However, the basic principles are essentially the same. If T cells are stimulated through the IS, Ca^{2+} release and Ca^{2+} entry through CRAC/ORAI1 channels are initiated following focal stimulation of the TCR by antigen or antibodies through the same signaling cascades (25, 56–62).

By inducing Ca^{2+} signals, the IS interestingly also influences its own stability, because the Ca^{2+} signal feeds back on the synapse. Two-photon studies in intact lymphatic tissue have shown that a rise of Ca^{2+} enhances synapse stability by arresting the cells (63–65).

Polarization and shape matter

Comparing the physiological stimulation of T cells through the IS with non-physiological stimuli like thapsigargin, antibodies in solution, or lectins, there are obvious differences which, of course, are not observed with the non-physiological stimuli: IS formation is paralleled by a dramatic cell polarization including the redistribution of many receptors or other signaling molecules and a dramatic shape change of the cell. These rearrangements affect the interplay between the molecules and organelles involved in T-cell activation and lead to highly coordinated Ca^{2+} signals. Ca^{2+} can act in several ways at the same time within a cell, depending on its subcellular distribution, its source, its proximity to Ca^{2+} binding effector proteins, and the magnitude and duration of changes in the Ca^{2+} concentration. The Ca^{2+}-dependent transcription factors NFAT and NFκB, for example, have different activation requirements. NFκB can be activated by shorter and higher rises in the Ca^{2+} concentration, because of its low sensitivity and slow inactivation, while NFAT can be activated selectively by lower, long-lasting Ca^{2+} signals (49, 66). Ca^{2+} is thus not a simple on/off switch but a very versatile messenger.

Polarization is thus a very important parameter for T-cell activation. Many receptors, molecules, and organelles are polarized during T-cell migration and T-cell activation. We

discuss our current understanding of the polarization of the key players which generate Ca^{2+} signals in T cells: CRAC/ORAI1 channels which are the source for Ca^{2+} entry, K^+ channels which set the membrane potential for Ca^{2+} entry, plasma membrane Ca^{2+} ATPases (PMCA) which export Ca^{2+} across the plasma membrane, the ER with its IP_3 receptors to release Ca^{2+}, with the SERCA to pump Ca^{2+} back into the ER, and with STIM1 to sense the store filling and to activate ORAI1 channels, and finally mitochondria which act as Ca^{2+} buffers and distributors.

CRAC/ORAI1 polarization

STIM1 and ORAI1 have been found to accumulate in puncta following depletion of Ca^{2+} stores (4, 5, 67–72). However, there are only two publications addressing the localization of ORAI1 and STIM1 proteins relative to the IS. Lioudyno *et al.* (73) showed that STIM1 and ORAI1 were accumulated at the IS following its formation, and Barr *et al.* (74) observed the localization of STIM1 and ORAI1 in puncta and dynamic caps often in the neighborhood of the IS but also at the distal pole of T cells. Lioudyno *et al.* (73) found that ORAI1 and STIM1 accumulated at the IS independently of Ca^{2+} signaling. Their accumulation, however, favored higher Ca^{2+} signals in the subcellular areas closer to the IS. They concluded that Ca^{2+} entry at the IS was enhanced compared with distal sites. Barr *et al.* (74) in some cases also reported enrichment of STIM1 and ORAI1 in puncta or caps close to the IS. In addition, their data provide insight into ORAI1 and STIM1 dynamics. Using high-resolution microscopy, they showed that STIM1 and ORAI1 localization at the IS is clearly distinct from the signaling complexes that form around TCR, which is in contrast to Lioundyno *et al.* (73). Moreover, Barr *et al.* (74) found that STIM1 and ORAI1 often clustered in caps that were localized at the distal pole and not at the IS. The caps were able to move to sites close to the IS. They speculated that the caps could be used to assemble pre-existing ORAI1–STIM1 complexes (together with other proteins) ready to be used at newly established synapses. Alternatively, the caps could also prevent STIM1–ORAI1 complexes to participate actively in Ca^{2+} entry outside the IS. In our model (*Fig. 1A*), ORAI1 enrichment at the IS is not necessarily needed to provide local Ca^{2+} influx at the IS, because the mitochondrial accumulation at the IS guaranties local Ca^{2+} entry at the IS anyway (61) (see below).

K^+ channel polarization

The membrane potential is a parameter that influences Ca^{2+} entry through ORAI1 channels and subsequent Ca^{2+} signals. The membrane potential is dependent on all ion channels that are open at any given time. Like in most other cells, the membrane potential of T cells is controlled by K^+ and Cl^- channels (75, 76). A depolarization decreases Ca^{2+} entry, whereas hyperpolarization increases Ca^{2+} entry through ORAI1 channels. Whereas very little is known about the functions of Cl^- channels following IS formation, K^+ channels have been a major focus as T-cell modulators. The two highly expressed and best studied K^+ channels are voltage-dependent channel Kv1.3 and the intermediate-conductance Ca^{2+}-activated potassium channel KCa3.1 (76–83). Both channels have been shown to play important roles during T-cell activation through their control of the membrane potential.

KCa3.1 is upregulated during T-cell activation (84) and is enriched at the IS after its formation. It has been shown that KCa3.1 compartmentalizes with F-actin and CD3ε at the IS but remains evenly distributed within the plasma membrane if no stimulus is provided to the T cells (78). As Ca^{2+} influx appears to be concentrated at the IS, this finding opens an interesting possibility of a positive feedback loop during Ca^{2+} entry: activation of ORAI1 channels leads to Ca^{2+} influx, increasing the activity of KCa3.1 channels, which hyperpolarize the plasma membrane and thereby further increase the driving force for Ca^{2+} entry through ORAI1 channels. The distance between KCa3.1 and ORAI1 channels would be one way to control Ca^{2+} entry, as local Ca^{2+} microdomains close to ORAI1 channels could be sensed by KCa3.1 channels. At the moment, nothing is known about a potential interaction between KCa3.1 and CRAC in T cells; however in other cell types, functional interactions between K^+ and Ca^{2+} channels have been described (85–88). The co-localization between Ca^{2+} influx and KCa3.1 at the IS thus provides a positive feedback loop for Ca^{2+}-dependent T-cell activation.

Kv1.3 has also been shown to localize to the IS following TCR engagement (77, 79, 81). Kv1.3 is not only enriched at the IS but also upregulated in postmortem inflammatory infiltrates of the brain of patients with multiple sclerosis (80) and in autoreactive T cells from patients with rheumatoid arthritis or diabetes mellitus (81). In T-helper cells, Kv1.3 was co-localized with different proteins at the IS including Kvβ.2 and CD4. While it obviously makes sense that the Ca^{2+}-activated KCa3.1 are enriched at the IS to provide a positive feedback loop for Ca^{2+} entry, it is not so obvious what function the enrichment of Kv1.3 at the IS may have. A local membrane hyperpolarization at the IS induced by a local Kv1.3 channel accumulation would dissipate on a time scale of much <1 s, because the membrane potential would almost instantaneously spread out over the whole plasma membrane. An

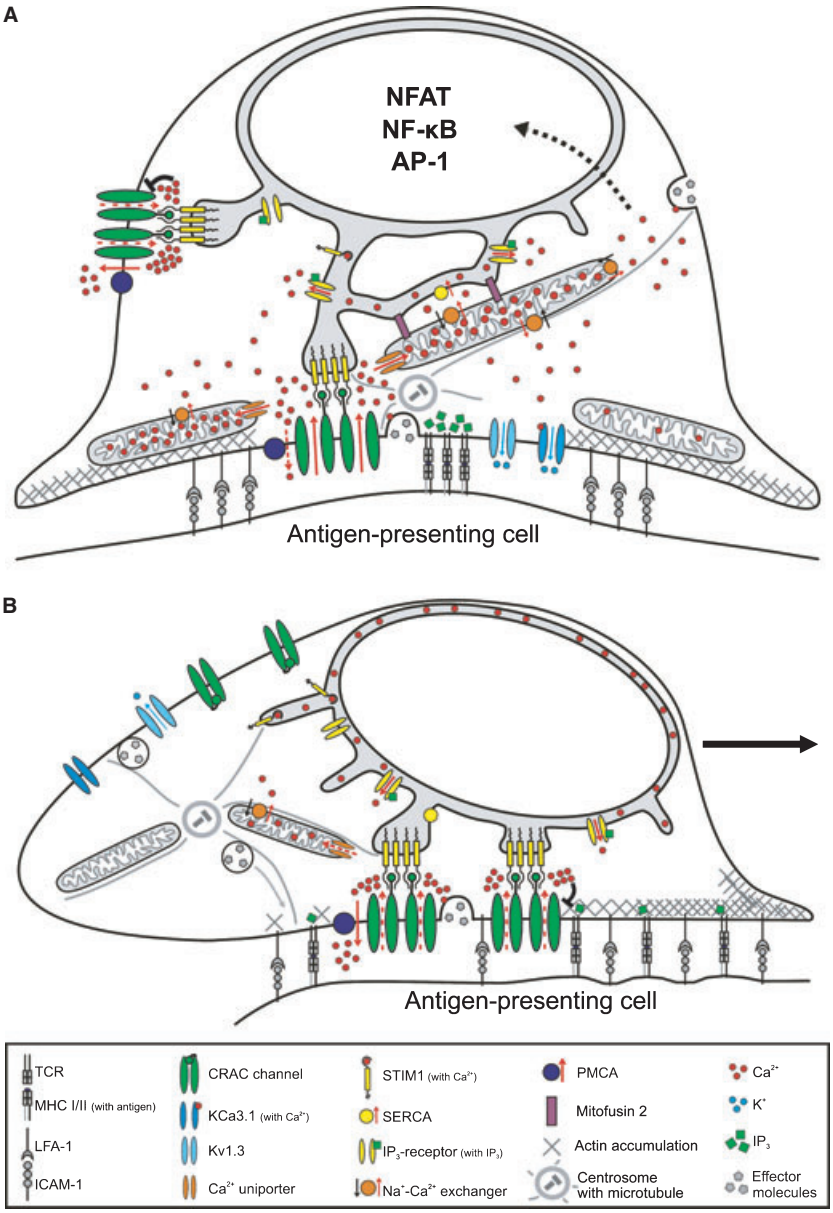

***Fig. 1.* Polarization and Ca^{2+} signals.** Polarization and Ca^{2+} signals are shown for a stable long-lasting synapse (A) and for a transient kinapse (B).

enrichment of voltage-gated channels at the IS would therefore most likely not induce local potential changes in T cells that could affect Ca^{2+} entry through ORAI1 channels.

We propose a different hypothesis of how enrichment of Kv1.3 could influence T-cell function. K^+ channels have been shown to be susceptible to redox regulation (89). Following the initial discovery, it was found that many K^+ channels are influenced by redox regulation and the metabolic state of cells (90, 91), including Kv1.3 in T cells which is inhibited by H_2O_2 (92). The production of reactive oxygen species (ROS), which leads to an elevation of H_2O_2, may therefore induce a depolarization of the plasma membrane and decrease Ca^{2+} entry through ORAI1 channels. ROS can be produced by different sources including the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and mitochondria (93). Whereas nothing is known about the polarization of the NADPH oxidase during T-cell activation, we have shown that mitochondria accumulate very close to the IS (61). The IS might therefore control local ROS production, which could influence the activity of Kv1.3 channels and possibly also other channels at the IS. In addition to local Ca^{2+} domains, local ROS domains may thus also play a crucial role for T-cell activation by modulating Ca^{2+} signals.

PMCA polarization

The PMCA constitutes the major Ca^{2+} extrusion mechanism in T cells (94). Whereas the localization of Ca^{2+} and K^+ channels within the IS would have a positive feedback on local Ca^{2+} entry, expression of the PMCA within the IS would counteract the Ca^{2+} entry. Polarization of PMCA has been reported in some cell types including neurons and epithelial cells (95–97), in which they may influence localized Ca^{2+} signals and signaling. However, nothing is known about the polarization of PMCA relative to the IS. In B cells, the surface protein CD22 was found to inhibit Ca^{2+} efflux by PMCA (probably by PMCA4a and PMCA4b) following B-cell receptor cross-linking. In T cells, Bautista *et al.* (94) reported that PMCA activity increased in two phases following the activation of Ca^{2+} entry through CRAC/ORAI1 channels: a rapid increase followed by a further Ca^{2+}-dependent increase of up to approximately fivefold over 10–60 s, which the authors termed modulation. This modulation increased the dynamic range of PMCA activity by enhancing its Ca^{2+} sensitivity and pump rate. While the upmodulation of PMCA activity could protect T cells from Ca^{2+} overload during stimulation, it would also limit Ca^{2+} influx and waste 'energy' by cycling Ca^{2+} back and forth over the plasma membrane in combination with active ORAI1 channels. The subsequent analysis of the interaction of PMCA and CRAC channels by Bautista *et al.* (98) suggested that a Ca^{2+} microdomain close to CRAC channels promotes upmodulation of PMCA activity. The authors proposed intimate coupling between CRAC channels and PMCA, which would greatly influence the magnitude of Ca^{2+} signals in T cells. This intimate coupling between CRAC channels and PMCA would even enhance cycling of Ca^{2+} across the plasma membrane between CRAC channels and PMCA and waste more energy. To avoid such Ca^{2+} cycling at the IS, we therefore propose that PMCA are not enriched at the IS (see model below).

Cytoskeleton

Upon the interaction with an APC and formation of the IS, T cells undergo pronounced changes in the dynamics of their cytoskeleton. Actin polymerization close to the IS and polarization of the secretory apparatus are key events in this process. The formation of the SMACs is dependent on the cytoskeleton, as homogenously distributed molecules need to organize spatially and temporally. While recent work provides a picture of increasing accuracy of these cytoskeletal rearrangements and the signaling cascades which initiate them (reviewed in 99–101), much less is known about the functional outcome of the rearrangements and how the cytoskeleton feeds back to signaling and in particular to Ca^{2+} signaling.

One possible way in which the actin cytoskeleton can regulate Ca^{2+} signals is through the spatial rearrangement of ORAI1, Kv1.3, KCa3.1, or PMCA localization. Proteins of the ezrin/radixin/moesin (ERM) family have been shown to link plasma membrane receptors or ion channels with the cytoskeleton. In T cells, they are involved in coupling several cell surface proteins to the cytoskeleton (37). Ezrin transiently locates to the IS upon T-cell activation, but at later stages, both ezrin and moesin move to the distal pole complex. They are inactivated by a Vav1–Rac1 dependent pathway, which results in a disanchoring of the cortical actin cytoskeleton from the plasma membrane supporting IS stability (102). Their downregulation also reduces PLC activation, Ca^{2+} release, and interleukin-2 (IL-2) production (103). There are currently no reports that ERM proteins are involved in the localization of ORAI1, Kv1.3, KCa3.1, or PMCA in T cells. We have found that another Ca^{2+} permeable ion channel, transient receptor potential channel 4 (TRPC4), binds to the ezrin-binding scaffold protein EBP50 (ezrin/moesin/radixin-binding phosphoprotein 50). The interaction between EBP50 and TRPC4 is necessary for the correct insertion of the channel into the plasma membrane and its proper localization (104). The reported enrichment of ORAI1 at the IS (73) and at the distal part of T cells (74) could in principle be mediated by the cytoskeleton. However, the same process could also depend on trapping of ORAI1 by STIM1 clusters (4, 5, 67–72).

T cells deficient in actin-regulatory proteins often display impaired Ca^{2+} mobilization, as reported for Vav (105, 106), Wiskott–Aldrich syndrome protein (WASp) (107), WASp family verprolin-homologous protein 2 (WAVE2) (108), coronin 1 (109), and HS1 (110). As the localization of many signaling proteins involved in the early steps of T-cell activation depends on the cytoskeleton, it is no surprise that changes in actin dynamics can have severe impacts on TCR-dependent Ca^{2+} release, especially at low antigenic densities (111). Any process leading to impaired IP_3 production will influence Ca^{2+} signaling upstream of store depletion. One exception is the study of Nolz *et al.* (108), who showed that WAVE2 not only controls TCR-mediated signaling cascades by abrogating actin polymerization and subsequent formation of a signalosome but also ORAI1 channel activity downstream of Ca^{2+} store depletion. WAVE2-depleted T cells had a reduced influx of Ca^{2+} following store depletion by both TCR ligation and, importantly, also by thapsigargin (TG). TG depletes the stores irreversible by blocking Ca^{2+} re-uptake into the ER through

SERCA and thus short-circuits the TCR cascade, leading to maximal ORAI1 activation. This observation indicates that proteins that regulate actin polymerization can modulate Ca^{2+} influx through ORAI1 channels. Up to this point it has however not been shown by patch-clamp experiments that ORAI1 channels are directly affected by WAVE2 (108). It is therefore possible that other modulators of Ca^{2+} entry (K^+ channels, mitochondria, etc.) are affected by WAVE2.

A compound often used to study the actin dependence of T-cell activation is cytochalasin D, a mycotoxin which inhibits actin polymerization. Cytochalasin D has been shown to disrupt ongoing Ca^{2+} signaling following stimulation of a T cell by an APC (111). The reduction of Ca^{2+} signals observed in cells treated with actin polymerization disrupting drugs cannot be explained as a direct block of ORAI1 channels, because both Ca^{2+} currents (112, 113) and ORAI1/STIM1 puncta were unaffected (69, 70). We have recently shown that inhibition of actin polymerization during the formation of an artificial IS inhibits the close apposition of mitochondria and the plasma membrane which is mediated by T-cell shape changes during a focal stimulation (62). Ca^{2+} signals are therefore reduced because of impaired buffering of Ca^{2+} influx by mitochondria and ongoing ORAI1 channel inactivation (see below).

In addition to the actin cytoskeleton, microtubules have important functions organizing protein and organelle localization during T-cell activation. Following IS formation, the microtubule-organizing center (MTOC) is re-localized from the rear end of the cell (uropod) to the IS within the first 30 min (40, 114, 115). As STIM1 has been shown to bind to the microtubule-plus-end tracking protein EB1 (116), it may be involved in remodeling the ER motor-based transport and may thus cause polarization of the ER at the IS.

ER polarization

As STIM1 is the major activator of ORAI1 channels and STIM1 is mostly ER bound (4–6), it is trivial that some part of the ER has to be close to the IS to activate ORAI1 channels, if ORAI1 channels at the synapse contribute to Ca^{2+} entry or are even the major source of Ca^{2+} entry as has been proposed (73).

While very little is currently known about the polarization of the ER at the IS, STIM1 and ORAI1 enrichment in puncta is well documented. The depletion of ER Ca^{2+} triggers the oligomerization of STIM1 (68, 117–119) and its subsequent accumulation in well-defined areas (=puncta) within 25 nm of the plasma membrane (69). Binding of STIM1 to ORAI1 through its CRAC activation domain induces clustering and activation of ORAI1 channels (72, 117). Whether or not the clustering between STIM1 and ORAI1 preferentially occurs at the IS is currently not clear.

Mitochondria polarization

When we were setting out to analyze the function of mitochondria in T cells, we assumed that like in many other cells tested before, mitochondria would act as a Ca^{2+} sink, thereby decreasing cytosolic Ca^{2+} signals. To our surprise, the opposite was the case in T cells. An inhibition of mitochondrial Ca^{2+} uptake would not lead to the predicted rise of cytosolic Ca^{2+} signals but instead greatly diminished cytosolic Ca^{2+} signals (120). These experiments also indicated that Ca^{2+} uptake into mitochondria and subsequent export of Ca^{2+} from mitochondria could enhance cytosolic Ca^{2+} signals in T cells. At that time, it was already known that CRAC channels were inactivated by Ca^{2+} in different ways (8, 9, 15, 16). We tested the hypothesis that the local Ca^{2+} domain at CRAC channels, which was known to influence fast inactivation of CRAC channels (15) and probably their slow inactivation as well (16), was somehow responsible for the 'strange' mitochondrial effect on CRAC channels. We finally arrived at the conclusion that mitochondria had to be close to CRAC channels, thereby decreasing the microdomain of Ca^{2+} at the channel and preventing part of the Ca^{2+} dependent CRAC inactivation. This in turn led to enhanced CRAC activity and finally increased cytosolic Ca^{2+} signals in T cells. This model was further supported by a series of patch-clamp recordings that demonstrated directly that slow Ca^{2+}-dependent inactivation was indeed influenced by mitochondria (121). The tight functional interaction between mitochondria and Ca^{2+} channels was later reported in many cell types and not only restricted to CRAC channels but also found for voltage-gated Ca^{2+} channels (122–126).

A key question now was whether or not the mitochondrial regulation of CRAC activity was just present 'by chance', because in T cells mitochondria are on average closer to the plasma membrane than in many other cell types because of the very large nucleus, or if T cells 'actively' positioned mitochondria close to Ca^{2+} channels. This question was resolved when we showed that mitochondria were actively translocated towards the plasma membrane upon T-cell activation (127) and that they were accumulated at the IS, inducing a very intimate contact between mitochondria and CRAC channels at the synapse (61) (*Fig. 1A*). At the plasma membrane and the IS, mitochondria prevent the Ca^{2+}-dependent inactivation of CRAC channels, most likely because they decrease the local Ca^{2+} microdomain close to CRAC channels. Indeed, mitochondria beneath the IS take up more Ca^{2+} than mitochondria

localized far away from the IS. The imported Ca^{2+} is then released from mitochondria at other locations. By this method, mitochondria distribute the inflowing Ca^{2+} away from the CRAC channels to other locations in the cytosol. One could picture the mitochondria as a broken vacuum cleaner, which sucks Ca^{2+} in at one place but releases it in all directions because of a broken dust catcher. Movement of mitochondria to the plasma membrane was found to be dependent on Ca^{2+} entry, resulting in a double positive feedback: mitochondria come closer to the plasma membrane thereby enhancing Ca^{2+} influx through CRAC channels. Whereas a modest rise in cytosolic Ca^{2+} is needed for mitochondrial movement to the plasma membrane in T cells (127), higher Ca^{2+} concentrations have been shown to inhibit mitochondrial motility (128, 129). Thus, Ca^{2+} influx may direct mitochondria to the plasma membrane and to the IS in T cells, where mitochondria experience high Ca^{2+} microdomains that finally inhibit their motility anchoring them close to the ORAI1 channels where they are needed to drive Ca^{2+}-dependent T-cell activation. The molecular mechanism of transport of mitochondria to the IS is not well understood in T cells; however, it is clear that the cytoskeleton and Ca^{2+} are important players. In neurons, it has been recently shown that the EF-hand motifs of the mitochondrial adapter protein Miro mediate the Ca^{2+}-dependent arrest of mitochondria (130). The Ca^{2+}–Miro interaction permits Miro to interact with kinesin-1, which is present on all mitochondria. Binding of Ca^{2+}–Miro to kinesin-1 prevents the interaction of motors with microtubules limiting mitochondrial mobility. This model has to be tested in T cells.

An important aspect during T-cell activation is its shape change during and after formation of the IS, which allows a perfect fit with the APC and is also important for T-cell polarization. We found that the shape change of T cells following synapse formation further enhances the coupling between mitochondria and the IS (62). The shape change-dependent mitochondrial localization close to the plasma membrane prevented CRAC channel inactivation even in T cells in which motor protein-dependent mitochondria movements towards the plasma membrane were completely abolished, highlighting the importance of the shape change-dependent control of Ca^{2+} influx. We conclude that morphological changes of T cells not only facilitate an efficient contact with APCs but strongly modulate Ca^{2+}-dependent T-cell activation through the coupling between mitochondria and ORAI1 channels.

A hypothetical model

We propose here a model for how the IS controls local and global Ca^{2+} signals through polarization. As duration of the IS is an important parameter for T-cell activation (33), we focus first on a longer lasting IS (*Fig. 1A*), which is required for the clonal expansion and maturation of naive T cells (38). These synapses have to be stable for several 10s of minutes up to several hours (potentially days). All receptor/organelle polarizations discussed above have been analyzed after the induction of such stable synapses. Thus, we assume that a stable long-lasting IS is fully polarized and potentially reaches 'steady state'. The localization of receptors, channels, and organelles as shown in *Fig. 1A* visualizes such a steady state fully polarized IS. Independent of whether or not ORAI1 is enriched at the IS (73, 74), we have shown that Ca^{2+} entry occurs preferentially at the IS because only there mitochondria are enriched and can prevent Ca^{2+}-dependent inactivation of ORAI1 channels (61). According to our model, mitochondria should decrease the Ca^{2+} microdomain right at the IS, thereby inhibiting Ca^{2+}-dependent inactivation of ORAI1 channels. At all other plasma membrane sites, Ca^{2+} microdomains should be higher and limit ORAI1 channel activity until a steady state between the microdomain Ca^{2+} concentration and the ORAI1 inactivation has been reached. In these areas, PMCA activity would counter balance the Ca^{2+} entry. Only close to the IS mitochondria may out compete the PMCA and thereby redistribute Ca^{2+} deeper into the cytosol. However, mitochondria cannot be as close to ORAI1 channels as the ER, because the physical STIM1–ORAI1 interaction is needed for ORAI1 activity. The mitochondria may thus just sit next to the ER close to the IS and close to the ORAI1 channels at the IS.

Only mitochondria but not the ER can enhance the global Ca^{2+} concentration through the broken vacuum cleaner-like Ca^{2+} transport away from the IS into the cytosol, because refilling of the ER deactivates ORAI1. The ER could be involved however in anchoring mitochondria next to ORAI1 channels by mitofusin 2, which has been recently shown to tether the ER with mitochondria (131). The tethering between the ER and mitochondria would thus ensure global Ca^{2+} rises required for clonal T-cell expansion and maturation.

The polarization of KCa3.1 to the IS helps to ensure a stable Ca^{2+} entry through ORAI1 channels. The role of Kv1.3 enrichment at the IS is less clear. We do not believe that enrichment itself can change the membrane potential significantly; however, the potential regulation of Kv1.3 by ROS through mitochondria adds another regulatory mechanism.

If PMCA would be enriched at the IS, which has not been shown and which in our opinion does not make sense, they on one hand would also reduce Ca^{2+}-dependent inactivation of ORAI1 channels, but on the other hand, every Ca^{2+} ion

transported out of the cell by PMCA would be lost for signaling. Competition for the incoming Ca^{2+} ions by mitochondria and PMCA is simply controlled by their relative localization towards ORAI1 channels at the IS. In our model, mitochondria are clearly the 'winners' in this competition model, because their localization very close to the ORAI1 channels prevents the generation of large Ca^{2+} microdomains at the IS and thereby inhibits non-productive Ca^{2+} recycling between ORAI1 channels and PMCA. In summary, we postulate that T cells need an intimate coupling between the IS and mitochondria to ensure extensive Ca^{2+} signaling during a stable long-lasting IS. Changing mitochondrial positioning offers an exciting tool to fine-tune T-cell responses.

The other extreme of an IS is the kinapse, a kinetic synapse that is not stable but may be moving during contact with a target cell. Much less is known about T-cell polarization under such conditions. A short-lived IS is depicted in *Fig. 1B*. Under these conditions, full polarization of receptors, organelles, etc. to the IS cannot be achieved. Let us assume that such a contact is stable for up to several minutes. TCR activation within seconds induces a small and transient rise of IP_3, which leads to partial Ca^{2+} store depletion, partial CRAC activation (132, 133), and a subsequent small rise in the cytosolic Ca^{2+} concentration. An important feature of this cascade is its localization. IP_3 is generated only at the IS for a short amount of time, which may result in preferential depletion of Ca^{2+} from ER Ca^{2+} stores in the immediate vicinity of the IS. This would favor activation of ORAI1 channels at the IS close to the depleted ER. Ca^{2+} entry in this model would be restricted to the IS and induce a high Ca^{2+} microdomain at the IS. This Ca^{2+} microdomain would not extend far into the cytosol, as not many mitochondria are present at the IS within the very first minutes of IS formation (61). ORAI1 channels would be inactivated by the high Ca^{2+} microdomain and deactivated by the Ca^{2+} uptake into the ER. The result would be a transient high microdomain of Ca^{2+} at the IS for a few minutes. Interestingly, slow Ca^{2+}-dependent inactivation of CRAC channels operates exactly on this time scale (16, 121, 127). We propose that the cytosolic Ca^{2+} concentration is only high enough at the IS for a reasonable time and not high enough at any other site in the cytosol necessary to induce gene expression, clonal expansion, and maturation of a naive T cell. Only Ca^{2+}-dependent processes localized right at the IS could be supported in this model. For effector T cells, such a scenario makes perfect sense, because they need to activate release of vesicles at the IS. In case of effector T-helper cells, it has been shown that different mechanisms exist to release vesicles restricted either to the IS only or anywhere at the plasma membrane (134). The transient unstable synapse would favor directed release of vesicles at the IS, whereas longer more stable synapses would in addition facilitate non-directed release of vesicles. For CTLs, a similar scenario can be envisioned. Perforin and probably also granzymes have to be released at the IS (40). A transient unstable IS would favor directed release and killing of the target cell through IS formation. Thus, the local microdomains of high Ca^{2+} induced by a transient unstable IS would facilitate a fast killing restricted to the IS as is desired to avoid killing of non-target cells which happen to be close to the CTL just by chance. In this model, killing of the target cell would be fast and would not require a stable synapse, as has also been proposed by Purbhoo *et al.* (41). However, in this model there is not enough time to bring perforin vesicles to the synapse by reorientation of the centrosome, as was suggested by Stinchcombe *et al.* (40). Other mechanisms would be needed to guarantee perforin release at the IS. We propose that the Ca^{2+} influx at the IS may facilitate transport of perforin vesicles to the IS and fusion at the IS. We have already shown that a rise of cytosolic Ca^{2+} was required for transport of another organelle, mitochondria, to the plasma membrane (127). As the mass of a perforin vesicle is much smaller than the mass of a mitochondrion, it is reasonable to assume that perforin vesicles could be transported much faster to the plasma membrane than mitochondria. We propose that a perforin vesicle can only be docked and primed at the IS, and it can only be released there, as only at the IS is the required Ca^{2+} concentration high enough. A transient unstable IS may therefore be well suited to kill target cells quickly and may thus increase the efficiency of the immune system. The hypothesis on the role of local Ca^{2+} domains at the IS to induce directed vesicle release in helper and killer T cells needs to be challenged through high resolution time-resolved live-cell imaging under conditions as physiological as possible.

In our hypothetical model, synapse stability plays a very important role for Ca^{2+}-dependent T-cell function. How synapse stability is regulated is not fully understood. However, there are certain signals that induce a 'stop' of T cells and thus a higher chance for stable synapses, and there are signals that induce a 'go' of T cells and thus a higher chance for unstable synapses, as reviewed by Dustin (33). The cytosolic Ca^{2+} concentration itself also determines IS duration: high Ca^{2+} has been shown to serve as a stop signal for T-cell motility, thus enhancing the probability of a stable synapse (63–65). These findings hint to a positive feedback loop: once enough Ca^{2+} has entered the T cell, it stabilizes the IS, thereby allowing more Ca^{2+} entry needed for clonal T-cell expansion and

maturation. A short transient Ca^{2+}entry, however, is not sufficient to stabilize the IS. The positive Ca^{2+}-dependent feedback needed for a long-lasting stable IS therefore requires mitochondrial translocation to the IS to prevent Ca^{2+}-dependent ORAI1 inactivation. How exactly Ca^{2+} facilitates the induction of long-lasting stable synapses is currently not known.

We hypothesize that spontaneous activity of the actin cytoskeleton plays a crucial role in the formation of contact sites between T cells and APCs as well as in the maturation of such a site into either a synapse or a kinapse. This view is based on two recent discoveries of actin dynamics *in vivo*. In various cell types the transient formation of glycosyl-phosphatidylinositol-anchored protein nanoclusters has been found to depend on cortical actin (135). Among others, these nanoclusters are associated with signaling functions of Ras proteins (136). Myosin activity as well as actin polymerization drives the formation of these nanoclusters, possibly through the spontaneous transient formation of actin asters. A similar mechanism might be responsible for the formation of initial TCR clusters that nucleate IS formation.

The second discovery supporting the 'actin hypothesis' concerns the dynamics of actin in motile cells. An emerging view is that spontaneous polymerization and/or contraction waves provide a principle of cytoskeletal organization during cell motility. It has been observed that the protein Hem-1 forms propagating waves that co-localize with the leading edge of motile human neutrophils (137). Hem-1 is part of the Scar (suppressor of cyclic AMP receptor)/WAVE2 complex that intervenes with actin polymerization through Rac. Computational modeling has shown that these waves can emerge spontaneously from the reciprocal interaction of Hem-1 with treadmilling actin filaments (137, 138). When impinging on an obstacle, e.g., another cell, Hem-1 waves either die out or move laterally along the obstacle, similarly to lateral waves along the leading edge of spreading fibroblasts (139).

Combining these findings, we speculate that actin-driven, spontaneous formation of TCR clusters is the first molecular structure forming at the contact site of a motile T cell with an APC (44, 140). Depending on the degree of cytoskeletal activity and the strength of the interaction between TCR and MHC-bound antigens, the nanoclusters can on one hand mature into a symmetric long-lasting synapse. On the other hand, a propagating actin wave running over the cluster can induce an asymmetric protein distribution in the forming SMAC, resulting in a kinapse. According to this view which is supported by the observation that disruption of the actin cortex from the plasma membrane stabilizes an IS (102), the actin cortex dynamics would determine IS duration [together with other factors (33)].

Testing the model

How can the model presented in above be tested? We believe that the most important technology to test the local and global Ca^{2+} control by IS-induced cell polarization is fast, high resolution live-cell imaging. Recent years have shown amazing advances by increasing the resolution of fluorescence microscopy well below the diffraction barrier (reviewed in 141). While currently used mostly for fixed samples, it will be a matter of time until these techniques will be used as standard methods in live-cell imaging. Fast epifluorescence microscopy with subsequent deconvolution, confocal microscopy, and total internal reflection microscopy are mostly used to image the IS and IS-dependent Ca^{2+} signals. These techniques and, in the future, high-resolution microscopy will facilitate the testing of certain aspects of the model. However, microscopy will always have its limitations. Therefore, we believe that computational/theoretical analysis should be combined with live-cell microscopy to fully understand the proposed model and its predictions.

As discussed above, the upregulation of the cytosolic Ca^{2+} concentration for several hours during T-cell activation involves a complex interplay of several Ca^{2+}-containing compartments – cytosol, ER, and mitochondria – and clusters of CRAC channels and Ca^{2+} pumps in a small region at the IS. Fluorescence microscopy techniques allow the visualization of Ca^{2+} signals, but since the spatial resolution of fluorescent images is usually only hundreds of nanometers, it is impossible to resolve the temporal and spatial dynamics of Ca^{2+} concentrations within the region of activated Ca^{2+} channels. This problem has triggered a huge body of simulation studies with the attempt to calculate the expected time course of Ca^{2+} concentration increases and their spatial extent. Initial studies were focused on a single opened Ca^{2+} pore in the presence of multiple mobile and (homogeneously distributed) immobile buffers (142, 143). The best studied Ca^{2+} channel in the theoretical literature is the IP_3 receptor in the ER membrane, for which De Young and Keizer (144) formulated a probabilistic model involving several activating and inhibiting subunits resulting in a stochastic opening behavior. Models for the spatiotemporal Ca^{2+} dynamics based on Ca^{2+} release from the ER usually involve one or several clusters of 10–30 IP_3 receptors (145–150) and a standard reaction-diffusion scheme, describing the diffusion and chemical reaction of Ca^{2+} and mobile buffers, and physical Ca^{2+} fluxes through opened channels

and active pumps (reviewed in 151). Recently, large scale simulations of intracellular Ca^{2+} dynamics have been carried out (152, 153).

The key players involved in a quantitative model for the spatiotemporal Ca^{2+} dynamics during T-cell activation are four Ca^{2+}-containing compartments, the cytosol, ER, mitochondria, and the extracellular space (*Fig. 2A*), and the channels and pumps that transfer Ca^{2+} from one compartment into the other, the IP_3 receptor, SERCA, CRAC channels, PMCA, mitochondrial uniporters, and mitochondrial Na^+/Ca^{2+} exchangers. Only for the IP_3 receptor do detailed theoretical models exist (144, 151). SERCA are beginning to attract attention of theoretical modelers (154), but not much modeling work has been performed for CRAC channels and PMCA, which is why the corresponding Ca^{2+} fluxes are usually described in a phenomenological way. Within the biological model for the formation of the IS in T cells, mitochondria play an important role – not simply as immobile Ca^{2+} buffers but as 'Ca^{2+} conductors' that collect cytosolic Ca^{2+} at the Ca^{2+} entry site, i.e., close to the CRAC channels, and distribute it smoothly throughout the cytosol. Thus the position and functional performance of parts of the mitochondrial network with respect to the position of the CRAC channels is an important ingredient of a quantitative model.

A sketch of a specific arrangement of a single mitochondria (M) filament together with parts of the ER within the cytosol underneath a CRAC channel within the plasma membrane (PM) is shown in *Fig. 2A*. The mitochondrial filament is considered as a closed tube, aligned perpendicular to the plasma membrane and represented by a single spatial coordinate (z). Multiple channels and filaments as sketched in *Fig. 2B* provide a model for the organelle configurations at different stages of the formation of the IS. That a quasi-one-dimensional description of intra-mitochondrial Ca^{2+} diffusion is justified was discussed, experimentally and theoretically, by Gerencser and Adam-Vizi (155). If we neglect the presence of mobile buffers in the mitochondria, then within a single filament the z-dependent Ca^{2+} concentration, $c_M(z)$, is defined by the one-dimensional diffusion equation

$$\frac{\partial c_M}{\partial t} = D_M \frac{d^2 c_M}{dz^2} + j_M(\Delta c(z))$$

where D_M is the Ca^{2+} diffusion constant within mitochondria and j_M the (z-dependent) Ca^{2+} flux through the mitochondrial membrane. This approach subsumes the effect of uniporters and Na^+/Ca^{2+} exchangers and depends on the local Ca^{2+} concentration difference $\Delta c(z)$ between mitochondrial Ca^{2+} concentration, $c_M(z)$, and cytosolic Ca^{2+} concentration, c_{cyt} at the spatial coordinate r(z) within the cytosol (note that when the top of the mitochondrial tube is at the position r_M within the cytosol, then r(z) is given by r_M plus a shift by z in the z-direction (*Fig. 2B*). Net Ca^{2+} entry into mitochondria occurs at sites, at which cytosolic Ca^{2+} is high whereas Ca^{2+} is exported otherwise.

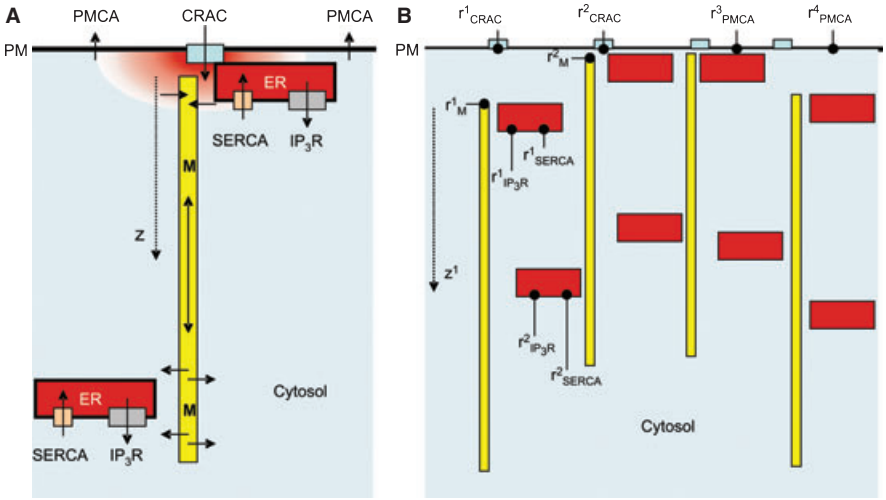

***Fig. 2.* A model for the spatiotemporal Ca^{2+} dynamics and for the organelle configurations at different stages of the formation of the IS.** (A) Sketch of the individual organelles within the cytosol, channels, and pumps at the compartment boundaries involved in the model. Arrows indicate Ca^{2+} fluxes. PM, plasma membrane; PMCA, plasma membrane ATPase; CRAC, Ca^{2+} release-activated Ca^{2+} channel; ER, endoplasmatic reticulum; SERCA, sarco-endoplasmatic reticulum Ca^{2+} ATPase; IP_3R, IP_3 receptor; M, motochondria. (B) Sketch of a compartment arrangement relevant for a stage in the primary T-cell activation. The positions of individual channels, pumps, and filaments within the cytosol as used in the model are indicated, for CRAC channels r^i_{CRAC} ($i = 1,\ldots,N_{CRAC}$), IP_3 receptors r^i_{IP3R} ($i = 1,\ldots,N_{IP3R}$), SERCA pumps (r^i_{SERCA}, $i = 1,\ldots,N_{SERCA}$), PMCAs r^i_{PMCA} ($i = 1,\ldots,N_{PMCA}$), and the top of the individual mitochondria filaments r^i_M ($i = 1,\ldots,N_M$). The filament coordinate z^1 of the first mitochondrium is also indicated.

The three-dimensional diffusion equation for Ca^{2+} in the cytosol (c_{cyt}) is

$$\frac{\partial c_{cyt}}{\partial t} = D_{cyt}\nabla^2 c_{cyt} + J_{ER} + J_M + J_{PM}$$

where J_{ER}, J_M, and J_{PM} denote the Ca^{2+} fluxes between the cytosol and the ER, the mitochondria M, and the plasma membrane PM, respectively. D_{cyt} is the diffusion constant for Ca^{2+} within the cytosol. For the Ca^{2+} concentration within the ER, c_{ER}, we assume all ER compartments to be interconnected, although they are sketched as separate boxes in the two-dimensional sketch in *Fig.* 2. The Ca^{2+} concentration within the ER is high, between 0.1 and 1 mM, and if we neglect spatial gradients, we can assume:

$$\frac{\partial c_{ER}}{\partial t} = \tilde{J}_{ER}$$

J_{ER} is composed of two contributions, $J_{ER} = J_{IP3R} - J_{SERCA}$, the influx from the IP_3 receptors, J_{IP3R}, and the Ca^{2+} pumped back into the ER through the SERCAs, J_{SERCA}. J_{IP3R}, and J_{SERCA} are sums of all contributions of the individual IP_3 receptors and SERCA pumps located at positions r^i_{IP3R} and r^i_{SERCA} in space, respectively. The contribution j_{IP3R} of a single IP_3 receptor involves IP_3 activation, Ca^{2+} activation, and a retarded Ca^{2+} inhibition (144, 151). Similarly, the contribution of a single SERCA pump located at a position r^i_{SERCA} depends on the cytosolic Ca^{2+} concentration at this position $c(r^i_{SERCA})$ and increases in a sigmoidal way as soon as $c(r^i_{SERCA})$ is increased (154). $\tilde{J}_{ER}$, the Ca^{2+} influx from the cytosol into the ER, is the sum of all contributions j_{IP3R} and j_{SERCA}, without the spatial dependence that J_{ER} has (because c_{ER} is assumed to be homogeneous within the ER).

J_{PM} can also be composed into two contributions, $J_{PM} = J_{CRAC} - J_{PMCA}$, the influx from the CRAC channels, J_{CRAC}, and the Ca^{2+} pumped out of the cytosol through the PMCAs, J_{PMCA}. Both are again sums of all contributions of the individual CRAC channels and PMCA located at positions r^i_{CRAC} and r^i_{PMCA} in space, respectively. The CRAC influx is coupled to the Ca^{2+} concentration within the ER (provided that extensions of the ER are already close to the CRAC channels and the STIM1-ORAI1 interaction is established). It depends on the deviation of the Ca^{2+} concentration in the ER from its 'normal' value c^0_{ER}, and it increases in a sigmoidal way once the ER is depleted by more 30%, i.e., once c_{ER} is smaller than 0.7 c^0_{ER}. Moreover, an increased Ca^{2+} concentration around the CRAC channel will inhibit, with a time delay of around 1–3 min (slow CRAC inactivation), Ca^{2+} influx through the CRAC channel, which should be incorporated into the model in case not only the stationary Ca^{2+} distribution is of interest but also the dynamics on the scale of seconds to minutes.

J_{PMCA} describes the Ca^{2+} removal from the cytosol through PMCA. Defining the cytosolic Ca^{2+} concentration at the position r^i_{PMCA} (the ith PMCA) as $c = c_{cyt}(r^i_{PMCA})$, the contribution of an individual PMCA depends in a sigmoidal way on c.

This mathematical model combines the experimental and theoretical knowledge about intracellular Ca^{2+} dynamics with the experimental observations of organelle translocations and channel activities during primary T-cell activation. It provides a comprehensive tool for calculating the spatial inhomogeneities of intracellular Ca^{2+} concentrations for a given organelle arrangement and allows predictions for quantities like channel, receptor, and mitochondrial filament densities or spatial organelle distances relevant for the establishment of Ca^{2+} concentrations necessary for T-cell activation.

Conclusions

We have proposed that T cells 'use' the duration of the IS as a tool to regulate Ca^{2+}-dependent T-cell functions. Synapse duration and polarization greatly influence local and global Ca^{2+} signals. Transient unstable synapses favor localized Ca^{2+} microdomains at the IS and may be beneficial to drive Ca^{2+}-dependent processes localized at the IS, the perfect example being vesicle fusion restricted to the IS. Long-lasting stable synapses favor Ca^{2+} entry at the IS, decrease Ca^{2+} microdomains at the IS, but induce long-lasting global Ca^{2+} elevations. These do not favor vesicle fusion restricted to the IS but induce vesicle secretion of different vesicles everywhere in the T cell, leading probably to a generalized activation of the immune system. They also lead to the T-cell activation required for clonal expansion and maturation of the T cells.

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