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Functional Anatomy of T Cell Activation and Synapse Formation

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Abstract

T cell activation and function require a structured engagement of antigen-presenting cells. These cell contacts are characterized by two distinct dynamics in vivo: transient contacts resulting from promigratory junctions called immunological kinapses or prolonged contacts from stable junctions called immunological synapses. Kinapses operate in the steady state to allow referencing to self-peptide-MHC (pMHC) and searching for pathogen-derived pMHC. Synapses are induced by T cell receptor (TCR) interactions with agonist pMHC under specific conditions and correlate with robust immune responses that generate effector and memory T cells. High-resolution imaging has revealed that the synapse is highly coordinated, integrating cell adhesion, TCR recognition of pMHC complexes, and an array of activating and inhibitory ligands to promote or prevent T cell signaling. In this review, we examine the molecular components, geometry, and timing underlying kinapses and synapses. We integrate recent molecular and physiological data to provide a synthesis and suggest ways forward.

Keywords

immunological synapse; kinapse; microcluster; TCR triggering

Introduction

T cells have evolved a highly efficient mechanism for finding and discriminating antigen while integrating multiple environmental cues to determine the context of these signals. Efficiency is important for T cells for several reasons: **T cell receptors (TCRs)** must be able to recognize a few activating **peptide-MHC (pMHC)** complexes (~10) in a sea of self-pMHC on the surface of an **antigen-presenting cell (APC)**. Furthermore, the difference between an activating and nonactivating pMHC complex can be a single conservative amino acid change, sometimes translating into minor differences in affinity. Next, antigen recognition in vivo occurs on the fly as cells brush past each other at surprisingly rapid speeds. These connections, or hapsis, are necessary because the TCRs and pMHC are both membrane-anchored molecules that span a

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combined length of nearly 15 nm. We refer to these interactions as immunological **kinapses** when fleeting and immunological synapses (IS) when longer lived. In theory, each APC could be expressing different arrays of pMHC, adding to the complexity problem. Finally, TCRs are unique and clonal, generated by random swapping and editing of DNA segments prior to any exposure with cognate pMHC. Each individual must evolve their own repertoire of receptors over a period of a few weeks to optimize the thermodynamic receptor-ligand engagement process in their particular genetic background. Although the starting TCR repertoire is not entirely random and has some bias toward recognizing pMHC (1), this is still a remarkably ad hoc process compared with other receptor-ligand systems that have evolved over millions of years. Nevertheless, for the most part, these systems are highly capable of identifying rare pathogen-associated ligands while learning to ignore self and benign foreign antigens. The consequences of errors are profound: autoimmunity resulting from inappropriate recognition of self, allergies and hypersensitivity from inappropriate recognition of benign foreign antigens, and chronic infection or death because of a failure to recognize or mount an appropriate response to pathogen-associated antigens.

Therefore, and not surprisingly, the mechanisms and mystery of T cell activation have received great attention in various fields in biology and medicine for quite some time. In recent years, the ability to image T cell activation by high-resolution in vitro methods and under physiological conditions in vivo has boosted our understanding of these processes and has helped to unify a diverse but often disjointed body of biophysical and functional data. In this review, we deal with the molecular reorganization that occurs during activation, the degeneracy of this phenomenon in other synapses, and the impacts on physiological conditions in vivo. We also try to integrate how the in vitro lessons instruct us on the cellular behaviors observed in vivo.

To understand T cell activation, we must first visit the steady-state behavior of T cells in vivo. The advent of two-photon laser scanning microscopy (TPLSM) imaging of lymphocytes in vivo has exposed the physiological behavior of these cells, which have long been studied ex vivo. It has also provided a metric to evaluate the physiological relevance of divergent in vitro observations. Early studies by Cahalan, Miller, and colleagues (2,3) illustrated that naive T cells are in constant motion, scanning the lymph node at high rates (10–15 μm/min average, 25 μm/min burst speeds) in search of antigen and danger signals and capable of contacting 5000 dendritic cells (DCs) in 1 h.

Lymphoid tissue contains a complex but stereotyped array of cells and signals that are highly compartmentalized but fluid at the same time. A common characteristic is adjacent zones dominated by T or B cells, each tethered to distinct stromal networks. T cells kinapse with fibroblastic reticular cell networks and appear to use these to access DCs that form an interdigitating network and process past or stream with other lymphocytes via transient adhesive interactions coupled to the actin cytoskeleton (4–6). These surfaces are covered with stop, go, and exit signals that T cells must integrate to decide what to do (7,8). A major stop signal is agonist pMHC on a DC. Three phases have been described in T cell interaction with pMHC (9). Phase 1 is initial transient T cell–DC interactions characterized by continued rapid T cell migration that can last from 30 min to 8 h depending on the pMHC density. Signals in phase 1 are integrated through kinapses. Phase 2 is a period of stable T cell–DC interactions lasting ~12 h, during which cytokines such as IL-2 are produced. Signals in phase 2 are integrated through IS. Phase 3 is a return to transient T cell–DC interaction and rapid T cell migration during which the T cell divides multiple times and then exits the lymphoid tissue. The correct interpretation of these stop and go signals is critical for generation of effector and memory T cells (10). This is the environment in which T cells engage antigen. We return to the in vivo view after developing the in vitro details.

From high-resolution imaging of in vitro T cell–APC conjugates and from imaging of T cells interacting with supported bilayers, we have been able to illuminate the molecular organization of T cell activation. Fixed-cell imaging studies by Kupfer (11) revealed the formation of a bull's eye pattern with a central cluster of TCR-pMHC, defined as the **cSMAC** (central supramolecular activation complex), surrounded by a ring of the cognate integrin LFA-1 (lymphocyte function–associated antigen 1) and its immunoglobulin superfamily ligand ICAM-1 (intercellular adhesion molecule 1), defined as the **pSMAC** (peripheral SMAC). The region outside the pSMAC, which appeared to be rich in CD45, was referred to as the dSMAC (distal SMAC) (12). Contemporaneous studies with supported planar bilayers revealed a similar stable configuration of small and large adhesion molecules in activated T cell contacts, suggesting an IS (13). Dynamic studies with planar bilayers further showed that the IS is formed through a nascent intermediate in which activating TCR clusters form first in the dSMAC and then move to the cSMAC region in an F-actin-dependent process in a few minutes to form the pattern described by Kupfer (11). Formation of the IS, regardless of the size of the cSMAC, correlates with full T cell activation over a timescale of hours, leading to an initial characterization that the IS is important for sustained TCR signaling (14). We initially thought that the TCR translocation from the dSMAC to cSMAC was a one-time event driven by actinomyosin contraction (15). However, it is now clear that TCR signaling is sustained by TCRs (**MCs**) that are continually forming in the dSMAC and moving to the cSMAC (16). This ongoing process of cluster formation and transport often ends in TCR signal termination (16), but not always (17,18). We discuss the sequence of T cell activation by first examining the life cycle of TCRs.

The Life Cycle of TCRS During T Cell Activation

TCR Triggering

T cells can coordinate responses to as few as 10 agonist peptides presented on an **APC** (19). The exact mechanism of initial **TCR** triggering is unknown and remains hotly disputed. An intriguing study recently demonstrated that nonphosphorylated immunoreceptor tyrosine-based activation motif (**ITAM**) tyrosines are buried in the inner leaflet of the plasma membrane based on synergy with a positively charged N-terminal sequence that interacts with acidic lipids in the inner leaflet of the plasma membrane (20). Under normal conditions the phosphorylation of the ITAMs is inhibited unless the negative charge of the plasma membrane is reduced to allow the ITAM to dissociate from the membrane and become accessible to phosphorylation. This electrostatic switch that exposes the ITAMs may be an important early event in TCR triggering.

To achieve high sensitivity, however, T cells must overcome the low affinity of TCR-**pMHC** interactions (21,22). Although it is generally accepted that higher affinities tend to correlate with activating peptides, strict correlations between TCR-pMHC affinity and T cell reactivity have been described predominantly in thymocytes (23) and peripheral CD8 T cells (24). In peripheral CD4 T cells, there remains a partial discord between affinity (or off rates) of TCR to pMHC and the functional outcome for activation, and this prevents complete resolution. One example of this divergence comes from recent work from the Shaw lab that has characterized altered peptide ligands (APLs) of the cognate antigen moth cytochrome *c* (MCC) peptide, which is recognized by the AND TCR (17,25). In these papers, the investigators showed that a peptide variant, K99A, with lower affinity for the AND TCR than cognate antigen for the AND TCR, nevertheless can induce equivalent or even elevated levels of T cell proliferation when presented by the same B cell APC as the higher-affinity $MCC_{88–103}$-I-E^k complex. Understanding TCR triggering is further complicated by a variance in the threshold affinities for pMHC by different TCRs for activation (26). Moreover, the orientation and footprint for binding can vary among TCR/pMHC complexes, with some alloreactive TCRs binding pMHC interfaces in unconventional orientations (27). Physical

models for relating solution affinity to functional triggering are still not fully predictive, suggesting that direct measurements of interactions in contact areas are needed (see sidebar and Figure 1).

Measuring 2-D Affinity and Kinetics

Much of the thermodynamic and biophysical data on TCR-pMHC interactions have been measured in solution or, to put it differently, in systems where six degrees of kinetic freedom (from x, y, and z translation and x, y, and z rotation) are lost upon binding. In theory, two apposed cell membranes should constrain TCR and pMHC movement such that only three kinetic degrees of freedom (x, y translation and z rotation) are lost on binding (Figure 1). We quantified the 2-D interactions of CD2-CD58 adhesion molecules, which form a large central aggregate, much as the TCR forms a cSMAC (28). Within the central cluster, we determined a 2-D dissociation constant (Kd) of 1.7 molecules/μm^2 (29–31). Photobleaching experiments yielded an off rate of 0.074 s^{-1} and an on rate of 0.044 μm^2/s (32). The off-rate value was ~100-fold slower than that predicted by solution measurements, possibly due to constrained diffusion leading to cycles of rebinding of CD2 and CD58 prior to exchange. Whether similar rebinding effects govern TCR-pMHC interaction is unknown. Estimating 2-D Kd for the 2B4 TCR interaction with I-E^k with MCC peptide 88–103 yields a value of 10 molecules/μm^2, five-fold weaker than the CD2-CD58 interaction, and the TCR also displayed slower photobleaching recovery rates, suggesting a relatively long effective lifetime compared with CD2-CD58 (14). The effective lifetime of the TCR-pMHC complex with rebinding is likely to be the important parameter for signaling through the TCR.

Microcluster Formation

Various experimental models have revealed the subtleties of TCR activation (summarized in Table 1). One pathway to T cell activation is based on induction of TCR clustering by cross-linking with bivalent IgG and secondary antibodies. Surface adsorbed, bivalent anti-CD3 antibodies induce actin-dependent TCR clusters (33–35). Anti-CD3 tethered to planar bilayers can also induce **MCs** and mature IS (36). Whether these MCs and cSMACs are the same as those induced by pMHC is not yet clear. In solution, chemically defined pMHC class II dimers with optimal spacing are the minimal soluble stimuli for CD4 T cell activation (37).

How pMHC presented by APCs or on other surfaces triggers T cell signaling is unclear. Recent studies have shown transient Ca^{2+} elevations in response to a single pMHC (19) and sustained Ca^{2+} elevation in response to chemically defined heterodimers of agonist pMHC and endogenous pMHC (38). Planar bilayers presenting pure agonist pMHC and ICAM-1 induce formation of TCR MCs within seconds of ligand engagement (14,39). Titration studies suggest that MCs require a single pMHC complex to nucleate, and the pMHC may serially engage several TCR complexes within the same MC (40). This may be analogous to the rebinding process described above for CD2-CD58 interactions. Therefore, although a single pMHC may trigger the TCR, a multivalent TCR complex seems to form regardless. The TCR clusters may also preexist and become stabilized or rearranged in some manner by ligation (41). Transient and actin-dependent TCR microclustering is observed in the absence of pMHC by imaging (16). It is unknown if formation of nanoscale, short-lived TCR clusters would increase the avidity of initial TCR-pMHC interactions or provide intrinsic cortical tension to support low-valency interactions. Ligand may convert transient MCs that are specialized for pMHC capture into more stable MCs that are specialized for signaling and pMHC retention. Recent electron microscopy and superresolution optical imaging studies reveal a non-homogeneous distribution of TCR and the transmembrane adapter linker of activation in T cells (LAT) (42). Activation results in a convergence of discrete TCR and LAT domains (42).

Although the stoichiometry of the TCR-pMHC interaction is 1:1, we do not know how rapid binding and dissociation, combined with cytoskeletal interactions, influence the effective valency in the TCR MCs. The question of how these low-valency, low-affinity TCR-pMHC interactions lead to cluster formation is also unknown, but we know of two mechanisms by which signal initiation within MCs might be supported. First, cluster formation and T cell activation are critically dependent on the actin cytoskeleton (16,33,35,39,40). Secondly, early signal integration within clusters might be supported by size-dependent exclusion of large phosphatases such as CD45 for the life of the MC, a process now known as kinetic segregation. Springer (43) was the first to speculate that size-dependent exclusion of CD45 could be a basis for initiation of TCR signaling. Choudhuri et al. (44) provided the best evidence for the importance of this process in TCR signal integration, and Varma et al. (16) directly demonstrated exclusion of CD45 from TCR MCs.

TCR MCs are the signaling units of T cell activation. Staining of T cells interacting with planar bilayers containing agonist pMHC and ICAM-1 shows enrichment of phosphorylated Lck, ζ-associated protein 70 (ZAP-70), LAT, Src-homology 2 (SH2) domain-containing leukocyte protein of 76 KDa (SLP-76), and growth factor receptor-bound protein 2 (Grb2) signaling kinases within MCs (33,42,45), correlating with increasing ITAM phosphorylation. When TCR ligands are laterally mobile in a membrane, all these components move together in a MC. Soon after formation, TCR MCs transit radially and accumulate to form the cSMAC (16). MCs travel at a rate of ~1–5 μm/min along the T cell surface. Acute treatment with anti-MHC during T cell activation, which blocks new MC formation, extinguishes signaling and Ca^{2+} influx after 2 min (16). This latency correlates well with the time needed for existing MCs (unaffected by anti-MHC) to reach the cSMAC and terminate signaling (16). This result suggests that signaling is sustained only during the lifetime of these discrete MCs (16). Sustained signaling requires continued MC formation and continued actin polymerization.

When TCR ligands are fixed to the substrate, the TCR and ZAP-70 MCs remain fixed in place and spawn SLP-76 domains that move toward the IS center (46). Recent data have demonstrated that coupling costimulation of very late antigen-4 (VLA-4) integrin retards the cytoskeletal movements that ultimately results in the centralization and inactivation of given SLP-76 domains (47). These findings identify a third domain of MCs enriched in SLP-76 that can persist independent of TCR MCs.

cSMAC and Signal Termination

Upon reaching the cSMAC, the signaling molecules dissociate from the TCR MCs, and signal termination and TCR degradation begin (16,39,45). The first clue regarding the mechanism came from detection of lysobisphosphatidic acid, which marks multivesicular bodies (MVBs) (16), at the cSMAC. Signal termination in MVBs is achieved via segregation of receptors to limiting membranes within the MVB and via fusion with lysosomes, thus isolating them from downstream kinases and adaptor proteins as soon as MVBs are formed (48). A family of proteins known as the endosomal sorting complex required for transport (ESCRT) proteins (48) are implicated in this process (S. Vardhana, K. Choudhuri, R. Varma, and M. Dustin, manuscript submitted). TSG101, the critical component of the upstream ESCRT complex ESCRT-I, is required for identification of ubiquitinated TCR MCs, for sorting them into the cSMAC compartment in the planar bilayer model, and for TCR downregulation in cellular models.

The behavior of ESCRT complexes within the IS is both domain and substrate specific. Sorting of TCR for termination of signaling and degradation occurs exclusively at the cSMAC. TCRs that do not interact with TSG101 are unable to enter the cSMAC. This is in line with prior reports demonstrating specific recruitment of MVBs to the cSMAC (16) and elevated signal transduction when engagements are restricted to the periphery, either by physical barriers

(49) or by immobilized ligands (50). This is the first demonstration that ESCRT functions have been linked to protein organization within spatially segregated domains of a polarized cell interface and provides new possibilities for ESCRT proteins to respond to polarized stimuli. Importantly, ESCRT-mediated TCR sorting into the cSMAC also critically depends on ubiquitination, as neither ubiquitin-depleted nor ubiquitin-noninteracting ESCRT-I can induce cSMAC formation. It is not known how TCR signaling is sustained in the cSMAC in some situations, particularly in $CD8^+$ cytotoxic T lymphocytes (CTLs) where this signaling may play an important role in cytotoxic granule targeting to the secretory domain adjacent to the cSMAC (51). This process could be controlled at the level of ubiquitin ligases, deubiquitinating enzymes, or ubiquitin recognition, to name only three potential control points. The consequence of disabling ESCRT-I leads to chronic TCR signaling, which could be deleterious if left unchecked. Thus, the TCR signaling cascade is likely modulated rather than completely inactivated in cells displaying cSMAC-associated signaling such as CTLs.

Additional Players of the Synapse and T Cell Activation

Adhesion and Costimulatory Molecules

LFA-1 engagement of ICAM-1 is essential for IS formation in vitro and in vivo and acts in several ways. Initially, these molecules serve as adhesion molecules, tethering opposing T cell and **APC** membranes within tens of nanometers, facilitating **TCR-pMHC** interactions. Engaged LFA-1 quickly consolidates into an enriched pSMAC network, which may provide the positional stability that allows it to enhance T cell sensitivity to antigen 100-fold as compared with the situation in the absence of LFA-1 engagement (52).

TCR activation induces Rap1 activation through ADAP (adhesion and degranulation–promoting adaptor protein) and SKAP55, which leads to a clustered higher-affinity LFA-1 binding of ICAM-1, referred to as inside-out signaling (53,54). Engagement of high-affinity LFA-1 generates positive feedback on TCR-induced Ras activation by shifting Ras activation from the Golgi to the plasma membrane (55). In the thymus, this process may significantly affect negative selection, which is characterized by similar shifting of Ras activation from the Golgi to the plasma membrane (23). Linking of integrins to actin is generated by focal adhesions in many cells and involves myosin-driven recruitment and activation of force-sensitive substrates such as p130Cas, fyn, and other mediators of integrin scaffolding (56). LFA-1 microclustering and pSMAC formation are critically dependent on Talin activity but also appear to depend on myosin IIA–based contraction and may involve a role for force-sensitive substrates such as p105CasL (57; S. Vardhana, L. Santos, M. Sheetz, and M. Dustin, unpublished observations). One model for integrin activation suggested that LFA-1 was transiently released from actin to facilitate diffusion and interaction with ligands, but recent high-resolution imaging of the formation of integrin-mediated adhesion suggests that integrins are preclustered by an F-actin bundle in the lamellipodium prior to ligand binding (58).

TCR **MC**s also serve as sites for initiation of costimulation. The critical costimulatory receptor CD28, when engaged by its primary ligand CD80 (B7.1), is highly enriched in TCR MCs (59). Incorporation of CD28 into MCs is completely independent of its signaling, occurring in the absence of a cytoplasmic region of CD28 (59) where PKCθ (protein kinase Cθ) binds. This finding is consistent with phosphorylation-independent formation of TCR MCs (42). The CD28-CD80 complexes are transported in TCR MCs to the cSMAC, at which point they array in an annular cluster around the cSMAC, segregated from TCRs (Figure 2). This CD28-PKCθ compartment is actively maintained. Fluorescence recovery after photobleaching (FRAP) experiments with PKCθ or CD28 have shown that it rapidly recovers after photobleaching, indicating a dynamic enrichment in this compartment (59), and application of anti-CD3 rapidly induced loss of CD28-CD80 clusters in T cell–DC interfaces (60). This is in contrast to TCR in the cSMAC, which does not recover quickly after photobleaching (14) or

dissipates upon addition of anti-pMHC antibodies (16). Although it is unclear what maintains the CD28-PKCθ annular ring around the cSMAC, it may be related to the fact that the structure is situated at the boundary between F-actin-rich and F-actin-depleted zones of the IS. Activated PKCθ is necessary for formation of cytoplasmic Bcl10/MALT1-rich foci that lead to NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) translocation into the nucleus (61).

CD2 functions equally as an adhesion and costimulatory molecule. We have recently observed the generation of novel signaling domains in response to activation of the T cell costimulatory molecule CD2 by its murine ligand, CD48. CD2 functions prominently as a costimulatory molecule by lowering the threshold for T cell activation (62). CD2 engagement induces unique phosphorylation of phospholipase γ1 (PLCγ1) via Fyn kinase (63), potentiating intracellular calcium levels above those achieved in response to TCR triggering alone. CD2 also can facilitate T cell adhesion to APCs (62). Because it spans a similar intermembrane distance as TCR-pMHC, CD2-CD58 interactions may facilitate the construction of closely apposed domains for facilitation of TCR-mediated signaling in loci that exclude phosphatases such as CD45 with large extracellular domains (28). CD2 had been described during IS formation as a large accumulation only within the cSMAC (64). However, we have found that, although a proportion of engaged CD2 is quickly routed to the cSMAC, the remainder consolidates into distinct foci within the dSMAC (S. Vardhana and M. Dustin, unpublished observations). These outer foci remain anchored in the periphery and are not translocated to the cSMAC. We have found that CD2 associates with the signaling molecule Fyn in the periphery but is largely silent in the cSMAC. Thus, engaged CD2 contributes to T cell activation in discrete ways that depend on its localization within the IS.

Inhibitory Receptors and Cosignaling

T cell activation and IS formation can be constrained by the action of inhibitory receptors. The classic example is expression of cytotoxic T lymphocyte antigen-4 (CTLA-4, CD152) on T cells after activation, which dampens signaling by a variety of mechanisms. CTLA-4 can outcompete CD28 for binding to costimulatory ligands B7-1 (CD80) and B7-2 (CD86) (65). CTLA-4 localization in synapses depends on ligand engagement (66), and ligand dosage and its engagement reduces ZAP-70 recruitment to MCs (67). Despite CTLA-4's effect on MC signaling, it is unknown if it colocalizes with them. It recruits SHP2 and SHP1 and blocks Akt phosphorylation (in a CD28-dependent manner for the latter), inhibiting T cell activation. Although cross-linking CD3 on previously activated mouse and human T cells reduces cell motility, coligation of CTLA-4 results in a reversal of cell arrest, with cells moving at speeds similar to that of untreated cells. Rudd (68) has proposed a reverse–stop signal model to explain this phenomenon. By increasing the rate of motility, CTLA-4 coligation increases the threshold for TCR triggering, thus decreasing the likelihood of T cell activation. We revisit the relationship between T cell arrest and activation in a later section. Mice deficient for CTLA-4 develop an aggressive autoimmune disorder characterized by inflammation and overproliferation of T cells. New cancer immunotherapies have targeted CTLA-4, which has the capacity to boost the function of tumor-infiltrating lymphocytes in patients (69).

Another inhibitory molecule involved in cosignaling in T cells is the programmed-death 1 receptor (PD-1, CD279), and, as its name suggests, it was first identified as a proapoptotic factor on CD8 T cells (70). PD-1 is part of the CD28 family, which also includes CTLA-4, ICOS (inducible T cell costimulator; CD278), and BTLA (B and T lymphocytes attenuator; CD272) (71). PD-1 has two ligands---PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273)---although PD-L1 can also interact with CD80 to generate inhibitory signals (72).

Recently, PD-1 has become implicated as a marker for the “exhausted” phenotype of antigen-specific CTLs that is associated with chronic viral infections such as human immunodeficiency

virus (HIV), simian immunodeficiency virus (SIV), and lymphocytic choriomeningitis virus (LCMV) clone 13 in mice (73,74). Exhaustion has been characterized as an inability to produce effector cytokines, such as IFN-γ and IL-2, and by diminished proliferation. The level of expression of PD-1 positively correlates with the degree of functional exhaustion, but this phenotype is actively maintained by signaling. Blockade of PD-1 ligands can restore the functionality of the exhausted T cells and can curtail chronic viremia. As with a deficiency in CTLA-4, mice deficient for PD-1 also have an autoimmune disorder; however, it manifests later in life (75). PD-1 signaling inhibits CD28-dependent PI3K activation (76) and thus blocks Akt activation. The role played by PD-1 in TCR MCs is unclear. As with CTLA-4 in cancer, blockade of PD-1 signaling is being explored as a new therapy for patients with chronic viral infections (77).

Many questions remain regarding cosignaling of these receptors. For example, does PD-1 expression impair T cell arrest, as does CTLA-4 expression? From imaging experiments and affinity measurements, there seems to be a crosstalk between members of the CD28 receptor family and ligands of the B7 family. Furthermore, various receptors have nontrivial affinities for contra-ligands, and PD-L1 and PD-1 are both expressed on T cells. This suggests that these interactions may take place in homotypic (T cell–T cell) interactions or even in *cis*, e.g., between membrane projections from the same cell. Moreover, where does PD-1 localize at the IS and is its spatial and temporal control important for exerting its negative regulatory role in T cell activation? Addressing these questions will provide additional insight into how T cell activation versus inhibition is controlled. Although the inhibitory action of CTLA-4 and PD-1 may sometimes be exploited by pathogens or tumors to escape the immune response, these mechanisms are likely important in averting immunopathology.

The Actin Cytoskeleton

Actin filaments (F-actin) play a critical role throughout the various stages of T cell activation (Figure 2). In the steady state, actin polymerization at the leading edge and cytoskeletal contraction at the uropod mediate rapid migration during scanning (78). Upon T cell engagement of an APC bearing agonist pMHC, the T cell's machinery for locomotion is recycled for synapse formation (Figure 2). Globally, actin polymerization continues as before, but without T cell displacement (79). The T cell spreads a lamellar sheet over the APC surface, inducing outward, radial actin polymerization. This is coupled to contraction and centripetal flux of F-actin toward the center of the IS, which eventually becomes the cSMAC (36). Dynamic protrusion and retraction produce contractile oscillations in the lamellipodium (80). These oscillations may allow the T cell to rake the APC surface for antigen as well as generate force transduction on the TCR, inducing triggering (81). Valitutti et al. (40) also commented on this F-actin-dependent scanning of the APC surface by T cells.

TCR signaling also induces F-actin remodeling. TCR triggering leads to LAT and SLP-76 activation, which in turn recruit Vav1, the guanine exchange factor for the Rho GTPases Cdc42 and Rac1 (82). Activated Cdc42 and Rac1 interact with WASp (Wiskott-Aldrich Syndrome protein) and the WAVE2 complex, respectively, to recruit and activate the actin-related protein 2/3 (Arp2/3) complex, leading to dendritic nucleation of F-actin (82–84). However, MC formation is independent of Src kinase activity, which indicates that actin-dependent MC formation precedes TCR signaling.

TCR triggering and MC formation depend on F-actin. Disrupting actin filaments with latrunculin A (LatA) inhibits MC formation and signaling. Antigen sensitivity is elevated in the lamellipodium, where actin polymerization is concentrated (85,86). The stability and transit of MC to the cSMAC also depend on the actin cytoskeleton. Waves of F-actin, which can be seen flowing toward the cSMAC (87), seem to shuttle MCs to the cSMAC. The interaction of

TCR MCs with actin is discontinuous, as shown by the ability of MCs to translocate around F-actin barriers (88).

The nonmuscle actin motor protein, myosin IIA, is also a key player in MC movement and signaling. This myosin isoform is the only one expressed in mouse T cells, whereas human T cells also express myosin IIB (89,90). Myosin IIA knockdown CD4 T cells can form TCR MCs, but these MCs are barely mobile and cannot translocate efficiently to the cSMAC (91). In MCs formed in the absence of myosin IIA, there is a markedly diminished recruitment and activation of ZAP-70 and LAT (91).

Coincidentally, both MC signaling and the actin cytoskeleton terminate at the cSMAC. The mechanism that couples MC signaling to the cytoskeleton is still unclear. The void in F-actin in the cSMAC is important for localized exo- and endocytosis (92,93). It is unclear what maintains the F-actin-free zone in the cSMAC, but this may be important for vesicle trafficking.

The Microtubule Network

The microtubule organizing center (MTOC) and microtubule network of the cell provide a molecular highway for vesicle traffic and structural support for polarized cell functions. Within seconds after TCR stimulation, the MTOC mobilizes and polarizes to the IS in T cells (94, 95). Polarization is important for efficient trafficking and directed secretion of cytolytic granules and cytokines for secretion at the synapse (95,96).

The mechanism that initiates MTOC polarization is poorly understood. Signaling through TCR is required, with several downstream factors implicated, such as Lck, ZAP-70, LAT, SLP-76, and Ca^{2+} influx (97–99). Localized diacylglycerol production by PLCγ precedes MTOC polarization (100). The microtubule minus-end-directed motor dynein is recruited to the IS via an interaction with the adaptor ADAP, and knockdown of either protein abrogates MTOC polarization (101,102). SLP-76 may mediate and help localize the assembly of the dynein-ADAP complex (103). Actin polymerization is also involved in MTOC polarization. Two of the formin family members, formin-like 1 (FMNL1) and Diaphanous 1 (Dia1), colocalize with the centrosome, form a ring-like structure surrounding the MTOC, and control its polarization as well as cell-mediated killing. The formin family of proteins nucleates actin into linear filaments found in actin cables, filopodia, and stress fibers (104). Branched actin structures generated by the Arp2/3 complex may not be involved in polarization (105). The plasma membrane proximal localization of the centrosome at the IS is reminiscent of centrosome positioning in basal bodies of flagella and cilia, although leukocytes lack the primary cilium that acts as sensory structures in epithelial cells. However, an evolutionary connection between the IS and sensory cilium is suggested by the recent finding that intraflagellar transport proteins play a critical role in sustaining the IS and T cell signaling (106).

Questions remain regarding the molecular mechanism and the dynamics of MTOC polarization in various aspects of the IS. How these structures function in CD4 cytokine versus CTL granule secretion is unclear. Additionally, MTOC polarization is likely involved in asymmetric cell division and segregation of polarity proteins.

Calcium and T Cell Activation

Ca^{2+} signaling is required for full T cell activation and function (107). TCR activation triggers a transient Ca^{2+} flux through release of ER stores. This opens CRAC (Ca^{2+}-release activated Ca^{2+}) channels on the plasma membrane, leading to a sustained influx of Ca^{2+} or store-operated Ca^{2+} entry (SOCE). Recently, Lewis and colleagues (108) demonstrated that stromal interaction molecule (STIM) and Orai proteins are the major players in SOCE. In response to ER store depletion, STIM proteins (primarily STIM1) form clusters that localize to sites of ER

membrane association with the plasma membrane, so-called puncta (109–111). Sites of STIM1 clusters overlap with Orai1 clusters and mediate localized Ca^{2+} influx (112,113). Direct binding of STIM1 to the cytoplasmic domain of Orai1 appears to mediate opening of the Orai channels (114,115). In T cells, Orai1 and STIM1 are recruited to the IS in response to antigen-bearing DCs, resulting in increased local Ca^{2+} entry at the T cell–APC interface (116). STIM1 and Orai1 interact in a stable complex in puncta at the IS of Jurkat T cells, interacting with anti-CD3-coated glass (117). However, these clusters do not colocalize with TCR clusters. STIM1 and Orai1 recruitment to the IS is mediated through proximal TCR signals and is independent of sustained Ca^{2+} signaling (116,117). Although STIM and Orai have not been directly linked to the cytoskeleton, there is evidence that their function depends on cytoskeletal components. WAVE2 is required for sustained Ca^{2+} increase (118). This may be due to a defect in localization of Ca^{2+} signaling components at the IS. In addition to STIM1 and Orai1, molecules indirectly involved in Ca^{2+} signaling translocate to the IS. Potassium channels (Kv1.3 and KCa3.1) that regulate membrane potential localize to the IS (119,120). Mitochondria that buffer local Ca^{2+} levels also localize to the IS (121). STIM1 and Orai1 also localize to TCR-dependent cap-like structures that form outside the IS or at the distal pole. Barr et al. (117) have proposed that the formation of caps might serve as a repository for preformed channels, allowing rapid responses to additional APCs.

Overall, the function of STIM1 and Orai1 recruitment and localized Ca^{2+} entry at the synapse may serve to propagate Ca^{2+}-dependent TCR signals and facilitate long-term activation of T cells. Indeed, Ca^{2+} signaling is implicated in promoting long-lived interactions between T cells and APCs in vitro and in vivo (85,122–124).

Asymmetric Cell Division and the IS

Although synapse formation is critically important in the initial signaling events and the activation of T cells, we and others have proposed that termination of the IS plays a role in the fate selection of effector and memory cell differentiation via asymmetric cell division (125). Asymmetric cell division is an evolutionarily conserved mechanism important in the generation of diverse cell types from a common progenitor (126). Polarity complex proteins are required to partition cell-fate determinants asymmetrically in a cell undergoing mitotic division (127, 128).

Polarity network proteins are expressed in T cells. These include proteins from the Par, Crumbs, and Scribble polarity complexes (129–132). The Par complex consists of the proteins Par3 and Par6 (127,133) and an atypical protein kinase C-ζ (PKCζ) (134), whereas the Scribble complex is composed of the proteins Scribble, Dlg, and Lgl (128,135). The Crumbs complex is composed of the proteins Crumbs, Pal1, and PatJ (136). The expression of these proteins during T cell activation plays an important role in both migration and the functional properties of T cells (129–131,136). Chang and colleagues (129) demonstrated that prior to the first cell division of an activated T cell, Scribble was associated with CD3 and CD8 localization at the IS, whereas PKCζ segregated to the distal pole (opposite the IS). Upon the first division of activated $CD8^+$ T cells, the parental cells underwent asymmetric cell division with one daughter cell proximal and the other distal to the IS, and the progeny retained the unequal inheritance of the proteins, with the proximal cell inheriting greater amounts of LFA-1 and CD8 and the distal cell inheriting greater amounts of PKCζ. The proximal daughter cell had an effector phenotype, whereas the distal cell had a memory phenotype in vivo. Teixeiro et al. (137) demonstrated that T cells with a mutation in the transmembrane domain of TCRβ had defects in polarity protein distribution and could proliferate and differentiate into effector T cells, but they did not generate memory T cells. The Scribble binding partner Crtam (MHC class I–restricted T cell–associated molecule), an Ig superfamily transmembrane protein (138), may play a role in T cell polarity during the critical period prior to the first cell division (132).

Collectively, these data suggest that segregation of cell polarity proteins during T cell activation may provide a means to generate divergent cell subtypes from a single naive T cell.

Although asymmetric cell division in lymphocytes can give rise to distinct lineages of T cells (i.e., effector and memory precursors) (129), it is not clear whether the daughter cells that undergo subsequent divisions continue down the pathway of asymmetric division or whether there is a molecular switch that programs the cells to divide symmetrically. Furthermore, Bannard et al. (139) have recently shown, through the use of a granzyme B cell–fate mapping system, that memory cells can arise from effector cells. This does not preclude the possibility that asymmetric cell division could occur after the first cell division in an effector precursor, giving rise to a memory cell. Alternatively, this may also indicate that there are multiple mechanisms in place for the differentiation of effector and memory cells. Understanding these aspects of dividing lymphocytes will greatly aid in the development of adoptive immunotherapy to generate antigen-specific effector cells for immediate effector function as well as long-term protection through the generation of memory cells. It will be interesting to see if these mechanisms can be exploited by means of manipulating the IS in vitro to enhance the generation of memory T cells or to identify and isolate effector or memory precursors early during the immune response for further expansion ex vivo.

Effector Synapses

Many studies on the synapse have been performed with naive or resting $CD4^+$ T cells to study induction of long-term processes such as cytokine production and proliferation. Acutely activated effector T cells may also form distinct shorter-lived interactions to execute effector programs with prestored or rapidly generated proteins. The IS forms in other cell types, such as B cells, natural killer (NK) cells, and CTLs. Here we focus only on recent progress on cytotoxic, regulatory, and virological synapses (summarized in Table 2).

Cytotoxic T Lymphocyte Synapses

The classical view of a CTL is as a serial killer that forms a well-organized IS that is short lived (in vivo and in vitro), with a life span of 20–30 min. Similar to the $CD4^+$ T cell IS, the CTL synapse consists of the same pattern of SMACs, but it also contains a distinct secretory domain (92). The pSMAC also contributes to CTL cytolytic function in several ways. Blocking LFA-1 (CD11a/CD18) and ICAM-1 (CD54) interactions leads to decreased sensitivity and ability of CTL to lyse target cells (93,140). Forming a complete pSMAC ring is important for CTL efficiency, and failure to do so decreases killing efficiency at least threefold (51,140). Thus, a kinapse is not expected to be an efficient configuration for target killing.

Both actin and microtubules play a key role in cytolytic function. Upon antigen recognition on the target cell, MTOC polarizes rapidly toward the synapse. Lytic granules are trafficked to the IS along microtubules and accumulate at the interface just below the plasma membrane (51). A void of cortical actin opens up just below the cSMAC, which allows for lytic granule delivery to the target. The MTOC transiently contacts the plasma membrane at the cSMAC and directly delivers the lytic granules to the synapse (92). **TCR** signaling contributes to MTOC polarization through Lck and Fyn (97,141). Lck can be found in CD8 T cell cSMACs (11). The actin cytoskeleton rearrangements upon CTL IS formation may be linked to MTOC polarization through the interaction of Cdc42 with IQGAP1 (103), a cytoskeletal regulator, which interacts with both actin and the plus-ends of microtubules (92,142,143). This may connect actin reorganization at the IS with MTOC polarization in a single mechanism.

Regulatory T Cell Synapses

$CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) play a central role in the maintenance of tolerance to self-antigens and immune homeostasis. Tregs suppress the function of multiple immune cell types including conventional $CD4^+$ (Th) and $CD8^+$ cells, B cells, and DCs through an antigen receptor and cell contact–dependent mechanism (144,145). Tregs decrease the stability of in vivo T cell–DC synapses formed by conventional T cells and other Tregs (146, 147).

Comparison of the ability of Tregs and Th cells to form an IS on supported planar bilayers revealed that human Tregs form a more stable IS than do Th. The Treg IS has less phosphorylated Src family kinases and a near absence of PKCθ and Carma-1 (A. Zanin-Zhorov, Y. Ding, M. Attur, K. Hippen, M. Brown, B. Blazar, S. Abramson, J. Lafaille, and M. Dustin, manuscript submitted). The higher IS stability seems to be mediated by the exclusion of PKCθ from the IS, which leads to attenuated NF-κB activation. NF-κB activation appears to antagonize Treg function, which is consistent with recent evidence that TNF-α directly suppresses Treg function through TNFRII signaling, which activates NF-κB and downmodulates Foxp3 expression (148). Recent studies have reported that both PKCθ and NF-κB are critical for regulating the development and expansion of Tregs but not for their suppression function in mice (149,150).

Another example of altered TCR-induced signaling pathways in Tregs is provided by a recent study demonstrating that human Tregs fail to phosphorylate AKT upon TCR-mediated activation and that restoration of AKT activity in Tregs reversed their suppressive capacity (151). Thus, the defect in the ability of Tregs to fully activate AKT contributes to their unique suppressive function.

Synapse stabilization may enhance Treg function, as suggested by recent evidence that the IS plays an important role in Treg effects mediated through DCs (144,152). Thus, tight regulation of NF-κB activation could be critical for Treg function, and further detailed characterization of this unique signaling induced upon IS formation in Tregs may offer new therapeutic strategies in inflammatory disease.

Virological Synapses

Viruses such as HIV-1, human T cell lymphoma virus-1 (HTLV-1), and herpes simplex virus (HSV) (153–155) have co-opted components of the IS to form a similar structure referred as the infectious or virological synapse (VS). These processes have not been subdivided in the literature, but it is convenient to define an infectious synapse as a junction through which a virion harbored by a noninfected cell such as a DC is transferred and infects a CD4 T cell (156). The VS could then be defined as a junction for transfer of virus from an infected T cell to a noninfected T cell (153,157). We discuss the organization and structure of the VS for HIV-1 and a model system that we have constructed in which the infected cell is replaced by a supported planar bilayer.

VS formation occurs when the HIV-1 envelope protein, gp120, is expressed on the plasma membrane of the infected cell and engages CD4 along with either CCR5 (CD195) or CXCR4 (CD184) chemokine receptors on the target cell (157–159). HIV-1 gp120 presented with ICAM-1 in a supported planar bilayer induces assembly of a VS with IS-like supramolecular structures: gp120 clusters to form a cSMAC-like structure and segregate from LFA-1-ICAM-1 that forms a pSMAC (160). A VS is transient, persisting for about 15 min (160). Soluble gp120 (and cell-free virion) can activate various intracellular signaling events in T cells and macrophages (161–164), including activation of focal adhesion kinase (FAK) (165,166), Pyk2 (167), and the mitogen-activated protein kinase (MAPK) pathway (168,169), as well as Ca^{2+}

influx in T cells (170) and translocation of nuclear factor of activated T cells (NFAT) to the nucleus (171). T cell signaling triggered by gp120 in the context of the VS has never been studied but would be important because viral life cycle depends on T cell activation. TCR machinery is recruited and activated in the VS, but its spatial-temporal organization differs greatly from the one in the IS. A better understanding of the molecular details and biological consequences of VS may lead to the development of novel intervention strategies that block HIV-1 cell-to-cell spread within an infected host.

Synapse Stability and Kinapses

We have observed that while naive T cells are forming stable synapses on supported planar bilayers, they routinely break pSMAC symmetry, migrate away, and reform synapses elsewhere with a periodicity of about 20 min (80). This interconversion between stable and migrating morphologies is reciprocally regulated by PKCθ and WASp pathways. PKCθ-deficient T cells form hyperstable synapses, whereas WASp-deficient T cells cannot reform a symmetrical IS after initial destabilization. This is consistent with Tregs having hyperstable synapses and trace levels of PKCθ at the synapse. We have suggested that PKCθ promotes destabilization via myosin IIA contraction, which favors motility. In migrating cells, myosin IIA promotes fast amoeboid locomotion by inactivating LFA-1 at the trailing edge. Similar activity may produce breaking of the LFA-1-ICAM-1-dominated pSMAC. Contraction can reduce contact surface, leading to asymmetry in the F-actin flow leading to motion. Thus, the balance between polymerization and contraction of the actin cytoskeleton seems to control the transition between symmetric synapses and asymmetric kinapses.

The transition from migratory to stopped T cell is still poorly understood. We know Ca^{2+} signals and antigen are important for stopping. But how do these signals affect locomotion? How do antigenic signals reset actin polymerization from a migrating to a gathering orientation? Can a single **MC** induce stopping in vivo? Woolf et al. (172) demonstrated that solid-phase CCL21 chemokine at >100 molecules/μm^2 drives rapid migration of naive T cells in vitro without adhesion molecules. This finding enables the incorporation of a physiological go signal into in vitro models of synapse formation. We still must translate the in vitro lessons of T cell activation to the in vivo environments of the immune system.

In Vivo Synapses and T Cell Activation

The in vivo image of T cell activation is a bit more complex. Von Andrian and colleagues (9) developed an experimental system that uses subcutaneous injection of labeled, LPS-activated DCs followed by intravenous injection of naive transgenic $CD8^+$ T cells. Using intravital TPLSM, they tracked the behavior of these cells and observed a three-phase model for T cell activation: (9) During the first 8 h after entering the lymph node, the T cells establish only short-lasting contacts with DCs while upregulating activation markers. The following 12 h are characterized by the formation of stable, longer-lasting (up to 1 h or more) T cell–DC contacts and the production of cytokines. After 24 h, T cells return to their fleeting, motile behavior and start to proliferate.

In a subsequent study, they investigated the contributions of peptide quality and quantity on T cell behavior. They found that reducing the antigenic stimulus---either by using APL with lowered **TCR** affinities, by reducing the density of **pMHC** complexes per **APC**, or by reducing the number of antigen-bearing APCs---had the same effect of increasing the length of phase 1 (173).

In collaboration with the Nussenzweig laboratory, we utilized a system in which the antigen is targeted to endogenous DCs by coupling the peptide to an anti-DEC-205 antibody under priming (in the presence of anti-CD40 antibody) or tolerizing (without anti-CD40) conditions

(174). We imaged naive T cell behavior by TPLSM and found rapid arrest after encountering antigen during the first 6 h, often near HEV sites of entry. T cells regained their motility after 18–24 h, correlating to von Andrian's phases 2 and 3, respectively. Under both tolerizing and priming conditions, T cells formed conjugates with DCs. The use of APL in this model revealed that upregulation of CD69 and retention in the lymph node was independent of the potency of the pMHC complex (124). However, only engagement with high-potency pMHC complexes leads to a Ca^{2+} fluxing and complete T cell arrest.

Several groups have established experimental model systems to study the nature of T cell–DC interactions in vivo, and differences in observations clearly reflect differences in the setup and level of stimulation established (175,176). For example, if T cells encountered a large number of cognate pMHC complexes, they engaged more rapidly with DCs in tight contacts, which may be one explanation for the difference in our system, in which T cells arrested almost immediately after transfer. Von Andrian and colleagues (173) suggest that T cells integrate their received signals while sampling the APCs during phase 1, which leads to transition to phase 2 and T cell activation upon reaching a certain threshold. But in this context, T cells may arrest on the very first DC they encounter (177). Also, the in vivo situation is complicated by the fact that T cell arrest can be negatively regulated by CTLA-4 (178) or the presence of Tregs (146) and that chemokines present in the microenvironment (e.g., CCL21) influence the kinetics of T cell–DC interactions (179).

Effector T Cell Synapses In Vivo---Viral Activation

Viruses have adapted many immune escape mechanisms to guarantee their own propagation and spreading. Several studies have demonstrated the impact of virus infection on the formation of the IS. Most of these findings were derived from in vitro studies, which, as in the case of naive T cell activation, leave room for questioning their significance in the in vivo setting. The advent of high-resolution imaging techniques will allow us in the near future to address the role of IS formation during the immune response and during viral escape directly. A key finding was provided by Barcia et al. (180), who investigated a model of adenoviral infection of astrocytes in the central nervous system. They used Confocal imaging of brain sections from infected rats to demonstrate that the formation of the SMAC precedes the clearance of infected astrocytes. Initially, CD8 T cells exhibited a staining pattern in which phosphor-Lck and phosphor-ZAP-70 polarized toward target astrocytes. Later, TCR surrounded by an LFA-1 ring-like structure was found at contact areas with infected cells. This strongly suggests the presence of an IS in vivo.

HIV-1 also modifies the assembly of the IS (181). Most relevant for the in vivo situation, Thoulouze et al. (182) showed that HIV-1-infected T cells poorly conjugated with B cells, which serve as APCs in this setting, and TCR clustering at the site of the synapse is severely reduced. Interestingly, this was predominantly attributable to the presence of the viral protein Nef, which has a central role in HIV-1 pathogenesis. It was therefore proposed that this provides a countermechanism that prevents hyperactivation of HIV-1-infected T cells, which will lead to apoptosis and an end to the virus life cycle (181).

Another example of viral interference with the IS is respiratory syncytial virus (RSV), a common respiratory pathogen in children that impairs adaptive immune responses in vivo (183). In an in vitro system, González et al. (184) showed that RSV-infected DCs cannot stimulate T cells because of impaired IS assembly as assessed by Golgi polarization.

In Vivo T Cell–B Cell Contacts

A series of multiphoton microscopy studies have recently shed light on the peculiarities of synapses at each of these stages within intact lymphoid organs, as well as on the similarities

and differences that exist between T cell–B cell and T cell–DC synapses (185–187). T cell–B cell synapses are classically thought to occur in two spatially and temporally distinct settings: (*a*) the T:B border phase, which takes place during the first few days of an immune response, and (*b*) the germinal center (GC), which peaks at about one week later. Both stages of interaction involve previously activated T cells. During the initial phase, T cells and B cells engage in extended contacts at the border between T zone and follicle (185), some lasting for over 40 min. Similar to T cell–DC synapses, T cells also arrest on B cells, but the B cells continue to migrate, carrying the T cells with them (185). These motile conjugates have been described as serially monogamous: T cells engage a single B cell at a time but can hop to a new B cell (188). It is unclear why these conjugates continue migrating and what consequence this has on signal integration for the B and T cells. One possibility is that motility of a B cell–T cell pair may increase the likelihood that the T cell will encounter another B cell of higher affinity for the antigen (and therefore with higher surface peptide density), thus promoting partner exchange for B cells with higher-affinity B cell receptor (BCR). A caveat of this study is the use of B cells with extremely high affinity (the HEL-specific MD4 BCR) as well as on a relatively high dose of antigen; it would therefore be interesting to determine whether such extended interactions also occur in lower affinity/avidity settings.

In contrast to the extended contacts observed at the T:B border, synapses between T and B cells in the GC are much shorter lived (186). Synapses in the GC are rare and short, seldom lasting longer than 5 min (only 4%). T cells are critical to maintaining GCs, given that treatment targeting CD40-CD40L interaction between GC B and T cells extinguishes the GC rapidly (189). At the molecular level, the role of antigen-specific interactions in the GC is unclear, particularly in regulating competition between B cells expressing higher- or lower-affinity receptors. The first in vivo insight into the molecular mechanism of T cell–B cell interactions was provided by Germain and colleagues (187), who described a role for signaling lymphocyte activation molecule (SLAM)-associated protein (SAP), a T cell adaptor protein downstream of the SLAM pathway, in determining the length of T cell–B cell contacts at the T:B border. Although the duration of contacts between wild-type and SAP-deficient T cells and DCs was equivalent, knockout T cells selectively lose their ability to form extended contacts with cognate B cells between 1 and 3 days postimmunization. SAP-deficient T cells spent less time in GCs compared with wild-type T cells. SLAM receptors engage in homotypic interactions and are upregulated on GC B cells but not on DCs. The SLAM/SAP pathway may enhance the efficiency of cognate T cell–B cell engagements uniquely.

In Vivo Cytotoxic T Lymphocyte Contacts

CTLs appear to use synapse or kinapse modes of interaction with target cells in tumors or viral infection sites, respectively. CTLs interacted with tumor targets that were killed, as detected by a caspase biosensor, after ~6 h of stable contact (190). This surprisingly long duration of contact may reflect an unfavorable tumor environment. CTLs specific for LCMV were slowed in contact with infected meningeal fibroblasts but did not arrest during an immune response, leading to fatal meningitis (191). Previous histological studies in the same model capture IS-like molecule patterns (192), but this may have been misleading in the absence of dynamic information. CTLs forming kinapses are likely to be inefficient in killing, although we did not directly evaluate target killing in this study. The use of kinapses by the antiviral CTLs may reflect an initial strategy to activate innate antiviral defenses rather than directly kill the target. In contrast, innate defenses may be less effective against tumor cells, leaving killing of the tumor cells or tumor-supporting normal cells as the only effective control mechanism.

Reconciling Differences Between in vivo and in Vitro Systems

How does the information obtained from the in vitro systems apply to in vivo T cell activation and synapse formation? Despite a handful of static images showing synapse-like

accumulations, there has been no satisfactory demonstration of the classic in vitro bull's eye by dynamic TPLSM intravital imaging. Two reasons are given for this discrepancy. The first is technical. TPLSM imaging requires high levels of fluorescent signal (millions of green fluorescent proteins), either chemical dyes or fluorescent proteins, and has worked best in cytoplasmic distributions. To visualize in situ synapses requires labeling of factors controlling T cell activation, which are present in tens of thousands of copies per cell. These have not been bright enough to visualize. But with newer generation TPLSM technology and the development of new molecular tools, the visualization of the IS at subcellular levels in vivo is in sight.

The second reason may be that the classic bull's eye is an in vitro amplification of what occurs at much smaller scales in vivo. We know that peptide dose affects the size of the cSMAC linearly (14,45). Levels of **pMHC** on endogenous DCs may be lower than the levels used in our in vitro models. The rigid and flat nature of the glass-supported bilayer may also drive kinetic size segregation. Chemokinetic signals may often be absent or excessive in simplified in vitro systems. In vitro cell:cell conjugates tend to have multifocal synapses (193). Finally, we must recognize that the **APC** is contributing mechanical and functional contributions to IS surfaces. Recent studies have identified new roles for the APCs in modulating synapse structure and function (194,195). We are careful to use the word amplification rather than artifact; the biology and signaling is the same, but we need to remind ourselves that the overall size/structure we see is tunable to the factors and constraints we introduce.

Concluding Remarks and Future Questions

In this review, we have tried to focus on the studies that have changed our view of the T cell synapse in the past few years. But questions remain. With the identification of the **TCR MC** as the quantum of signaling, the microstructure and regulation of MCs are still unknown. But with newer superresolution optical techniques, it should be possible to investigate. In vivo, the kinapse seems to rule, empirically, during early stages of signal integration and in late effector stages, when T cells seem to migrate even in the presence of antigen. The membrane protein organization of the kinapse is unknown, as is how the balance between stop and go signals is transduced. From what we know already, these pathways are redundant and reciprocal, with the balance finely tuned based on the context. We are still trying to resolve the complexity of cues and systems biology of the downstream signaling pathways that coordinate the actions of an elaborate yet elegant T cell.

Literature Cited

1. Marrack P, Scott-Browne JP, Dai S, Gapin L, Kappler JW. Evolutionarily conserved amino acids that control TCR-MHC interaction. Annu Rev Immunol 2008;26:171–203. [PubMed: 18304006]
2. Miller MJ, Wei SH, Parker I, Cahalan MD. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. Science 2002;296:1869–73. [PubMed: 12016203]
3. Miller MJ, Wei SH, Cahalan MD, Parker I. Autonomous T cell trafficking examined in vivo with intravital two-photon microscopy. Proc Natl Acad Sci USA 2003;100:2604–9. [PubMed: 12601158]
4. Lindquist RL, Shakhar G, Dudziak D, Wardemann H, Eisenreich T, et al. Visualizing dendritic cell networks in vivo. Nat Immunol 2004;5:1243–50. [PubMed: 15543150]
5. Bajenoff M, Egen JG, Koo LY, Laugier JP, Brau F, et al. Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. Immunity 2006;25:989–1001. [PubMed: 17112751]
6. Beltman JB, Maree AF, Lynch JN, Miller MJ, de Boer RJ. Lymph node topology dictates T cell migration behavior. J Exp Med 2007;204:771–80. [PubMed: 17389236]
7. Dustin ML. Stop and go traffic to tune T cell responses. Immunity 2004;21:305–14. [PubMed: 15357942]

8. Pham TH, Okada T, Matloubian M, Lo CG, Cyster JG. $S1P_1$ receptor signaling overrides retention mediated by $G\alpha_i$-coupled receptors to promote T cell egress. Immunity 2008;28:122–33. [PubMed: 18164221]
9. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature 2004;427:154–59. [PubMed: 14712275]
10. Scholer A, Hugues S, Boissonnas A, Fetler L, Amigorena S. Intercellular adhesion molecule-1-dependent stable interactions between T cells and dendritic cells determine $CD8^+$ T cell memory. Immunity 2008;28:258–70. [PubMed: 18275834]
11. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. Nature 1998;395:82–86. [PubMed: 9738502]
12. Freiberg BA, Kupfer H, Maslanik W, Delli J, Kappler J, et al. Staging and resetting T cell activation in SMACs. Nat Immunol 2002;3:911–17. [PubMed: 12244310]
13. Dustin ML. Making a little affinity go a long way: a topological view of LFA-1 regulation. Cell Adhes Commun 1998;6:255–62. [PubMed: 9823476]
14. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, et al. The immunological synapse: a molecular machine controlling T cell activation. Science 1999;285:221–27. [PubMed: 10398592]
15. Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. Nat Immunol 2000;1:23–29. [PubMed: 10881170]
16. Varma R, Campi G, Yokosuka T, Saito T, Dustin ML. T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. Immunity 2006;25:117–27. [PubMed: 16860761]
17. Cemerski S, Das J, Giurisato E, Markiewicz MA, Allen PM, et al. The balance between T cell receptor signaling and degradation at the center of the immunological synapse is determined by antigen quality. Immunity 2008;29:414–22. [PubMed: 18760640]
18. Beal A, Anikeeva N, Varma R, Cameron TO, Norris PJ, et al. Protein kinase Cθ regulates stability of the peripheral adhesion ring junction and contributes to the sensitivity of target cell lysis by CTL. J Immunol 2008;181:4815–24. [PubMed: 18802085]
19. Irvine DJ, Purbhoo MA, Krogsgaard M, Davis MM. Direct observation of ligand recognition by T cells. Nature 2002;419:845–49. [PubMed: 12397360]
20. Xu C, Gagnon E, Call ME, Schnell JR, Schwieters CD, et al. Regulation of T cell receptor activation by dynamic membrane binding of the CD3ε cytoplasmic tyrosine-based motif. Cell 2008;135:702–13. [PubMed: 19013279]
21. Matsui K, Boniface JJ, Reay PA, Schild H, Fazekas de St Groth B, Davis MM. Low affinity interaction of peptide-MHC complexes with T cell receptors. Science 1991;254:1788–91. [PubMed: 1763329]
22. Corr M, Slanetz AE, Boyd LF, Jelonek MT, Khilko S, et al. T cell receptor-MHC class I peptide interactions: affinity, kinetics, and specificity. Science 1994;265:946–49. [PubMed: 8052850]
23. Daniels MA, Teixeiro E, Gill J, Hausmann B, Roubaty D, et al. Thymic selection threshold defined by compartmentalization of Ras/MAPK signaling. Nature 2006;444:724–29. [PubMed: 17086201]
24. Zehn D, Lee SY, Bevan MJ. Complete but curtailed T-cell response to very low-affinity antigen. Nature 2009;458:211–14. [PubMed: 19182777]
25. Cemerski S, Das J, Locasale J, Arnold P, Giurisato E, et al. The stimulatory potency of T cell antigens is influenced by the formation of the immunological synapse. Immunity 2007;26:345–55. [PubMed: 17346997]
26. Rudolph MG, Luz JG, Wilson IA. Structural and thermodynamic correlates of T cell signaling. Annu Rev Biophys Biomol Struct 2002;31:121–49. [PubMed: 11988465]
27. Nicholson MJ, Hahn M, Wucherpfennig KW. Unusual features of self-peptide/MHC binding by autoimmune T cell receptors. Immunity 2005;23:351–60. [PubMed: 16226501]
28. Kaizuka Y, Douglass AD, Vardhana S, Dustin ML, Vale RD. The coreceptor CD2 uses plasma membrane microdomains to transduce signals in T cells. J Cell Biol 2009;185:521–34. [PubMed: 19398758]
29. Dustin ML, Golan DE, Zhu DM, Miller JM, Meier W, et al. Low affinity interaction of human or rat T cell adhesion molecule CD2 with its ligand aligns adhering membranes to achieve high physiological affinity. J Biol Chem 1997;272:30889–98. [PubMed: 9388235]

30. Zhu DM, Dustin ML, Cairo CW, Thatte HS, Golan DE. Mechanisms of Cellular Avidity Regulation in CD2-CD58-Mediated T Cell Adhesion. ACS Chem Biol 2006;1:649–58. [PubMed: 17168569]

31. Zhu DM, Dustin ML, Cairo CW, Golan DE. Analysis of two-dimensional dissociation constant of laterally mobile cell adhesion molecules. Biophys J 2007;92:1022–34. [PubMed: 17085486]

32. Tolentino TP, Wu J, Zarnitsyna VI, Fang Y, Dustin ML, Zhu C. Measuring diffusion and binding kinetics by contact area FRAP. Biophys J 2008;95:920–30. [PubMed: 18390627]

33. Bunnell S, Hong DI, Kardon JR, Yamazaki T, McGlade CJ, et al. T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. J Cell Biol 2002;158:1263–75. [PubMed: 12356870]

34. Bunnell SC, Barr VA, Fuller CL, Samelson LE. High-resolution multicolor imaging of dynamic signaling complexes in T cells stimulated by planar substrates. Sci STKE 2003;2003:PL8. [PubMed: 12684528]

35. Douglass A, Vale RD. Single-molecule microscopy reveals plasma membrane microdomains created by protein-protein networks that exclude or trap signaling molecules in T cells. Cell 2005;121:937–50. [PubMed: 15960980]

36. Kaizuka Y, Douglass AD, Varma R, Dustin ML, Vale RD. Mechanisms for segregating T cell receptor and adhesion molecules during immunological synapse formation in Jurkat T cells. Proc Natl Acad Sci USA 2007;104:20296–301. [PubMed: 18077330]

37. Cochran JR, Cameron TO, Stern LJ. The relationship of MHC-peptide binding and T cell activation probed using chemically defined MHC class II oligomers. Immunity 2000;12:241–50. [PubMed: 10755611]

38. Krogsgaard M, Li QJ, Sumen C, Huppa JB, Huse M, Davis MM. Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. Nature 2005;434:238–43. [PubMed: 15724150]

39. Campi G, Varma R, Dustin ML. Actin and agonist MHC-peptide complex-dependent T cell receptor microclusters as scaffolds for signaling. J Exp Med 2005;202:1031–36. [PubMed: 16216891]

40. Valitutti S, Dessing M, Aktories K, Gallati H, Lanzavecchia A. Sustained signaling leading to T cell activation results from prolonged T cell receptor occupancy. Role of T cell actin cytoskeleton. J Exp Med 1995;181:577–84. [PubMed: 7836913]

41. Schamel WW, Arechaga I, Risueno RM, van Santen HM, Cabezas P, et al. Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. J Exp Med 2005;202:493–503. [PubMed: 16087711]

42. Lillemeier BF, Mörtelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM. TCR and LAT occur in separate domains on T cell membranes, and concatenate during activation. Nat Immunol. 2009 In press.

43. Springer TA. Adhesion receptors of the immune system. Nature 1990;346:425–34. [PubMed: 1974032]

44. Choudhuri K, Wiseman D, Brown MH, Gould K, Van Der Merwe PA. T-cell receptor triggering is critically dependent on the dimensions of its peptide-MHC ligand. Nature 2005;436:578–82. [PubMed: 16049493]

45. Yokosuka T, Sakata-Sogawa K, Kobayashi W, Hiroshima M, Hashimoto-Tane A, et al. Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. Nat Immunol 2005;6:1253–62. [PubMed: 16273097]

46. Bunnell S, Singer AL, Hong DI, Jacque BH, Jordan MS, et al. Persistence of cooperatively stabilized signaling clusters drives T-cell activation. Mol Cell Biol 2006;26:7155–66. [PubMed: 16980618]

47. Nguyen K, Sylvain NR, Bunnell SC. T cell costimulation via the integrin VLA-4 inhibits the actin-dependent centralization of signaling microclusters containing the adaptor SLP-76. Immunity 2008;28:810–21. [PubMed: 18549800]

48. Williams RL, Urbe S. The emerging shape of the ESCRT machinery. Nat Rev Mol Cell Biol 2007;8:355–68. [PubMed: 17450176]

49. Mossman KD, Campi G, Groves JT, Dustin ML. Altered TCR signaling from geometrically repatterned immunological synapses. Science 2005;310:1191–93. [PubMed: 16293763]

50. Shen K, Thomas VK, Dustin ML, Kam LC. Micropatterning of costimulatory ligands enhances CD4$^+$ T cell function. Proc Natl Acad Sci USA 2008;105:7791–96. [PubMed: 18505845]

51. Stinchcombe JC, Bossi G, Booth S, Griffiths GM. The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity 2001;15:751–61. [PubMed: 11728337]
52. Bachmann MF, McKall-Faienza K, Schmits R, Bouchard D, Beach J, et al. Distinct roles for LFA-1 and CD28 during activation of naive T cells: adhesion versus costimulation. Immunity 1997;7:549–57. [PubMed: 9354475]
53. Dustin ML, Springer TA. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature 1989;341:619–24. [PubMed: 2477710]
54. Wang H, Moon EY, Azouz A, Wu X, Smith A, et al. SKAP-55 regulates integrin adhesion and formation of T cell-APC conjugates. Nat Immunol 2003;4:366–74. [PubMed: 12652296]
55. Mor A, Campi G, Du G, Zheng Y, Foster DA, et al. The lymphocyte function-associated antigen-1 receptor costimulates plasma membrane Ras via phospholipase D2. Nat Cell Biol 2007;9:713–19. [PubMed: 17486117]
56. Sawada Y, Tamada M, Dubin-Thaler BJ, Cherniavskaya O, Sakai R, et al. Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell 2006;127:1015–26. [PubMed: 17129785]
57. Smith A, Carrasco YR, Stanley P, Kieffer N, Batista FD, Hogg N. A talin-dependent LFA-1 focal zone is formed by rapidly migrating T lymphocytes. J Cell Biol 2005;170:141–51. [PubMed: 15983060]
58. Choi CK, Vicente-Manzanares M, Zareno J, Whitmore LA, Mogilner A, Horwitz AR. Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. Nat Cell Biol 2008;10:1039–50. [PubMed: 19160484]
59. Yokosuka T, Kobayashi W, Sakata-Sogawa K, Takamatsu M, Hashimoto-Tane A, et al. Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters and protein kinase C θ translocation. Immunity 2008;29:589–601. [PubMed: 18848472]
60. Tseng SY, Waite JC, Liu M, Vardhana S, Dustin ML. T cell-dendritic cell immunological synapses contain TCR-dependent CD28-CD80 clusters that recruit protein kinase C θ. J Immunol 2008;181:4852–63. [PubMed: 18802089]
61. Rossman JS, Stoicheva NG, Langel FD, Patterson GH, Lippincott-Schwartz J, Schaefer BC. POLKADOTS are foci of functional interactions in T-cell receptor-mediated signaling to NF-κB. Mol Biol Cell 2006;17:2166–76. [PubMed: 16495340]
62. Bachmann MF, Barner M, Kopf M. CD2 sets quantitative thresholds in T cell activation. J Exp Med 1999;190:1383–92. [PubMed: 10562314]
63. Espagnolle N, Depoil D, Zaru R, Demeur C, Champagne E, et al. CD2 and TCR synergize for the activation of phospholipase Cγ1/calcium pathway at the immunological synapse. Int Immunol 2007;19:239–48. [PubMed: 17220479]
64. Singleton K, Parvaze N, Dama KR, Chen KS, Jennings P, et al. A large T cell invagination with CD2 enrichment resets receptor engagement in the immunological synapse. J Immunol 2006;177:4402–13. [PubMed: 16982875]
65. Collins AV, Brodie DW, Gilbert RJ, Iaboni A, Manso-Sancho R, et al. The interaction properties of costimulatory molecules revisited. Immunity 2002;17:201–10. [PubMed: 12196291]
66. Pentcheva-Hoang T, Egen JG, Wojnoonski K, Allison JP. B7-1 and B7-2 selectively recruit CTLA-4 and CD28 to the immunological synapse. Immunity 2004;21:401–13. [PubMed: 15357951]
67. Schneider H, Valk E, da Rocha Dias S, Wei B, Rudd CE. CTLA-4 up-regulation of lymphocyte function-associated antigen 1 adhesion and clustering as an alternate basis for coreceptor function. Proc Natl Acad Sci USA 2005;102:12861–66. [PubMed: 16126897]
68. Rudd CE. The reverse stop-signal model for CTLA4 function. Nat Rev Immunol 2008;8:153–60. [PubMed: 18219311]
69. Sarnaik AA, Weber JS. Recent advances using anti-CTLA-4 for the treatment of melanoma. Cancer J 2009;15:169–73. [PubMed: 19556898]
70. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 1992;11:3887–95. [PubMed: 1396582]
71. Riley JL. PD-1 signaling in primary T cells. Immunol Rev 2009;229:114–25. [PubMed: 19426218]

72. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26:677–704. [PubMed: 18173375]
73. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 2006;443:350–54. [PubMed: 16921384]
74. Kaufmann D, Walker BD. PD-1 and CTLA-4 inhibitory cosignaling pathways in HIV infection and the potential for therapeutic intervention. J Immunol 2009;182:5891–97. [PubMed: 19414738]
75. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science 2001;291:319–22. [PubMed: 11209085]
76. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 2005;25:9543–53. [PubMed: 16227604]
77. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682–87. [PubMed: 16382236]
78. Giannone G, Dubin-Thaler BJ, Rossier O, Cai Y, Chaga O, et al. Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell 2007;128:561–75. [PubMed: 17289574]
79. Bunnell S, Kapoor V, Trible RP, Zhang W, Samelson LE. Dynamic actin polymerization drives T cell receptor-induced spreading: A role for the signal transduction adaptor LAT. Immunity 2001;14:315–29. [PubMed: 11290340]
80. Sims T, Soos TJ, Xenias HS, Dubin-Thaler B, Hofman JM, et al. Opposing effects of PKCθ and WASp on symmetry breaking and relocation of the immunological synapse. Cell 2007;129:773–85. [PubMed: 17512410]
81. Ilani T, Vasiliver-Shamis G, Vardhana S, Bretscher A, Dustin ML. T cell antigen receptor signaling and immunological synapse stability require myosin IIA. Nat Immunol 2009;10:531–39. [PubMed: 19349987]
82. Billadeau DD, Nolz JC, Gomez TS. Regulation of T-cell activation by the cytoskeleton. Nat Rev Immunol 2007;7:131–43. [PubMed: 17259969]
83. Barda-Saad M, Braiman A, Titerence R, Bunnell SC, Barr VA, Samelson LE. Dynamic molecular interactions linking the T cell antigen receptor to the actin cytoskeleton. Nat Immunol 2005;6:80–89. [PubMed: 15558067]
84. Gomez T, Billadeau DD. T cell activation and the cytoskeleton: you can't have one without the other. Adv Immunol 2008;97:1–64. [PubMed: 18501768]
85. Negulescu PA, Krasieva TB, Khan A, Kerschbaum HH, Cahalan MD. Polarity of T cell shape, motility, and sensitivity to antigen. Immunity 1996;4:421–30. [PubMed: 8630728]
86. Wei X, Tromberg BJ, Cahalan MD. Mapping the sensitivity of T cells with an optical trap: polarity and minimal number of receptors for Ca^{2+} signaling. Proc Natl Acad Sci USA 1999;96:8471–76. [PubMed: 10411899]
87. Kaizuka Y, Douglass AD, Varma R, Dustin ML, Vale RD. Mechanisms for segregating T cell receptor and adhesion molecules during immunological synapse formation in Jurkat T cells. Proc Natl Acad Sci USA 2007;104:20296–301. [PubMed: 18077330]
88. DeMond AL, Mossman KD, Starr T, Dustin ML, Groves JT. T cell receptor microcluster transport through molecular mazes reveals mechanism of translocation. Biophys J 2008;94:3286–92. [PubMed: 18199675]
89. Jacobelli J, Chmura SA, Buxton DB, Davis MM, Krummel MF. A single class II myosin modulates T cell motility and stopping, but not synapse formation. Nat Immunol 2004;5:531–38. [PubMed: 15064761]
90. Morin NA, Oakes PW, Hyun YM, Lee D, Chin EY, et al. Nonmuscle myosin heavy chain IIA mediates integrin LFA-1 de-adhesion during T lymphocyte migration. J Exp Med 2008;205:195–205. [PubMed: 18195072]
91. Ilani T, Vasiliver-Shamis G, Vardhana S, Bretscher A, Dustin ML. TCR signaling and immunological synapse stability requires myosin IIA. Nat Immunol 2009;10:531–39. [PubMed: 19349987]
92. Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM. Centrosome polarization delivers secretory granules to the immunological synapse. Nature 2006;443:462–65. [PubMed: 17006514]

93. Beal AM, Anikeeva N, Varma R, Cameron TO, Norris PJ, et al. Protein kinase C θ regulates stability of the peripheral adhesion ring junction and contributes to the sensitivity of target cell lysis by CTL. J Immunol 2008;181:4815–24. [PubMed: 18802085]
94. Geiger B, Rosen D, Berke G. Spatial relationships of microtubule-organizing centers and the contact area of cytotoxic T lymphocytes and target cells. J Cell Biol 1982;95:137–43. [PubMed: 6982900]
95. Kupfer A, Dennert G, Singer SJ. Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. Proc Natl Acad Sci USA 1983;80:7224–28. [PubMed: 6359165]
96. Kupfer A, Mosmann TR, Kupfer H. Polarized expression of cytokines in cell conjugates of helper T cells and splenic B cells. Proc Natl Acad Sci USA 1991;88:775–79. [PubMed: 1825141]
97. Lowin-Kropf B, Shapiro VS, Weiss A. Cytoskeletal polarization of T cells is regulated by an immunoreceptor tyrosine-based activation motif-dependent mechanism. J Cell Biol 1998;140:861–71. [PubMed: 9472038]
98. Spitaler M, Emslie E, Wood CD, Cantrell D. Diacylglycerol and protein kinase D localization during T lymphocyte activation. Immunity 2006;24:535–46. [PubMed: 16713972]
99. Kuhne MR, Lin J, Yablonski D, Mollenauer MN, Ehrlich LI, et al. Linker for activation of T cells, ζ-associated protein-70, and Src homology 2 domain-containing leukocyte protein-76 are required for TCR-induced microtubule-organizing center polarization. J Immunol 2003;171:860–66. [PubMed: 12847255]
100. Quann EJ, Merino E, Furuta T, Huse M. Localized diacylglycerol drives the polarization of the microtubule-organizing center in T cells. Nat Immunol 2009;10:627–35. [PubMed: 19430478]
101. Combs J, Kim SJ, Tan S, Ligon LA, Holzbaur EL, Kuhn J, Poenie M. Recruitment of dynein to the Jurkat immunological synapse. Proc Natl Acad Sci USA 2006;103:14883–88. [PubMed: 16990435]
102. Martin-Cofreces NB, Robles-Valero J, Cabrero JR, Mittelbrunn M, Gordon-Alonso M, et al. MTOC translocation modulates IS formation and controls sustained T cell signaling. J Cell Biol 2008;182:951–62. [PubMed: 18779373]
103. Huse M, Quann EJ, Davis MM. Shouts, whispers and the kiss of death: directional secretion in T cells. Nat Immunol 2008;9:1105–11. [PubMed: 18800163]
104. Faix J, Grosse R. Staying in shape with formins. Dev Cell 2006;10:693–706. [PubMed: 16740473]
105. Gomez TS, Kumar K, Medeiros RB, Shimizu Y, Leibson PJ, Billadeau DD. Formins regulate the actin-related protein 2/3 complex-independent polarization of the centrosome to the immunological synapse. Immunity 2007;26:177–90. [PubMed: 17306570]
106. Finetti F, Rossi Paccani S, Riparbelli MG, Giacomello E, Perinetti G, et al. Intraflagellar transport is required for polarized recycling of the TCR/CD3 complex to the immune synapse. Nat Cell Biol. 2009 In press.
107. Feske S. Calcium signaling in lymphocyte activation and disease. Nat Rev Immunol 2007;7:690–702. [PubMed: 17703229]
108. Lewis RS. The molecular choreography of a store-operated calcium channel. Nature 2007;446:284–87. [PubMed: 17361175]
109. Muik M, Frischauf I, Derler I, Fahrner M, Bergsmann J, et al. Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. J Biol Chem 2008;283:8014–22. [PubMed: 18187424]
110. Stathopulos PB, Li GY, Plevin MJ, Ames JB, Ikura M. Stored Ca^{2+} depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: An initiation mechanism for capacitive Ca^{2+} entry. J Biol Chem 2006;281:35855–62. [PubMed: 17020874]
111. Wu MM, Buchanan J, Luik RM, Lewis RS. Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. J Cell Biol 2006;174:803–13. [PubMed: 16966422]
112. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. J Cell Biol 2006;174:815–25. [PubMed: 16966423]
113. Xu P, Lu J, Li Z, Yu X, Chen L, Xu T. Aggregation of STIM1 underneath the plasma membrane induces clustering of Orai1. Biochem Biophys Res Commun 2006;350:969–76. [PubMed: 17045966]

114. Vig M, Kinet JP. Calcium signaling in immune cells. Nat Immunol 2009;10:21–27. [PubMed: 19088738]
115. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, et al. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. Cell 2009;136:876–90. [PubMed: 19249086]
116. Lioudyno MI, Kozak JA, Penna A, Safrina O, Zhang SL, et al. Orai1 and STIM1 move to the immunological synapse and are up-regulated during T cell activation. Proc Natl Acad Sci USA 2008;105:2011–16. [PubMed: 18250319]
117. Barr VA, Bernot KM, Srikanth S, Gwack Y, Balagopalan L, et al. Dynamic movement of the calcium sensor STIM1 and the calcium channel Orai1 in activated T-cells: puncta and distal caps. Mol Biol Cell 2008;19:2802–17. [PubMed: 18448669]
118. Nolz JC, Gomez TS, Zhu P, Li S, Medeiros RB, et al. The WAVE2 complex regulates actin cytoskeletal reorganization and CRAC-mediated calcium entry during T cell activation. Curr Biol 2006;16:24–34. [PubMed: 16401421]
119. Panyi G, Vamosi G, Bacso Z, Bagdany M, Bodnar A, et al. Kv1.3 potassium channels are localized in the immunological synapse formed between cytotoxic and target cells. Proc Natl Acad Sci USA 2004;101:1285–90. [PubMed: 14745040]
120. Nicolaou SA, Neumeier L, Peng Y, Devor DC, Conforti L. The Ca^{2+}-activated K^+ channel KCa3.1 compartmentalizes in the immunological synapse of human T lymphocytes. Am J Physiol Cell Physiol 2007;292:C1431–39. [PubMed: 17151145]
121. Quintana A, Schwindling C, Wenning AS, Becherer U, Rettig J, et al. T cell activation requires mitochondrial translocation to the immunological synapse. Proc Natl Acad Sci USA 2007;104:14418–23. [PubMed: 17726106]
122. Bhakta NR, Oh DY, Lewis RS. Calcium oscillations regulate thymocyte motility during positive selection in the three-dimensional thymic environment. Nat Immunol 2005;6:143–51. [PubMed: 15654342]
123. Wei SH, Safrina O, Yu Y, Garrod KR, Cahalan MD, Parker I. Ca^{2+} signals in $CD4^+$ T cells during early contacts with antigen-bearing dendritic cells in lymph node. J Immunol 2007;179:1586–94. [PubMed: 17641025]
124. Skokos D, Shakhar G, Varma R, Waite JC, Cameron TO, et al. Peptide-MHC potency governs dynamic interactions between T cells and dendritic cells in lymph nodes. Nat Immunol 2007;8:835–44. [PubMed: 17632517]
125. Dustin ML, Chan AC. Signaling takes shape in the immune system. Cell 2000;103:283–94. [PubMed: 11057901]
126. Horvitz HR, Herskowitz I. Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. Cell 1992;68:237–55. [PubMed: 1733500]
127. Kemphues KJ, Priess JR, Morton DG, Cheng NS. Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. Cell 1988;52:311–20. [PubMed: 3345562]
128. Assemat E, Bazellieres E, Pallesi-Pocachard E, Le Bivic A, Massey-Harroche D. Polarity complex proteins. Biochim Biophys Acta 2008;1778:614–30. [PubMed: 18005931]
129. Chang JT, Palanivel VR, Kinjyo I, Schambach F, Intlekofer AM, et al. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. Science 2007;315:1687–91. [PubMed: 17332376]
130. Giagulli C, Scarpini E, Ottoboni L, Narumiya S, Butcher EC, et al. RhoA and ζ PKC control distinct modalities of LFA-1 activation by chemokines: critical role of LFA-1 affinity triggering in lymphocyte in vivo homing. Immunity 2004;20:25–35. [PubMed: 14738762]
131. Ludford-Menting MJ, Oliaro J, Sacirbegovic F, Cheah ET, Pedersen N, et al. A network of PDZ-containing proteins regulates T cell polarity and morphology during migration and immunological synapse formation. Immunity 2005;22:737–48. [PubMed: 15963788]
132. Yeh J, Sidhu SS, Chan AC. Regulation of a late phase of T cell polarity and effector functions by Crtam. Cell 2008;132:846–59. [PubMed: 18329370]
133. Etemad-Moghadam B, Guo S, Kemphues KJ. Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early *C. elegans* embryos. Cell 1995;83:743–52. [PubMed: 8521491]

134. Tabuse Y, Izumi Y, Piano F, Kemphues KJ, Miwa J, Ohno S. Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. Development 1998;125:3607–14. [PubMed: 9716526]

135. Iden S, Collard JG. Crosstalk between small GTPases and polarity proteins in cell polarization. Nat Rev Mol Cell Biol 2008;9:846–59. [PubMed: 18946474]

136. Humbert PO, Dow LE, Russell SM. The Scribble and Par complexes in polarity and migration: friends or foes? Trends Cell Biol 2006;16:622–30. [PubMed: 17067797]

137. Teixeiro E, Daniels MA, Hamilton SE, Schrum AG, Bragado R, et al. Different T cell receptor signals determine $CD8^+$ memory versus effector development. Science 2009;323:502–5. [PubMed: 19164748]

138. Kennedy J, Vicari AP, Saylor V, Zurawski SM, Copeland NG, et al. A molecular analysis of NKT cells: identification of a class-I restricted T cell-associated molecule (CRTAM). J Leukoc Biol 2000;67:725–34. [PubMed: 10811014]

139. Bannard O, Kraman M, Fearon DT. Secondary replicative function of $CD8^+$ T cells that had developed an effector phenotype. Science 2009;323:505–9. [PubMed: 19164749]

140. Anikeeva N, Somersalo K, Sims TN, Thomas VK, Dustin ML, Sykulev Y. Distinct role of lymphocyte function-associated antigen-1 in mediating effective cytolytic activity by cytotoxic T lymphocytes. Proc Natl Acad Sci USA 2005;102:6437–42. [PubMed: 15851656]

141. Martin-Cofreces NB, Sancho D, Fernandez E, Vicente-Manzanares M, Gordon-Alonso M, et al. Role of Fyn in the rearrangement of tubulin cytoskeleton induced through TCR. J Immunol 2006;176:4201–7. [PubMed: 16547257]

142. Fukata M, Watanabe T, Noritake J, Nakagawa M, Yamaga M, et al. Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell 2002;109:873–85. [PubMed: 12110184]

143. Lansbergen G, Akhmanova A. Microtubule plus end: a hub of cellular activities. Traffic 2006;7:499–507. [PubMed: 16643273]

144. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133:775–87. [PubMed: 18510923]

145. Shevach EM. Mechanisms of $Foxp3^+$ T regulatory cell-mediated suppression. Immunity 2009;30:636–45. [PubMed: 19464986]

146. Tadokoro CE, Shakhar G, Shen S, Ding Y, Lino AC, et al. Regulatory T cells inhibit stable contacts between $CD4^+$ T cells and dendritic cells in vivo. J Exp Med 2006;203:505–11. [PubMed: 16533880]

147. Tang Q, Adams JY, Tooley AJ, Bi M, Fife BT, et al. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. Nat Immunol 2006;7:83–92. [PubMed: 16311599]

148. Valencia X, Stephens G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE. TNF downmodulates the function of human $CD4^+CD25^{hi}$ T-regulatory cells. Blood 2006;108:253–61. [PubMed: 16537805]

149. Gupta S, Manicassamy S, Vasu C, Kumar A, Shang W, Sun Z. Differential requirement of PKC-θ in the development and function of natural regulatory T cells. Mol Immunol 2008;46:213–24. [PubMed: 18842300]

150. Lu LF, Gondek DC, Scott ZA, Noelle RJ. NFκB-inducing kinase deficiency results in the development of a subset of regulatory T cells, which shows a hyperproliferative activity upon glucocorticoid-induced TNF receptor family-related gene stimulation. J Immunol 2005;175:1651–57. [PubMed: 16034105]

151. Crellin NK, Garcia RV, Levings MK. Altered activation of AKT is required for the suppressive function of human $CD4^+CD25^+$ T regulatory cells. Blood 2007;109:2014–22. [PubMed: 17062729]

152. Sarris M, Andersen KG, Randow F, Mayr L, Betz AG. Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. Immunity 2008;28:402–13. [PubMed: 18328743]

153. Igakura T, Stinchcombe JC, Goon PK, Taylor GP, Weber JN, et al. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. Science 2003;299:1713–16. [PubMed: 12589003]

154. Jolly C, Sattentau QJ. Retroviral spread by induction of virological synapses. Traffic 2004;5:643–50. [PubMed: 15296489]

155. Aubert M, Yoon M, Sloan DD, Spear PG, Jerome KR. The virological synapse facilitates herpes simplex virus entry into T cells. J Virol 2009;83:6171–83. [PubMed: 19339346]

156. McDonald D, Wu L, Bohks SM, KewalRamani VN, Unutmaz D, Hope TJ. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science 2003;300:1295–97. [PubMed: 12730499]

157. Jolly C, Kashefi K, Hollinshead M, Sattentau QJ. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. J Exp Med 2004;199:283–93. [PubMed: 14734528]

158. Chen P, Hubner W, Spinelli MA, Chen BK. Predominant mode of human immunodeficiency virus transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. J Virol 2007;81:12582–95. [PubMed: 17728240]

159. Jolly C, Mitar I, Sattentau QJ. Adhesion molecule interactions facilitate human immunodeficiency virus type 1-induced virological synapse formation between T cells. J Virol 2007;81:13916–21. [PubMed: 17913807]

160. Vasiliver-Shamis G, Tuen M, Wu TW, Starr T, Cameron TO, et al. Human immunodeficiency virus type 1 envelope gp120 induces a stop signal and virological synapse formation in noninfected CD4 $^{+}$ T cells. J Virol 2008;82:9445–57. [PubMed: 18632854]

161. Cicala C, Arthos J, Martinelli E, Censoplano N, Cruz CC, et al. R5 and X4 HIV envelopes induce distinct gene expression profiles in primary peripheral blood mononuclear cells. Proc Natl Acad Sci USA 2006;103:3746–51. [PubMed: 16505369]

162. Cicala C, Arthos J, Selig SM, Dennis G Jr, Hosack DA, et al. HIV envelope induces a cascade of cell signals in nonproliferating target cells that favor virus replication. Proc Natl Acad Sci USA 2002;99:9380–85. [PubMed: 12089333]

163. Lee C, Liu QH, Tomkowicz B, Yi Y, Freedman BD, Collman RG. Macrophage activation through CCR5- and CXCR4-mediated gp120-elicited signaling pathways. J Leukoc Biol 2003;74:676–82. [PubMed: 12960231]

164. Weissman D, Rabin RL, Arthos J, Rubbert A, Dybul M, et al. Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor. Nature 1997;389:981–85. [PubMed: 9353123]

165. Cicala C, Arthos J, Rubbert A, Selig S, Wildt K, et al. HIV-1 envelope induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4^{+} T cells. Proc Natl Acad Sci USA 2000;97:1178–83. [PubMed: 10655504]

166. Cicala C, Arthos J, Ruiz M, Vaccarezza M, Rubbert A, et al. Induction of phosphorylation and intracellular association of CC chemokine receptor 5 and focal adhesion kinase in primary human CD4^{+} T cells by macrophage-tropic HIV envelope. J Immunol 1999;163:420–26. [PubMed: 10384144]

167. Davis CB, Dikic I, Unutmaz D, Hill CM, Arthos J, et al. Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5. J Exp Med 1997;186:1793–98. [PubMed: 9362541]

168. Del Corno M, Liu QH, Schols D, de Clercq E, Gessani S, et al. HIV-1 gp120 and chemokine activation of Pyk2 and mitogen-activated protein kinases in primary macrophages mediated by calcium-dependent, pertussis toxin-insensitive chemokine receptor signaling. Blood 2001;98:2909–16. [PubMed: 11698270]

169. Lee C, Tomkowicz B, Freedman BD, Collman RG. HIV-1 gp120-induced TNF-α production by primary human macrophages is mediated by phosphatidylinositol-3 (PI-3) kinase and mitogen-activated protein (MAP) kinase pathways. J Leukoc Biol 2005;78:1016–23. [PubMed: 16081599]

170. Melar M, Ott DE, Hope TJ. Physiological levels of virion-associated human immunodeficiency virus type 1 envelope induce coreceptor-dependent calcium flux. J Virol 2007;81:1773–85. [PubMed: 17121788]

171. Cicala C, Arthos J, Censoplano N, Cruz C, Chung E, et al. HIV-1 gp120 induces NFAT nuclear translocation in resting CD4^{+} T-cells. Virology 2006;345:105–14. [PubMed: 16260021]

172. Woolf E, Grigorova I, Sagiv A, Grabovsky V, Feigelson SW, et al. Lymph node chemokines promote sustained T lymphocyte motility without triggering stable integrin adhesiveness in the absence of shear forces. Nat Immunol 2007;8:1076–85. [PubMed: 17721537]

173. Henrickson SE, Mempel TR, Mazo IB, Liu B, Artyomov MN, et al. T cell sensing of antigen dose governs interactive behavior with dendritic cells and sets a threshold for T cell activation. Nat Immunol 2008;9:282–91. [PubMed: 18204450]
174. Shakhar G, Lindquist RL, Skokos D, Dudziak D, Huang JH, et al. Stable T cell-dendritic cell interactions precede the development of both tolerance and immunity in vivo. Nat Immunol 2005;6:707–14. [PubMed: 15924144]
175. Henrickson SE, von Andrian UH. Single-cell dynamics of T-cell priming. Curr Opin Immunol 2007;19:249–58. [PubMed: 17433876]
176. Celli S, Garcia Z, Beuneu H, Bousso P. Decoding the dynamics of T cell-dendritic cell interactions in vivo. Immunol Rev 2008;221:182–87. [PubMed: 18275482]
177. Celli S, Lemaitre F, Bousso P. Real-time manipulation of T cell-dendritic cell interactions in vivo reveals the importance of prolonged contacts for $CD4^+$ T cell activation. Immunity 2007;27:625–34. [PubMed: 17950004]
178. Schneider H, Downey J, Smith A, Zinselmeyer BH, Rush C, et al. Reversal of the TCR stop signal by CTLA-4. Science 2006;313:1972–75. [PubMed: 16931720]
179. Friedman RS, Jacobelli J, Krummel MF. Surface-bound chemokines capture and prime T cells for synapse formation. Nat Immunol 2006;7:1101–8. [PubMed: 16964261]
180. Barcia C, Thomas CE, Curtin JF, King GD, Wawrowsky K, et al. In vivo mature immunological synapses forming SMACs mediate clearance of virally infected astrocytes from the brain. J Exp Med 2006;203:2095–107. [PubMed: 16923851]
181. Fackler OT, Alcover A, Schwartz O. Modulation of the immunological synapse: a key to HIV-1 pathogenesis? Nat Rev Immunol 2007;7:310–17. [PubMed: 17380160]
182. Thoulouze MI, Sol-Foulon N, Blanchet F, Dautry-Varsat A, Schwartz O, Alcover A. Human immunodeficiency virus type-1 infection impairs the formation of the immunological synapse. Immunity 2006;24:547–61. [PubMed: 16713973]
183. Vallbracht S, Unsold H, Ehl S. Functional impairment of cytotoxic T cells in the lung airways following respiratory virus infections. Eur J Immunol 2006;36:1434–42. [PubMed: 16708402]
184. Gonzalez PA, Prado CE, Leiva ED, Carreno LJ, Bueno SM, et al. Respiratory syncytial virus impairs T cell activation by preventing synapse assembly with dendritic cells. Proc Natl Acad Sci USA 2008;105:14999–5004. [PubMed: 18818306]
185. Okada T, Miller MJ, Parker I, Krummel MF, Neighbors M, et al. Antigen-engaged B cells undergo chemotaxis toward the T zone and form motile conjugates with helper T cells. PLoS Biol 2005;3:e150. [PubMed: 15857154]
186. Allen CD, Okada T, Tang HL, Cyster JG. Imaging of germinal center selection events during affinity maturation. Science 2007;315:528–31. [PubMed: 17185562]
187. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal center formation. Nature 2008;455:764–69. [PubMed: 18843362]
188. Cahalan MD, Parker I, Wei SH, Miller MJ. Real-time imaging of lymphocytes in vivo. Curr Opin Immunol 2003;15:372–77. [PubMed: 12900266]
189. Takahashi Y, Dutta PR, Cerasoli DM, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. V. Affinity maturation develops in two stages of clonal selection. J Exp Med 1998;187:885–95. [PubMed: 9500791]
190. Breart B, Lemaitre F, Celli S, Bousso P. Two-photon imaging of intratumoral $CD8^+$ T cell cytotoxic activity during adoptive T cell therapy in mice. J Clin Invest 2008;118:1390–97. [PubMed: 18357341]
191. Kim JV, Kang SS, Dustin ML, McGavern DB. Myelomonocytic cell recruitment causes fatal CNS vascular injury during acute viral meningitis. Nature 2009;457:191–95. [PubMed: 19011611]
192. McGavern DB, Christen U, Oldstone MB. Molecular anatomy of antigen-specific $CD8^+$ T cell engagement and synapse formation in vivo. Nat Immunol 2002;3:918–25. [PubMed: 12352968]
193. Brossard C, Feuillet V, Schmitt A, Randriamampita C, Romao M, et al. Multifocal structure of the T cell – dendritic cell synapse. Eur J Immunol 2005;35:1741–53. [PubMed: 15909310]
194. Bloom O, Unternaehrer JJ, Jiang A, Shin JS, Delamarre L, et al. Spinophilin participates in information transfer at immunological synapses. J Cell Biol 2008;181:203–11. [PubMed: 18411312]

195. Fooksman DR, Shaikh SR, Boyle S, Edidin M. Cutting edge: phosphatidylinositol 4,5-bisphosphate concentration at the APC side of the immunological synapse is required for effector T cell function. J Immunol 2009;182:5179–82. [PubMed: 19380760]

196. Yoon ST, Dianzani U, Bottomly K, Janeway CA Jr. Both high and low avidity antibodies to the T cell receptor can have agonist or antagonist activity. Immunity 1994;1:563–69. [PubMed: 7600285]

197. Bekoff M, Kubo R, Gray HM. Activation requirements for normal T cells: accessory cell-dependent and -independent stimulation by antireceptor antibodies. J Immunol 1986;137:1411–19. [PubMed: 3489033]

198. Wolff CH, Hong SC, von Grafenstein H, Janeway CA Jr. TCR-CD4 and TCR-TCR interactions as distinctive mechanisms for the induction of increased intracellular calcium in T-cell signaling. J Immunol 1993;151:1337–45. [PubMed: 8335930]

199. Cochran JR, Cameron TO, Stone JD, Lubetsky JB, Stern LJ. Receptor proximity, not intermolecular orientation, is critical for triggering T-cell activation. J Biol Chem 2001;276:28068–74. [PubMed: 11384988]

200. Yokoyama WM, Koning F, Kehn PJ, Pereira GM, Stingl G, et al. Characterization of a cell surface-expressed disulfide-linked dimer involved in murine T cell activation. J Immunol 1988;141:369–76. [PubMed: 2838547]

Terms and Definitions

APC	antigen-presenting cell
cSMAC	central supramolecular activation complex
Immunoreceptor tyrosine-based activation motif (ITAM)	a sequence motif in the cytoplasmic domain of antigen receptors with the form $YxxLx_{(7–12)}YxxL$
Kinapse	a migratory, transient T cell–APC contact with information transfer
MC	T cell receptor microcluster
pMHC	peptide-major histocompatibility complex
pSMAC	peripheral supramolecular activation complex
TCR	T cell receptor

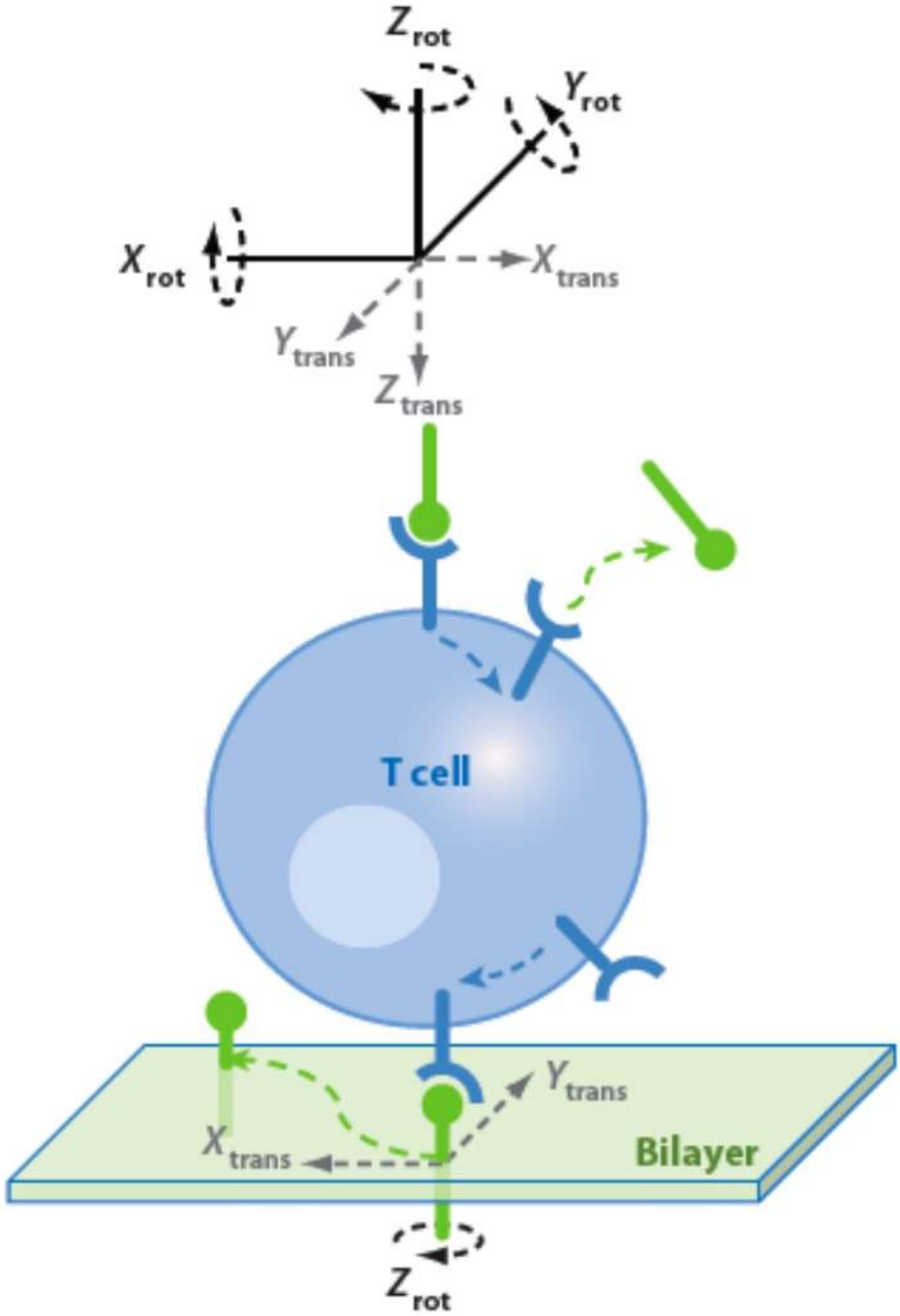

Figure 1.
Receptor-ligand kinetics in solution versus membranes. In theory, dissociation constants (Kd) for bound ligands in solution are calculated based on dissociation occurring in 3-D space, with six degrees of freedom. However, some of these receptor-ligand interactions occur when two opposed cell membranes (or in this case, cell membrane and artificial lipid bilayer) are interacting. Under these circumstances, ligands are confined to 2-D translation and 1-D rotation, which may stabilize and prolong these interactions. Also, rebinding by *cis*-receptors (such as clustered TCR) can further trap ligands and generate longer binding than would be predicted in solution.

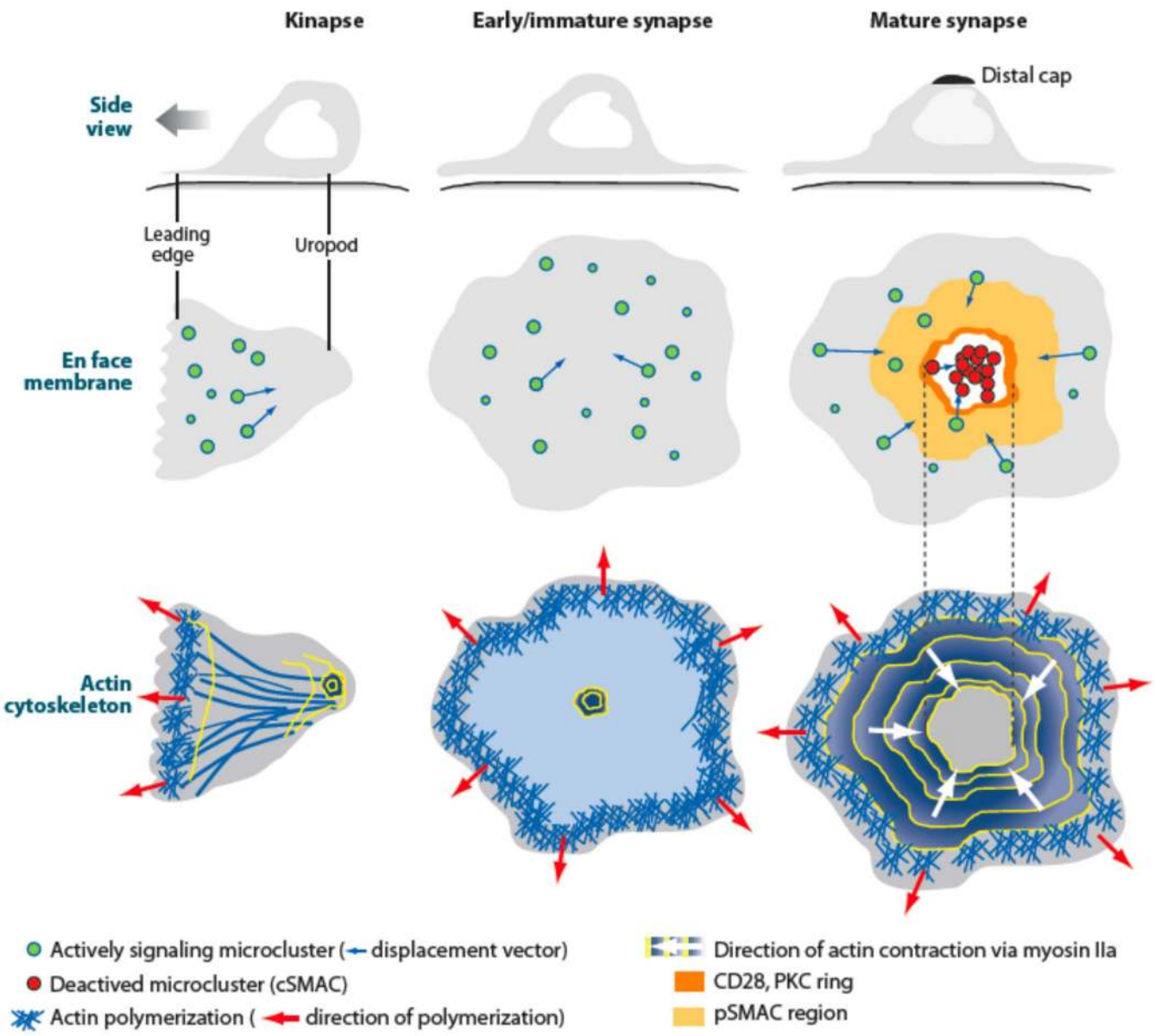

Figure 2.

Spatiotemporal map of synapse formation. Side and en face views of the T cell as it engages an APC. The migrating cell has a polarized cell body with actin polymerization at the leading edge and myosin contraction at the uropod. The membrane topology in the migrating cell is poorly understood. The early/immature synapse has symmetrical actin polymerization radiating outward from the center along the perimeter of the contact surface and cytoskeletal contractions through myosin radially inward to the center. The early synapse only contains microclusters, and after a few minutes these accumulate and form the cSMAC.

Table 1

Comparison of TCR stimuli and responses

TCR stimulus	Ligand:TCR[a]	TCR activation	Comments	Reference
Soluble				
Anti-TCR F'ab	1:1	No activation	Weak μM Kd, might not be stable; no force transduction either	196
pMHC monomers	1:1	No activation	No activation despite binding of monomers to TCR	37
Anti-CD3 antibody	2:2	Partial	Signaling not blocked by actin depolymerization drugs	197,198
Agonist pMHC dimers	2:2	Full and sustained	intermolecular distance, not pMHC orientation, is critical	37,199
Agonist/endogenous pMHC heterodimers	2:2	Full and sustained	CD4 required for activation	38
Cross-linked anti-CD3	2:2+	Full and transient	CD69 upregulation	197
Plate-bound				
Anti-CD3	2:2++	Full and sustained	Stable bivalent ligand induces clustering, may generate force for mechanotransduction	33
Membrane associated				
pMHC monomers + ICAM-1 on planar bilayers	1:1++	Full and sustained if >0.2 pMHC/μm^2. Less sensitive without ICAM-1	Requires F-actin and myosin IIA	14,16,81
Anti-CD3 linked to Fc on APC surface	2:2++	Full and sustained	F-actin required?	200
Single pMHC on APC surface	1:1+++	Full and transient if <10 pMHC; full and sustained if >10 pMHC	Full activation achieved by 10 agonist peptides in a CD4-dependent manner (CD4 blockade raises threshold to 25 agonist peptides)	19

[a] Stoichiometry of engagement.

+ Higher-order cross-linking at optimal ratio of anti-CD3 and secondary antibody.

++ Higher-order interactions owing to distribution of receptor-ligand interactions across a 2-D interface.

+++ Higher-order interaction owing to distribution of receptor-ligand interactions across a 2-D interface and the presence of weak self-ligand interactions.

Table 2

Comparison of various immunological synapses

Synapse	Minimum requirements for synapse formation	Site of signaling	Stability	Function, consequences, and in vivo behavior
Naive T cells	TCR-pMHC LFA-1-ICAM-1 CD28-CD80	In the periphery within MC	Periodic (break and reform IS)	Asymmetric cell division Duration of contacts with APC depends of antigen concentration
Effector CD4	TCR-pMHC LFA-1-ICAM-1	In the periphery within MC	Prolonged	Directed cytokine secretion Long-lived contacts with APC
Effector CD8 (CTL)	TCR-pMHC LFA-1-ICAM-1	cSMAC	Transient (lytic) Prolonged (stimulatory)	Release of cytolytic granules Cytokine secretion (stimulatory synapse)
Treg	TCR engagement LFA-1-ICAM-1	In the periphery within MC	Prolonged	Suppression of effector-DC contacts and effector function
HIV-1 VS	HIV-1 env gp120-CD4/ chemokine receptor LFA-1-ICAM-1	Initiated in gp120 MC and sustained in VS cSMAC	Transient	Direct secretion of viral particles and efficient Viral spread