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T cell–dendritic cell immunological synapses

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Dendritic cells (DCs) are myeloid lineage cells that are imprinted by their environment and that mature in response to microbial products. A crucial role of the DC is to impart this context-specific information to T cells as well as to present self and foreign MHC–peptide complexes through formation of an immunological synapse. The structure of the T cell–DC immunological synapse departs from the canonical structure formed with B cells or with supported planar bilayers in that it has multiple foci of T-cell receptor interactions rather than a central focus. Recent studies on model systems provide insight into the mechanisms and biological consequences of the unique T cell–DC synaptic patterns.

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Introduction

The partnership between dendritic cells (DCs) and T lymphocytes (T cells) defends the body against viruses, bacterial pathogens, eukaryotic parasites and abnormal host cells [1,2]. DCs develop from myeloid precursors that enter the tissues and are then exposed to tissue-specific factors that influence differentiation and synergize with pathogen-derived innate signals during maturation. Tissue-derived DCs can then direct the development of antigen-specific T cells to bias them towards the appropriate tissue homing pattern and effector phenotype [3]. This communication takes place across the immunological synapse (IS) between the T cells and DCs — a specialized cell–cell adhesive junction characterized by stability and directed secretion [4]. The structure of the T cell–DC IS has been a matter of controversy, and evidence is emerging that it departs from the bull's eye pattern established for T cell–B cell and T cell–planar bilayer interactions. It has been proposed that the DC cytoskeleton plays an active role in shaping the T cell–DC immunological synapse, and there is direct evidence

that the antigen-presenting cell (APC) cytoskeleton can modify the location of key molecules. These recent findings require a broadening of the definition of the IS and a consideration of mechanisms and biological outcomes associated with different synaptic patterns.

In this commentary we will discuss recent discoveries regarding sustained signaling in the immunological synapse and relate these to recent findings on T cell–DC synaptic patterns.

New model for sustained signaling through the immunological synapse

The initial conception that the IS formed as a stable structure to sustain signaling has evolved into a more dynamic concept. The classical definition of the IS is based on a cell–cell interface in which leukocyte function-associated antigen 1 (LFA-1)–intercellular adhesion molecule 1 (ICAM-1) interactions and talin form a ring around a central cluster of TCR–MHC_p (T-cell receptor–major histocompatibility complex molecule–peptide complex) interactions and protein kinase C- θ [5–7]. These structures were defined as supramolecular activation clusters (SMACs). The TCR cluster marked the central (c)SMAC, whereas the LFA-1 ring marked the peripheral (p)SMAC. Correlative evidence was provided that TCR signaling was initiated and sustained by the cSMAC [5,7].

The cSMAC was later shown to form by convergence of TCR microclusters formed in the periphery of the nascent synapse in a co-receptor-dependent process [8–10]. TCR signaling is initiated before the cSMAC forms [8,9,11] and T cells can be fully activated without appearing to form a cSMAC [12,13]. Furthermore, the cSMAC has relatively low levels of phosphotyrosine, activated phospho-Lck 394 or activated phospho-zeta-associated protein (ZAP)-70 319 after 5 minutes, all of which are located in the periphery of the IS [11,12,14]. Early (\sim 30 s) TCR microclusters contain up to \sim 150 TCRs each [15^{**}], which are readily detectable by wide-field and confocal fluorescence microscopy. However, after the cSMAC forms at 5 minutes, the TCR microclusters that are subsequently generated contain in the range of 11–17 TCRs each and can only be detected by low background total internal reflection fluorescence microscopy [15^{**},16^{**}]. New TCR microclusters form continuously in the periphery of the IS and move toward the cSMAC at a rate of 1–2 μ m/min. The role of sustained TCR microcluster formation in signaling was supported by the demonstration that peripheral microclusters were stained with anti-phosphotyrosine, anti-phospho-Lck 394 and anti-phospho-ZAP-70 319 antibodies and they

recruit ZAP-70–green fluorescent protein (GFP) and Src homology 2 domain containing linker protein (SLP)-76–GFP [15^{••},16^{••}] (see supplementary material online for movie and legend). This result is significant as it shows that TCR microclusters concentrate the activated kinases and adapter proteins emblematic of early TCR signaling. Elimination of the TCR microclusters, but not of the cSMAC, correlates temporally with loss of Ca²⁺ signaling [17^{••}]. The cSMAC accumulates lysobisphosphatidic acid, which indicates that it is a site of TCR sorting for degradation [17^{••},18].

This picture of a compartmentalized IS that has signaling microclusters and sorting cSMACs is clearest for the T cell–supported planar bilayer IS; the precise relationship of this to the different types of T cell–APC IS has not yet been explored. The current understanding of the T cell–planar bilayer model system predicts the presence of a dynamic T cell–APC IS in which signaling is sustained by continuous formation of TCR microclusters.

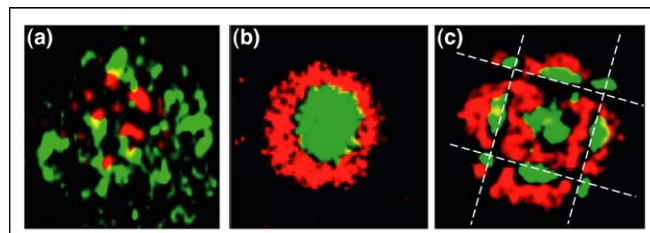
The dendritic cell immunological synapse

It is risky to generalize about the T cell–DC IS based on the relatively small number of experiments that have been performed with cultured or primary DCs. The most interesting distinctions between T cell–DC IS and the more intensively studied T cell–B cell and T cell–planar bilayer IS to date are the important functional contributions of the DC cytoskeleton to T cell activation and the distinctive pattern of the DC IS. Treatment of DCs, but not of B cells, with cytochalasin D impairs their ability to present antigen to T cells [19]. Although fluorescence studies show that the T cell–DC IS may sometimes form with a clear cSMAC and pSMAC [11,20], this has been a controversial claim and some of the same authors have presented evidence that DCs preferentially form multifocal IS [21^{••}]. This latter conclusion was based on comparison of the T cell–B cell IS with the T cell–DC IS by electronic microscopy; these results are consistent

with a large central close contact for the T cell–B cell IS, but tens of smaller close contacts for the T cell–DC IS. It is tempting to relate the multifocal DC IS to the dynamic TCR microclusters observed with the supported planar bilayers [22]. However, the fluorescence images of T cells with primary DCs reveal a multifocal pattern, in which the TCR clusters are much larger than 11–17 TCR (Figure 1a) (SYT and MLD, unpublished). Mechanistic insights into the origin of this T cell–DC pattern might be gained from recent studies with other adherent APCs and nanopatterned supported planar bilayers.

The APC cytoskeleton has been proposed to play a role in optimal CD28-mediated costimulatory signaling through interactions with the cytoplasmic domain of CD80 [23,24]. When full-length and cytoplasmic tail deleted CD80 are expressed in CHO cells along with I-E^k for antigen presentation to naïve CD4⁺ T cells it is revealed that the cytoplasmic domain of CD80 mediates segregation of CD28–CD80 interactions from regions of TCR–MHCp interactions [25^{••}]. This micron-scale spatial segregation is correlated with increased IL-2 production in responding T cells. The sites within CD80 that are important for the functional effects include a binding site for an ezrin-radixin-moesin family member and a putative phosphorylation site [24]. Although we have not performed experiments with cytoplasmic domain deleted forms of CD80 in DCs, we have observed that TCR–MHCp and CD28–CD80 clusters (and the associated protein kinase C- θ) are segregated in the multifocal T cell–primary DC IS (Figure 1a, red). Experiments with a supported planar bilayer suggest that T cells naturally form a cSMAC/pSMAC IS when there are no cytoskeletal interactions to influence movement of molecules in the APC (Figure 1b). The formation of multifocal patterns of TCR–MHCp interactions and the segregation of TCR–MHCp from CD28–CD80 interactions suggest that there are selective barriers to MHCp and CD80 transport that act in the DC.

Figure 1



IS patterns in T cell–DC and model systems. **(a)** Fluorescence view of fixed T cell–DC IS, in which TCR is labeled green and PKC θ is labeled red. PKC- θ colocalizes with CD28 clusters. A multifocal TCR cluster pattern can be seen. The TCR clusters each contain on the order of 100 TCRs. **(b)** IS formed by T cells and supported planar bilayers that lack any diffusion barriers. TCR is labeled green and ICAM-1 is labeled red. Note the well-formed cSMAC and pSMAC. **(c)** IS formed on by a T cell on a patterned planar bilayer in which chrome lines (dashed lines) disrupt diffusion of agonist MHCp and ICAM-1 in the bilayer. TCR is green and ICAM-1 is red. Note that the pSMAC still forms in a relatively normal manner, but eight additional large TCR clusters are formed in addition to the cSMAC. (b) and (c) are adapted with permission from [26^{••}]. The scaling of the TCR clusters formed on this patterned bilayer is similar to the multifocal TCR clusters formed with a DC. This suggests that some kind of diffusion barrier imposed by the DC might be involved in generating the multifocal pattern.

Spatial mutations induced by nanopatterned bilayers

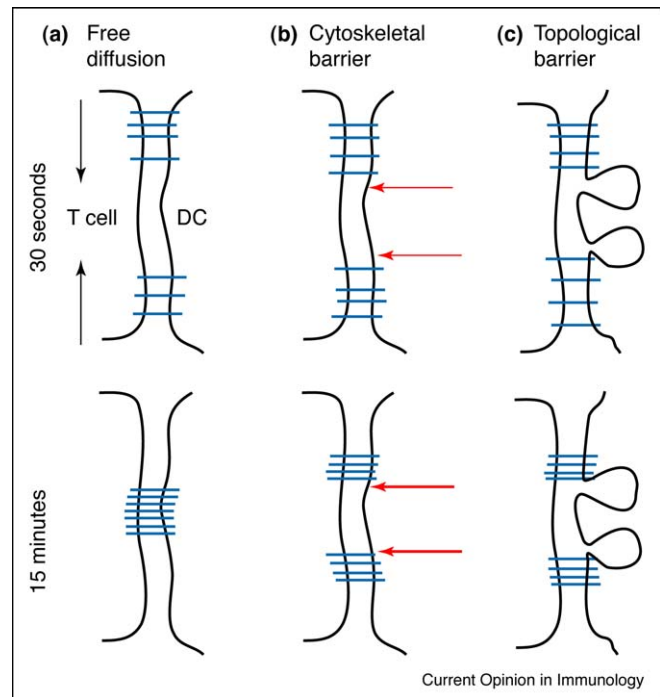
A model for simple non-selective cytoskeletal barriers has been developed in the supported planar bilayer system based on using electron beam lithography to write chrome lines 100 nm wide and 5 nm high on glass substrates [26^{••}]. These lines can be formed in any pattern, and they disrupt the planar bilayer to generate non-selective barriers to diffusion and transport of MHCp and ICAM-1. These barriers disrupt the movement of TCR microclusters in the plane of the T-cell membrane such that multifocal patterns can be elicited from T cells. It was found that these enhance signaling by preventing transport of TCR to the cSMAC compartment [26^{••}].

The appearance of patterns formed on a $2\ \mu\text{m} \times 2\ \mu\text{m}$ spaced square array of lines (Figure 1c, green) shows a striking similarity to the multifocal patterns of TCR–MHCp clustering in the T cell–DC interface (Figure 1a, green). This simple experiment, together with other studies on microclusters, suggests a number of interesting characteristics of TCR clusters in the IS. First, that TCR clusters and the precursor microclusters are too stable to disengage MHCp when they encounter a barrier. In contrast, LFA-1–ICAM-1 cluster transport does not appear to be impeded by the barriers, perhaps because the individual clusters are more dynamic. Second, that the location of TCR clusters is important, with more active signaling in the periphery of the IS. TCR clusters thus have stable and dynamic characteristics. They are dynamic in that microclusters form continuously and feed into larger clusters, but they are stable in that once formed they do not readily dissociate, but appear to terminate activity by joining larger clusters from which they can be sorted by mechanisms involving lysobisphosphatidic acid.

How does the dendritic cell cytoskeleton regulate the immunological synapse?

Based on our understanding of TCR microcluster dynamics and stability we can propose at least two mechanisms by which the APC cytoskeleton could selectively regulate the translocation of receptor clusters in the IS (Figure 2). In the absence of barriers, actin flow on the T cell side of the IS induces transport of TCR clusters to the cSMAC, where their signaling activity is terminated and they can be sorted for degradation (Figure 2a). If the APC responds to clustering of MHCp by forming local interactions between its cytoskeleton and the MHCp clusters it can prevent this translocation and enhance the duration of signaling from the TCR cluster by delaying access to inactivating mechanisms in the cSMAC region (Figure 2b). These interactions might have a selective effect on the molecular clusters depending upon either sequence specificity in the cytoplasmic domains or the kinetics of the extracellular interactions, which allow more dynamic receptor clusters to evade the barriers. Another mechanism to stop transport of clusters is

Figure 2



Possible mechanisms for generation of multifocal IS. Simple schematic in which T cell and DC membranes are indicated by vertical curved lines (T cell on left and DC on right) and TCR–MHCp interactions are indicated by short straight blue horizontal lines that bridge the membranes and extend into the T cell and DC cytoplasm. The black arrows indicate the direction of actin flow in the T cell, which is the same in all cases. (a) The lack of a barrier in DCs allows formation of cSMAC by 15 minutes. This has been observed with some DC preparations. (b) Cytoskeletal barriers (red arrows) in the DC block the translocation of clusters. Although this is possible, additional mechanisms must exist because cytoskeletal poisons did not result in cSMAC with DCs that form multifocal IS. (c) Folding of DC membrane, which might still take place in presence of partial cytoskeletal disruption, could also interrupt TCR cluster transport and result in the generation of multifocal IS. A third mechanism that is not depicted is that signals from the DC to the T cells could disrupt the actin flow pattern in the T cells and remove the drive to form a cSMAC.

topological. If the APC membrane is not flat, but is infolded, then the stable receptor clusters that reach the folds will stop translocating (Figure 2c). This mechanism is non-selective for sequences in the cytoplasmic domain of the APC molecules and would even act on glycosylphosphatidylinositol-anchored ligands. It might be evaded if the receptor clusters are sufficiently dynamic to disengage ligand on one side of the barrier and re-engage them on the other side.

An interesting prediction of this model is that clusters might segregate based on the kinetics of their extracellular interactions. For example, CD28 and cytotoxic T lymphocyte antigen 4 (CTLA-4) both interact with CD80 and CD86, which contain cytoskeletal binding sites in their cytoplasmic domains [24]. Whereas the monovalent

interactions of CTLA-4 and CD28 with CD80 or CD86 are transient, with half-lives of less than a second [27], structural studies and binding studies with multivalent ligands suggest that CTLA-4 can form higher-order structures that are more stable, whereas CD28 can't [28,29]. If clusters that involve both CTLA-4 and CD28 are transported across a barrier based on the interaction of CD80 with the cytoskeleton of the APC, then CTLA-4 might be selectively trapped on the barrier whereas CD28 might be able to disengage in the trapped cluster and form a new cluster in the adjacent membrane. This effect might influence the competition between CD28 and CTLA-4 and provide some advantage to CD28 as long as free ligands are available near the trapping sites in the IS.

Conclusions

The structure of the T cell-DC IS is likely to vary depending upon the origin and activation history of the DC. Therefore, it is too early to generalize about the DC IS. It is known that DCs can form multifocal IS. Parallel studies in model systems show that preventing movement of TCR clusters to the cSMAC might delay inactivation. Furthermore, segregating CD28-CD80 interactions from TCR-MHCp interactions enhances costimulation. These results reinforce the idea that regulation of the DC cytoskeleton is important for IS patterning and function. Another area that has been explored relatively little is the impact of other adhesion and signaling systems on IS organization. For example, chemokines released by DCs might enhance or hinder stable IS formation [30-32]. Integrating these factors in DCs from different anatomical sites will be important to gain a full understanding of the complex partnership between adaptive and innate immunity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.coi.2006.05.017](https://doi.org/10.1016/j.coi.2006.05.017).

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