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Review

T cell antigen receptor activation and actin cytoskeleton remodeling ☆

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ABSTRACT

T cells constitute a crucial arm of the adaptive immune system and their optimal function is required for a healthy immune response. After the initial step of T cell-receptor (TCR) triggering by antigenic peptide complexes on antigen presenting cell (APC), the T cell exhibits extensive cytoskeletal remodeling. This cytoskeletal remodeling leads to the formation of an "immunological synapse" [1] characterized by regulated clustering, segregation and movement of receptors at the interface. Synapse formation regulates T cell activation and response to antigenic peptides and proceeds *via* feedback between actin cytoskeleton and TCR signaling. Actin polymerization participates in various events during the synapse formation, maturation, and eventually its disassembly. There is increasing knowledge about the actin effectors that couple TCR activation to actin rearrangements [2,3], and how defects in these effectors translate into impairment of T cell activation. In this review we aim to summarize and integrate parts of what is currently known about this feedback process. In addition, in light of recent advancements in our understanding of TCR triggering and translocation at the synapse, we speculate on the organizational and functional diversity of microfilament architecture in the T cell. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters.

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1. Introduction

Recognition of antigenic peptides bound to major histocompatibility complex (pMHC) molecules by the TCR is the key step in T cell activation. While as few as 10 pMHC are capable of actuating a T cell response [4], this is only possible due to the concerted action of accessory interactions that mediate adhesion and supplemental signals known collectively as co-stimulation. The T cell must first find the antigenic pMHC-bearing cells through a process of active migration in lymphoid tissues at speeds up to several cell diameters per minute. This process of pMHC sampling relies heavily on lamellipodial dynamics of the T cell. Upon locating antigenic pMHC the T cell must transform rapidly from loosely adherent and highly motile to a tightly adherent and arrested cell in a matter of seconds. This rapid change demands determinative cytoskeletal alterations, achieved *via* major actin and microtubule remodeling. Once activated, TCR signaling induces actin polymerization, which then feeds back for optimal TCR triggering and T cell–APC contact expansion, generating a stable interface or immunological synapse. Actin dynamics has been envisaged to participate extensively from the very first step of TCR triggering to the completion of a successful activation cycle, serving as highly versatile machinery. Initial TCR ligation sets up robust signaling cascades to achieve actin polymerization, rearrangement and dynamics. Diverse sets of molecules including TCR-associated kinases, GTPases and adaptor molecules orchestrate this process. The role and regulation of filamentous actin (F-actin) at the T cell synapse has been a focus of extensive investigation for over a decade. Ectopic expression studies, genetic lesions affecting F-actin integrity, as well as advent of better visualization tools have greatly enhanced our knowledge of molecular regulation of actin polymerization at the synapse. There are many reviews summarizing the role of actin effectors in T cell development, differentiation and activation [3,5–9], as well as several recent insights into the signaling modules that could selectively regulate the actin architectures. In this review we will discuss the diversity of TCR-responsive molecular regulators and potential role in specific steps of T cell activation. Although we are far from understanding the precise spatio-temporal events occurring between TCR activation and establishment of mature synaptic actin cytoskeleton, recent findings provide significant clues towards a better understanding of the structural and functional heterogeneity within F-actin at the synapse.

2. Tools to study actin in T cell activation

2.1. Perturbation tools

Cell-permeable pharmacological reagents can be used to perturb F-actin and its formation. Latrunculin A binds G-actin monomers and prevents polymerization and cytochalasin D (cytoD) results in an increase in ADP-bound G-actin through acceleration of ATP hydrolysis in actin dimers. Both of the drugs lead to F-actin depolymerization by reducing the amount of ATP-bound G-actin that can be added to filaments after an initial increase in F-actin. In addition, Jasplakinolide causes stabilization of existing filaments. As discussed in the later sections, initial insights into the role of F-actin in T cell activation were deduced using these actin-targeting drugs. Since the mechanism of action of these inhibitors on F-actin is well characterized, these were also employed to discern between actin depolymerization *vs.* polymerization regulating TCR microcluster movement at the synapse [10–13]. The small molecule inhibitor of actin motor myosin activity, blebbistatin, has been utilized to study myosin-mediated actin rearrangements upon T cell activation [14]. Another class of inhibitors reported recently, Arp2/3 and formin inhibitors [15,16], target these specific actin nucleation factors selectively. Although not yet utilized to investigate actin dynamics in T cells, these are powerful tools to dissect the molecular machinery responsible for actin polymerization at the synapse. Further understanding of the molecular regulation of actin polymerization was deduced using genetic tools. These studies involved the analysis of T cells derived from gene targeted mice, siRNA-mediated silencing, or ectopic expression of dominant negative proteins. In addition, human T cells derived from patients with immunodeficiencies such as Wiskott–Aldrich syndrome (WAS) [17] and a subset of common variable immunodeficiency [18], that have defects in the actin-regulatory proteins WASP and Vav1, respectively, have provided significant clues into the regulation of actin polymerization at the synapse, and its consequence in T cell activation (see Section 4).

2.2. Visualization tools

Much of the studies investigating F-actin at the synapse in fixed T cells have been carried out using fluorescently labeled fungal toxin phalloidin. Phalloidin labels F-actin in fixed cells, enhancing the contrast of microfilament detection even in the presence of large amounts of G-actin. Actin has been monitored in live Jurkat T cells following transfection with DNA constructs encoding β-actin tagged with green fluorescent protein (GFP) [19]. However, the actin-GFP system can lack contrast due to the equivalent detection of F-actin and G-actin. Two F-actin reporters that can be used in live cells have been established as useful tools. A 17 amino acid peptide derived from actin crosslinking protein ABP120, referred to as LifeAct, binds to F-actin selectively and with sufficiently low affinity that it appears not to affect F-actin while allowing visualization with excellent contrast. LifeAct has been utilized to visualize F-actin in live T cells [20]. The actin-targeting domain (residues 9–52) of the enzyme inositol trisphosphate 3-kinase, referred to as F-tractin, also binds F-actin with low affinity [10,21], enabling visualization of the native F-actin in live cells when fused to a monomeric fluorescent protein. In addition to reporters, the development of advanced microscopy methodology (reviewed in [22]), analysis and tracking tools, and use of reconstituted systems (as discussed in Section 2.3) have greatly enhanced the knowledge of actin distribution and dynamics at the T cell synapse.

2.3. T cell activation systems

A variety of T cell activation tools have been used in studies of F-actin at the synapse. For a quantitative estimate of actin polymerization at the synapse, in initial studies T cell–APC conjugates were fixed and labeled with phalloidin and analyzed using flow cytometry or confocal microscopy. However, in this setup the details of F-actin distribution in the plane of synapse and underlying qualitative features were missing. To overcome these limitations and visualize F-actin in greater detail, a range of reconstituted planar TCR activation systems have been developed. These include glass coverslips coated with anti-CD3 antibody [23] and supported lipid bilayers (SLB) reconstituted with an adhesion molecule like ICAM-1 plus antigenic pMHC or with anti-CD3 antibody [1]. Lipid anchored molecules in SLB are laterally mobile, which enables the translocation of ligated protein clusters and the formation of supramolecular synaptic patterns. Apart from providing a planar synapse orientation for better visualization, these systems also allow the assessment of the effect of individual T cell surface receptors in isolation and the control of antigen density for TCR triggering. SLB interspersed with nano-scale chromium barriers enables the creation of geometrically repatterned immunological synapses [24]. When T cells are incubated with these surfaces, the flow of the pMHC–TCR complex is discontinuous within the cells and is blocked at the diffusion barriers [25]. This system has been utilized to study the coupling between F-actin, in the cytosol, and TCR, at the T cell surface.

Total internal reflection fluorescent (TIRF) microscopy is routinely utilized to study membrane proximal behavior of F-actin at the synapse at cell interfaces with solid phase antibodies or SLB [26,27].

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