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Cell adhesion molecules and actin cytoskeleton at immune synapses and kinapses

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Abstract

The immunological synapse is a stable adhesive junction between a polarized immune effector cell and an antigen-bearing cell. Immunological synapses are often observed to have a striking radial symmetry in the plane of contact with a prominent central cluster of antigen receptors surrounded by concentric rings of adhesion molecules and actin rich projections. There is a striking similarity between the radial zones of the immunological synapse and the dynamic actinomyosin modules employed by migrating cells. Breaking the symmetry of an immunological synapse generates a moving adhesive junction that can be defined as a *kinapse*, which facilitates signal integration by immune cells while moving over the surface of antigen presenting cells.

Keywords

Synapse; T cell receptor; signaling; immunological synapse; actin; myosin

The adaptive immune response is initiated by interactions of T cell antigen receptors (TCR) with surface expressed antigenic complexes (e.g. major histocompatibility complex proteins with bound foreign peptides = MHCp) in a nanometer scale gap between T cells and the antigen presenting cells (APCs) [1]. Since very few MHCp ligands are typically available the sensitive recognition of a few such complexes depends critically upon adhesion molecules- a fact that drove the early description of adhesion receptors in the immune system [2,3]. How has the immune system adapted the basic tools of cell motility and receptor signaling to achieve high sensitivity and fidelity?

Signaling and adhesion structures in T cell activation

TCR signaling is based on a tyrosine kinase cascade that leads to rapid activation of phospholipase C γ [4]. The triggering of the cascade is based on recruitment and activation of Lck. A model incorporating feedback loops involving the activation of the serine/threonine kinase Erk and adapters including Unc 119 or TsAD, which increase activation of Lck in a agonist MHCp dependent fashion, and SHP-1, which in recruited in place of ZAP-70 to partially phosphorylated TCR and negatively regulates Lck, can account for the ability of TCR to kinetically discriminate agonist and antagonist MHCp [5]. This is a self limited process since

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components of the TCR signaling complex are ubiquitinated and are degraded in multivesicular bodies following a period of signaling [6]. Therefore, the manner in which TCR signaling is sustained seems to involve continuous re-engagement of TCR by small numbers of MHCp.

Members of the integrin and immunoglobulin families mediate T cell adhesion to APCs [2]. Costimulatory molecules also are configured as adhesion molecules and the line between adhesion and costimulation molecules is often blurry [7,8]. By definition, adhesion enhances the physical interaction of T cells with APC and the interaction of TCR and MHCp, while costimulation enhances TCR signaling or produces independent signals that integrate with the TCR signal to influence T cell activation. However, the major T cell adhesion molecules have co-stimulatory activity.

LFA-1 is the major integrin on T cells that mediates adhesion by binding to ICAMs [2,9]. These interactions increase sensitivity to MHCp 100-fold [10]. In addition to mediating adhesion, LFA-1 contributes to a characteristic organization of the actin cytoskeleton [11] and provides signals that enhance Ca^{2+} [12], and MAPK pathway activation [13]. Ras activation can take place on the Golgi apparatus and/or at the plasma membrane [14]. In mature T cells, TCR stimulation alone results in Ras activation on the Golgi apparatus only, whereas the combination of TCR and LFA-1 engagement results in Ras activation at the plasma membrane [13]. LFA-1 engagement by ICAM-1 was required for phospholipase D2 activation leading to generation of phosphatidic acid. Phosphatidic acid phosphatase was then required to generate diacylglycerol, which activated RasGRP at the plasma membrane. This is the first detailed mechanism that differentiates Ras activation at the plasma membrane and Golgi apparatus. Thus, spatial integration of TCR and LFA-1 signaling is likely to be a critical function in the IS.

The CD2-CD58 (LFA-2-LFA-3) system was the first cell-cell adhesion system for which direct receptor ligand interactions were established [15,16]. Purified CD58 was reconstituted in supported planar bilayers (SPB) that are formed from phospholipid liposomes on clean glass coverslips [17]. Transmembrane proteins in SPBs are laterally immobile, whereas a naturally GPI anchored form of CD58 are laterally mobile, an observation that paved the way to using SPB to reconstitute T cell activation [3,18]. CD2 augments T cell sensitivity to MHCp, particularly under conditions where CD28 is absent [19]. CD2 contributes to T cell signaling via multiple polyproline motifs that activate kinases like Fyn. CD2 also contributes to generation of TCR triggered microdomains in the membrane that recruit signaling molecules like Lck and LAT [20].

A striking feature of T cell-APC interface is the segregation of different receptor-ligand interactions. Springer predicted this phenomenon based on the different sizes of TCR-MHCp (~15 nm) and LFA-1-ICAM-1 (~40 nm) interactions [2]. Images of fixed cell-cell conjugates demonstrated a bull's eye pattern in which TCR-MHCp interactions formed a large central cluster 2-3 μm across surrounded by a ring of LFA-1-ICAM-1 interactions that is also 2-3 μm thick [21]. Kupfer and colleagues labeled these domains and central and peripheral supramolecular activation clusters abbreviated cSMAC and pSMAC, respectively [21]. Subsequently, an actin and CD45 rich ring outside the pSMAC, called the dSMAC, was defined [22]. A fourth compartment, called the distal pole complex, has also been described that contains PKC ζ , an important polarity regulating kinase [23]. Parallel studies with T cell adhesion to SPBs containing purified CD58 and ICAM-1 revealed patterns of submicron segregation that could be organized into larger concentric domains dependent upon interactions with the cytoskeleton via CD2AP [24]. Based on these studies the term immunological synapse was applied to the stable radially symmetric adhesive interfaces. Synapse is defined here as fastening (to fasten= haptein to -apse) together in place (together = syn-).

Continuous formation of TCR microclusters in periphery sustains signaling

TCR signaling is initiated prior to cSMAC formation [25,26]. The formation of the cSMAC takes place through convergence of TCR clusters that form during initial contact formation and are actively transported to the center [26]. Application of total internal reflection fluorescence microscopy (TIRFM) to the IS led to a striking discovery that this centripetal transport of TCR clusters is continuous during T cell activation [26-28]. Microclusters form at the outer edge of the IS and move at 1 $\mu\text{m}/\text{min}$ inward that that each microcluster lasts about 2 minutes prior to encountering the cSMAC [27]. Staining for active kinases and time resolved imaging of signaling after blocking new microcluster formation demonstrate that microclusters are the structures that sustain signaling [26,27]. What drive microcluster movement?

Relationship of SMACs to cell motility modules

Fibroblast spreading on fibronectin-coated glass has revealed the modular nature of cell migration [29]. The rapid phase of contact expansion is driven by actin polymerization at the outer edge while forming a radially symmetric sheet of f-actin close to the contact surface. Once the contact area reaches its maximal size the f-actin layer continues to undergo polymerization at the edge and this results in centripetal actin flow. At this stage a new f-actin layer forms on top of the first one at the leading edge and this pushes the edge outward by 2-3 microns before activation of myosin II at the back of this layer resulting in retraction. The contraction of this upper layer causes the lower layer to buckle and nascent integrin mediated adhesion are stabilized during this contraction event, perhaps in response to force mediated signaling. These “contractile oscillations” are observed in insect cells, mouse fibroblasts and in the immunological synapse [30] (Figure 1B). The lower actin layer corresponds to the lamella where as the upper layer combined with this lower layer defines the lamellapodium (Figure 1B). The lamellar actin layer contains more tropomyosin and talin, whereas the upper layer of lamellapodium actin contains more cofilin [31,32]. Talin is a classical marker of the pSMAC, suggesting that the pSMAC corresponds to the lamella. Tropomyosin is also stained in the pSMAC [33]. The outer edge of the synapse, in which TCR microclusters form, is rich in cofilin [33]. A striking feature of this system is that during contractile oscillations the two layers in the lamellapodium protrude together, but signaling at the back of the lamellapodium selectively contracts that top layer [29]. Microclusters of LFA-1 or TCR that have engaged ICAM-1 or pMHC at the tip of the protruding lamellapodium are subjected to lateral and vertical forces as the bottom layer is pulled back and up by the top layer. Sheetz and colleagues have suggested that these lateral and vertical forces trigger mechanotransduction, for example, by Cas unfolding to reveal new src family kinase phosphorylation sites [34]. It has also been observed that T cells adhering to round B cells actively form a flat cell-cell interface, which is consistent with the application of vertical forces by the T cell lamellapodium/dSMAC. Thus, we would suggest that the immunological synapse is composed of common cytoskeletal modules, which are adapted by T cells for signal/adhesion integration (Figure 1A).

The immunological synapse achieves stability by taking a dynamic, migration/force-sensing module and using symmetry to cancel motile forces. T cells can use symmetry breaking to regulate migration during antigen recognition. Wild type naive T cells on SPB containing ICAM-1, CD80 and agonist MHCp initially form symmetric synapses, but then periodically break and reform symmetry of the pSMAC to allow periods of stability and migration during antigen recognition [33]. PKC θ is required for symmetry breaking, while WASp is required to restore symmetry in a migrating T cell. T cells appear to efficiently integrate signals during migration by forming microclusters in the leading lamellapodium (R. Varma and MLD, unpublished observations). Recently we have observed that T cell integrate tolerogenic signals from dendritic cells bearing low potency MHCp ligands without any reduction in migration velocity, which in vivo is $\sim 10 \mu\text{m}/\text{min}$ [35]. Therefore, there are two distinct types of contact

areas through which T cells can integrate signals that are distinguished by the presence or absence of symmetry in the dynamic f-actin system.

Synapse is a good description of symmetric junctions that are stable, but not the asymmetric junctions that move. I suggest that the later polarized T cell-antigen presenting cell junction could be described as a *kinapse*- combining roots indicating movement (kin- from *kinesis*) and fastening (-apse from *haptein*). A kinapse has a leading lamellipodium in which TCR microclusters can form, an integrin rich lamella in which TCR microclusters and integrin clusters intermix and a trailing uropod in which adhesion and TCR structures disengage as the T cell moves (Figure 1A). This is qualitatively different from the synapse in which TCR accumulate in the cSMAC. Interestingly, some uropod associated molecules appear to segregated to either the cSMAC or the distal pole complex. This segregation may be an important distinction between a synapse and kinapse. Thus, we anticipate that there will be quantitative and qualitative differences in signal integration by kinapses and synapses. While synapses are symmetrical in the contact plane, they have strong polarity or the microtubule cytoskeleton perpendicular to the contact plane, which may be critical for asymmetric cell division in T cells (Figure 1A). In contrast, the kinapse has both its actin motility machinery and microtubule cytoskeleton co-polarized parallel to the cell-cell junction. This has implications for asymmetry of early cell divisions and may link early synapse stability to T cell differentiation [36,37].

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Figure 1. Schematic of actin cytoskeleton modules active in immunological synapse and kinapse. A. Schematic of Immunological synapse and kinapse emphasizing actin dynamic actin layers. Red- bottom “lamellar” actin layer; green- top “lamellipodial” actin layer; yellow- Golgi; blue and purple circles- exocytic and recycling vesicles; black lines-microtubules. In the synapse the dynamic actin structures are radially symmetric whereas in the kinapse the symmetry is broken and the cell migrates in the direction of the arrow. B. Schematic based on Giannone et al [29] that shows two outcomes of contractile oscillations cycles. From the top: The lamellipodial protrusion is mediated by polymer growth in both the bottom and top actin layers. Myosin II activation in the top layer mediated contraction of the lamellipodium which either buckles the bottom layer or lifts the lamellipodium if the APC membrane is flexible. Either way TCR-pMHC clusters forming during lamellipodial extension as subjected to forces that may be important for signal transduction.