Integrins Put the Brakes on Microcluster Dynamics at the Immunological Synapse

Janis K. Burkhardt1,*
1Department of Pathology and Laboratory Medicine, Children’s Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
*Correspondence: jburkhar@mail.med.upenn.edu
DOI 10.1016/j.immuni.2008.05.002

Integrin engagement costimulates T cell receptor signaling, but the underlying mechanisms are poorly understood. In this issue of Immunity, Nguyen et al. (2008) show that engagement of VLA-4 promotes sustained signaling by altering the dynamics of actin filaments and signaling molecules at the immunological synapse.

Productive T cell activation is clearly coupled to protein movements at the immunological synapse. Video analysis of living T cells shows that T cell receptor (TCR) engagement leads to the assembly of signaling molecules into microclusters at the cell periphery. These microclusters then move centripetally to converge in the central region of the immunological synapse (Bunnell et al., 2002; Varma et al., 2006; Yokosuka et al., 2005). Early tyrosine-phosphorylation events take place predominantly in peripheral microclusters. In contrast, central clusters are thought to represent sites where signaling is terminated by internalization and degradation of signaling complexes (Seminario and Bunnell, 2008). Several studies demonstrate that this relationship is more than just correlative. Biochemical or mechanical trapping of TCR-signaling complexes in the periphery leads to enhanced TCR signaling (Lee et al., 2003; Mossman and Bunnell, 2008). Conversely, conditions that either prevent new microcluster formation in the periphery or promote microcluster centralization lead to diminished T cell activation (Cemerski et al., 2007; Seminario and Bunnell, 2008). Interestingly, parameters such as peptide-MHC half-life can affect central supramolecular activation cluster (C-SMAC) formation (Cemerski et al., 2007), suggesting the existence of feedback pathways that balance T cell responses to a broad range of agonists. Thus, a new paradigm has recently emerged, in which the dynamics of microcluster formation and centralization determine the duration of TCR signaling and thereby control the outcome of T cell activation. In this issue of Immunity, Nguyen et al. (Nguyen et al., 2008) have added an important new element to this concept by showing that costimulatory signals from integrins can modulate microcluster dynamics.

On the basis of early studies using fixed T cell-B cell conjugates, the immunological synapse was originally described as a “bullseye” structure, with a peripheral region containing adhesion molecules (the peripheral supramolecular activation cluster, or P-SMAC) and a central region rich in TCR, downstream kinases, and adaptor proteins (C-SMAC). However, it is now known that immunological-synapse architecture is highly dynamic and heterogeneous. The patterns formed by synapse proteins vary substantially depending on many factors, including the nature of the T cell and of the antigen-presenting cell, the duration of their interaction, the agonist strength, the costimulatory interactions, and the tissue context in which the interaction is taking place (Burkhardt et al., 2008). Some of the best information about protein dynamics at the immunological synapse comes from simplified two-dimensional experimental systems in which the movements of fluorescently tagged signaling proteins are monitored by video microscopy in T cells responding to either anti-TCR-coated coverslips or lipid bilayers containing peptide-MHC complexes. These planar assay systems are highly artificial; in coverslip assays, ligands are immobilized on a rigid surface, whereas bilayer assays allow nearly infinite ligand mobility. Ligand mobility in the membrane of an antigen-presenting cell would lie between these two extremes and might well be actively regulated. Nonetheless, both planar assay systems are powerful because they permit investigators to view protein movements in a predetermined plane with optimal spatial and temporal resolution. Moreover, they make it possible to test the effects of ligating-specific receptors in the absence of other stimuli. These studies have shown that TCR engagement leads to spreading of the T cell and formation of an actin-rich lamellipodium around the circumference of the contact site. Signaling molecules form microclusters within this lamellipodium and move centripetally to converge in the central C-SMAC-like region (Bunnell et al., 2002; Varma et al., 2006; Yokosuka et al., 2005). Microcluster formation and movement are actin-dependent processes, most likely driven by the formation of branched actin filaments at the cell periphery, which is coupled to retrograde F-actin flow (Burkhardt et al., 2008). Peripheral and central microclusters are functionally distinct; peripheral microclusters are sites of active signaling, whereas central microclusters are sites for signal termination (Seminario and Bunnell, 2008). Thus, the rate of centripetal microcluster movement is linked to the duration of TCR signaling.

Here, Nguyen et al. (2008) demonstrate that costimulatory signaling by the integrin VLA-4 (α4β1) requires the adaptor protein SLP-76 and that VLA-4-induced costimulation occurs only under conditions that induce T cell spreading and SLP-76 microcluster formation. Using a coverslip-based assay to analyze the movement of SLP-76 microclusters, the authors make the striking observation that VLA4 engagement arrests centripetal movement of SLP-76 microclusters. They show that SLP-76 microclusters in T cells responding to coverslips coated with anti-CD3 and the VLA-4 ligand VCAM-1 move more slowly, traverse shorter distances,
and are longer-lived than those in T cells responding to anti-CD3 alone. Remarkably, many microclusters in VLA-4-costimulated cells persist for over 4 min. Importantly, αCD43, which also induces T cell spreading and microcluster formation but does not costimulate T cell activation in this system, does not arrest microcluster movement. This finding indicates that the important parameter for effective costimulation is the retardation of microcluster movement. Moreover, it suggests that “outside-in” signals from VLA-4 play a role in the control of microcluster dynamics.

Because it is known that tyrosine-phosphorylation events occur in peripheral microclusters, it makes sense that the retaining of SLP-76 in these clusters can strengthen and sustain signaling by prolonging the life of “signalosomes” that depend on tyrosine phosphorylation of this key adaptor protein for their stability. Although Nguyen et al. (2008) do not test this key adaptor protein for their stability, they show that costimulation by VLA-4 causes SLP-76 microclusters to remain in proximity to the ZAP-70-rich, phosphotyrosine-rich microclusters where they originate (Figure 1). In coverslip assays, these ZAP-70-rich microclusters remain in the periphery independent of costimulation, most likely because they represent sites where TCR is engaged with immobilized antibodies. How does VLA-4 alter the movement of SLP-76 microclusters? Clues to the answer of this question come from a set of experiments showing that retrograde flow of actin filaments within the lamellipodium is also retarded in VLA-4-costimulated cells. This suggests that the interaction of engaged integrins with actin filaments slows the cortical flow that drives microcluster movement. However, this is probably only part of the answer, because the rate at which actin filaments move inward always exceeds the rate of SLP-76 microcluster centralization. To explain this observation, Nguyen et al. propose that SLP-76 microclusters engage in additional interactions that slow their progress. These could be interactions with TCR-associated signaling molecules, such as LAT, as well as interactions with VLA-4-associated molecules.

This study opens the door to new ways of thinking about how costimulation works. It will be interesting to determine whether other costimulatory molecules have similar effects on microcluster dynamics. The idea that signaling can be modulated by the alteration of cytoarchitecture is particularly appealing with respect to integrins, which are already known to tether cortical cytoskeletal elements to extracellular ligands. Thus, it is especially important to ask whether engagement of LFA-1, the β2 integrin that mediates adhesion of T cells to ICAM-1 on antigen-presenting cells, also retards microcluster centralization. Using a bilayer-based assay in which T cells were responding to anti-TCR together with ICAM-1, Kaizuka et al. (2007) recently showed that microdomains containing ICAM-1 form at the periphery and move centripetally to the C-SMAC boundary in an F-actin-dependent fashion. Superficially, the behavior of LFA-1-ICAM-1 seems very different from the static behavior of VLA-4-VCAM-1 described in the Nguyen et al. study, but much of this could be attributable to the difference in ligand mobility on coverslips versus that on bilayers. Thus, it would be particularly illuminating to conduct parallel analysis of integrin effects with the use of coverslip- and bilayer-based assays. In the long run, of course, it will be important to ask whether integrin-dependent microcluster retardation occurs in T cell-APC conjugates and to assess the role of CD28 and other costimulatory signals. CD28 engagement activates PI3 kinase and CDC42 (Burkhardt et al., 2008), both of which promote actin polymerization at the cell periphery. Does this explain why CD28 engagement promotes PKCθ accumulation in the C-SMAC? If so, why is this engagement stimulatory rather than inhibitory?

Finally, the idea that sustained signaling in microclusters is driven by cortical actin polymerization, opposed by integrin-dependent actin tethers, could help to explain why actin-regulatory proteins are so important for T cell activation. If the function of actin-nucleation-promoting proteins like WASp, WAVE-2, and HS1 is balanced by the function of cortical-tethering proteins such as talin, ezrin, and moesin, then the set point for T cell activation could be altered by regulatory changes in this balance. Cytoskeletal control of ligand mobility in antigen-presenting cells might be equally important. In this regard, it is appealing to hypothesize that fascin, an actin-bundling protein that is recruited to the immunological synapse in dendritic cells, serves to more effectively stimulate T cells by decreasing ligand mobility.

After years of debate about how immunological-synapse structure relates to T cell-activation events, a unified paradigm is beginning to emerge from studies of molecular movements in living cells. Nguyen et al. (2008) have provided other costimulatory molecules have similar effects on microcluster dynamics.
important new insights into how costimulatory signaling by integrins can modulate these molecular movements. These insights will lead the way for future studies aimed at the understanding of how signal-dependent changes in molecular choreography at the immunological synapse translate into specific T cell responses.

REFERENCES


