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TCR SIGNALING: MECHANISMS OF INITIATION AND PROPAGATION

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Abstract

The mechanisms by which a T cell detects antigen using its T cell antigen receptor (TCR) are crucial to our understanding of immunity and harnessing T cells therapeutically. A hallmark of the T cell response is the ability of T cells to quantitatively respond to antigenic ligands derived from pathogens while remaining inert to similar ligands derived from host tissues. Recent studies have revealed exciting properties of the TCR and behaviors of its signaling effectors that are used to detect and discriminate between antigens. Here we highlight these recent findings, focusing on the proximal TCR signaling molecules Zap70, Lck and LAT, to provide mechanistic models and insights into the exquisite sensitivity and specificity of the TCR.

Keywords

TCR; Signaling; Kinase; Phosphatase; T cell; antigen

Introduction

The specificity of a T cell for antigen is defined by its T cell antigen receptor (TCR) which acts as an antigen detector. Because the TCR is generated through the rearrangement of genomic DNA segments during development, each T cell is endowed with a TCR of unique specificity – providing a clonal identity. Each naive T cell clone uses its TCR to survey for antigenic ligands, short peptide fragments bound to MHC class I or class II molecules (pMHC), that are novel or have not been previously encountered (FIGURE 1A). Three dimensional TCR affinities for such agonist pMHCs are remarkably low, typically 1–10 μM. Although each T cell clone can in principle possess a TCR of unique specificity, that TCR may interact to varying extents with many different pMHC ligands [1]. Indeed, the TCR also interacts with endogenous or self pMHC and such interactions are required for positive selection during T cell development in the thymus and for peripheral naive T cell survival. Differences in TCR affinities for agonist pMHC and self pMHC are very small, typically

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only 10-fold weaker [2]. Despite these small differences in ligand affinities, T cells must distinguish between agonist and self pMHC which has led to the evolution of a specialized signaling apparatus and downstream machinery (BOX 1).

BOX 1

TCR Signaling Events

When a TCR engages a relevant pMHC ligand (or other stimulus such as anti-TCR crosslinking), TCR signaling is initiated (FIGURE 1B). Each clonally distributed TCR is sufficient for pMHC recognition, however, the associated invariant CD3 and ζ chains are required for signaling to occur. The cytoplasmic domains of CD3 and ζ contain sequence motifs called immunoreceptor tyrosine-based activation motifs (ITAMs). Upon pMHC binding to the TCR, Lck is recruited to the TCR complex by the colocalization of CD4 or CD8 coreceptors to pMHC molecules where Lck can phosphorylate ITAM signaling motifs. ITAM motifs each contain two tyrosines that, when phosphorylated, create binding sites for the tandem SH2 domains of the Zap70 kinase. Prior to TCR engagement, Zap70 predominately resides within the cytoplasm where it is autoinhibited. By binding to phosphorylated ITAMs Zap70 is recruited to the plasma membrane and its autoinhibited conformation is disrupted. The active conformation of Zap70 is further stabilized through phosphorylation of its interdomain linker and activation loop by Lck.

Once recruited and activated, Zap70 is then able to propagate signaling events from the TCR. Specifically, Zap70 phosphorylates the linker for activation of T cells (LAT) which serves as a signaling hub [3, 4]. LAT contains four major Zap70 phosphorylation sites: Y132, Y171, Y191 and Y226. Phospho-Y132 recruits PLC 1 to provide for calcium and Ras/MAPK pathway activation, whereas phospho-Y171, -Y191, and -Y226 are responsible for the recruitment of Grb2 and Gads, adaptors that bind SOS and SLP-76 which can lead to Ras, Rac, Rho GTPase activation, among other effector responses. [3, 5–7]. In addition to assembly of the LAT signalosome, the TCR and the CD28 costimulatory molecule activate PI3K. PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP_2) to yield phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) which can recruit proteins to the inner leaflet of the plasma membrane, such as ITK kinase [8]. ITK binds to PIP_3 through its PH domain and SLP-76 through its SH3 domain which localize it to the plasma membrane and cause its activation. Activated ITK can phosphorylate PLC 1, which in addition to membrane recruitment, is important for its activation. PLC 1 is responsible for hydrolyzing PIP_2 to generate the secondary messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 is free to diffuse within the cytoplasm where it is bound by its receptor (IP_3R) located in the endoplasmic reticulum (ER). Binding of IP_3 to IP_3R causes the release of Ca^{2+} stores from the ER which then causes the influx of extracellular calcium through channels in the plasma membrane (e.g., Orai1). Elevated levels of cytosolic Ca^{2+} activates many proteins including the transcription factor NFAT. In contrast to IP_3, DAG remains within the plasma membrane where it activates protein kinase C (PKC) and RasGRP, which can activate Ras. In addition, recruitment of SOS to LAT via Grb2 results in the activation of the RAS pathway. The combined actions of RasGRP and SOS lead to a rapid, bistable amplification of Ras activation. Ras-mediated activation of Raf, leads to activation of

MEK, and ultimately the MAP kinase ERK [9]. The MAP kinases respond to diverse signaling inputs to activate transcriptional regulators which culminate in T cell activation.

Models and observations: The initiation of TCR signaling

Despite years of intensive study, the mechanism by which ligand recognition triggers TCR signaling remains enigmatic. Questions surround how TCR-pMHC binding initiates signaling and how these signaling events can quantitatively discriminate between pMHC complexes with small incremental differences in affinity. To explain how pMHC binding initiates signaling, several models have been proposed. It is important to note that because these models address or explain experimental observations they are not necessarily mutually exclusive. Our goal here is to highlight recent findings and their implications for the initiation of TCR signaling.

In vivo, a higher affinity pMHC agonist will generally induce a stronger response than a weaker agonist and below a specific affinity threshold no response will occur [10, 11]. To establish a threshold for responsiveness at the single cell level, it has been proposed that a series of signaling events must accumulate over time and initiate a critical event prior to dissociation of the TCR:pMHC complex, a process referred to as kinetic proofreading [12]. Kinetic proofreading is generally agnostic to the phenomena that initiates TCR signaling and has been supported by recent computational studies [13, 14]. Many proposed models for the initiation of TCR signaling are kinetically sensitive and therefore relevant to the kinetic proofreading framework (reviewed in [15–17]). Recently the kinetic proofreading framework has been expanded to more specifically incorporate a mechanism for the initiation of TCR signaling [18] (FIGURE 2A). In T cells, the CD8 and CD4 coreceptors bind weakly to non-polymorphic regions of MHC I and II molecules, respectively, through their extracellular domain and within the cell via their cytoplasmic tails to the Src family kinase (SFK) Lck [19–21]. The CD4/CD8 coreceptors can therefore recruit Lck to the bound TCR:pMHC where it can phosphorylate ITAM tyrosines in the CD3 and ζ chains of the TCR complex and also Zap70. However, only a small proportion of coreceptors are associated with Lck. Moreover, the associated Lck kinases can be in either an active or inactive conformation. Stepanek and colleagues propose a ‘coreceptor scanning’ model whereby the CD4/CD8 coreceptors rapidly exchange or “scan” the TCR:pMHC. Coreceptors that recruit active Lck will result in the phosphorylation of the TCR complex (and Zap70). Sufficient TCR complex phosphorylation must occur prior to TCR:pMHC dissociation to initiate a cellular response. Consistent with this model, the authors were able to increase a T cell response to weaker pMHC agonists by stabilizing the interaction between Lck and the CD8 coreceptor [18]. Moreover, in support of this model, it has also been independently observed that increasing the abundance of active Lck can potentiate T cell responses to lower affinity pMHC agonists [22]. These results imply that the earliest step in kinetic proofreading could be phosphorylation of the TCR complex by Lck. However, Lck has additional substrates and it is likely that subsequent signaling events may be required prior to TCR:pMHC dissociation. Studies have reported that constitutive ζ chain phosphorylation and bound Zap70 can be detected in ex vivo T cells, the result of interactions of the TCR (and presumably coreceptors) with self-pMHC [23, 24]. Thus, a

second set of kinetically sensitive steps have been predicted to involve Zap70 and its phosphorylation and activation by Lck [25, 26]. Phosphorylation of Zap70 stabilizes its active conformation and also creates a docking site (phospho-Y319) to which the Lck SH2 domain can bind. Because the loss of the Lck-Zap70 docking site attenuates TCR signaling, the interaction between Lck to Zap70 is reported to be important for the assembly of a TCR signalosome [27]. Therefore, following ITAM phosphorylation, the formation of a TCR-Zap70-Lck complex can comprise a subsequent kinetically sensitive step that must occur [25]. Moreover, the engagement of the Lck SH2 domain by phospho-Y319 would be expected to prevent Lck from adopting a closed, inactive conformation, consistent with a positive feedback loop that might contribute to signal amplification.

Additional models for the initiation of TCR signaling also describe kinetically sensitive steps. T cells can respond to perhaps a single agonist pMHC complex [28] and it has been proposed that such sensitivity requires the serial engagement of multiple TCRs by a single pMHC complex [29]. Within the serial engagement model, a pMHC binds to a TCR and causes a degree of signaling before the TCR:pMHC complex dissociates and the pMHC binds to an additional receptor - a process that can be repeated to accumulate sufficient TCR signaling for T cell activation to occur. Rather than serially engaging multiple TCRs, re-binding of pMHC to a single TCR may be a critical to TCR antigen recognition. Such re-binding of pMHC to the same TCR may increase the effective half-life or “confinement time” of a TCR:pMHC interaction [30]. Others have also reported that TCR:pMHC rebinding through fast pMHC on-rates can explain how pMHC with relatively short-dwell times cause T cell activation [31]. Interestingly, it has been recently observed using single molecule tracking of TCR:pMHC complexes that at low antigen densities positive cooperativity through active feedback occurs [32]. Such cooperativity results in an apparent increase in TCR:MHC affinity at low antigen densities and may facilitate antigen discrimination.

When a T cell recognizes antigen, it forms a synapse with the antigen presenting cell (APC). Within the synapse, over time, the TCR becomes concentrated at the center and larger molecules such as the receptor-like protein tyrosine phosphatase CD45 is excluded to the periphery [40]. CD45 is a critical regulator of TCR signaling and is required for T cell function. Interestingly, CD45 has been described to play both positive and negative roles in TCR signaling and these are highlighted in BOX 2. Because synapse formation is antigen-dependent, binding of pMHC to the TCR has been proposed to initiate signaling by segregating signaling components within the plasma membrane (FIGURE 2B). Within the kinetic segregation model, CD45 exclusion from the bound TCR:pMHC causes an apparent increase in Lck activity and TCR complex phosphorylation occurs [41]. Interestingly, formation of an immune synapse can be recapitulated in a minimal heterologous cell-based reconstituted system [42]. The phosphorylation of the TCR complex and Zap70 recruitment in this minimal system coincides with the segregation of CD45. Kinetic segregation requires the CD45 extracellular domain be large and rigid and this has been confirmed by recent structural analysis [43]. The extended extracellular structure is comprised of an alternatively spliced N-terminal mucin-like segment, followed by a structured cysteine-rich domain and three fibronectin-type 3 domains. Analysis of these domains confirmed that they can extend away from the plasma membrane. When mapped to the CD45 structure, sequence

conservation is greatest at the domain interfaces which suggests that structural rigidity is important for CD45 function and has been conserved through evolution. A hurdle for the kinetic segregation model has been the observation that a mature synapse forms over the course of several minutes whereas early TCR signaling events can be detected much more rapidly (< 1 minute) [44]. However, more rapid microsynapses have also been observed and occur on a time scale compatible with the initiation of TCR signaling [45, 46]. Excitingly, recent findings report CD45 is excluded from submicron-sized regions of the plasma membrane where contact between surfaces occurs, termed ‘close contacts’ [43]. These ‘close contacts’ form spontaneously in the absence of antigen which indicates that contact between surfaces is sufficient to exclude CD45. Perhaps more controversially, it is also reported that close contacts cause signaling in the absence of antigen. This finding suggests that the exclusion of CD45 alone is sufficient to initiate TCR signaling, without a TCR ligand, which has been challenging to demonstrate [43]. The segregation additional cellular signal transduction molecules with respect to the agonist-bound TCR have also been observed. The redistribution of these signaling molecules within lipid microdomains has also been proposed to be important for the initiation of TCR signaling [47, 48].

BOX 2

The receptor-type protein tyrosine phosphatase (RPTP) CD45 and its critical role in T cell function

The phosphatase CD45 is among the most abundant proteins resident within the T cell plasma membrane. CD45 possesses a large extracellular domain followed by a membrane spanning transmembrane domain and two intracellular protein tyrosine phosphatase homology domains (PTP). Interestingly, only the first such PTP domain (D1) is catalytically active and the role of the second domain (D2) remains unclear [33]. Genetic evidence has revealed a critical role for CD45 in T cell function [34, 35]. In mice, loss of CD45 (*ptprc*$^{-/-}$) causes an accumulation of immature thymocytes during T cell development. The inability of CD45-deficient cells to mature is due to an inability to signal through their TCR. CD45 is required to activate the SFK Lck by dephosphorylating its inhibitory C-terminal tail (Y505) (FIGURE 3A) [36–38]. In the absence of CD45 activity, Lck is hyperphosphorylated at this site and predominately autoinhibited. Because SFK activity is required to phosphorylate the TCR complex, loss of CD45 impairs beta-selection which requires signaling through the pre-TCR complex and the positive selection of double positive thymocytes [39]. These findings reveal a positive role for CD45 in activating SFKs, notably Lck, which is necessary to phosphorylate the TCR complex and the recruited Zap70 kinase. However, in vitro studies have suggested that CD45 can also act to dephosphorylate signaling components such as the TCR complex. Consistent with a negative regulatory role, CD45 is segregated from the TCR during the formation of an immune synapse. Such observations have suggested that during antigen encounter it may be necessary to exclude CD45 in order to achieve robust phosphorylation of the TCR complex [16]. Overall, CD45 plays a critical role in T cell function as evidenced by both genetic and cell-based studies.

When ligands bind to many other cell surface receptors they often induce conformational changes that are transmitted across the plasma membrane to then initiate signaling events within the cell. However, comparison of the bound and unbound αβ TCR structures revealed mostly localized conformational changes within the TCR loops comprising the pMHC binding site [49, 50]. Despite the absence of an obvious conformational switch distal to the binding site within the αβ TCR itself, evidence has suggested that pMHC binding could alter the conformation of the TCR complex through reorientation of the TCR relative to its associated CD3 and ζ chains [51–54]. A recent study using NMR has provided updated insight into the relative orientations of the CD3 subunits and the TCR [55]. In contrast to previous models where CD3 subunits are located on one side of the TCR, these recent findings support a model where the αβTCR is flanked on either side by the CD3εγ and CD3εδ subunits [51, 55]. Although it remains unclear how antigen binding alters this complex, recent evidence suggests that the TCR complex can respond to mechanical force [53, 56–58]. Specifically, the interface of the T cell and APC acts to exert force upon the TCR:pMHC. When force is exerted on the TCR complex it is proposed to undergo a conformational change that causes signaling (FIGURE 2C). Using optical tweezers, it was found that lateral forces potentiate TCR signaling and causes a conformational transition within the TCR complex [58, 59]. Perhaps consistent with a mechanosensitive mechanism, it has been observed that ‘catch bonds’, or interactions that are stabilized by the application of external force, are formed between TCR:pMHC [60]. Recently, evidence for a mechanical switch within the transmembrane helices of the of the TCR-associated ζ chain has been reported [61]. Using several in situ proximity assays, it was observed that the two ζ chains are spread apart when assembled into the TCR complex and that TCR engagement caused their reorientation (FIGURE 2D).

Because the TCR complex lacks intrinsic kinase activity, how a conformational change can be communicated to the signaling motifs which causes their phosphorylation by Lck is not clear. It has been proposed that heterodimerization of a TCR with coreceptor (CD4/8) or through pseudodimerization of TCR complexes may be sufficient [62]. Within the pseudodimerization model, TCR bound to agonist pMHC forms pseudodimers with other nearby TCRs bound weakly to self pMHC complexes to augment TCR sensitivity [63–65]. There is also evidence to suggest that the accessibility of the ITAM signaling motifs is regulated. It has been observed that the disordered signaling chains containing ITAMs can associate with acidic phospholipids [66]. Association of these signaling chains with the membrane appears to stabilize an alpha helical secondary structure and bury the tyrosine residues within the plasma membrane to preclude their phosphorylation [67]. Conformational changes within the TCR complex may cause the dimerization or aggregation of TCR complexes [68, 69] which could expose the ITAM residues by altering the lipid environment [48]. Alternatively, more direct conformational changes within the TCR complex may occur (FIGURE 2E). It has also been observed that upon TCR:pMHC binding a proline-rich sequence (PRS) within CD3ε becomes exposed. The exposed PRS can be bound by the SH3 domain of Nck and is reported to amplify weak TCR signals [70, 71]. Overall, however, a structural understanding of the changes that occur within the bound TCR complex that facilitates its phosphorylation remains a major challenge.

Exchanging currencies: Conversion of TCR:pMHC binding into an intracellular signal by Lck

Lck is critical to the initiation of TCR signaling because it converts an extracellular recognition event, pMHC binding, into an intracellular biochemical signal by phosphorylating the TCR complex and Zap70. Importantly, models for the initiation of TCR signaling need to account for the availability of active Lck for phosphorylation of the TCR. Lck activity is controlled through the conformation of its catalytic kinase domain which is regulated by phosphorylation [72, 73] (FIGURE 3A). It has been observed that a substantial amount of Lck is basally active in resting unstimulated T cells. To estimate the abundance of active Lck, specific Lck phosphoforms were quantified using immunodepletion [74]. These findings suggested that up to ~ 50% of Lck could be active. However, another study has provided a much lower estimate, ~2% [75]. Recent studies using an Lck FRET reporter have confirmed a pre-existing pool of Lck that is an open conformation and presumably active [76]. It was also noted that a subpopulation of Lck molecules does undergo a conformational change consistent with its activation upon TCR stimulation. A subsequent study using an improved FRET reporter confirmed these findings and also suggested that Lck autophosphorylation is required [77]. The phosphatase CD45 activates Lck and its loss in mice blocks T cell development due to an inability to signal through the TCR during positive selection (BOX 2). Mice that express a series of CD45 mutant alleles were used to titrate CD45 expression amounts on thymocytes and T cells. Altering the amount of CD45 caused changes in the amount of activated Lck, as assessed by changes in regulatory phosphorylation sites. In these experiments, TCR responsiveness was best correlated with phosphorylation of the Lck activation loop which peaked at intermediate amounts of CD45 in resting cells, indicating that the initial amount of active Lck is important [78]. In a more recent study, the initial pool of Lck was manipulated by inhibiting the cytoplasmic kinase Csk, a negative regulator of Lck, and the impact of increasing the abundance of active Lck on antigen discrimination was assessed. CD8 T cell responses to lower affinity antigens were potentiated while activation caused by high affinity antigens remained unchanged [22]. Therefore, the ability of the TCR to discriminate between antigens based on their affinities was altered by manipulating the size of the active Lck pool (FIGURE 3B).

An important question emerges from these findings: how does a T cell control the abundance of active Lck molecules to enforce antigen discrimination? Quantitative mass spectrometry revealed that inhibition of the Lck substrate and downstream kinase, Zap70, causes an apparent increase in Lck activity [79]. Negative feedback pathways were therefore predicted to regulate active Lck abundance. A Zap70-dependent phosphorylation site, Y192, within the SH2 domain of Lck was found to control its association and activation by CD45, which dephosphorylates the C-terminal negative regulatory tyrosine [80]. Because phosphorylation of Y192 in Lck responds to Zap70 inhibition, it is proposed to regulate the activation of Lck by CD45 as part of a negative regulatory loop (FIGURE 3C). Interestingly, this site is conserved amongst other SFKs and its phosphorylation is reported in several hematopoetic malignancies [80, 81]. In addition to regulating Lck activation by CD45, other regulatory loops are predicted to act concert to control the amount of active Lck. Adaptor proteins, such as PAG, can recruit Csk to the plasma membrane where it can inhibit Lck by

phosphorylating its inhibitory C terminal tail [82] (Figure 3D). In many cases the loss of these adaptors in mice causes only subtle phenotypes which suggests that their loss can be compensated for. However, it has recently been observed that deletion of PAG can alter the reactivity of effector T cells [83]. Therefore, looking at the loss of negative regulatory pathways in specific contexts may be important to unravelling their functions.

A molecular handshake: Zap70 activation and assembly of the LAT signalosome

T cells require fewer than 10 agonist pMHC interactions to trigger a full T cell response [2, 44, 65]. How can T cells exhibit such sensitivity toward foreign antigens while maintaining their quiescence toward self-pMHC? To address this question, it is necessary to consider the intracellular effectors employed by the TCR to propagate signaling events. Because the kinases Lck and Zap70 control the initiation of proximal TCR signaling, their activities together must be tightly coordinated to enforce the exquisite sensitivity and discrimination demonstrated by the TCR [84, 85]. Importantly, Lck and Zap70 exist in a strict hierarchy with Lck lying upstream of Zap70 [26] (FIGURE 4A). Although other non-receptor tyrosine kinases, such as Syk, can play initiating roles in ITAM-containing receptor signaling in other cells of the immune system, this does not occur in T cells [86]. How then is a signaling hierarchy enforced in T cells? Lck is ideally suited to play the initiating role in TCR responses to pMHC. First, it is basally active and associates with the cytoplasmic tails of CD4 and CD8 coreceptors. Because CD4 and CD8 recognize non-polymorphic regions of MHC molecules, Lck is ensured to be positioned in proximity of a TCR that has bound a self or agonist pMHC. In contrast, Zap70 resides within the cytoplasm in its autoinhibited conformation until recruited to phosphorylated ITAM residues. As detailed in BOX 1 and FIGURE 2B, Lck-dependent phosphorylation of ITAM motifs in the CD3 and ζ chains is required to initiate TCR signaling, and leads to the recruitment of Zap70 via its tandem SH2 domains with high affinity and specificity. In addition to ITAM binding, full activation of Zap70 activation requires its phosphorylation by Lck [87]. Because both ITAM phosphorylation and phosphorylation of Zap70 require Lck activity, active Lck must reside near the pMHC:TCR complex for sufficient duration for this to occur. Specifically, Lck phosphorylates Zap70 on Y315 and Y319 which destabilizes the Zap70 autoinhibited conformation and promotes its adoption of an open active conformation. The active conformation of the kinase domain is further stabilized by the Lck-mediated phosphorylation of its activation loop (Y493) (FIGURE 4B).

In addition to their specific mechanisms of activation, it is also necessary to consider how Lck and Zap70 recognize their substrates. In contrast to the related and more ubiquitously expressed Syk kinase, Zap70 is unable to phosphorylate ITAM residues and cannot phosphorylate additional Zap70 molecules. Additionally, despite residing at the plasma membrane with Lck, the critical adaptor LAT is a poor Lck substrate and requires Zap70 to phosphorylate it. Because Zap70 expression is restricted to T and NK cells, these observations suggest it has evolved unique properties critical for T cell function. Recent analysis of the substrate bias of Lck and Zap70 using bacterial display libraries has revealed the unique features of Zap70 substrate recognition [88]. Specifically, Zap70 only efficiently

phosphorylated peptides that contained tyrosines flanked by acidic residues. The presence of nearby basic residues prevented phosphorylation and were largely absent in regions surrounding known Zap70 phosphorylation sites. This selectivity is attributable to a highly basic substrate binding site in the Zap70 kinase domain (FIGURE 4C). This basic region acts as an electrostatic filter for potential Zap70 substrates. This electrostatic mechanism also prevents Zap70 from *trans*-autophosphorylating its activation loop or phosphorylating ITAM motifs within the TCR complex. In contrast, the tyrosine residues within Zap70 (including Y315, Y319, Y493) and TCR complex ITAMs all meet the criteria for Lck substrates [88]. Thus, the substrate preferences for Zap70 and the broader Lck specificity (and its basal activity) provide a logic for the ordered and sequential roles that these kinases play in TCR signaling.

The strict localization of active Zap70 could present a hurdle to initiating and amplifying a TCR signal when a bona-fide agonist pMHC is encountered. Katz el al. have suggested that Zap70 may cycle through “catch-and-release” modes [89]. In this model, activation of Zap70 by Lck also causes its release from the ITAM motifs of the TCR complex. The released-yet-still-active Zap70 was observed to be mobile. Active Zap70 was able to translocate to adjacent protein islands where LAT was present and induced its phosphorylation (FIGURE 4D). Interestingly, the release of Zap70 from ITAMs is proposed to occur through phosphorylation of Y126 which is located within the linker that connects the SH2 domains of Zap70. Phosphorylation of this site was identified by mass spectrometry analysis and is reported to trigger the release of activated Zap70 from phosphorylated ITAM motifs [89]. In principle, this allows other not-yet-activated Zap70 molecules to bind and become activated. The “catch-and-release” model envisions the engaged TCR complexes as a “catalytic unit” that produces activated Zap70 which can go on to phosphorylate LAT. However, the translocation of Zap70 from TCR complexes to neighboring LAT clusters requires confirmation by microscopic imaging studies. If Zap70 is released from the TCR complex how far can it meander before it is inactivated (by phosphatases or ubiquitin ligases, for instance)? Such parameters would be critical constraints for TCR signaling because continued ligation of the TCR is required for T cell responses.

LAT and SLP-76, when phosphorylated by Zap70, provide docking sites for specific SH2 domain-containing proteins. These SH2 domain containing proteins are assembled onto LAT or SLP-76 to promote the formation of oligomeric signalosomes. Recently, proximal TCR signaling, including assembly of a LAT signalosome, have been reconstituted in vitro using supported lipid bilayers [90, 91]. These studies demonstrate that LAT clusters observed in T cells are caused by multivalent interactions of associated proteins such as Grb2 and SOS which can bridge multiple LAT molecules. Importantly, these clusters were found to be dynamic and could be disassembled through dephosphorylation of LAT. When proximal signaling components, including Lck, the ζ chain, Zap70, and CD45 were also added, LAT was able to assemble into dynamic clusters in the presence of Gads, Grb2, SOS and SLP-76. Interestingly, these clusters appeared to enrich Zap70 and exclude the phosphatase CD45 [90] (FIGURE 5A). LAT clustering also promoted actin assembly when Nck and N-WASP were incorporated. Together these findings are consistent with LAT-based microclusters acting as foci of TCR signaling that augment sensitivity toward weak TCR stimuli [90].

Moreover, these findings highlight the unique properties of proteins that comprise the LAT signalosome which allow it to act as a hub of TCR signaling.

Interestingly, a recent study suggested clusters of LAT in T cells may have heterogeneous compositions and by extension form distinct signaling hubs [92]. In addition to the canonical LAT-Gads-SLP-76 signalosomes, another set of LAT-based clusters containing Grb2 and SKAP1 were identified. These latter clusters appeared to play a role in terminating conjugate formation between a T cell and an antigen-presenting cell by inactivating the integrin LFA-1. The assembly of these LAT-Grb2-SKAP1 clusters was exclusively dependent on phosphorylation of Y171 of LAT. Unlike the canonical LAT-Gads-SLP-76 signalosomes, assembly of the LAT-Grb2-SKAP1 signalosomes were initiated by crosslinking of LFA-1 which promoted the activation of FAK1 kinase which then phosphorylated LAT Y171 (FIGURE 5B). The presence of two distinct LAT-based signalosomes suggests interesting possibilities for the regulation and function of the T cell-APC synapse. It is interesting to speculate whether the composition of a LAT signalosome is dependent upon its proximity to an engaged TCR complex or whether it is influenced by additional co-inhibitory or co-stimulatory signals.

Concluding remarks

Early insights into TCR signaling have shaped our understanding of the T cell response and made possible the emerging use of T cells therapeutically. The successful design of cancer-targeting chimeric antigen receptors (CARs), as an example, were derived from understanding the signaling motifs which recruit Zap70 to evoke TCR signaling and therefore elicit a T cell response [93]. We have highlighted recent advances in understanding how the TCR, and the proximal signaling proteins to which it is coupled, can detect antigens and drive a T cell response; however, many questions remain. For example, the relative contributions of TCR proximal signaling events to models of kinetic proofreading remain poorly defined. Such efforts to define how specific signaling events quantitatively contribute to the remarkable sensitivity and specificity of the TCR remain ongoing. Efforts to understanding how T cells signal in different contexts and integrate multiple cues will also be critical to therapeutically exploiting the emerging role of T cells in maintaining tissue homeostasis [94]. How these signals induce responses at the single cell and population levels will be crucial. Many questions surround how observed heterogeneity in single cell T cell responses translates to a population based response in vivo [28, 95].

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GLOSSARY/TERMS

Adaptive immunity

The branch of the immune system that can respond (or adapt) to novel antigens that have not been previously encountered

Adaptor protein
A protein that lacks catalytic activity but possess binding domains (e.g., SH2 domain) and/or binding sites to recruit signaling proteins. Often act as signaling hubs by recruiting effectors and substrates

Antigen
A molecule or compound that can elicit an immune response

CD4+ T cell
Broadly defined as “helper” T cells which express CD4 and recognize pMHC class II complexes. Produce cytokines to coordinate the adaptive immune response

Antigen presenting cells
Professional antigen presenting cells include dendritic cells, B cells, and macrophages. These cells express MHC I and II, and can process and present self or foreign antigens within pMHC complexes to be specifically recognized by T cells

Autoinhibition
A common feature of signaling proteins where the inactive conformation is stabilized through intramolecular interactions that must be relieved prior to activation

CD8+ T cell
Mediate cellular immunity upon activation and differentiation into cytotoxic T lymphocytes (CTLs) which can induce cell death in target cells. Express CD8 and recognize pMHC class I complexes

Coreceptor
A receptor that does not typically signal on its own but influences the engagement or signaling of other receptors (e.g., CD4/CD8)

ITAM
Immunoreceptor tyrosine-based activation motif, a conserved sequence found in many antigen receptors which can recruit Zap70 or Syk when phosphorylated

Peptide-MHC complexes (pMHC)
Protein complexes that contain peptide fragments, which are derived from within the cell, and then displayed on the cell surface where they can be detected by a T cell antigen receptor (TCR). Those MHC complexes presenting peptides derived from the self proteins are referred to as self pMHC, whereas those MHC complexes presenting peptide derived from foreign antigens and able to trigger immune responses are agonist pMHC antigens

Signaling effector
Typically intracellular enzymes (e.g., kinases) that modify a substrate to propagate a biochemical signal within the cell

TCR complex
The αβTCR and its associated CD3 subunits (CD3ε, CD3γ, CD3δ) and ζ chains

References

1. Sewell AK. Why must T cells be cross-reactive? Nat Rev Immunol. 2012; 12(9):669–77. [PubMed: 22918468]
2. Juang J, et al. Peptide-MHC heterodimers show that thymic positive selection requires a more restricted set of self-peptides than negative selection. J Exp Med. 2010; 207(6):1223–34. [PubMed: 20457759]
3. Balagopalan L, et al. The LAT story: a tale of cooperativity, coordination, and choreography. Cold Spring Harb Perspect Biol. 2010; 2(8):a005512. [PubMed: 20610546]
4. Wange RL. LAT, the linker for activation of T cells: a bridge between T cell-specific and general signaling pathways. Sci STKE. 2000; 2000(63):re1.
5. Zhang W, et al. Association of Grb2, Gads, and phospholipase C-gamma 1 with phosphorylated LAT tyrosine residues. Effect of LAT tyrosine mutations on T cell angigen receptor-mediated signaling. J Biol Chem. 2000; 275(30):23355–61. [PubMed: 10811803]
6. Liu SK, et al. The hematopoietic-specific adaptor protein gads functions in T-cell signaling via interactions with the SLP-76 and LAT adaptors. Curr Biol. 1999; 9(2):67–75. [PubMed: 10021361]
7. Yablonski D, et al. Uncoupling of nonreceptor tyrosine kinases from PLC-gamma1 in an SLP-76-deficient T cell. Science. 1998; 281(5375):413–6. [PubMed: 9665884]
8. Andreotti AH, et al. T-cell signaling regulated by the Tec family kinase, Itk. Cold Spring Harb Perspect Biol. 2010; 2(7):a002287. [PubMed: 20519342]
9. Das J, et al. Digital signaling and hysteresis characterize ras activation in lymphoid cells. Cell. 2009; 136(2):337–51. [PubMed: 19167334]
10. Zehn D, et al. Complete but curtailed T-cell response to very low-affinity antigen. Nature. 2009; 458(7235):211–4. [PubMed: 19182777]
11. Davis MM, et al. Ligand recognition by alpha beta T cell receptors. Annu Rev Immunol. 1998; 16:523–44. [PubMed: 9597140]
12. McKeithan TW. Kinetic proofreading in T-cell receptor signal transduction. Proc Natl Acad Sci U S A. 1995; 92(11):5042–6. [PubMed: 7761445]
13. Lever M, et al. Phenotypic models of T cell activation. Nat Rev Immunol. 2014; 14(9):619–29. [PubMed: 25145757]
14. Lever M, et al. Architecture of a minimal signaling pathway explains the T-cell response to a 1 million-fold variation in antigen affinity and dose. Proc Natl Acad Sci U S A. 2016; 113(43):E6630–E6638. [PubMed: 27702900]
15. Choudhuri K, van der Merwe PA. Molecular mechanisms involved in T cell receptor triggering. Semin Immunol. 2007; 19(4):255–61. [PubMed: 17560121]
16. van der Merwe PA, Dushek O. Mechanisms for T cell receptor triggering. Nat Rev Immunol. 2011; 11(1):47–55. [PubMed: 21127503]
17. Malissen B, Bongrand P. Early T cell activation: integrating biochemical, structural, and biophysical cues. Annu Rev Immunol. 2015; 33:539–61. [PubMed: 25861978]
18. Stepanek O, et al. Coreceptor scanning by the T cell receptor provides a mechanism for T cell tolerance. Cell. 2014; 159(2):333–45. [PubMed: 25284152]
19. Shaw AS, et al. Short related sequences in the cytoplasmic domains of CD4 and CD8 mediate binding to the amino-terminal domain of the p56lck tyrosine protein kinase. Mol Cell Biol. 1990; 10(5):1853–62. [PubMed: 2109184]
20. Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature. 1987; 330(6145):256–9. [PubMed: 2823150]
21. Norment AM, et al. Cell-cell adhesion mediated by CD8 and MHC class I molecules. Nature. 1988; 336(6194):79–81. [PubMed: 3263576]
22. Manz BN, et al. Small molecule inhibition of Csk alters affinity recognition by T cells. Elife. 2015:4.
23. van Oers NS, et al. ZAP-70 is constitutively associated with tyrosine-phosphorylated TCR zeta in murine thymocytes and lymph node T cells. Immunity. 1994; 1(8):675–85. [PubMed: 7600293]

24. Mandl JN, et al. T cell-positive selection uses self-ligand binding strength to optimize repertoire recognition of foreign antigens. Immunity. 2013; 38(2):263–74. [PubMed: 23290521]
25. Thill PA, et al. Phosphorylation of a Tyrosine Residue on Zap70 by Lck and Its Subsequent Binding via an SH2 Domain May Be a Key Gatekeeper of T Cell Receptor Signaling In Vivo. Mol Cell Biol. 2016; 36(18):2396–402. [PubMed: 27354065]
26. Chakraborty AK, Weiss A. Insights into the initiation of TCR signaling. Nat Immunol. 2014; 15(9):798–807. [PubMed: 25137454]
27. Di Bartolo V, et al. Tyrosine 315 determines optimal recruitment of ZAP-70 to the T cell antigen receptor. Eur J Immunol. 2002; 32(2):568–75. [PubMed: 11828374]
28. Huang J, et al. A single peptide-major histocompatibility complex ligand triggers digital cytokine secretion in CD4(+) T cells. Immunity. 2013; 39(5):846–57. [PubMed: 24120362]
29. Valitutti S, et al. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature. 1995; 375(6527):148–51. [PubMed: 7753171]
30. Aleksic M, et al. Dependence of T cell antigen recognition on T cell receptor-peptide MHC confinement time. Immunity. 2010; 32(2):163–74. [PubMed: 20137987]
31. Govern CC, et al. Fast on-rates allow short dwell time ligands to activate T cells. Proc Natl Acad Sci U S A. 2010; 107(19):8724–9. [PubMed: 20421471]
32. Pielak RM, et al. Early T cell receptor signals globally modulate ligand:receptor affinities during antigen discrimination. Proc Natl Acad Sci U S A. 2017; 114(46):12190–12195. [PubMed: 29087297]
33. Hermiston ML, et al. CD45: a critical regulator of signaling thresholds in immune cells. Annu Rev Immunol. 2003; 21:107–37. [PubMed: 12414720]
34. Koretzky GA, et al. Tyrosine phosphatase CD45 is essential for coupling T-cell antigen receptor to the phosphatidyl inositol pathway. Nature. 1990; 346(6279):66–8. [PubMed: 2164155]
35. Pingel JT, Thomas ML. Evidence that the leukocyte-common antigen is required for antigen-induced T lymphocyte proliferation. Cell. 1989; 58(6):1055–65. [PubMed: 2550143]
36. Mustelin T, et al. Rapid activation of the T-cell tyrosine protein kinase pp56lck by the CD45 phosphotyrosine phosphatase. Proc Natl Acad Sci U S A. 1989; 86(16):6302–6. [PubMed: 2548204]
37. Ostergaard HL, et al. Expression of CD45 alters phosphorylation of the lck-encoded tyrosine protein kinase in murine lymphoma T-cell lines. Proc Natl Acad Sci U S A. 1989; 86(22):8959–63. [PubMed: 2530588]
38. Sieh M, et al. CD45 specifically modulates binding of Lck to a phosphopeptide encompassing the negative regulatory tyrosine of Lck. EMBO J. 1993; 12(1):315–21. [PubMed: 8428589]
39. van Oers NS, et al. alpha beta T cell development is abolished in mice lacking both Lck and Fyn protein tyrosine kinases. Immunity. 1996; 5(5):429–36. [PubMed: 8934570]
40. Anton van der Merwe P, et al. Cytoskeletal polarization and redistribution of cell-surface molecules during T cell antigen recognition. Semin Immunol. 2000; 12(1):5–21. [PubMed: 10723794]
41. Davis SJ, van der Merwe PA. The kinetic-segregation model: TCR triggering and beyond. Nat Immunol. 2006; 7(8):803–9. [PubMed: 16855606]
42. James JR, Vale RD. Biophysical mechanism of T-cell receptor triggering in a reconstituted system. Nature. 2012; 487(7405):64–9. [PubMed: 22763440]
43. Chang VT, et al. Initiation of T cell signaling by CD45 segregation at ‘close contacts’. Nat Immunol. 2016; 17(5):574–582. [PubMed: 26998761]
44. Huse M, et al. Spatial and temporal dynamics of T cell receptor signaling with a photoactivatable agonist. Immunity. 2007; 27(1):76–88. [PubMed: 17629516]
45. Hashimoto-Tane A, Saito T. Dynamic Regulation of TCR-Microclusters and the Microsynapse for T Cell Activation. Front Immunol. 2016; 7:255. [PubMed: 27446085]
46. Krummel MF, et al. Differential clustering of CD4 and CD3zeta during T cell recognition. Science. 2000; 289(5483):1349–52. [PubMed: 10958781]
47. Horejsi V. Lipid rafts and their roles in T-cell activation. Microbes Infect. 2005; 7(2):310–6. [PubMed: 15715974]

48. Filipp D, et al. Lck, Membrane Microdomains, and TCR Triggering Machinery: Defining the New Rules of Engagement. Front Immunol. 2012; 3:155. [PubMed: 22701458]
49. Garcia KC. Molecular interactions between extracellular components of the T-cell receptor signaling complex. Immunol Rev. 1999; 172:73–85. [PubMed: 10631938]
50. Reiser JB, et al. A T cell receptor CDR3beta loop undergoes conformational changes of unprecedented magnitude upon binding to a peptide/MHC class I complex. Immunity. 2002; 16(3): 345–54. [PubMed: 11911820]
51. Kuhns MS, Davis MM. TCR Signaling Emerges from the Sum of Many Parts. Front Immunol. 2012; 3:159. [PubMed: 22737151]
52. Wucherpfennig KW, et al. Structural biology of the T-cell receptor: insights into receptor assembly, ligand recognition, and initiation of signaling. Cold Spring Harb Perspect Biol. 2010; 2(4):a005140. [PubMed: 20452950]
53. Brazin KN, et al. Structural Features of the alphabetaTCR Mechanotransduction Apparatus That Promote pMHC Discrimination. Front Immunol. 2015; 6:441. [PubMed: 26388869]
54. Natarajan K, et al. An allosteric site in the T-cell receptor Cbeta domain plays a critical signalling role. Nat Commun. 2017; 8:15260. [PubMed: 28508865]
55. Natarajan A, et al. Structural Model of the Extracellular Assembly of the TCR-CD3 Complex. Cell Rep. 2016; 14(12):2833–45. [PubMed: 26997265]
56. Kim ST, et al. The alphabeta T cell receptor is an anisotropic mechanosensor. J Biol Chem. 2009; 284(45):31028–37. [PubMed: 19755427]
57. Ma Z, et al. The receptor deformation model of TCR triggering. FASEB J. 2008; 22(4):1002–8. [PubMed: 17984179]
58. Feng Y, et al. Mechanosensing drives acuity of alphabeta T-cell recognition. Proc Natl Acad Sci U S A. 2017
59. Das DK, et al. Force-dependent transition in the T-cell receptor beta-subunit allosterically regulates peptide discrimination and pMHC bond lifetime. Proc Natl Acad Sci U S A. 2015; 112(5):1517–22. [PubMed: 25605925]
60. Liu B, et al. Accumulation of dynamic catch bonds between TCR and agonist peptide-MHC triggers T cell signaling. Cell. 2014; 157(2):357–368. [PubMed: 24725404]
61. Lee MS, et al. A Mechanical Switch Couples T Cell Receptor Triggering to the Cytoplasmic Juxtamembrane Regions of CD3zetazeta. Immunity. 2015; 43(2):227–39. [PubMed: 26231119]
62. Trautmann A, Randriamampita C. Initiation of TCR signalling revisited. Trends Immunol. 2003; 24(8):425–8. [PubMed: 12909455]
63. Lo WL, Allen PM. Self-peptides in TCR repertoire selection and peripheral T cell function. Curr Top Microbiol Immunol. 2014; 373:49–67. [PubMed: 23612987]
64. Krogsgaard M, et al. Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. Nature. 2005; 434(7030):238–43. [PubMed: 15724150]
65. Irvine DJ, et al. Direct observation of ligand recognition by T cells. Nature. 2002; 419(6909):845–9. [PubMed: 12397360]
66. Aivazian D, Stern LJ. Phosphorylation of T cell receptor zeta is regulated by a lipid dependent folding transition. Nat Struct Biol. 2000; 7(11):1023–6. [PubMed: 11062556]
67. Xu C, et al. Regulation of T cell receptor activation by dynamic membrane binding of the CD3epsilon cytoplasmic tyrosine-based motif. Cell. 2008; 135(4):702–13. [PubMed: 19013279]
68. Reich Z, et al. Ligand-specific oligomerization of T-cell receptor molecules. Nature. 1997; 387(6633):617–20. [PubMed: 9177351]
69. Alam SM, et al. Qualitative and quantitative differences in T cell receptor binding of agonist and antagonist ligands. Immunity. 1999; 10(2):227–37. [PubMed: 10072075]
70. Tailor P, et al. The proline-rich sequence of CD3epsilon as an amplifier of low-avidity TCR signaling. J Immunol. 2008; 181(1):243–55. [PubMed: 18566390]
71. Mingueneau M, et al. The proline-rich sequence of CD3epsilon controls T cell antigen receptor expression on and signaling potency in preselection CD4+CD8+ thymocytes. Nat Immunol. 2008; 9(5):522–32. [PubMed: 18408722]

72. Liaunardy-Jopeace A, et al. Encoding optical control in LCK kinase to quantitatively investigate its activity in live cells. Nat Struct Mol Biol. 2017
73. Boggon TJ, Eck MJ. Structure and regulation of Src family kinases. Oncogene. 2004; 23(48): 7918–27. [PubMed: 15489910]
74. Nika K, et al. Constitutively active Lck kinase in T cells drives antigen receptor signal transduction. Immunity. 2010; 32(6):766–77. [PubMed: 20541955]
75. Ballek O, et al. The pool of preactivated Lck in the initiation of T-cell signaling: a critical re-evaluation of the Lck standby model. Immunol Cell Biol. 2015; 93(4):384–95. [PubMed: 25420722]
76. Stirnweiss A, et al. T cell activation results in conformational changes in the Src family kinase Lck to induce its activation. Sci Signal. 2013; 6(263):ra13. [PubMed: 23423439]
77. Philipsen L, et al. De novo phosphorylation and conformational opening of the tyrosine kinase Lck act in concert to initiate T cell receptor signaling. Sci Signal. 2017; 10(462)
78. Zikherman J, et al. CD45-Csk phosphatase-kinase titration uncouples basal and inducible T cell receptor signaling during thymic development. Immunity. 2010; 32(3):342–54. [PubMed: 20346773]
79. Sjolin-Goodfellow H, et al. The catalytic activity of the kinase ZAP-70 mediates basal signaling and negative feedback of the T cell receptor pathway. Sci Signal. 2015; 8(377):ra49. [PubMed: 25990959]
80. Courtney AH, et al. A Phosphosite within the SH2 Domain of Lck Regulates Its Activation by CD45. Mol Cell. 2017; 67(3):498–511. e6. [PubMed: 28735895]
81. Jin LL, et al. Tyrosine phosphorylation of the Lyn Src homology 2 (SH2) domain modulates its binding affinity and specificity. Mol Cell Proteomics. 2015; 14(3):695–706. [PubMed: 25587033]
82. Simeoni L, et al. Adaptors and linkers in T and B cells. Curr Opin Immunol. 2004; 16(3):304–13. [PubMed: 15134779]
83. Davidson D, et al. The Csk-Associated Adaptor PAG Inhibits Effector T Cell Activation in Cooperation with Phosphatase PTPN22 and Dok Adaptors. Cell Rep. 2016; 17(10):2776–2788. [PubMed: 27926878]
84. Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. Cell. 1994; 76(2): 263–74. [PubMed: 8293463]
85. Smith-Garvin JE, et al. T cell activation. Annu Rev Immunol. 2009; 27:591–619. [PubMed: 19132916]
86. Mukherjee S, et al. Monovalent and multivalent ligation of the B cell receptor exhibit differential dependence upon Syk and Src family kinases. Sci Signal. 2013; 6(256):ra1. [PubMed: 23281368]
87. Yan Q, et al. Structural basis for activation of ZAP-70 by phosphorylation of the SH2-kinase linker. Mol Cell Biol. 2013; 33(11):2188–201. [PubMed: 23530057]
88. Shah NH, et al. An electrostatic selection mechanism controls sequential kinase signaling downstream of the T cell receptor. Elife. 2016:5.
89. Katz ZB, et al. A cycle of Zap70 kinase activation and release from the TCR amplifies and disperses antigenic stimuli. Nat Immunol. 2017; 18(1):86–95. [PubMed: 27869819]
90. Su X, et al. Phase separation of signaling molecules promotes T cell receptor signal transduction. Science. 2016; 352(6285):595–9. [PubMed: 27056844]
91. Huang WY, et al. Phosphotyrosine-mediated LAT assembly on membranes drives kinetic bifurcation in recruitment dynamics of the Ras activator SOS. Proc Natl Acad Sci U S A. 2016; 113(29):8218–23. [PubMed: 27370798]
92. Raab M, et al. LFA-1 activates focal adhesion kinases FAK1/PYK2 to generate LAT-GRB2-SKAP1 complexes that terminate T-cell conjugate formation. Nat Commun. 2017; 8:16001. [PubMed: 28699640]
93. Barrett DM, et al. Chimeric antigen receptor therapy for cancer. Annu Rev Med. 2014; 65:333–47. [PubMed: 24274181]
94. Marchingo JM, et al. T cell signaling. Antigen affinity, costimulation, and cytokine inputs sum linearly to amplify T cell expansion. Science. 2014; 346(6213):1123–7. [PubMed: 25430770]

95. Au-Yeung BB, et al. IL-2 Modulates the TCR Signaling Threshold for CD8 but Not CD4 T Cell Proliferation on a Single-Cell Level. J Immunol. 2017; 198(6):2445–2456. [PubMed: 28159902]

96. Garcia KC, et al. An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. Science. 1996; 274(5285):209–19. [PubMed: 8824178]

97. Garcia KC, et al. Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen. Science. 1998; 279(5354):1166–72. [PubMed: 9469799]

98. Rudolph MG, et al. How TCRs bind MHCs, peptides, and coreceptors. Annu Rev Immunol. 2006; 24:419–66. [PubMed: 16551255]

TRENDS

The interface of a T cell and APC is thought to exert force upon the TCR and increasing evidence indicates the TCR acts is mechanosensitive. During antigen detection, mechanical force may be important for antigen discrimination.

Lck is basally active and phosphorylates the TCR complex and Zap70 upon pMHC binding. Active Lck abundance and its coupling to the TCR during antigen encounter is able to influence affinity discrimination.

A hallmark of TCR signaling is its sensitivity and specificity. How the strict TCR signaling hierarchy is enforced is continuing to emerge through structural insight into the substrate selectivity of Lck and Zap70 and how the activation of Zap70 is controlled.

The emerging properties of the LAT signalosome include its assembly into liquid-like phase separated clusters that contain phospho-LAT and its binding partners which can enhance actin polymerization. These emerging properties reveal how LAT can function as a foci of TCR signaling.

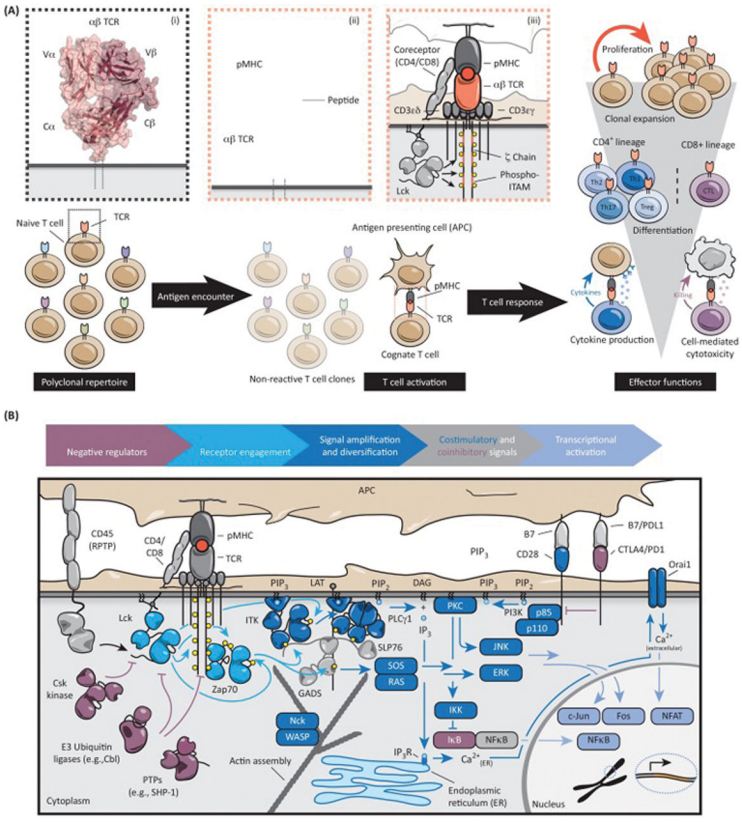

FIGURE 1. An overview of antigen recognition by the TCR

(**A**) In humans, the TCR is comprised of predominately αβ heterodimers (but also γδ chains) that assemble into the TCR (i. PDB 1TCR) [96]. Crucial for the ability of the adaptive immune system to respond to novel antigens, each T cell possesses a TCR with unique pMHC binding properties (ii. PDB 2AK4). In cells the αβ TCR is associated with the CD3εγ, CD3εδ, and a ζ chain homodimer (ζζ) in a 1:1:1:1 stoichiometry (iii.) [97, 98]. Binding of the TCR to antigen causes T cell activation and leads to the initiation of a T cell response. Upon activation, a naive T cell undergoes expansion and differentiation into effector subsets. These effector subsets carry out specialized functions that are responsible for coordinating the adaptive immune response. (**B**) When TCR:pMHC binding occurs it is communicated across the T cell plasma membrane when the TCR complex is phosphorylated by the kinase Lck. Phosphorylated signaling motifs within the TCR complex recruit the Zap70 kinase. Recruitment of Zap70, and its phosphorylation by Lck, causes its activation. Zap70 then phosphorylates the adaptor protein LAT which recruits additional signaling effectors that become activated (BOX 1). In this way, the binding of the TCR to pMHC antigen is converted into a biochemical signal. A TCR signal causes global cellular changes within the T cell ranging from the activation of transcriptional regulators and protein synthesis to the reorganization of the cytoskeleton and altered metabolism and are necessary for a naive T cell to undergo clonal expansion and differentiation into effector subsets.

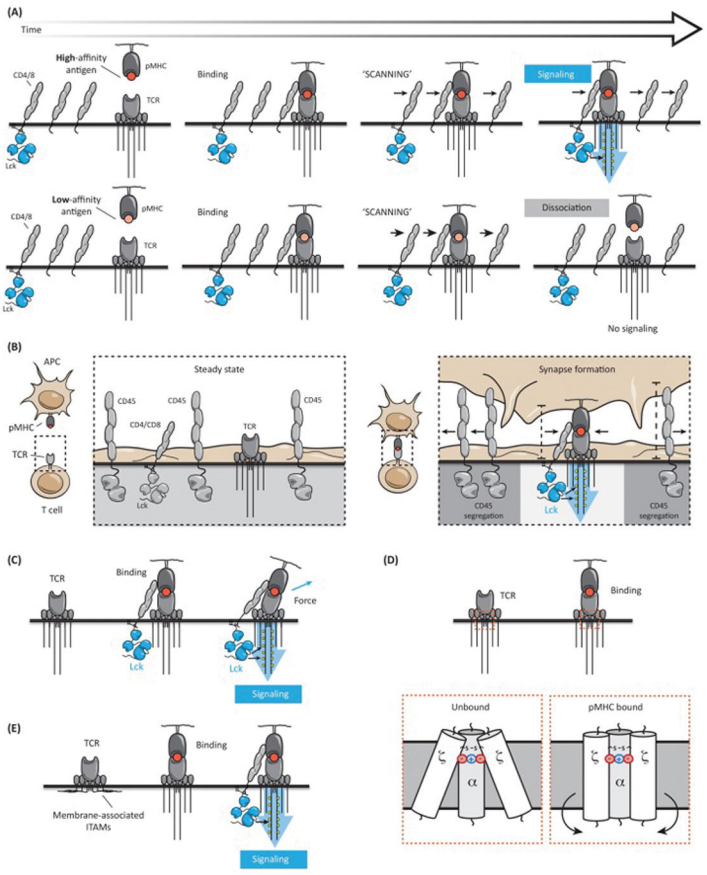

FIGURE 2. Models for the initiation of TCR signaling

(**A**) A 'coreceptor scanning' model for kinetic proofreading proposes that T cell activation occurs when the TCR complex is phosphorylated by recruited Lck prior to TCR:pMHC dissociation. Dissociation of the TCR:pMHC prior to achieving an activation threshold disrupts signaling and the accumulated signaling events are reversed. (**B**) Kinetic segregation proposes that the kinase Lck randomly encounters the TCR complex and phosphorylates it. These phosphorylation events are counteracted by the activities of protein tyrosine phosphatases, such as CD45. To initiate TCR signaling, the steady state balance between Lck and CD45 is disrupted by TCR:pMHC binding which causes the segregation of signaling components within the T cell plasma membrane. (**C**) Mechanical force has been observed to potentiate TCR signaling and has been proposed to alter the conformation of the TCR to initiate TCR signaling. (**D**) In situ proximity reporters have revealed that the transmembrane domain of the ζ chain homodimer can undergo a conformational change upon pMHC binding. (**E**) The signaling motifs of the TCR complex can associate with acidic phospholipids within the inner leaflet of the plasma membrane suggesting a mechanism for their release and phosphorylation upon pMHC binding.

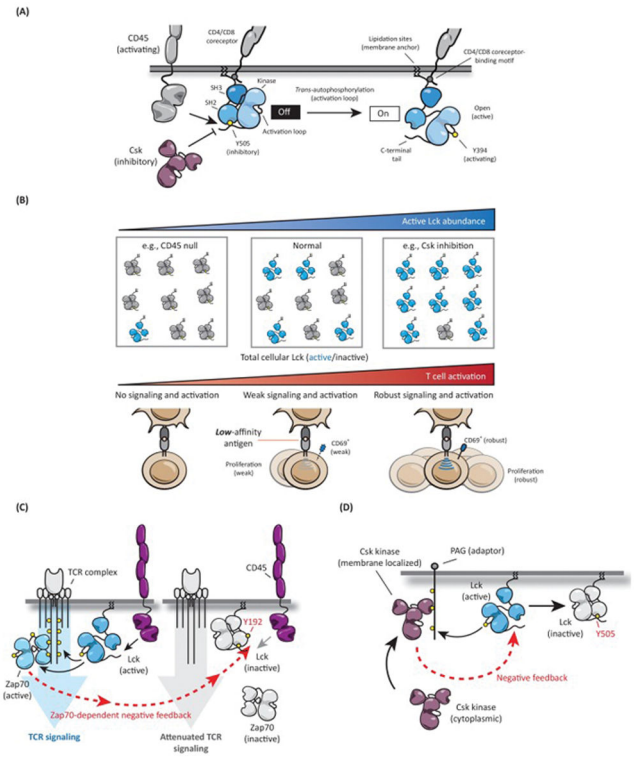

FIGURE 3. Regulation of Lck and negative feedback pathways

(**A**) Lck is a Src family kinase (SFK) and is localized to the inner leaflet of the plasma membrane through lipidation of its N-terminus where it associates with the cytoplasmic domain of the CD4/CD8 coreceptors. Lck possess a catalytic kinase domain, two regulatory domains, and several sites of phosphorylation. The activity of the catalytic kinase domain is controlled by regulating its conformation. Conformational control is achieved through phosphorylation of Lck regulatory sites. The C-terminal tail of Lck, when phosphorylated, is bound by the SH2 regulatory domain through an intramolecular interaction to stabilize the inactive autoinhibited conformation. In contrast, the active conformation of Lck is stabilized by *trans*-autophosphorylation of the activation loop within the kinase domain. (**B**) Recent studies have indicated that a pool of Lck is basally active in resting T cells. Increasing the abundance of active Lck molecules can alter affinity discrimination and potentiate T cell activation in response to low affinity ligands. (**C**) Inhibitory feedback pathways are proposed to regulate the abundance of active Lck molecules in a T cell. Phosphorylation of Y192 within the SH2 domain of Lck is proposed to disrupt the activation of Lck by CD45 and therefore reduce the abundance of active Lck and TCR sensitivity. (**D**) Because the inhibitory kinase Csk is cytoplasmic, recruitment of Csk to the plasma membrane through the Lckdependent phosphorylation of inhibitory adaptor proteins (e.g., PAG) can decrease active Lck abundance.

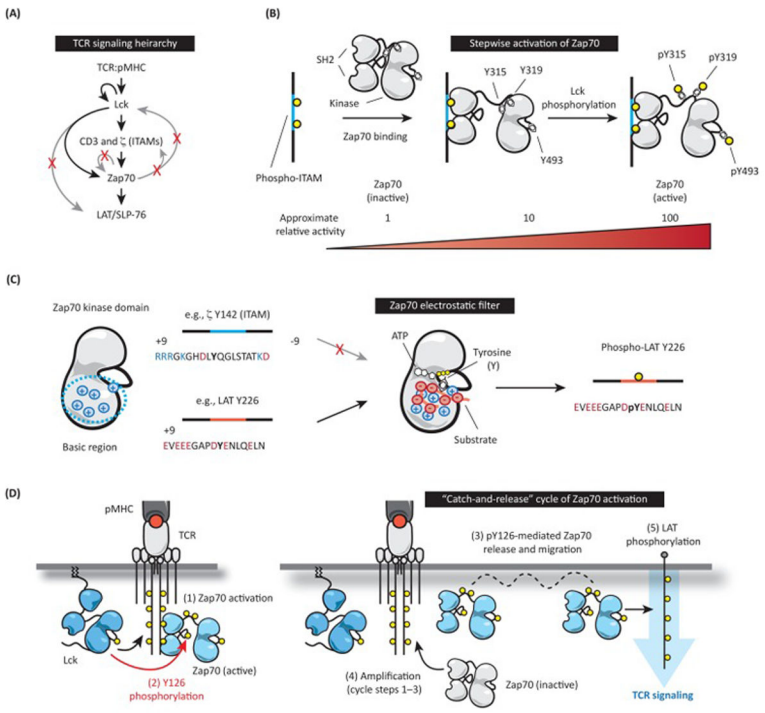

FIGURE 4. Zap70 activation and propagation of TCR signaling

(**A**) Lck and Zap70 exist in a strict signaling hierarchy. (**B**) Zap70 undergoes stepwise activation which requires its recruitment to phosphorylated ITAMs within the TCR complex and its phosphorylation by Lck. (**C**) Zap70 contains a highly basic region that is important for its rigid substrate specificity. The basic region of Zap70 acts as an electrostatic filter to ensure specificity for phosphosites within LAT and SLP-76 and prevents phosphorylation of ITAMs and the Zap70 activation loop. (**D**) Phosphorylation of Zap70 Y126 is proposed to cause its release from the TCR complex such that it can migrate and encounter LAT to phosphorylate it. Meanwhile, additional Zap70 molecules are able to bind the TCR complex and become activated suggesting a mode of TCR signal amplification.

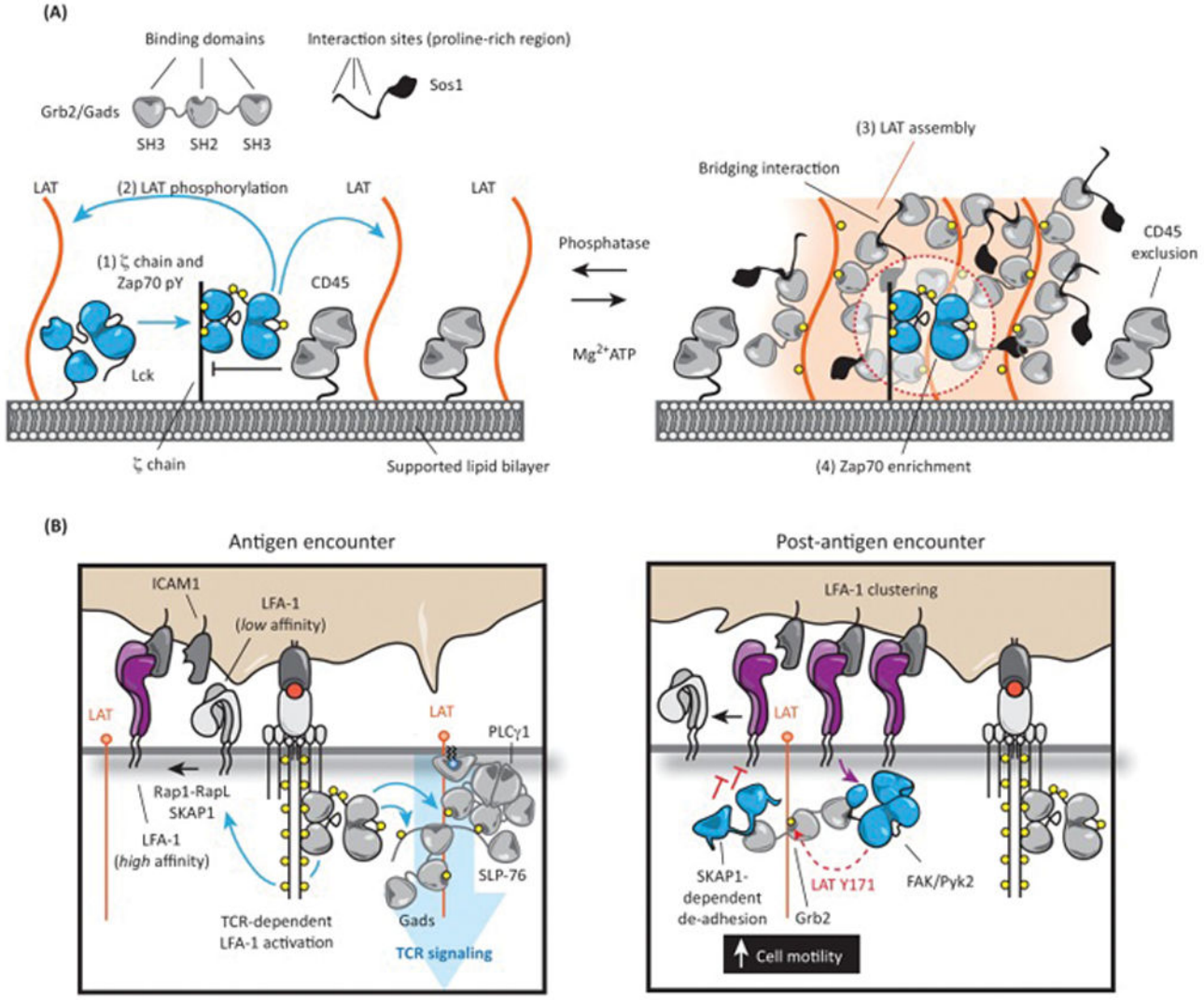

FIGURE 5. Assembly of the LAT signalosome

(**A**) In vitro reconstitution studies have revealed that multivalent interactions between adaptors such as Grb2/Gads and SOS1 molecules can bridge LAT adaptors to assemble liquid-like phase separated clusters. These clusters are dynamic and can be reversed through dephosphorylation by protein tyrosine phosphatases. Interestingly, these LAT clusters have been observed to enrich Zap70 and exclude the phosphatase CD45. (**B**) The assembly of distinct LAT signalosomes has been observed to occur upon crosslinking of the LFA-1 integrin. These LAT signalosomes are formed through the activation of FAK kinase which phosphorylates only LAT Y171 which recruits a Grb2/SKAP1 complex. These LAT-Grb2-SKAP1 signalosomes are reported to mediate de-adhesion of LFA-1 and regulate cell motility.