The TCR signalosome: a dynamic structure with expanding complexity
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Signal transduction in T cells is a dynamic process involving a large number of membrane and cytosolic proteins. The TCR macromolecular complex (signalosome) is initiated by receptor occupancy and becomes more elaborate over time. This review describes how ‘vertical’ displacement mechanisms and lateral coalescence of lipid-raft-associated scaffold proteins combine to form distinct signalosomes, which control signal specificity.

Introduction

The detection of pathogenic antigens depends on their presentation by MHC molecules and their recognition by specific TCRs. The αβ TCR is composed of a Fab-like clonotypic αβ heterodimer, which is noncovalently associated to an invariant, multimeric CD3 complex comprising a ζζ chain, phospholipase Cγ-1 (PLCγ-1) a protein kinase A (PKA), peptide–MHC (pMHC), tyrosine-phosphorylated 21 kDa CD3ζ isoform (pp21ζ), tyrosine-phosphorylated 23 kDa isoform (pp23ζ), tyrosine-phosphorylated 21 kDa CD3ζ isoform (pp21ζ), and the 23 kDa isoform (pp23ζ), which ultimately modulate T cell biology.

The formation of specific signalosomes is essential for quantitative and qualitative control of TCR signaling. The TCR signalosome is composed of a variety of proteins with different signaling functions including co-receptors, kinases, phosphatases and adaptors such as CD8, Lck, the Src homology 2 (SH2)-containing tyrosine phosphatase 1 (SHP-1) and the linker for activation of T cells (LAT), respectively [4,7,8•]. This macromolecular complex connects events on the plasma membrane to distal signaling cascades, which ultimately modulate T cell biology.

This review documents the recent advances made in understanding the molecular mechanisms of TCR triggering. We discuss how ‘vertical’ signals elicited by receptor engagement and lateral amplification by lipid raft coalescence combine to form the TCR signalosome. It should be stressed that the signalosomes discussed in this review are important elements of the immunological synapse [3].

Structural features and mechanisms initiating TCR triggering

Binding of ligands to the TCR triggers the activation of various receptor-associated protein tyrosine kinases (PTKs) of the Src family, such as Lck and Fyn, leading to the rapid tyrosine phosphorylation of numerous proteins [9]. The phosphorylation of the conserved immunoreceptor tyrosine-based activation motifs (ITAMs), which are located in the cytoplasmic tails of each CD3 ζ, ε, γ and ζ chain, generates binding sites for proteins bearing SH2 domains such as the cytosolic PTK, ζ-associated protein 70 (ZAP-70) [3,4,9]. Recruitment of ZAP-70 to phosphorylated ITAMs of CD3ζ allows enhanced activation of that kinase, which in turn phosphorylates components of distinct downstream signaling pathways [10•,11•,12].

The sequential phosphorylation of the three ITAMs located in each CD3ζ chain initiates the formation of various phospho-CD3ζ polypeptides with distinct molecular masses [13,14]. Agonist stimulation forms the tyrosine-phosphorylated 21 kDa CD3ζ isoform (pp21ζ) and the 23 kDa isoform (pp23ζ), whereas only pp21ζ is observed upon partial agonist binding [13,15•]. Thus, it has been argued that specific and sequential phosphorylation of CD3ζ ITAMs may function as a molecular ‘sensor’, which ‘detects’ the...
binding of different pMHC ligands to the TCR and transduces the signal to the intracellular compartment \[13,16\].

Interestingly, several recent reports have shown that TCR triggering and T cell activation can occur normally in the absence of CD3ζ ITAMs \[17\] or without the specific formation of pp21ζ and pp23ζ \[18,19**\]. It is possible that the various ITAMs on the different CD3 polypeptides are redundant \[20\] and that signal transduction is dependent on the phosphorylation of only a minimal number of ITAMs \[19**\]. Additional CD3 phosphorylation may then modulate signaling kinetics by recruiting dispensable components, which activate synergistic or inhibitory pathways. In this regard, it has been reported that prolonged stimulation of T lymphocytes with a partial agonist can trigger a similar regard, it has been reported that prolonged stimulation of T cell development \[24**\], suggesting that the extracellular fragment of the CD3εγζ heterodimer \[21**\].

**Vertical displacement at the TCR and CD3 interface**

Whereas a great deal of research has been undertaken to understand the role of CD3 ITAM phosphorylation in controlling TCR signaling, other domains of the CD3 polypeptides have been less well studied. A recent publication describes the solution structure of an ectodomain fragment of the CD3εγζ heterodimer \[21**\]. It appears that the extracellular immunoglobulin-like domains of CD3ε and γ form a stable and rigid heterodimeric complex. Because of the rigid ‘rod-like’ shapes of the TCR β chain \[1,22\] and the CD3εγζ heterodimer, the authors speculate that TCR triggering involves a piston-type displacement mechanism of the TCR and CD3 modules \[21**\]. This movement may induce a distortion of the CD3εγζ transmembrane helices, which may allow the recruitment of other intracellular components. A similar model of intrasubunit piston-type motion, where one transmembrane helix moves downward relative to the other, has been proposed to trigger signaling of the bacterial aspartate receptor \[23\]. Although the existence of such a signal transduction mechanism has yet to be established, it is an attractive model, in which a vertical displacement may precede CD3 phosphorylation.

Given the structural similarities between CD3γε and CD3δ, a comparable mechanism could be relevant for the CD3δεζ heterodimer, as well. In this regard it is noteworthy that the expression of a tail-less mutant of CD3δ in a CD3δεζ− background rescued the block in positive selection during T cell development \[24**\], suggesting that the extracellular and/or transmembrane domains of CD3δ bestow the TCR with unique features for signal transduction.

**Lateral amplification by TCR oligomerization**

Although dimerization triggers the activation of numerous receptors that are endowed with intrinsic tyrosine kinase activity \[25\], the question of whether TCR oligomerization regulates signal transduction remains \[26\]. The study of TCR–pMHC ectodomain complexes in solution failed to provide significant evidence for oligomerization \[27\]. Perhaps, as suggested by Baker and Wiley \[27\], membrane-anchored, fully assembled TCR–CD3 complexes should be used to test this hypothesis. The membrane-proximal stalk motif of CD3δ, ε and γ \(\text{R}x\text{CxxCxE}\), using single-letter code for amino acids, with x representing any amino acid), which stabilizes heterodimer pairing, also facilitates the insertion of the TCR–CD3εδ heterodimers into the lipid bilayer and hence into glycolipid-enriched rafts \[21**\]. It is conceivable that lipid rafts regulate receptor oligomerization by facilitating lateral movements of the TCR signalosome, bringing several receptors into close proximity \[28\].

A similar mechanism is believed to induce heterodimerization of the TCR with the CD8 or CD4 co-receptors upon binding to the same pMHC ligand \[28,29*,30\]. Several independent reports have recently documented that CD4 as well as CD8 can partition into glycolipid-enriched microdomains \[31,32*\]. Coalescence of lipid rafts may influence the interaction between the TCR–CD3 and CD4 or CD8 and hence drive their heterodimerization. It remains controversial whether TCR–CD3–co-receptor signalosomes are constitutive or induced upon ligand binding. New evidence obtained using the fluorescence resonance energy transfer technique showed that CD3δ is brought into close proximity of CD8 independently of observable phosphorylation events, suggesting that TCR–co-receptor heterodimerization may occur prior to the activation of PTKs \[33\].

**Lateral amplification by signalosome expansion**

Once triggered by ligand binding to the TCR, raft-associated CD3γε and CD3δεζ heterodimers may then promote the lateral reorganization of distinct signalosomes \[7,8*,34\]. We have shown that the mutation of an essential motif within the TCR’s α-chain connecting peptide domain (α-CPM) blocks the transduction of signals controlling positive, but not negative, selection \[35**\]. In response to low-affinity ligands, which trigger positive selection in wild-type animals, the mutation prevents the organization of a proximal signalosome, which involves CD3δ \[35**\]. Interestingly, even though CD3γε was expressed and associated with the α-CPM-mutant TCR it failed to functionally replace CD3δ, suggesting that CD3γε and CD3δ polypeptides control the formation of distinct signalosomes. As the targeted disruption of CD3δ also abrogates the transduction of signals required for positive selection \[24**\], it may be inferred that CD3δ plays a unique role in transducing signals controlling this step in T cell development.

TCR triggering induces the relocalization of distinct signalosomes composed of proximal and intermediary
signaling components to receptor-associated scaffolds [5–7, 24••, 35••, 36]. Some molecules such as ZAP-70 can amplify the signal by specifically phosphorylating downstream components such as LAT [37•] or phospholipase Cγ-1 (PLCγ-1) [38, 39]. In contrast, the PTK, Csk — which relocates to rafts by docking to the transmembrane adaptor, Cbp (also known as PAG) [40••, 41•–43•] — and the phosphatase SHP-1 [44, 45] limit signals generated by TCR engagement. Importantly, proximal signal amplifier or repressor molecules have a common feature: they partition...
with glycolipid-enriched rafts. Palmitoylation constitutively embeds several components such as Lck and LAT into these lipid micro-domains, whereas others such as ZAP-70 relocalize into rafts upon TCR engagement [5,6,35*,36].

The mechanism by which binding of distinct ligands to the TCR-co-receptor induces the formation of specific proximal signalosomes remains elusive. Recent findings place several protein adaptors [46], and LAT in particular [47], as central switches in translating the quality, quantity and duration of signals into the correct activation of specific downstream pathways.

LAT contains nine tyrosine residues, which when phosphorylated may serve as docking domains for numerous signaling components bearing SH2 motifs such as PLCγ-1 and the adaptors Grb2 and Gads [37*,48*,49*,50**]. It is noteworthy that over-expression of a soluble LAT mutant markedly decreases T cell activation by competing with endogenous membrane localized LAT [51], suggesting that phosphorylated LAT recruits various downstream signaling effectors to membrane signalosomes. For example, LAT interacts with Gads, which is associated with SLP-76, and together they form a macromolecular scaffold, which stabilizes the interaction of PLCγ-1 with the TCR signalosome [39,48*,49*,52*,53*]. In this way the signalosome expands in molecular complexity and amplifies the TCR initiated signal. A physical interaction of LAT with the intracellular tail of CD8β could be observed [54], underlying LAT's role as a TCR-signalosome-associated scaffold molecule. Remarkably, LAT can also bind proteins that negatively regulate TCR signaling. It has been reported that endogenous SHP-1 is found to associate with LAT upon TCR stimulation [44] and may prevent further phosphorylation of the adaptor by ZAP-70, suggesting a potential conversion from an 'activating' to an 'inhibiting' signalosome.

In summary, recent observations documenting the initial events that trigger TCR signaling upon binding of pMHC ligands lead to the following tentative mechanisms (Figure 1). It has been suggested that a vertical piston-like displacement between the TCR and CD3 heterodimers exposes protein-docking sites on CD3 chains and induces the lateral movement of lipid signalosomes [21*]. Concomitant binding of CD4 or CD8 to the same ligand molecule bind the TCR amplifies raft coalescence [32*,33] and brings Lck and other co-receptor-interacting components such as LAT to the TCR signalosome [29*,30,36,54]. Mobilization of the tyrosine phosphatase, CD45, would bring about Lck activation and subsequent phosphorylation of the CD3 complex [8*,32*,55] and the activation of ZAP-70 [10*,11*]. This is followed by the docking of SH2-containing proteins to the phosphorylated tyrosine residues and the expansion of signalosomes in which scaffolds [46], such as LAT, recruit distinct components and hence control the activation of specific signaling pathways [24*,35*,39,47,48*,49*,50*,52*,53*]. The downregulation of the signal is controlled in part by the activation and directed relocalization of SHP-1 [44,45] and Csk [40*,41*–43*] to the TCR signalosome. In the TCR signalosome, Csk was found to be phosphorylated by co-localized cAMP-dependent protein kinase A (PKA) [43*]. Activated Csk terminates the signal by phosphorylating Lck and Fyn on negative regulatory tyrosine residues.

Some of these events may be redundant, whereas others are absolutely required [10*,11*,19*,20,24*,35*,36,50*,52*,53*]. In the light of these putative mechanisms, it still remains to be unraveled whether different pMHC ligands induce distinct signaling cascades. On the basis of several recent reports one can speculate that high and low affinity ligands induce similar proximal signalosomes, but the intensity and duration of the signals produced by these ligands vary [15*,35**]. High affinity ligands trigger signals as a result in fast responses due to rapid formation of signalosomes. The barely detectable responses induced by low affinity ligands may be due to minimal signalosome coalescence, which occurs with delayed kinetics. However, measured over a longer time-span, these responses can be as powerful as the ones elicited by strong agonists [15**]. Although it can be argued that distinct ligands induce different conformational changes of the TCR, similar receptor structures have been reported when a TCR is bound to an agonist or an antagonist ligand [1,2]. Thus, rather than inducing distinct structural modifications at the pMHC-TCR interface, kinetic differences in the formation of proximal signalosomes may provide a direct means by which the TCR discriminates the affinity of a specific ligand.

**Conclusions**

The cloning of ubiquitous and T cell specific signaling molecules has burgeoned in the last 20 years. More recently, the focus has been to characterize the intricate connections between these molecules, to elaborate the signaling networks and to decipher the mechanisms whereby distinct cytosolic components are associated with transmembrane receptors. A real breakthrough was the discovery that lateral compartmentalization of the plasma membrane bilayer into sphingolipid/cholesterol-enriched micro-domains provides a dynamic mechanism that regulates signal transduction.

Because of the observation that numerous proteins associate with lipid rafts upon receptor triggering, signal transduction can no longer be viewed as a linear process (vertical transduction). With the integration of vertical and lateral signaling events, three-dimensional mechanisms have to be considered. A future leap will be to add the kinetic dimension. Indeed, whereas a great body of evidence has been generated that establishes how TCR signaling is transduced via the formation of signalosomes, the kinetic aspects of these processes remain largely unknown. This will certainly be a focus of future research.

One would also like to know whether the binding of different ligands to the TCR-co-receptor triggers the formation of structurally distinct signalosomes, which results
in specific cellular responses. Alternatively it may be the kinetics of signalosome expansion and not the organization of distinct signalosomes that control the specificity of a T cell response towards a particular ligand.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest

8. A very interesting review, which discusses various aspects of lipid raft formation and their particular role in the transduction of immunoreceptor-triggered signals.
Together with [11*], this paper reports that distinct tyrosine residues of ZAP-70 control the activation of this kinase and hence thymic selection. It was shown that phosphorylation of Tyr 315 and Tyr 319 augment the activity of ZAP-70 and T cell responses, whereas phosphorylation of Tyr 292 attenuates TCR signaling. In addition, TCR-triggered phosphorylation of Tyr 292 and Tyr 315 does not occur in docking sites for Cbl and Vav, two proteins that had been suggested to regulate the function of ZAP-70.
See annotation to [10*].
By analyzing the kinetics of T cell activation triggered by high- and low-affinity ligands, this paper shows that although both ligands induce similar T cell responses, the one mediated by the low-affinity ligand was delayed by several hours. Whereas the low-affinity ligand only induced a very faint pp21C increase and no measurable downstream signaling events, the high-affinity ligand induced a tremendous increase in pp21C and pp23C, as well as transient Cal** mobilization and ERK phosphorylation. The authors propose a revised kinetic proofreading/discrimination model, in which signals ‘trickle through’ the early activation cascade and accumulate to induce later T cell responses.
Using mass-spectrometry, and transgenic mice expressing distinctly mutated CD3C ITAMs obtained by site-directed mutagenesis, this paper describes a molecular mechanism for the formation of pp21C and pp23C isoforms. The pp21C form is generated by phosphorylation of four tyrosines located in the second and third ITAMs, whereas pp23C is formed by full phosphorylation of all three ITAMs. Wherever the stable formation of both isoforms requires the binding of ZAP-70, TCR-triggered signaling can occur without the generation of pp21C and pp23C isoforms.
This paper describes a biochemical and crystallographic analysis of a heterodimeric CD3Cyc ectodomain. The mutation of the linker domains as well as the membrane stalk motif provides evidence as to why noncovalently linked CD3Cγ and CD3Cδ homodimers as well as CD3Cγδ heterodimers cannot exist. More importantly the authors suggest that the rigidity of CD3C polypeptides may modulate TCR-based signal transduction via a vertical piston-type mechanism.
This paper, together with [35*], describes the formation of a distinct TCR signalosome, which controls positive selection specifically via the activation of the ERK signaling module. The generation of this signalosome is abrogated in CD3δ deficient thymocytes, whereas the expression of a tailless CD3 δ rescues ERK activation and positive selection, implying that the extracellular and/or transmembrane domains of CD3δ bestow the TCR with unique features for signal transduction.
Lymphocyte activation and effector functions


This paper reports that membrane-bound CD8ββ, but not CD8αα or soluble CD8β, substantially increases mP4C lipid binding to the TCR, and T cell activation. This effect is mediated by the interaction of the cytoplasmic tail of CD8β and CD3, which drives the relocation of TCR–CD3 to lipid rafts that contain CD8. The formation of the CD8–TCR–CD3 signalosome induces the activation of CD8-associated Lck, which in turn phosphorylates the CD3-ITAMs.


This paper describes that TCR engagement of Th1 or Th2 cells induces distinct patterns of lipid compartmentalization into rafts, which may specifically control their different functional roles. The formation of raft-associated TCR signalosomes, which control Ca2+ mobilization, has been observed in Th1 but not in Th2 cells. CD4 is believed to regulate in part TCR recruitment to rafts as well as TCR and CD8 signaling aggregation.


In this paper it is shown that a mutation of a particular domain of the TCR α-chain, the α-C'PM, profoundly affects positive, but not negative, selection by specifically impairing ERK but not JNK or p38 activation. This mutant TCR failed to interact with CD3δ and failed to trigger the formation of a distinct TCR signalosome, which is similar to the results described in [24**]. Interestingly, in thymocytes expressing a wild-type TCR, kinetics of ERK activation, might also be regulated.


Using site-directed mutagenesis, this paper, together with [48-49*••••], reports that the phosphorylation of distinct tyrosine residues of LAT controls the recruitment and activation of PLC-γ1, Vav, p85α/110 PI3K, Grb2 and Gads. The recruitment of specific components controls distal signaling events such as ERK activation and Ca2+ mobilization.


This paper, together with [41-43*••••], describes the cloning and characterization of the C-terminus binding protein, Cbp, as well as the mechanism by which this scaffold protein modulates the activity of the PTK, Csk. Cbp is a transmembrane protein, which is tyrosine-phosphorylated and specifically interacts with the SH2-domains of Csk. This interaction constitutively localizes Csk to rafts in resting cells. TCR triggering induces the dephosphorylation of Cbp and the dissociation of Csk, which becomes cytoplasmic. Rephosphorylation of Cbp restores raft association of Csk, which relocalizes to the TCR signalosome and limits the transduction of the TCR-based signal by negatively regulating the activities of Src-PTK. These papers present evidence on how a transmembrane scaffold might regulate an enzyme by mediating its subcellular localization.


See annotation to [40*].


See annotation to [40*].


This paper shows that cAMP-dependent PKA, which is located in the TCR signalosome, phosphorylates Csk on Ser364, leading to an increase in the enzymatic activity of the PKA. This is a newly described mechanism by which cAMP downregulates T cell activation through Csk.


See annotation to [37*].


This paper shows that tyrosine development requires the four distinct tyrosine residues of LAT. Using a knock-in strategy, it was found that the mutation of Tyr136, Tyr175, Tyr195 and Tyr235 arrested tyrosine maturation at the C4-D4-C8- stage. This implies that the recruitment of PLC-γ1, Grb2 and Gads, which probably leads to the activation of the ERK cascade, is essential for pre-TCR signaling and T cell development.


This is a very elegant paper, which describes the role of specific domains of SLP-76 in mediating signals required for thymopoiesis and T cell activation. Interestingly, the expression of various SLP-76 mutants on a SLP-76 null
background showed a differential requirement for the adaptor in the regulation of T cell development and function. The mutation of the three tyrosine residues abrogates thymopoiesis, whereas a mutation of the SH2 domain leads to significant defects in mature T cell function. Mutation of the proline-rich domain inhibits the interaction with Gads and in agreement with (53•) suggests its requirement for optimal signaling in T cell development and function. These findings suggest that the different protein-docking domains of SLP-76 endow this adaptor with a unique role in regulating the composition of signalosomes.


This paper documents the phenotype of a Gads-deficient mouse strain generated by targeted gene disruption. Even though the authors observed severe defects in selection and proliferation of thymocytes, thymopoiesis is normal in Gads-deficient animals and mature T cells populate the periphery. The signaling defects observed in thymocytes and mature T cells may be due to the uncoupling of SLP-76 and LAT, which is mediated by Gads in wild-type mice.
