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The Immunological Synapse: a Dynamic Platform for Local Signaling

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Abstract

The immunological synapse (IS) as a concept has evolved from a static view of the junction between T cells and their antigen-presenting cell partners. The entire process of IS formation and extinction is now known to entail a dynamic reorganization of membrane domains and proteins within and adjacent to those domains.

Discussion The entire process is also intricately tied to the motility machinery—both as that machinery directs “scanning” prior to T-cell receptor engagement and as it is appropriated during the ongoing developments at the IS. While the synapse often remains dynamic in order to encourage surveillance of new antigen-presenting surfaces, cytoskeletal forces also regulate the development of signals, likely including the assembly of ion channels. In both neuronal and immunological synapses, localized Ca^{2+} signals and accumulation or depletion of ions in microdomains accompany the concentration of signaling molecules in the synapse. Such spatiotemporal signaling in the synapse greatly accelerates kinetics and provides essential checkpoints to validate effective cell–cell communication.

Keywords T lymphocyte · ion channel · Ca^{2+} signaling · STIM1 · Orai1

Introduction

The immune system relies upon molecular signaling and cellular communication initiated by direct cell to cell contact. Coined by Sherrington from the Greek “syn” (together) and “haptein” (to clasp) to signify neuronal cell to cell junctions that communicate electrically through ion channel activity, the term immunological synapse (IS) has come to represent a wide variety of cell to cell junctions that vary in molecular structure and dynamics. A dozen years ago, Kupfer and colleagues originally described T-cell–B-cell synapses as having a characteristic bull’s-eye pattern with centrally localized T-cell receptors engaging peptide major histocompatibility complex (pMHC) and the co-receptor CD28 engaging CD80/86 in the central supramolecular activating complex (c-SMAC), surrounded by a peripheral zone (p-SMAC) with adhesion molecules lymphocyte function-associated antigen-1 (LFA-1) engaging intercellular adhesion molecule-1 (ICAM-1) [1]. Yet it was quickly realized that the term encompasses a wide variety of molecular assemblies, depending on the cell types [2]. Moreover, synapses can form transiently (kinapses) and promote signaling [3], and even the relatively stable interactions evolve over time, including undergoing continuous surveillance of a chosen antigen-presenting cell (APC).

Our purpose in this mini-review is to point out some organizing principles that promote localized signaling and feedback loops involving ion channels, calcium influx, and the cytoskeleton. Both neuronal and immunological synapses serve to bring molecules into close proximity or direct contact, both laterally within the

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plasma membrane of pre- and post-synaptic elements and across the synaptic cleft. By focusing signaling into two-dimensional microdomains, synapses raise the effective concentrations of kinases, adapter molecules, and ion channels, thereby promoting local signaling and greatly accelerating the kinetics of reaction networks. Receptor–ligands interactions such as the T-cell receptor (TCR)–pMHC also appear to have fundamentally higher avidities for one another in the two-dimensional synaptic environment, as compared to the same pairs measured in solution phase [4]. Finally, cell motility is an integral part of the formation of these junctions, and thus, a treatment of the process from scanning to calcium signaling must include consideration of the activities of the cytoskeleton.

Synapse Ultrastructure and Microcluster Dynamics

The IS assembles and disassembles over the course of minutes to hours: a much more dynamic contact as compared to neurological synapses. However, over the relatively short period of its existence, it is engaged to produce the same degrees of efficiency and fidelity as compared to the more stable neuronal structure.

The ultrastructural view of a T cell–dendritic cell (DC), T cell–B cell, or cytotoxic T lymphocyte (CTL) target contacts [5, 6] reveals that the IS is indeed synaptic (see Fig. 1a, b)—namely that the distance between apposing cells varies from approximately 15 nm at their closest to distances on the order of 100 nm. This latter extracellular opening between cells, termed a synaptic cleft, is thought to be the region into which cytolytic granules and cytokines are released, much like neurotransmitters are released into the synaptic space in that structure. The intermembrane distances at the close appositions are then those presumably underlying the T-cell receptor contacts and, at the edges, likely receptor ligand pairs such as LFA-1/ICAM-1 [6–8]. While it has also been proposed that ICAM/LFA-enriched domains must be separated by approximately 40 nm, as this is the intermolecular distance calculated for the LFA-1/ICAM pair from crystal structures [7, 8], ultrastructural analyses failed to find distinct zones matching this outer spacing [6]. This suggests either that ICAM “bends” to accommodate a closer apposition or that these ligands must be densely concentrated at the edge of the contacting region, giving rise to its apparent accumulation there as visualized by fluorescence microscopy. The spacing between membranes in the synaptic clefts is highly variable (averages around 100 nm) but has been observed to account for upwards of 80% of the total length of the membrane in the synapse [6]. The arrangement of these closely and distantly apposed domains is also likely dynamic in nature although this has been difficult to ascertain experimentally.

Interestingly, the synaptic nature of the contact, particularly the relatively minimal contact region, is likely absent in one of the most highly studied model for the immunological synapse, the supported lipid bilayer. The use of these bilayers, in which high densities of pMHC complexes and ICAM are seeded, has been tremendously important for revealing the fine details of molecular rearrangements in signaling structures, due to its ability to be imaged in the shallow illumination fields of total internal reflection (TIRF) microscopy. However, it is notable that cells form a c-SMAC-like structure in these systems in >90% of cases, whereas T–B-cell contacts do so with lower frequencies varying from 30% as measured by electron microscopy (EM) to 50–80% as observed by confocal microscopy [9]. This existence of “multifocal” synapses by both EM and in microscopy suggests that at least one parameter limiting complete “zippering” of membranes between T cells and APCs must be missing from the supported lipid bilayer models. Good candidates for such would be the highly sialylated glycocalyx of APCs or rigidity and curvature of the opposing antigen-presenting cell membrane.

Despite this pitfall, the benefits of the bilayer system in revealing dynamic signaling assemblies have been extensive. While *en face* views of IS assembly first characterized “clusters” of approximately 1 μm in minimum diameter that formed during signaling onset [10], TIRF imaging of T cells on bilayers revealed much smaller “microclusters” whose dimensions are on the order of a few hundred nanometers or less in diameter and which coalesce to form larger aggregates, often in the c-SMAC [11, 12]. In lipid bilayers, the microclusters “stream” toward the center of the contact, dependent upon an intact actin cytoskeleton [11, 12]. At the same time as the TCRs are moving inward, ICAM clusters are confined to the exterior of the contact where they remain in the p-SMAC. Such absence of streaming of ICAM may be a function of cluster size as artificially large ICAM clusters can induce ICAM–LFA-1 clusters to also stream toward the center [13]. Given the discrepancies in topology between the systems noted, it is tempting to imagine that each contact at a cell–cell junction may be a small microcosm of what is exemplified in the single larger and flatter contact that is formed in the lipid bilayer system (Fig. 1b).

Insights from In Vivo Imaging: Dynamics of Cellular Interactions and Synapse Formation

Within the past decade, two-photon imaging has permitted real-time visualization of cellular interactions in lymphoid organs and in peripheral tissues as T cells encounter APCs. T cells are more robustly motile *in vivo* than *in vitro* and come into contact with DC dendrites in the diffuse cortex of lymph

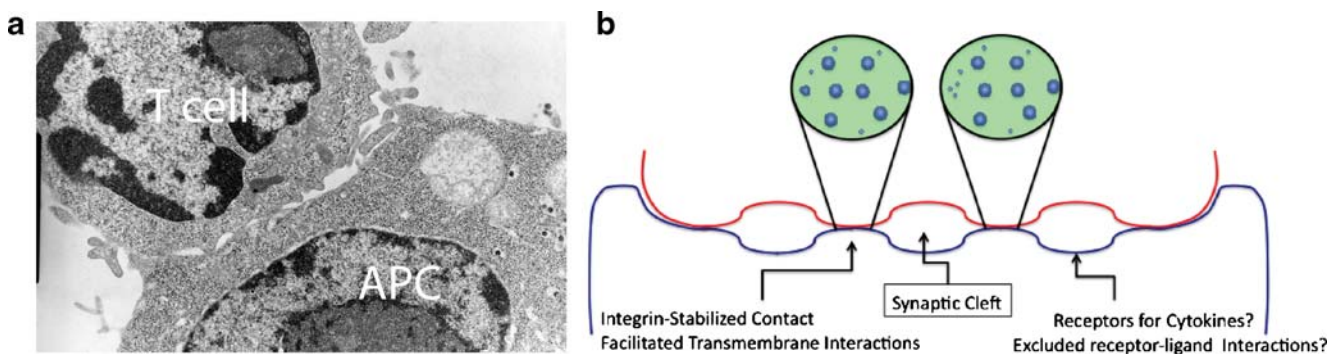


Fig. 1 Multifocal and uniform models of IS assembly. **a** TEM of D10 CD4⁺ T cell interacting with CH27 APC in the presence of 1 μ M antigenic peptide, 15 min after the onset of coupling. Note relative paucity of contact area compared to synaptic regions. **b** Models for

synaptic membrane configurations in cell–cell synapse and T-cell–bilayer synapse models. In this model, each contact region may be thought of as a microcosm of a bilayer contact in which microclusters of TCRs move and coalesce in a membrane patch (*expanded views*)

nodes. In the absence of antigen, DCs make frequent but brief contacts with T cells, at a rate of 5–10,000 cells per hour with contact durations of 2–3 min, enabling efficient scanning of T cells of varying antigen specificity by a stochastic mechanism [14]. In the presence of antigen, CD4⁺ or CD8⁺ T-cell interactions with DCs evolve in distinct stages [15–17]: (1) prolonged but still intermittent interactions, (2) stable interactions lasting hours, and (3) release and swarming followed by episodes of T-cell proliferation. During stage 1, kinapses are formed with multiple DCs, and Ca²⁺ signals are evoked in the T cells by TCR engagement of pMHC on the DC surface. During stage 2, stable synapses are formed and interleukin-2 gene expression begins. During stage 3, T cells again make contacts with multiple DCs and a fresh wave of DC can modulate the outcome by increasing cytokine production [18].

An entirely different set of interactions takes place between helper CD4⁺ T cells and B cells [19]. B cells initially are localized to the follicle region of the lymph node, where they migrate randomly at a slower pace (8 μ m/min) than T cells (12–15 μ m/min) and are segregated from T cells in the surrounding diffuse cortex. Following antigen engagement, B cells up-regulate functional expression of the chemokine receptor CCR7 and migrate by chemotaxis to the follicle edge, following a gradient of CCL19 and CCL21. At the follicle edge, they encounter and begin to interact with activated helper T cells. Immediately following the initial contact, B cells and T cells begin to migrate as stable conjugate pairs, with B cells leading the way while dragging rounded up T cells behind. This second synaptic encounter is primarily monogamous, in contrast to T cell–DC encounters that may involve up to a dozen T cells forming stable contacts with a single DC. Moreover, the stability of T-cell–B-cell interaction allows migration as a conjugate pair. Although partner exchange can occur, such events are relatively

infrequent. Migration may allow the B cell to direct the colonization of germinal centers while ensuring that a captive T cell provides help from the rear. Moreover, it is tempting to speculate that pairwise migration ensures widespread distribution of T-cell cytokines secreted from the rear of the cell.

Activated effector memory T (T_{EM}) cells lose the ability to home into lymphoid organs and instead migrate to sites of inflammation. Imaging in spinal cord during autoimmune-mediated demyelination [20] and during delayed-type hypersensitivity [21] has shown that T cells initially arrest at the site of antigen presentation, enlarge as they become re-activated in the tissue environment, and then resume their motility. A similar choreography of thymocytes has been observed during positive selection in the thymus [22–24].

Recently, one of the T-cell subsynaptic molecules, linker for activation of T cells (LAT; tagged with eGFP), was imaged as T cells encountered DCs or B cells presenting antigen [25]. Under steady-state conditions, LAT was found distributed primarily in the trailing uropod of motile T cells. Upon contact with antigen-bearing DCs, recently activated T cells tended to form kinapses with DCs, whereas the same T cells formed motile conjugates with antigen-bearing B cells. T–B synapses were found to be more stable than T–DC synapses, and redistribution of LAT to the synapse was more apparent. Similar types of studies have been performed in one of our labs using TCR transgenic mice in which the alpha chain was tagged with GFP. Interestingly, these provide direct evidence for TCR internalization, indicative of T-cell signaling, at very transient contacts and in the absence of a stable c-SMAC-like structure (Friedman et al., in submission). These results show the feasibility of imaging synaptic molecules in vivo and reinforce previous imaging results indicating diverse IS characteristics depending upon the cell type.

Motility, Synapse Dynamics, and the Underlying Cytoskeleton

Unlike the neurological synapse, the IS must form, dissolve, form again, and so on through the lifespan of the T cell. It was first observed that during initial signaling, Ca^{2+} influx resulted in cortical relaxation of T cells and “rounding” [26–28]. In addition, there is then feedback between cell morphology changes as mitochondria pivot toward the synapse to support Ca^{2+} influx and buffering [29]. The initial Ca^{2+} rise, as a result of TCR signaling and discussed in further detail below, may function through a number of mechanisms including subsequent phosphorylation of ERM proteins that results in the detachment of the actin cytoskeleton from membrane proteins [30] (and reviewed in [31]) and/or phosphorylation of myosin heavy chains, resulting in loss of membrane tension [32]. The net result is that most IS are “motile” in the sense that there is continuous movement of the contact, particularly at the edge. To understand this requires a brief review of the motility mechanisms of T cells prior to engagement.

As noted above, the basal state for T-cell motility is one of stochastic scanning in which T cells dynamically move through the T-cell zone [33]. Similar scanning is also observed within the thymus during T-cell development [34]. During these behaviors, the T-cell cytoskeleton is appropriated for amoeboid motion in various ways. Most notably, lymphocytes appear to have integrin-dependent and independent modes of motion, with the latter predominating for lymphocyte motion *in vivo* [35, 36]. *In vitro*, two different modes can be directly observed (Fig. 2a), the integrin-independent mode being faster, myosin-dependent, and negatively regulated by the presence of integrin ligands. In contrast, the slower mode uses integrins as part of motility, is slower, and involves greater surface contacts. It is hypothesized that this latter mode may be used in the presence of more inflammatory milieu (chemokines and integrins) and enhanced by TCR-induced inactivation of myosin tension.

Thus, T cells can always live in two modes—one which is optimized for navigation and one which is optimized for surface contact. Upon antigen engagement *in vivo*, T cells probably obligately convert to the latter but then appear to take one of two possible paths. On the one hand, they may simply slow their motility and more actively scan the milieu, repeatedly engaging the same APCs (serial scanning, akin to simply adopting the mesenchymal mode, described above). Alternatively, their dynamic motility may fully arrest as T cells round up and form the synapse with an APC [26–28], as illustrated in Fig. 2b. In multiple studies, cells often start with the former approach and then only later proceed to the more stable T/DC interactions [15–17], and it is now tempting to hypothesize that the

serial scanning mode is produced by a change in motility mode, from the fast integrin-independent to the more adherent mode triggered by increased adhesion and decreases in cortical tension. It is notable though that increased stability of the T–APC synapse dynamics are quite generally observed: including in T/B interactions [19, 25], in T_{EM} cells during DTH [21], and in autoimmune models [20].

A possible explanation for these variations in the motile state of the IS is suggested by the observation that the actin cytoskeleton is undergoing continuous polymerization and inward “streaming” in to the IS, even when the contact is relatively stable as mediated by a uniform lipid bilayer [37]. Thus, the cytoskeleton is not at all at rest, even when cells are “arrested”. Such streaming is likely to be mediated by actin polymerization mediated by TCR activation of WAVE or WASP complexes that subsequently trigger the Arp2/3 complex which nucleates actin assembly. At sites where strong stimuli are engaged, it can then be supposed that TCR microclusters themselves “slip” and trigger local integrin activation which prevents polymerization from inducing net forward movement. One attractive hypothesis then is simply that the IS always contains such motility-encouraging elements but that, in a uniform surface such as a lipid bilayer or a highly activated APC loaded with high densities of pMHC, the forces generated by this streaming are balanced such that the cell does not move. In contrast, when a single edge of an IS finds above average levels of pMHC or integrin, an imbalance of traction (relative to the other sites on the IS where traction is lower) results in net movement into that area.

Tying It All Together: Local Ca^{2+} Signaling Sustained by Ion Channels at the Synapse

The above discussion focuses highly upon the cytoskeletal relaxation that may accompany the transition to signaling, largely in motile synapses. But, what synapse features give rise to microcluster assemblies in the IS? The simplest answer is that stable and motile actin arrays underlie microclusters. Actin has been shown to modulate the ability of specific proteins to interact within the membrane [38] and further has been implicated in generating “island”-like domains in which multiple proteins can co-aggregate [39]. As noted, the actin cytoskeleton undergoes retrograde flow toward the center of individual contacts [37], suggesting that specific strands of actin may generate a flow to which microclusters can couple in order to reach the center of contact zones. The actin formed in the IS is also likely to be responsible for integrin-mediated adhesions which keep the IS from dissolving until signaling is ceased and these bonds are broken.

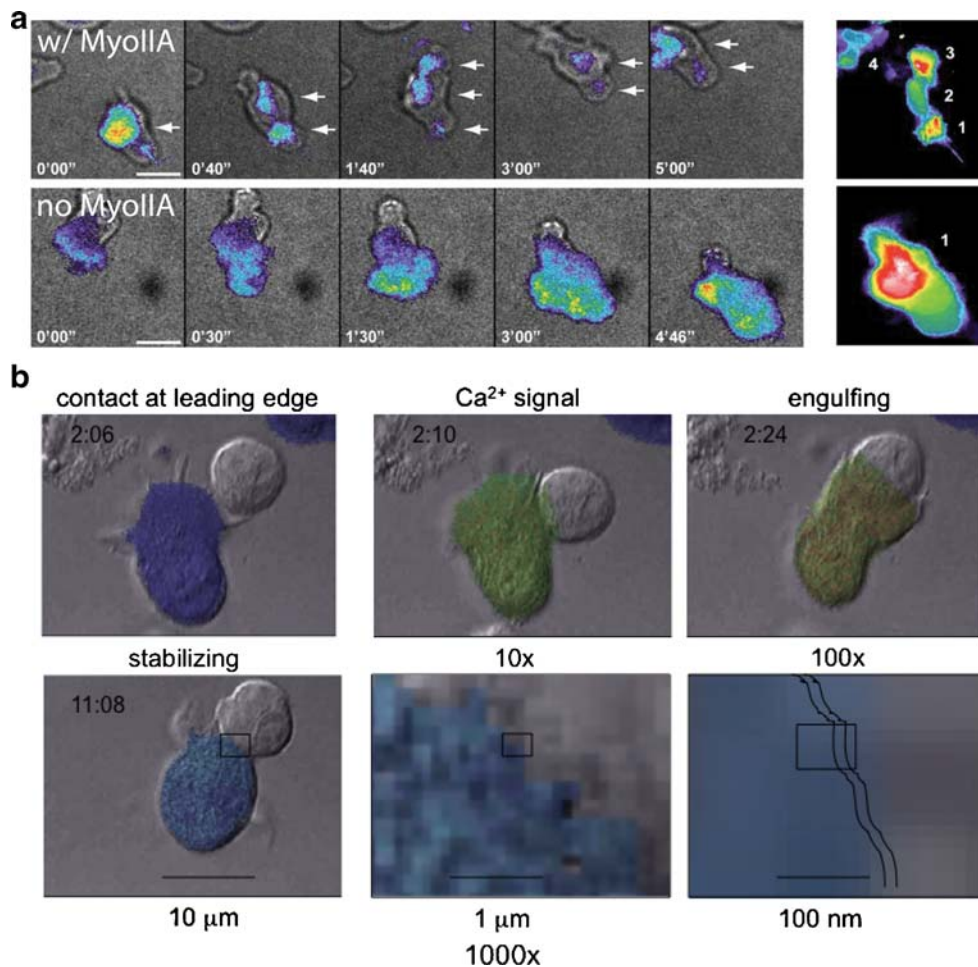


Fig. 2 Motility modes for T cells and control of membrane-membrane contacts and control by calcium signaling. **a** Two modes of cell-substrate interaction during motility as observed in Jacobelli et al. [58]. Individual timepoints of contacts of actin-GFP bearing cells as visualized in TIRF, to visualize the contact surface, are shown. The right-hand panel is a time-sum of all the positions that the cell made with the surface during the entire time sequence. In the *top* (“w/ MyoIIA”): In a “walking” mode that gives rise to faster motility rates, T cells make multiple, sequential, and discrete contacts with the substrate. *Bottom* (“no MyoIIA”): In a “sliding” mode more akin to how mesenchymal cells move, cells make a continuous and large contact with the substrate, gliding along during propagation. **b** Formation of an immunological synapse. T-cell (fura-

2 dye-loaded) and B-cell images from Negulescu et al. [26]. *Colors* indicate T-cell cytosolic Ca^{2+} (dark blue ~ 50 nM to red >1 μM); times shown in each frame are minute:second. Contact is initiated by the leading edge of the T cell. The leading edge probes the APC for 20 s to several minutes before the contact area expands. Ca^{2+} signaling begins 10 s later. The Ca^{2+} signal can be oscillatory, transient, or sustained, as Ca^{2+} enters through CRAC channel Orai1 pore-forming subunits. The signal is sustained by K^{+} channel (Kv1.3 or KCa3.1) activity. Ca^{2+} must remain elevated for synapse stabilization, inhibition of motility, and NF-AT gene expression responses. *Scale bars and boxes in lower panels* indicate “zooming” into the synaptic region after initial stabilization of the synapse. *Jagged lines* indicate the approximate width of the synaptic cleft

An as-yet unappreciated aspect of the cytoskeleton, however, is its role in delivering specific contents to the IS, in order to assist, reinforce, or attenuate signaling. To this extent, both the actin and tubulin cytoskeleton may act as rails upon which specific intracellular proteins may be delivered to nascent microclusters. Such has already been observed for the proximal kinase Ick, for which a large intracellular pool has been observed relocalizing to the IS, albeit late in the response [40]. What other players need to be moved to the IS?

T lymphocytes express ion channels with a diverse repertoire of biophysical, molecular, and cellular functions [41]. Ca^{2+} signaling in T cells is linked to cell function in several ways including the rapid inhibition of motility (Fig. 2b) and delayed activation of gene expression via the cyclosporin-dependent calcineurin pathway. The default condition of resting T cells is robust motility with actively probing pseudopodia; active motility is inhibited rapidly by only a three- to sixfold increase in cytosolic Ca^{2+} [26]. To trigger gene expression responses, the Ca^{2+} signal must

reach a cytosolic concentration tenfold higher than resting and be sustained for at least 2 h [42, 43]. For this purpose, three ion channels in the T-cell plasma membrane (PM) sustain Ca^{2+} signaling. Most importantly, Ca^{2+} release-activated Ca^{2+} (CRAC) channels conduct Ca^{2+} into the cell following TCR engagement. Although vital for T-cell activation and described biophysically as an inwardly rectifying and Ca^{2+} -selective current more than 20 years ago [44], the CRAC channel eluded molecular identification and mechanistic understanding until recently. RNAi screening for molecules required for store-operated Ca^{2+} influx led first to the identification of stromal interaction molecule (STIM) proteins that functionally couple the endoplasmic reticulum (ER) to the plasma membrane and subsequently to Orai proteins that form the pore of the Ca^{2+} channel itself, as reviewed [45]. Upon depletion of Ca^{2+} from the ER lumen, STIM1 physically translocates from the bulk ER to activate Orai proteins in the plasma membrane. In primary resting T cells, STIM1 couples primarily to Orai1, but other isoforms are up-regulated following activation [46]. Two types of K^{+} channel provide an electrical counterbalance to the entry of Ca^{2+} . The rise in cytosolic Ca^{2+} rapidly opens a K^{+} channel composed of KCa3.1 tetramers with CaM bound to the C-terminus. Ca^{2+} binds to CaM and rapid conformational changes then open the channels within milliseconds. The resulting K^{+} efflux helps to keep the membrane potential negative, compensating electrically for Ca^{2+} influx to prevent PM depolarization. A voltage-gated K^{+} channel composed of Kv1.3 tetramers also keeps the membrane potential negative and sustains Ca^{2+} signaling by virtue of its intrinsic voltage dependence. Any tendency toward membrane depolarization opens this K^{+} channel, and the resulting K^{+} efflux restores the membrane potential toward rest. A key point discussed previously [41] is that the balance of KCa3.1 and Kv1.3 channels depends upon the state of activation. In recently activated cells, KCa3.1 is up-regulated and predominates in the role of sustaining Ca^{2+} influx. Chronically activated T cells, however, become T_{EM} cells with up-regulated levels of Kv1.3 and fewer KCa3.1 channels. This shift in channel expression results in T_{EM} cells being more sensitive to channel blockers, enabling T_{EM} cell-mediated autoimmune and inflammatory reactions to be targeted selectively in animal models [41].

Less is known about TRPM7, a ubiquitously expressed channel kinase. TRPM7 is expressed in T lymphocytes and is also regulated by intracellular Mg^{2+} ions by a surface charge screening mechanism [47]. Although it is Ca^{2+} -permeable, there is no evidence that it participates in generating the Ca^{2+} signal following TCR engagement. Remarkably for an ion channel, TRPM7 bears a functional α -kinase domain at its C-terminus that autophosphorylates and in turn may alter key cytoskeletal proteins such as

MyosinIIA [48]. TRPM7 knockouts are embryonic—lethal and conditional deletion in thymocytes, driven by CD4-CRE, leads to impaired thymopoiesis and block of T-cell development.

CRAC channels, formed from Orai1 and triggered by STIM1, and the two lymphocyte K^{+} channels, Kv1.3 and KCa3.1, form a functional network that sustains the Ca^{2+} signal in T cells. Moreover, within the past 5 years, all three ion channels have been shown to redistribute to the zone of contact at the immunological synapse (Fig. 3a): Orai1/STIM1 [46], KCa3.1 [49], and Kv1.3 [50–55]. The colocalization of ion channels to the synapse may strongly influence signaling by several mechanisms. (1) Most importantly, Ca^{2+} influx localized to the synapse results in a higher localized concentration in microdomains within the subsynaptic region adjacent to the T-cell plasma membrane. This could alter the activity of Ca^{2+} -dependent enzymes and cytoskeletal elements. (2) Localized influx could produce significant depletion of Ca^{2+} from the synaptic cleft between the T cell and the APC, providing negative feedback to keep cytosolic Ca^{2+} from going too high. (3) Localized efflux of K^{+} into the synaptic cleft may increase extracellular K^{+} concentration, in turn promoting membrane potential depolarization of T cell, APC, or both. (4) Beta subunits (Kv β 2 and hDig/SAP97) of Kv1.3 may link these channels to β 1-integrin, ZIP proteins linking to PKC ζ , to GAKIN linking to other cytoskeletal elements, and via Lck to CD4 and the TCR. Kv β 2 also has enzyme activity as an aldo-keto reductase and binds NADP with high affinity. Furthermore, Kv1.3 trafficking to the IS is defective in T cells from patients with systemic lupus erythematosus and may result in excessive Ca^{2+} signaling [56]. (5) KCa3.1/CaM is coupled to a kinase (nucleoside diphosphate kinase, NDPK-B) and to a phosphatase (histidine phosphatase, PHPT-1), both of which modulate KCa3.1 channel function. NDPK-B interacts with the integrin-associated protein 1 alpha (ICAP-1 α), a molecule that may link KCa3.1 to β 1-integrin.

Overview and Future Questions

Ion channels have joined the array of molecules that localize at the synapse and have the potential—through local ionic signaling and physical links to receptors, enzymes, and cytoskeletal elements—to effect modulation of a network of other elements. Given the level of potential complexity, there is still much to be learned about the local signaling dynamics in the IS. Some of the key questions are: What are the mechanisms that enable ion channels to redistribute into the synapse? Figure 3b illustrates some of the key interactions that are currently known but where the exact cytoskeletal mechanisms

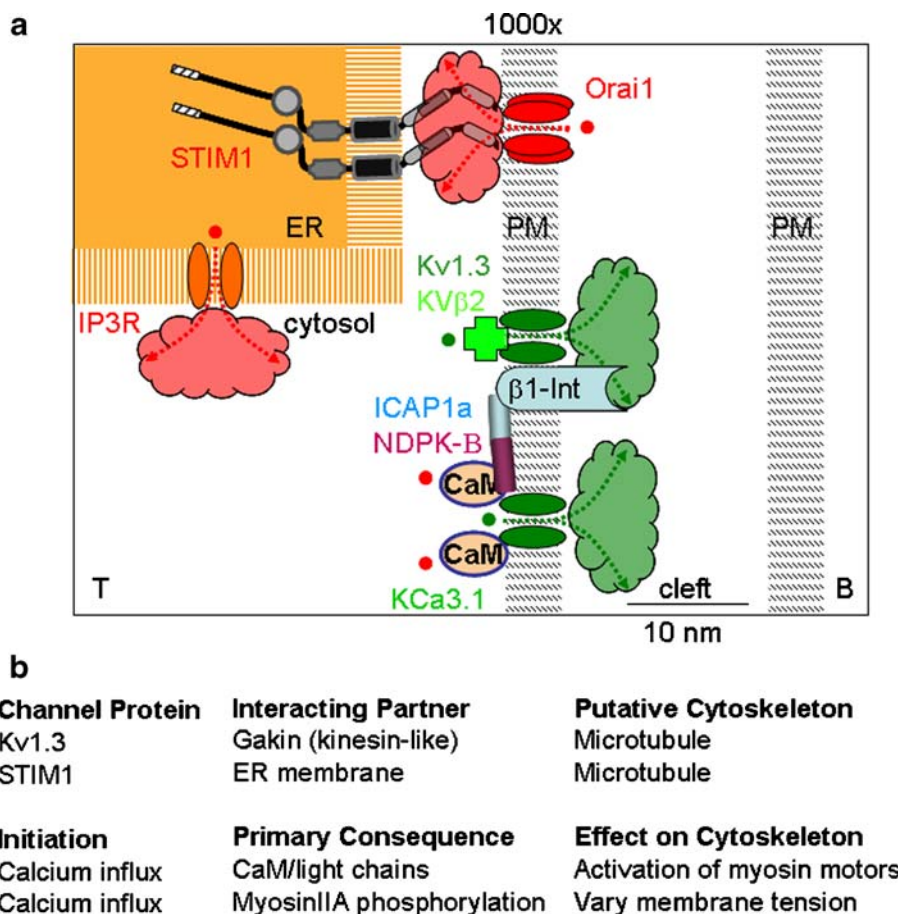


Fig. 3 Calcium channels and the cytoskeleton at the immunological synapse. **a** Zoomed-in schematic diagram of region shown by *box* in the *lower right panel* of Fig. 2a, showing T-cell molecules concentrated at the synapse, separated by a narrow synaptic cleft (~15–20 nm) from the B cell. Scales are approximate. A STIM1 dimer within the endoplasmic reticulum (ER) is shown activating a tetramer of Orai1 in the plasma membrane by direct contact. Protein domains of STIM1 include (from *left to right*): signal peptide, EF hand (without bound Ca^{2+}), and SAM domains in the ER lumen; a transmembrane segment, and bipartite coiled-coil in cytosol. Depletion of the ER Ca^{2+} store by release of Ca^{2+} ions (red) through the IP_3 receptor (IP_3R) initiates signaling via STIM1 to the PM. Ca^{2+} first unbinds from the EF hand; STIM1 then forms oligomers that

translocate to ER–PM junctions; Orai1 tetramers then open; Ca^{2+} ions enter the cell and begin to diffuse. Ca^{2+} opens KCa3.1/CaM ; membrane depolarization opens Kv1.3. K^+ ions (green) leave the cytosol and enter the cleft. Kv1.3 and KCa3.1 are linked through associated subunits to enzymes, to $\beta 1$ -integrin ($\beta 1\text{-Int}$), and to cytoskeletal elements. *Clouds* indicate diffusion of Ca^{2+} and K^+ ions and accumulation in nanodomains near Orai1 (red) and Kv1.3 and KCa3.1 channels (green). **b** Putative interplay between channels and the cytoskeleton. Channels themselves are likely assembled as a function of dynamic reorganization of cytoskeletal elements. Conversely, calcium transients and gradients almost certainly integrate at multiple levels to continue to stabilize or destabilize motility modes

remain undiscovered. Of particular note is the movement of the STIM1 subunit, which resides on the ER membrane, into close proximity with the Orai1 subunit, localized on the plasma membrane—might this ER translocation utilize a cytoskeletal rail system for its assembly? Conversely, what are the feedback loops between Ca^{2+} signaling and synapse stabilization, notably how does localized calcium gradients affect the local balances of forces that influence cell movement? For example, in motile fibroblasts, “flickers” of calcium are observed at the leading edge, likely in part regulated by TRPM7, perhaps functioning as a stretch-sensitive calcium-permeable channel [57]. Does “balanced” calcium flux at the IS maintain a stable (non-motile) IS and,

in contrast, does variegated microcluster formation lead to a destabilization and result in probing motility? Similar questions surround the cessation of signaling at the IS: Does Ca^{2+} depletion or K^+ accumulation in the synaptic cleft initiate or terminate signaling in DCs or B cells as the APC? Finally, how may ion channel modulators (blockers or openers) be harnessed for therapeutic action in autoimmunity and transplantation? As a promising example, Kv1.3 blockade *in vivo* by a peptide inhibitor has shown efficacy in experimental autoimmune encephalitis and in delayed type hypersensitivity responses, and it completely immobilizes T_{EM} cells in the periphery without altering the motility of naive T cells in the lymph node [21]. It is becoming clear

that answering these questions will require an integrated view extending from calcium channel function to localized motility and the action of the T-cell cytoskeleton.

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Conflict of interests None declared.

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