Review
Dynamic regulation of T cell activation and co-stimulation through TCR-microclusters

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Abstract

TCR-microclusters (MC) are generated upon TCR stimulation prior to the immune synapse formation independently of lipid rafts. TCR-MCs contain receptors, kinases and adaptors, and function as the signaling unit for T cell activation. The TCR complex, but not the signaling molecules, is transported to the center to form cSMAC. The co-stimulation receptor CD28 joins the signaling region of cSMAC and recruits PKCθ and Carma1. CTLA-4 accumulates in the same region and competes with CD28 for negative regulation of T cell activation. T cell activation is therefore mediated by two spatially distinct signaling compartments: TCR signaling by the peripheral TCR-MC and co-stimulation signal by the central signaling cSMAC.

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1. Introduction

T cells recognize antigen (Ag) as a peptide–MHC complex on Ag-presenting cells (APC) such as dendritic cells (DC) through direct cell–cell interactions. The T cell antigen receptor (TCR) binds to the Ag peptide–MHC complex and triggers T cell activation by recruiting various signaling molecules including the src-family kinase Lck and the syk-family kinase ZAP-70. Upon formation of the T cell–APC conjugate, T cells become polarized towards the APC and create a unique structure at the interface between the two cells, called the immune synapse (IS). Upon Ag recognition/activation, the TCR–CD3 complex accumulates at the center of the IS as the central (c-) supramolecular activation complex (cSMAC) and is surrounded by the integrin LFA1 as the peripheral (p-) SMAC [1,2]. When originally characterized, this structure appeared to support a model in which the cSMAC contained the TCR as the Ag recognition structure and the pSMAC contained the integrin to promote cell–cell adhesion. Together with accumulated evidence that various signaling molecules are recruited to the IS, this structure appeared to be the site of Ag recognition and signal transduction for T cell activation. However, the generation of the cSMAC and pSMAC of the IS takes 5–10 min after T cell-APC or T cell-MHC-containing planar bilayer interaction, kinetics that did not correspond at all to those of early activation events such as tyrosine phosphorylation, and intracellular Ca flux [3].

In addition, early analysis of the signaling complex responsible for T cell activation using Jurkat cells stimulated with immobilized anti-CD3 antibody (Ab) revealed that this intracellular complex, which includes adaptor proteins LAT and SLP-76/Gads as well as effector molecules such as PLCγ [4], is formed immediately upon stimulation. These imaging analyses using Jurkat cells were consistent with previous biochemical analyses of the well-established proximal signal transduction events upon TCR stimulation [5–7]; phosphorylation of the ITAMs of CD3 chains by Lck recruits ZAP-70 which induces phosphorylation of adaptor proteins LAT and SLP-76 followed by activation of downstream effector molecules. In normal T cells, Davis and Krummel first observed small cluster of CD3ε at the interface between T cells and B cell lines prior to cSMAC formation [8].

To precisely and dynamically analyze membrane proximal events upon stimulation of normal T cells with Ag peptide–MHC complexes, we have utilized the combination of Total Internal Reflection Fluorescence (TIRF) microscopy and a supported planar bilayer membrane containing mobile peptide–MHC and ICAM-1 as a pseudo-APC membrane for high-resolution imaging. Using this system, we found that the TCR complexes accumulate to form small clusters, termed TCR-microclusters (TCR-MC), immediately after the T cells attached to the membrane, which is much earlier than IS formation [9–11]. The analysis of generation and regulation...
of TCR-MCs has provided new insights into the molecular mechanism of T cell activation. In this article, we highlight our current understanding of the spatiotemporal regulation of T cell activation and co-stimulation through TCR- and CD28-MCs.

2. TCR-microclusters upon initial activation

When T cells become attached to the planar membrane containing peptide-MHC and ICAM-1, the TCR-MCs are generated first at the contact site. As T cells start spreading on the membrane, TCR-MCs are formed over the entire interface. After maximum spreading, T cells then start to contract and all of the TCR-MCs move towards the center of the interface to form the cSMAC. Quantitative analysis of the fluorescence intensity of individual TCR-MC revealed that a single TCR-MC contains approximately 50–300 CD3ε chains [9]. Some of the MCs fuse with each other as they move to the center from the periphery. By tracking the movement of TCR signaling molecules ZAP-70 and SLP-76 as representative kinase and adaptor proteins, respectively, we made two critical observations; first, a single TCR-MC contains both ZAP-70 and SLP-76, indicating that a TCR-MC represents a functional signaling complex. Second, although ZAP-70 and SLP-76 accumulate within the same TCR-MCs, unlike TCR-CD3 they do not move to the center of the interface but instead disappear on the way to the center. Thus, we noted that only the TCR complex is transported to the center to form the cSMAC.

TCR-MCs are continuously generated at the very edge of the contact site. As T cells start spreading on the membrane, TCR-MCs are formed over the entire interface. After maximum spreading, T cells then start to contract and all of the TCR-MCs are fixed and do not move nor form the cSMAC in Jurkat cells stimulated with immobilized Ab.

Whether the TCR-MCs detected in the planar bilayer are really the minimal unit of Ag recognition and cellular activation is a critical issue and remains to be resolved using various approaches. For example, the Davis group has shown that recognition of a single agonistic peptide-MHC by TCR induces Ca2+ influx and several peptide-MHCs are even sufficient to trigger effector function [12]. However, it is still possible that single agonist peptide with many other peptides including self-peptides are recognized by clustered TCR. Before Ag stimulation, it has been shown by electron microscopy that some small clusters of TCR or LAT were pre-formed and the TCR clusters are different from those of LAT, and that these two kinds of clusters will merge together upon Ag stimulation, probably to form TCR-MCs [13], suggesting that the clusters competent for signaling will be TCR-MCs.

3. Signaling through TCR-MC

The idea that the TCR-MC is the unit responsible for generating TCR activation signals came from the finding that every TCR-MC is stained with anti-phospho-tyrosine Ab and anti-phospho-ZAP-70 upon stimulation. Analysis of individual T cells revealed that the Ca2+ influx is induced in parallel with TCR-MC generation, which is a much earlier event than cSMAC formation. Thus, TCR-MCs represent a unit to transduce TCR recognition signals for activation. To compare kinetics of the known biochemical events of the TCR proximal signaling cascade, T cells were treated with the src-kinase inhibitor PP2. Although TCR-MCs were still induced even in the absence of Lck function, PP2 blocked the recruitment of ZAP-70 to the TCR-MCs and generation of the cSMAC.

To even more rigorously test the idea that the TCR-MC is a minimal unit of TCR activation, we analyzed the components of TCR-MCs using individual GFP-fused signaling molecules. Using this approach, we found that proximal signaling molecules such as CD3ζ, Lck, ZAP-70, LAT, SLP-76, Gads, Grb2, Itk, PLCγ, PI3K, Carm1, IKK, Vav, Nck, and WASP, are contained within TCR-MCs, but other molecules such as Ras, Rac, Sos, Erk, Akt, Pdk-1, and CD45 are not present. Therefore, consistent with the proposed role of TCR-MCs, the proximal signaling molecules to induce initial activation signals are the main components. These findings are almost entirely consistent with the signaling complex analyzed in Jurkat cells [4,14] except that the TCR-CD3 complex is not involved within the signaling complex since the TCR complex on the cell surface is fixed and does not move or form the cSMAC in Jurkat cells stimulated with immobilized Ab.

4. TCR-MCs for sustained T cell activation

It is well established that full activation of T cells, leading to cytokine production or cell proliferation, requires continuous stimulation for several hours [15]. In the planar bilayer model, TCR-MCs are continuously generated for hours at the periphery of the contact interface even after cSMAC formation. Newly generated TCR-MCs at the periphery also contain phosphorylated signaling molecules, including ZAP-70, which suggests that the peripheral TCR-MCs also induce signals for sustained activation. Phosphorylation of MCs in the periphery is in great contrast to the situation in the cSMAC, where very little tyrosine phosphorylation can be detected, suggesting that the sustained signal for T cell activation is induced through the peripheral TCR-MCs but not through the cSMAC. When Jurkat cells are stimulated with immobilized anti-TCR Ab, a SLP-76-nucleated signaling complex was formed and induced signals intracellularly at pre-Golgi, suggesting that sustained activation signal maybe induced in intracellular compartments [14,16]. By contrast, in normal T cells, this phosphorylated intracellular protein complex has not been observed, and the peripheral TCR-MCs are likely to mediate sustained as well as initial signaling.

Whereas the planar bilayer contains abundant Ag peptide-MHC, Ag concentration in vivo would obviously be more limited. Under stimulation condition with limited amounts of Ag peptide (under 0.1 μM) in the planar bilayer, T cells exhibit detectable TCR-MCs but no cSMAC. Thus, under conditions where peptide is limited such as in vivo, both the initial and sustained T cell activation will be induced via TCR-MCs in the absence of cSMAC formation or translocation of TCR-MCs to the center of the IS. The cSMAC will be formed and play a significant functional role only upon strong stimulation; either where there is high concentration of peptide, or strong co-stimulation signals. Such strong stimulation induces formation of the cSMAC, which then negatively regulates excessive T cell responses.

5. Lipid raft and TCR-MCs

Lipid rafts are specialized liquid-ordered membrane microdomains that are enriched in cholesterol and sphingolipids. Numerous studies have revealed that lipid rafts exist as small leaflets and float on the plasma membrane [17–19]. They are thought to function in protein sorting and cell activation as a platform for recruiting various signaling molecules such as src-family kinases, G proteins and adaptor proteins mainly through lipid-anchors such as palmitoylation. The functional significance of lipid rafts in signal transduction has been particularly well demonstrated for T cell activation [20,21]. Early studies indicated that crosslinking of the raft-associated ganglioside GM1 induced T cell activation [20], and that LAT with mutant palmitoylation sites, which no longer localized to lipid, rafts, failed to induce an activation signal [21].
Currently, lipid rafts are thought to serve as a platform for signaling molecules involved in T cell activation [22,23]. Since the most commonly used “raft marker” GM1 was found to accumulate at the IS upon stimulation, lipid rafts have been thought to accumulate at the cSMAC and serve as signaling platforms. However, TCR-MCs also recruit various signaling molecules, thus they too function as a kind of platform for T cell activation. Thus, the relationship between lipid raft clusters and TCR-MCs was addressed from the point of view of a signaling platform for T cell activation.

We performed imaging analysis to look for the co-localization of TCR-MCs and lipid rafts [24]. Lipid rafts were visualized by fluorescent raft probes defective in signaling capacity such as a truncated form of Lck as an inner membrane probe, LAT as a transmembrane probe and GM1 as an extracellular probe. No co-localization of these lipid raft probes with any TCR-MC molecules such as CD3ɛ, LAT or ZAP-70 was observed in either resting or stimulated cells. Considering that the lipid raft cluster is small (<100 nm) probably even after clustering, we used more sensitive FRET techniques to analyze molecular association between TCR-MCs and lipid raft probes. However, no increase of specific FRET between CD3ɛ and Lck- or LAT-based raft probes was observed in the region of TCR-MCs upon stimulation [24]. Therefore, our data indicate that lipid raft clusters are not involved in either the generation or signaling function of TCR-MCs but they are rather involved in intracellular protein trafficking, such as the transport of LAT to the plasma membrane. Lipid rafts therefore do not serve as the signaling platform to recruit signaling molecules. TCR-MCs accumulate mainly through protein interactions, consistent with recent report [25] suggesting that the plasma membrane is composed of protein-based islands that are formed independently of lipid raft clusters.

6. IS in migrating cells and cytoskeleton regulation

TCR activation through TCR-MCs induces the “stop signal” in T cells, by which IS between T cells and APC is stably formed for hours. However, T cells can also interact with APC during migration condition. Such dynamic interactions are driven by chemokines either on cell surface of APC or stroma cells. Often in vivo, chemokine-mediated migration dominates over the stop signals [49]. Therefore, TCR activation signals through TCR-MC have to overcome the migration signals mediated by chemokines in order to form IS, and their balance determines the fate of T cells, either movement or stable IS formation. When migration signals dominate, the interaction between migrating T cells and APC becomes short and T cells are not polarized toward APC. In this situation, instead of segregated IS, Dus-tin proposed that moving T cells form “immune kinapse” [50] to induce activation signal during the unstable and unorganized interaction. TCR accumulates at uropod while TCR-MCs is formed at leading edges as lamellipodium and LFA-1 at the lamella. In the T cells with kinapses as migrating cells, the coordinate action of actin and microtubule dynamics regulates the expansion and contraction at the leading edge and uropod of T cells.

Such dynamic regulation of TCR-MC movement in T cells either with synapse or kinapse is supported both by actin and microtubule cytoskeleton network. The targeting of vesicles containing LFA-1 integrin and GTPase Rap-1 together with Rap1 [51] and Talin induces translocation and activation of integrin, regulating T cell-APC adhesion during kinapse and synapse formation. The interplay between cortical actin and microtubules are critical for both stability and polarity of IS. The nucleation of TCR-MCs at IS relies on functional actin cytoskeleton. TCR-MCs appear to move towards the center of the interface by the force of actin retrograde flow [52]. In addition, the centripetal movement of the microclusters requires organized microtubules with motor protein dynein-mediated movement [53].

7. Co-stimulation and TCR-MCs

7.1. Positive regulation by CD28-MC

T cell activation and the consequent fate of T cells are positively and negatively regulated by several co-stimulation signals. The major co-stimulation receptor is CD28, whose ligands on APC are CD80 and CD86 [26,27]. It has been known for some time that in the absence of CD28-mediated co-stimulation, T cells become unresponsive, a status termed “anergy” [28,29]. The co-stimulation signal appears to be independent of TCR signals since independent stimulation, with no co-ligation of these receptors, significantly enhances T cell activation. In spite of extensive analysis of CD28-mediated signaling pathways, which have suggested a critical role of PI3K to mediate co-stimulation signals [30,31], the molecular nature and spatial relationship between CD28 and TCR remain elusive.

We analyzed the dynamics of CD28 and related signaling molecules, particularly to understand the spatial and signaling relationship between TCR-MCs and co-stimulation [32]. CD28 was found to be co-localized with TCR-MCs upon initial Ag stimulation. CD28-MCs moved to the center of the interface, similar to TCR-CD3, and when the cSMAC was formed, CD28 accumulated at the periphery of the cSMAC, but was still within the cSMAC since it was surrounded by the ring of LFA-1 representing the pSMAC. To identify molecule(s) mediating downstream events in CD28-mediated co-stimulation that move and function together with CD28 from the peripheral MC to the cSMAC, we have analyzed the dynamic movement of various signaling molecule as GFP-fusion proteins. For this analysis we chose molecules that are thought to be involved in CD28-mediated co-stimulation, including PI3K, Grb2, Gads, Itk, Vav, PP2A and PKCθ [33–36], although most of these molecules are also involved in the TCR-downstream signaling pathway. None of them except for PKCθ accumulated into the cSMAC upon stimulation. Indeed, PKCθ was co-localized in TCR-MCs upon initial activation, and then moved together with the TCR and then accumulated in the same region as CD28, CD28 and PKCθ not only move together, but they are also physically associated since we could co-immunoprecipitate them from normal T cells upon stimulation. The specific CD28 region that located at the periphery of the cSMAC is maintained dynamically because both the CD28 and PKCθ accumulated clusters recovered soon after photo-bleaching these specific regions. In addition, blocking of the CD28–CD80 interaction by CTLA-4-Ig abrogated the accumulation of not only CD28 but also PKCθ in that region, indicating that CD28 recruits PKCθ to this particular region of the cSMAC probably to mediate sustained co-stimulatory signals such as NF-κB activation [32] (Fig. 1). Indeed, we have now shown that Carma1, which forms the Carma1–Bcl10–Malt1(CBM) complex to induce NF-κB activation, also accumulates in the same region as CD28 and PKCθ [37]. We have begun to analyze CD28 mutants to identify the regions of the molecule responsible for CD28 cluster formation and PKCθ recruitment. Mutant CD28 lacking CD80-binding capacity cannot make clusters, whereas a tail-less CD28 mutant defective in signaling could induce clustering but failed to recruit and accumulate PKCθ, a defect that was correlated with defective co-stimulatory function [32]. Therefore, our analysis on CD28-mediated co-stimulation revealed an important mode of signal regulation during co-stimulation; the CD28 co-stimulatory receptor is initially accumulated in the TCR-MCs and then in the special region of the cSMAC. PKCθ is associated with and co-translocates with CD28 and
mediates sustained co-stimulation signals such as NF-kB activation.

7.2. Negative regulation by CTLA-4-MC

After analyzing the dynamic regulation of CD28-mediated positive co-stimulation, we investigated the negative regulation mediated by CTLA-4, a major negative co-stimulatory receptor. CTLA-4 has the same ligands, CD80 and CD86, as CD28 but, whereas CD28 is constitutively expressed on the cell surface, CTLA-4 is not until its expression is induced by TCR stimulation [38,39]. The CTLA4 gene is transcribed and translated upon stimulation, but the protein is retained and degraded within lysosomes in the absence of further stimulation through endocytosis by assembly with the AP2 complex [40–42]. Since CTLA-4 has a much higher affinity than CD28 for the same ligands, even low level expression of CTLA-4 on the cell surface can compete for ligand binding with CD28, which is thought to be the main mechanism of CTLA-4-mediated inhibition of T cell activation. Previous studies revealed that CTLA-4 is transported from storage in endosome/lysosomes to the plasma membrane upon further T cell stimulation, and that the induction of cell surface expression is only induced by strong activation whereas weak activation only leads to translocation of these vesicles to the vicinity of the plasma membrane [43]. However, how CTLA-4 blocks T cell activation has remained unclear.

When we analyzed spatiotemporal regulation of CTLA-4 expression, we found that CTLA-4 also forms microclusters, but these are not initially co-localized in TCR-MCs and instead directly accumulate in cSMAC [37]. The region of the cSMAC where the CTLA-4-MCs accumulate is exactly the same region where CD28 and PKCθ accumulate. Thus, CTLA-4-MCs, once translocated to the cSMAC, push CD28 and PKCθ away from a specific region of cSMAC, which results in the blockade of CD28-mediated co-stimulation (Fig. 1). To determine whether the accumulation of CTLA-4 in the cSMAC is required for CTLA-4-mediated inhibition of activation, we produced CTLA-4 chimeras with CD22 possessing various sizes of the CD22 ectodomain and the first ligand-binding Ig domain of CTLA4. The idea behind this experiment is that only short molecules with one or two Ig domains can accumulate in cSMAC whereas large molecules would be excluded and accumulate outside of the cSMAC. As expected, we found that the short ectodomain (one or two Ig domains)-bearing CTLA-4 co-localized with the TCR-MCs and accumulated later in the cSMAC whereas molecules with longer ectodomains failed to localize in either the TCR-MCs or the cSMAC. Functionally, only CTLA-4 with short ectodomains could inhibit T cell activation. These results proved the importance of its localization in the cSMAC for CTLA-4 to mediate inhibitory function [37].

8. Signaling at cSMAC

8.1. Bi-function of cSMAC

The cSMAC contains the concentrated TCR complex and this representative structure of the IS has been thought to be responsible for inducing activation signals. However, since tyrosine phosphorylation in the cSMAC was barely detectable, while abundant tyrosine phosphorylation was observed in individual TCR-MCs at the periphery, it has become clear that the cSMAC is not responsible for signal transduction. This concept was further supported by the finding that the cSMAC contains negatively regulating phosphatases and degradation machinery-related molecules [44,45] and that T cells from CD2AP-deficient mice, which have defective cSMAC formation, show enhanced TCR expression and cellular activation [3]. These findings all indicate that the cSMAC plays a role in negative regulation of T cell activation by promoting endocytosis and degradation of the TCR complex. However, recently several lines of analyses have provided evidence that the cSMAC also has signaling function, but in a way distinct from TCR-MCs. One, as described above, is in CD28-mediated co-stimulatory signaling, probably including NF-κB activation, and is mediated through the special region of cSMAC [32,46]. The other stems from the observation that TCR signals including phopho-CD3; may be actively induced under certain restricted conditions, particularly with weak stimuli [47].

Fig. 1. Spatiotemporal regulation of formation of TCR-microcluster and cSMAC and signal regulation through them. Upon recognition of antigen-MHC, a T cell exhibits sequential processes; spreading, contraction and immune synapse formation. As soon as a T cell attaches, TCR-MCs containing TCR, ZAP-70, SLP76 as well as CD28 and PKCθ are formed which induce initial activation signals. TCR-MCs then move towards the center of the interface to generate cSMAC while ZAP-70 and SLP-76 do not move and accumulate at the center. In contrast, both CD28 and PKCθ are accumulated in the specific region within cSMAC (CD3ε signaling cSMAC) to positively induce sustained co-stimulation signals. At late phase of activation, CTLA-4 is induced upon activation and accumulated not to TCR-MCs but directly to the same region of cSMAC as CD28 accumulated where CTLA-4 competes the ligand-binding with CD28. CD28-PKCθ is pushed out from the signaling cSMAC and consequently activation is inhibited.
Regarding the spatial regulation of signals in the cSMAC, we defined two distinct sub-regions based on our high-resolution microscopic analysis. The distinction between the two is based on the density of CD3; CD3-high (CD3$^{hi}$) and CD3-low (CD3$^{lo}$). The size of cSMAC is determined by TCR signal strength. In the absence of co-stimulation, the majority of cSMAC is occupied CD3$^{hi}$ region where extensive internalization and degradation of TCR takes place. In contrast, in the presence of strong co-stimulation, CD3$^{lo}$ region as dominate within cSMAC as “signaling cSMAC” which is responsible for co-stimulation signaling. Thus, the proportion of CD3$^{lo}$ vs. CD3$^{hi}$ is determined by the signal strength of TCR signal and co-stimulatory signal. TCR signal becomes strong by high dose of antigen or strong agonist while become weak with low concentration of antigen or weak agonist. Co-stimulatory signal becomes strong with high expression of CD28 on T cells or CD80/86 on APC, while become weak with low level of CD28 or CD80/86.

Fig. 2. Schematic model of functionally distinct subregions of cSMAC. cSMAC is segregated into two regions according to the density of CD3; CD3-high (CD3$^{hi}$) and CD3-low (CD3$^{lo}$). The size of cSMAC is determined by TCR signal strength. In the absence of co-stimulation, the majority of cSMAC is occupied CD3$^{hi}$ region where extensive internalization and degradation of TCR takes place. In contrast, in the presence of strong co-stimulation, CD3$^{lo}$ region as dominate within cSMAC as “signaling cSMAC” which is responsible for co-stimulation signaling. Thus, the proportion of CD3$^{lo}$ vs. CD3$^{hi}$ is determined by the signal strength of TCR signal and co-stimulatory signal. TCR signal becomes strong by high dose of antigen or strong agonist while become weak with low concentration of antigen or weak agonist. Co-stimulatory signal becomes strong with high expression of CD28 on T cells or CD80/86 on APC, while become weak with low level of CD28 or CD80/86.

Fig. 3. Possible implication of spatial and differential regulation of TCR- and co-stimulation signals through TCR-MCs and signaling cSMAC. Whereas the peripheral TCR-microclusters induce Ag recognition signal through activation of ZAP-70, LAT, SLP-76, co-stimulation signals are induced in the localized specialized cSMAC region “signaling cSMAC” through recruiting CD28, PKC$\theta$ and Carma-1. From the point of 2 signal model of T cell activation, these signals may represent “signal 1” and “signal 2”, respectively, which are therefore generated by spatially distinct signaling modules.

8.2. Subregions of the cSMAC

Regarding the spatial regulation of signals in the cSMAC, we defined two distinct sub-regions based on our high-resolution microscopic analysis. The distinction between the two is based on the density of CD3; CD3$^{hi}$ and CD3$^{lo}$ regions (Fig. 2) [32,46]. CD3$^{hi}$ represents the well-known standard cSMAC in which TCR-CD3 is highly accumulated. When analyzed using fluorescently labeled probes for MHC class II, we found that there was no overlap between the CD3$^{hi}$ region and the pMHC, however the CD3$^{lo}$ region correlated well with pMHC. This analysis indicates that TCR in the CD3$^{lo}$ region are associated with peptide-MHC on the cell surface, whereas TCR in the CD3$^{hi}$ region appear to be no longer assembled with pMHC, and are probably destined for internalization and degradation. Photobleaching experiments indicated that the CD3$^{lo}$ region is dynamically regulated whereas CD3$^{hi}$ is very rigid and did not recover after bleaching. Importantly, since CD28 and PKC$\theta$ as well as Carma-1 all accumulate in this CD3$^{lo}$ region, CD3$^{lo}$ is the region mediating signal transduction as the “signaling cSMAC”.

The relative dimensions of the CD3$^{lo}$ and CD3$^{hi}$ regions are regulated by the strength of the TCR and co-stimulation signals as shown in Fig. 2. A high dose of Ag induces a larger proportion of CD3$^{lo}$, which will be internalized/degraded, whereas a low dose of Ag results in a larger CD3$^{lo}$ region, which induces active
signaling. Conversely, strong co-stimulation increases the CD3lo weak co-stimulation increases the CD3hi regions, respectively. These studies lead to a new global and testable model for TCR signaling. Spatially differential regulation through peripheral TMC-TCR-MC and signaling CSMAC represent the site for “signal 1” and “signal 2” in classic co-stimulation models [29,48]. TCR-MCs induce Ag recognition signals through TCR as “signal 1” and signaling CSMAC (CD3lo CSMAC) induce co-stimulatory signals as “signal 2” (Fig. 3).

9. Concluding remarks

In this review, we describe dynamic feature of antigen recognition and activation of T cells from the analysis of newly defined microclusters. TCR-MC is generated, translocated and regulated dynamically by assembly and disassembly with various molecules. Since the regulation of cell activation by microcluster is similarly observed in B cells and NK cells, the dynamic regulation of receptor-mediated activation through microclusters is common mechanism among all lymphocytes. Probably not only lymphocytes but also other type of cells such as nerve cells may have similar regulatory system. Molecular imaging of single T cell made advance to understand molecular dynamics of signaling event. Many questions remain to be solved; where do signaling molecule such as ZAP-70 and SLP-76 disappear, how sustained activation is regulated with very low concentrations of antigen, how much extent do TMC-TCR-MC regulation reflect in vivo, and so on. Further analysis of spatiotemporal regulation of TMC-MCs within a cell and in vivo will unveil the complex signaling network for T cell activation and immune responses.

References


