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T Cell Receptor Signaling Kinetics Takes the Stage

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Abstract

It has been long surmised that the strength of stimulation of the T cell receptor (TCR) determines the robustness of TCR-mediated signaling and the magnitude of a T cell response. However, it is becoming evident that the signal from the TCR develops over time to approach its steady-state, affinity-determined maximal extent and that variations in this time have a substantial effect on the responsiveness of T cells. Here, I discuss data that show that the kinetics of signal propagation in various segments of the TCR signaling network can influence the spatiotemporal regulation of the effector functions of T cells and the quality of the T cell response.

Introduction

Productive engagement of the T cell antigen receptor (TCR) initiates the sequential activation of proximal and downstream signaling proteins, some of which act as intracellular enzymes, whereas others operate as adaptor proteins. Interactions between these proteins result in the formation of a comprehensive signaling network that enables the TCR-mediated signal to travel through various pathways that result in different outcomes. The flexibility of the signaling network is thought to account for the ability of T cells to exhibit various responses, each of which requires ligation of the TCR. Although many signaling pathways that lead to different T cell responses have been delineated, their cellular compartmentalization and temporal regulation are emerging as essential factors that influence the signaling outcome. Meanwhile, how the temporal and spatial regulation of various segments of the TCR signaling network mediate cytoskeletal function, protein segregation at the contact membrane between the T cell and its target, intracellular trafficking, secretion, and endocytosis are only beginning to be understood. Here, I discuss experimental data that extend our knowledge beyond the complexity of the comprehensive TCR signaling network. These data show that TCR signaling is initiated in distinct locations of the T cell contact membrane that are termed microclusters and that the magnitude of signaling in individual microclusters regulates the kinetics of downstream signaling. The difference in the kinetics of signal propagation in distinct segments of the TCR signaling network could be translated into spatial and temporal variations of intracellular events that control the quality of T cell responsiveness.

Proximal Signaling and Microcluster Formation

Within a few seconds, engagement of the TCR initiates proximal signaling and the development of secondary TCR-mediated events that result in the formation of TCR signaling microclusters (1–3). These represent an essential membrane-associated platform for the development and maintenance of TCR signaling. Similar microclusters that contain proximal signaling proteins are formed in response to ligation of the B cell receptor (BCR) (4), which suggests that the concentration of signaling proteins at the sites of ligated antigen receptors is a general feature of the mechanism of lymphocyte activation.

Engagement of the TCR leads to the formation of few microclusters at a small contact area between the T cell and its target, which is facilitated by actin polymerization (5). Thus, initiation of TCR-mediated signaling in microclusters occurs before the large-scale molecular segregation of the TCR and adhesion molecules and the formation of a bull's eye-like structure termed the immunological synapse (6). The number of microclusters then rapidly increases as a result of actin-dependent T cell spreading and a dramatic increase in the area of the contact membrane (7). Changes in actin dynamics seem to influence the content and signaling properties of microclusters, which is regulated by the activation of actin-regulatory proteins, including hematopoietic lineage cell-specific protein 1 (HS1) (7,8). As they signal, microclusters move from the periphery to the center of the immunological synapse and then usually disappear; most likely, they are taken up in a process that is believed to be necessary to quench signaling (3). It is thought that newly formed microclusters in the periphery of the synaptic interface function as the predominant sites for proximal signaling, whereas central microclusters are usually associated with decreased signaling (9).

Each microcluster contains tightly packed TCRs bound to peptide-major histocompatibility complex (pMHC) molecules (3). The packaging of the TCR-pMHC complexes in microclusters appears to be so tight that the addition of antibodies that can block the formation of new microclusters fails to destroy existing microclusters (3). The intensity of signaling in individual microclusters varies and depends on the intrinsic affinity of TCR-pMHC interactions that influences the number of engaged TCRs (10). The latter may also be modulated by the serial engagement of a TCR by a single pMHC (11), which results in the spreading of the signal from a few productively engaged TCR molecules to other TCRs within a single microcluster (12). The affinity of the BCR for antigen also determines the amount of proximal signaling proteins that are recruited into the BCR-containing microclusters (13), which suggests a common mechanism for the regulation of lymphocyte responsiveness by the affinity of antigen-specific receptors.

The role of serial engagement in the mechanisms that control T cell activation has become more apparent after measurements of the dissociation rates of strong and weak agonists from the TCR under conditions in which the TCR and pMHCs are embedded in closely opposing surfaces to interact in two-dimensional (2D) space, mimicking physiological conditions (14,15). As expected, the equilibrium affinity constants in 2D space are higher than those measured when at least one of the reactants is free in solution, as, for example, in 3D space. Contrary to expectations, dissociation of the pMHC from the TCR on opposing surfaces in two dimensions is much faster than in 3D space, and the increase in the 2D affinity is due to the substantially faster on-rate of the interactions. The faster off-rate of strong agonists is expected to facilitate serial engagement of TCRs in microclusters even under conditions in which there are very few stimulatory pMHCs per microcluster (16,17).

Primary signaling microclusters that are formed at the periphery of the immunological synapse can then give rise to the formation of secondary microclusters, which contain downstream signaling proteins and facilitate signal propagation (9). Consistent with this, subsynaptic intracellular vesicles beneath the IS that contain the adaptor molecule linker of activated T cells (LAT) can interact with microclusters that contain the adaptor protein Src homology 2 (SH2) domain-containing leukocyte phosphoprotein of 76 kD (SLP-76) at the immune synapse in a "touch-and-go" manner and that phosphorylated LAT (pLAT) can be exchanged between the vesicles and the microclusters (18). Moreover, phosphorylation of LAT appears to depend on the contact time between the LAT-containing vesicles and the SLP-76-containing microclusters. The formation of secondary microclusters and the exchange of content between different microclusters seem to be dynamic processes that likely serve to facilitate the rapid accumulation of activated signaling proteins at the synaptic

interface that regulate downstream signaling kinetics. This process may also influence the dynamics of a negative-feedback mechanism that could inhibit signaling in individual microclusters (19), which suggests that the microclusters likely become activating or nonactivating and, perhaps, even inhibitory. This is likely to be a digital process, consistent with the digital nature of proximal signaling, which is not evident below a threshold of antigen stimulation, but reaches a maximal extent at the threshold in a decisive “yes-or-no” manner (20–23).

Analysis of microclusters formed at the contact membrane of Jurkat cells (a human CD4⁺ T cell line) that interact with lipid bilayers shows that even at the initial stage of microcluster formation, the TCR and the integrin LFA-1 (lymphocyte function–associated antigen–1) are segregated into different microclusters and remain so as the microclusters move toward the central part of the contact area (24). Although the mechanism by which the TCR and LFA-1 are partitioned is not clearly understood, it occurs very early after engagement of the TCR and LFA-1 by their respective ligands, before the formation of supramolecular activating clusters (SMACs) (25). It is thought that the TCR and LFA-1 interact with different scaffolding proteins, which might explain the initial segregation of these molecules. Microclusters containing LFA-1 or TCR move with similar velocities in a process that is thought to be actin-driven. LFA-1 microclusters stop at the border between the central SMAC (cSMAC) and the peripheral SMAC (pSMAC) of the immunological synapse, whereas TCR-containing microclusters move across the border into the cSMAC. Because actin is displaced from the cSMAC, TCR-containing microclusters apparently can travel across the cSMAC-pSMAC border in an actin-independent manner. It is likely that microclusters of TCR, but not LFA-1, are stable without being attached to actin (24). TCR microclusters coalesce within the cSMAC and may contain activated signaling proteins (10,26). The biological relevance of residual TCR-mediated signaling in the cSMAC is unclear. However, Saito and colleagues found that the TCR and the CD28 coreceptor are initially located in the same microclusters, but that later these molecules are segregated and CD28 accumulates at the periphery of the cSMAC (27).

The Dynamics of Proximal Signaling

To analyze the dynamics of TCR-mediated signaling, Davis and colleagues developed a photoactivatable pMHC ligand that enabled them to define precisely the moment of productive TCR engagement and to measure the time required for the activation of selected signaling proteins (21). The appearance of microclusters containing a fusion of green fluorescent protein (GFP) and the adaptor protein Grb2 (Grb2-GFP), a marker of LAT activation, occurs 4 s after TCR triggering. It takes ~6 to 7 s for the recruitment of a fluorescence-labeled reporter [the C1 domain of protein kinase C θ (PKC- θ)] to the T cell contact membrane, which indicates the activation of phospholipase C- γ (PLC- γ) and the production of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃), which stimulates the release of Ca²⁺ from intracellular stores. Approximately the same time is necessary to detect an increase in the concentration of intracellular Ca²⁺ after TCR has been engaged.

Decreasing the strength of TCR stimulation, either by exposing the T cells to a lower density of cognate pMHC-II molecules or by blocking the co-receptor CD4, results in delayed mobilization of intracellular Ca²⁺; blocking CD4 appears to have the strongest effect. Weaker stimulation of the TCR with a lower density of pMHC-II does not change the kinetics of activation of LAT or PLC- γ ; however, blocking antibody against CD4 results in a decrease in the extent of LAT activation without changing the time required for its recruitment to the TCR. These data are in accordance with the important role of co-receptor in contributing to the intensity of the early signaling (12) and the kinetics of Ca²⁺ flux (10).

The proximal signaling cascade is localized at the T cell contact membrane, and the time required to accumulate a threshold amount of each signaling protein to activate the next protein in the pathway does not seem to depend on the number of engaged TCRs (21). This supports the notion that signal propagation through the proximal signaling cascade is a multistep digital process (22). In contrast, the generation of second messengers, such as Ca^{2+} or DAG, occurs in the cytoplasm and is apparently an integrated process, which enables the strength of TCR stimulation to determine changes in the kinetics of Ca^{2+} flux (10,21). It appears that the magnitude of proximal signaling controls the kinetics of downstream signaling (21), which could influence the temporal and spatial manifestation of intracellular molecular events and affect the quality of T cell effector functions (10).

Kinetics of Downstream Signaling and T Cell Responses

The TCR is intrinsically degenerate and recognizes various ligands, both self and nonself, during the life-span of a T cell (28). In particular, the TCR usually recognizes variants of a nominal agonist peptide presented by the same MHC protein, albeit with different sensitivities. For example, weak agonists can induce a T cell response that is similar in magnitude to that induced by a strong agonist, but only when the weak agonists are at a substantially higher concentration (29,30). For some T cell responses, however, it takes a longer time for a weak agonist to maximally stimulate a T cell compared with that required by a strong agonist. This has been observed by Jameson and colleagues (31) in the response of quiescent T cells. Specifically, the authors showed that freshly derived, transgenic CD8^+ OT-1 T cells increased the level of CD69, a marker of T cell activation, with different kinetics after stimulation with either strong or weak agonist pMHCs. They also demonstrated that, when the TCR is occupied to a similar extent by either strong or weak soluble pMHCs assembled on a streptavidin scaffold as pMHC-tetramers, the kinetics of Ca^{2+} mobilization induced by the strong agonist are much faster than that stimulated by the weak agonist. Thus, a strong agonist induces a T cell response faster than does a weak agonist, which provides an advantage to the host immune response.

We observed a difference in the kinetics of the cytolytic response of CD8^+ (which are most efficient) and CD4^+ (which are less efficient) cytotoxic T cells (CTLs) that recognize the same target cells, despite their exhibiting similar intensities of TCR-mediated Ca^{2+} flux at the plateau of the response (10) and releasing similar amounts of granules with equal potency under these conditions (32). Consistent with this, CD8^+ and CD4^+ CTLs exhibit very different patterns of granule polarization at the contact surface with glass-supported bilayers or live target cells (Animations 1 and 2). CD8^+ CTLs accumulate secretory vesicles near the center of the cytolytic synapse, whereas the vesicles in CD4^+ T cells are projected mostly to the periphery of the synapse. The same patterns of polarized granules, i.e., positioned centrally or peripherally within the cytolytic synapse, were observed in CD8^+ CTL recognizing strong and weak pMHC ligands, respectively (10,33). These data show that lytic granules are delivered to the secretory domain through different pathways and that the pathway chosen does not depend on the magnitude of TCR signaling at the plateau of the response.

From these experiments, the kinetics of signaling downstream of the TCR, rather than the magnitude of proximal signaling, has emerged as the most probable reason for the observed difference in the pathway of granule delivery. Indeed, quantum dots (QDs) that bear the appropriate pMHCs (12) stimulate profoundly different kinetics of Ca^{2+} mobilization in CD8^+ and CD4^+ CTLs (10). The Ca^{2+} flux elicited by QD-pMHC-I in CD8^+ CTLs is much more rapid and robust than that induced by QD-pMHC-II. Stimulation of CD8^+ and CD4^+ CTLs with CD3-specific antibodies reveals very similar Ca^{2+} flux in both cell types, which indicates that there is no intrinsic difference in the ability of either cell type to mobilize

Ca²⁺. The faster signaling kinetics in CD8⁺ CTLs was also linked to a more rapid release of granules and destruction of target cells.

These findings suggest that the kinetics of Ca²⁺-dependent signaling determine how rapidly granules reach the microtubule-organizing center (MTOC) (10). In addition, reorientation of the MTOC toward the contact interface is mediated by DAG-dependent signaling, which is independent of Ca²⁺ (21,34). Because DAG and IP₃, which initiates Ca²⁺ mobilization, are produced in parallel as a result of PLC- γ activity, we propose that the balance between Ca²⁺-dependent and DAG-dependent signaling influences the choice of pathway of granule delivery (10). Thus, if the kinetics of Ca²⁺ signaling is rapid, the granules are recruited to the MTOC before its polarization. Subsequent polarization of the MTOC delivers the granules directly to the secretory domain by the shortest path (Fig. 1). However, a slow kinetics of Ca²⁺ signaling enables the MTOC to polarize before the granules reach it. The granules are then rerouted to the periphery of the contact interface and travel across the adhesion ring (pSMAC) before their release into the secretory domain, which constitutes the long path of delivery (Fig. 1).

The delay in signaling kinetics in response to stimulation of the TCR with a weak agonist has been observed for both early and late T cell responses exercised by quiescent CD8⁺ T cells (31) and antigen-experienced, cloned CTLs (10), respectively. Thus, differences in TCR-mediated signaling kinetics are a critical determinant of the quality of T cell responses. In other words, the differences in temporal signaling within segments of TCR-mediated signaling network regulate the spatial dynamics of molecular events that are involved in the effector activities of T cells. The importance of the killing rate has been reiterated in a study that presents a 3D mathematical model that describes the efficacy of CTL killing and hunting for target cells in vivo (35).

Comparing the Kinetics and Magnitude of Signaling

The strength of stimulation of the TCR depends on the number of productively engaged receptors, and it regulates the magnitude and manifestation of T cell responsiveness. Various T cell responses have different sensitivities, which are largely determined by the density of epitopes on the target or antigen-presenting cells (APCs) (36,37). Epitopes on APCs vary from very few to many thousands per cell (16,17,38). Once the magnitude of the response approaches its maximum, the intensity of TCR signaling no longer depends on the number of engaged TCRs or the strength of TCR stimulation, regardless of the sensitivity of the response. In other words, at the plateau of the response, the signaling machinery of the T cell is mobilized to the maximal extent, beyond which a further increase in the number of activated TCRs does not lead to a higher magnitude of signaling. However, the kinetics of the approach to maximal signaling appear to be a decisive factor (10).

TCR-mediated signaling is initiated in the TCR-containing microclusters that harbor proximal signaling proteins. The signaling intensity in microclusters depends on the strength of engagement of the TCR, but the rate of accumulation of microclusters at the initial point of contact between the T cell and the target seems to be associated with the kinetics of downstream signaling. This indicates that both the magnitude of proximal signaling and the kinetics of downstream signaling control the outcome of T cell stimulation. We suggest that the magnitude of signaling mostly accounts for the sensitivity of a T cell response, whereas the changes in the rate of signal propagation downstream might serve as an essential mechanism that determines the efficiency of the T cell response. The development of T cell responses in a timely manner is often critical for a successful host immune response against pathogens. Thus, the quality of the T cell response can be determined at very early stages of TCR-mediated signaling and the kinetics of signaling appears to be an essential factor.

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Fig. 1.

The kinetics of signaling downstream of the TCR influences the balance between the long and the short pathways of granule delivery. Engagement of the TCR in more effective CTLs results in rapid and robust proximal TCR signaling, as opposed to the slower proximal signaling that is induced in less effective CTLs. In contrast to weak signaling, strong signaling is initiated by a greater number of activated signaling proteins per microcluster and leads to rapid downstream signaling kinetics, as determined by measurement of the rate of intracellular Ca^{2+} mobilization (10). Increasing the concentration of intracellular Ca^{2+} results in the activation of downstream signaling that regulates the velocity of granule movement to the MTOC. In contrast, translocation of the MTOC is independent of Ca^{2+} and is mediated by DAG-dependent signaling (21,34). This signaling dichotomy accounts for the mechanism that controls the two pathways of granule delivery to the secretory domain. A faster signaling kinetics stimulates the swift movement of granules toward the MTOC, and granules become concentrated near the MTOC before its polarization. The MTOC then directly delivers granules to the secretory domain, which is the short path of delivery. Slower signaling kinetics results in delayed recruitment of granules, which move along microtubules to the periphery of the synapse and then loop through the pSMAC to fuse at the outer edge of the secretory domain; this is the longer pathway. Both pathways can operate in a single cell, and the balance between them is regulated by the kinetics of TCR-mediated signaling. (See <http://stke.sciencemag.org/cgi/content/full/3/153/pe50/DC1> for Animations 1 and 2.)