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Review

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The dendritic cell side of the immunological synapse

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Abstract: Immune responses are initiated by the interactions between antigen-presenting cells (APCs), such as dendritic cells (DCs), with responder cells, such as T cells, via a tight cellular contact interface called the immunological synapse. The immunological synapse is a highly organized subcellular structure that provides a platform for the presentation of antigen in major histocompatibility class I and II complexes (MHC class I and II) on the surface of the APC to receptors on the surface of the responder cells. In T cells, these contacts lead to highly polarized membrane trafficking that results in the local release of lytic granules and in the delivery and recycling of T cell receptors at the immunological synapse. Localized trafficking also occurs at the APC side of the immunological synapse, especially in DCs where antigen loaded in MHC class I and II is presented and cytokines are released specifically at the synapse. Whereas the molecular mechanisms underlying polarized membrane trafficking at the T cell side of the immunological synapse are increasingly well understood, these are still very unclear at the APC side. In this review, we discuss the organization of the APC side of the immunological synapse. We focus on the directional trafficking and release of membrane vesicles carrying MHC molecules and cytokines at the immunological synapses of DCs. We hypothesize that the specific delivery of MHC and the release of cytokines at the immunological

synapse mechanistically resemble that of lytic granule release from T cells.

Keywords: antigen presentation; antigen-presenting cell; dendritic cell; immunological synapse; membrane trafficking; T cell.

Introduction

Immunology is the ecology of human physiology. Where ecologists inventorise ecosystems, immunologists characterize the biodiversity of the lymphocytes that populate our body. Much like animals in a forest, immune cells share a habitat and have symbiotic interactions with each other and with the other cells in our body. Especially interesting is how immune cells respond to disturbances that change these interactions such as infections or cancer (1). Whereas these communications can occur over long distances by means of cytokines and chemokines, in clear analogy with the urine trails laid out by some animals to attract or repel other animals, they can also occur via direct cellular contact by means of immunological synapses. Immunological synapses, a term derived from the morphologically similar neuronal synapse, are tight cellular contact interfaces between antigen presenting cells (APCs) and effector cells [T cells or natural killer cells (NKs)] (2, 3).

Immunological synapses are sites of polarized membrane transport where cytokines are locally released and membrane receptors are locally presented and recycled. Whereas this process is now well studied in T cells and NKs [reviewed in (4–7)], it is still less clear how localized membrane trafficking is regulated in APCs (8). In this review we provide an overview on the molecular cascades that lead to polarized trafficking of cargo molecules to the immunological synapse, with an emphasis on the trafficking events in dendritic cells (DCs). DCs are professional antigen presenting cells that prime naïve CD4⁺ helper and CD8⁺ killer T cells by means of an

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immunological synapse (1, 9). We focus on the mechanisms underlying polarized trafficking of antigen-loaded major histocompatibility complex (MHC) as well as of immunostimulatory cytokines from intracellular compartments to the immunological synapse, as this process is of great importance for the initiation of antigen-specific T cell responses.

Structure of the immunological synapse

Not only DCs and T cells, but also other APCs, such as B cells or infected cells, and other effector cells, such as NKs, form immunological synapses for intercellular communication as well as for the killing of (infected) target cells (6, 10–12). There are thus many different types of immunological synapses and these can be functionally divided into two categories (13). The first category consists of the so-called primary synapses, which are the cell-cell contacts that result in initial activation of T cells such as the synapses between DCs and T cells (8). In the T cell, the signals conferred at such an immunological synapse can ultimately lead to their activation and clonal expansion, depending on the type and activation state of the APC and on the efficiency of signal transduction (14, 15). The second category consists of the so-called secondary synapses that result from interactions established after initial priming such as activated T cells delivering stimulatory signals via, for example, CD40-CD40L interactions to B cells (1). This category also encompasses the synapses formed between NKs or cytotoxic T cells with their target APC where lytic granules are released to kill the APC (16). For both categories, the formation of immunological synapses can trigger intracellular signaling cascades in both the APC and the T cell that lead to reorganization of the cytoskeleton and rerouting of membrane trafficking.

Given the wide functional and structural variety of the different immunological synapses, their supramolecular organization is diverse and depends substantially on the interacting cell types, cellular activation states, as well as on antigen specificity involved (10, 11, 14, 17). Accordingly, an immunological synapse can arrange in a well-structured ‘bulls eye’ monocentric structure or can form more complex and heterogeneous polycentric arrangements. Furthermore, the time duration of cellular engagement varies widely for different synapses and can range from seconds up to several hours (13, 18).

The classical ‘bulls eye’ immunological synapse

Our knowledge of the structure of the immunological synapse was propelled by the functional reconstitution of immunological synapses with planar model membranes as surrogate APC [reviewed in ref. (6, 19, 20)]. With this technique the adhesion molecule ICAM-1 and MHC are functionally reconstituted in well-defined artificial bilayers and this suffices for synapse formation upon contact with T cells or NKs. Because the planar bilayer can be positioned directly on the surface of a microscope glass, this technique allows visualization of the synapse with total internal reflection fluorescence (TIRF) microscopy and other high resolution microscopy techniques. Synapses with APCs can also be visualized with high resolution microscopy approaches, for instance by reorienting these synapses with a micromanipulator parallel to the focal plane of the microscope (21). These and other techniques showed well-defined spatially segregated molecule clusters at the immunological synapse, and it is now well established that the immunological synapse can organize in a ‘bulls eye’ arrangement called the monocentric synapse (19, 22).

The monocentric synapse contains a distinct central region which is called the central supramolecular activation cluster (cSMAC) (22). The cSMAC was initially thought to be the location of T cell signaling due to the presence of the T cell receptor (TCR) at the cSMAC. However, this is no longer believed and the cSMAC is now considered to be a site of signal termination and receptor recycling (23, 24). This conjecture is supported by the finding that dissociation of signaling molecules, such as Lck, ZAP-70 and the adapter LAT, from the TCR microclusters occurs at the cSMAC (25). The peripheral SMAC (pSMAC) surrounds the cSMAC and contains adhesion molecules, such as the β 2-integrin LFA-1 and ICAM-1, on the T cell and APC (19, 22, 26). These adhesion molecules provide a mechanical scaffold for the immunological synapse and connect the plasma membranes and cytoskeletons of the APC and T cell together (27). The pSMAC is in turn surrounded by a more distally located SMAC (dSMAC) containing immune-inhibitory receptors such as CD43 and CD45 (28, 29). The dSMAC also contains microdomains of T cell receptors which (in contrast to cSMAC) are associated with signaling molecules, such as Lck, ZAP-70 and LAT, indicating that receptor signaling primarily occurs in dSMAC (25, 30). In the case of cytotoxic T cells and NK cells, the cSMAC of the monocentric synapse contains a distinct and separated secretory region where release of lytic granules occurs (4, 31).

The molecular cascades that lead to formation of the immunological synapse are well established and elaborately reviewed elsewhere (6, 10–12, 32). Importantly, synapse formation results in the reorientation of the microtubule organizing center (MTOC) towards the immunological synapse. This reorientation facilitates polarized membrane trafficking over microtubules to the synapse (33). The actin cytoskeleton also plays an important role in synapse formation, and retrograde actin transport drives the centripetal motion of many molecules to cSMAC, including that of the T cell receptor [reviewed in (7)]. However, not all molecules present at the immunological synapse move in a centripetal fashion, as the immune-inhibitory protein CD45 moves away from cSMAC within several minutes after synapse formation, which is proposed to promote T cell activation (29). The cSMAC is largely devoid of actin, although a residual cortical actin network may still be present. This actin poor region facilitates the release and recycling of trafficking vesicles (34, 35).

The non-classical multicentric synapse between DCs and T cells

As described above, the structure of immunological synapses strongly depends on the cell types involved, the presence and strength of antigen recognition, and additional co-stimulatory interactions (10, 11, 17). Especially for the immunological synapse between DCs and T cells, there is mounting evidence that the structure is aberrant from the classical monocentric synapse that is observed with other APCs (8). During the past decade, T cell priming by DCs has been studied by multiphoton imaging in explanted lymph nodes and by intravital imaging in live mice (17, 18, 36–40) [reviewed in ref. (41, 42)]. In the absence of cognate antigen, migrating T cells only interact briefly with DCs in lymph nodes (<3 min contact) (37). During this phase, DCs scan thousands of T cells per hour by extending agile dendrites that transiently contact the motile T cells (43), and this type of synapse is often referred to as the ‘immunological kinapse’. Using mouse DCs and a T cell line *in vitro*, it was found that the T cells migrating over the DC surface have different zones: an actin-rich leading zone driving migration, a mid-zone mediating TCR-induced signaling, and a rear uropod mediating MHC-independent signals (44). Upon detection of cognate antigen the contact duration between the DCs and T cells is prolonged but still in the order of minutes (~11–12 min) (18, 37). Provided the antigen dose is sufficiently high, this first phase of T cell priming is followed by a second phase, marked by the

formation of relatively stable clusters of DCs contacting multiple T cells simultaneously (18, 37, 38). This second phase is observed within 3 h after phase 1 (18, 37), although the precise onset depends on the antigen dose and the number of DCs presenting the cognate antigen (40). The DC/T cell clusters during this phase are very stable and can even be isolated from the lymph nodes (45). Although experimentally difficult to estimate (e.g. due to spatial drift of the microscope, photodamage, or migration of the cells away from the field of view), the second phase has an estimated duration of 3–5 h (46) and is followed by a third phase where T cells regain their motility and proliferate (18). Thus, it is now firmly established that T cell priming occurs in three distinct phases where the immunological synapse between DCs and T cells changes consecutively from (i) transient intermittent contacts through (ii) stable clusters to (iii) proliferation of the T cells.

Prolonged DC/T cell contacts are not required for T cell activation, and T cells can already get activated during the first phase of highly transient DC/T cell contacts (18, 40). The second phase of DC/T cell contacts coincides with IL-2 secretion from the T cells (37) and may facilitate development of effector cells and long-lived memory T cells (39, 40). The third phase is required for the development of T follicular helper cells (47). This corresponds with *in vitro* studies showing that activation of helper T cells by DCs does not require formation of a stable synapse, but short and sequential cellular interactions are sufficient for T cell activation (10, 13, 48). These interactions are too transient for complete formation of a well-defined monocentric synapse and the sizes of the cellular interfaces may also be too small to accommodate assembly of c- and p-SMACs (several μm) (37). In addition, DCs can actively prevent formation of a cSMAC by providing mechanical counter forces that keep the immunological synapse in a state where TCRs are not or only partially clustered and their distribution across the synapse is more scattered (10). Here, a multifocal or multicentric immunological synapse can be established where the T cell receptors interact with MHC molecules in multiple dispersed clusters at the interaction zone (49). In this case, the actin cytoskeleton of the DCs is polarized towards the immunological synapse in an antigen-specific manner, and this polarization is required for complete T cell activation (50, 51). Actin can also polarize in DCs upon synapse formation with NKs (52, 53).

A monocentric immunological synapse is not only unnecessary for activation of T cells, but its absence can even promote survival of the DC as cytolytic granules are not released from cytotoxic T cells and NKs (8, 11, 53). The actin cytoskeleton in DCs regulates the lateral mobility of ICAM-1 (but not of MHC class II) and this in turn opposes

forces of LFA-1 on the T cell surface which could inhibit cytolytic granule release (54). Indeed, it was shown that when cSMAC formation was delayed by a reduced lateral mobility of antibodies against CD3 incorporated in bilayers (as surrogate APC), this resulted in less signaling within the T cell (55). DCs not only counteract the centripetal forces conferred by the T cell cytoskeleton, but also receive signals themselves from T cells at the immunological synapse. Engagement of CD40L on the T cell with CD40 on the DC provides a pro-survival signal that protects the DC from undergoing apoptosis via the Akt1 pathway (8, 56). Moreover, the accumulation of tyrosine-phosphorylated proteins and of the lipid phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] at the DC side of the immunological synapse indicates that active signaling occurs within the DC (56, 57). PI(4,5)P₂ has many functions in cellular signaling, cytoskeletal attachment and membrane trafficking (58), and its accumulation correlates with increased DC survival (57). In mouse, formation and stabilization of the immunological synapse as well as functional T cell priming depends on the small GTPases Rac1 and Rac2 (36), the Wiskott-Aldrich syndrome protein WASp (59, 60), and the mammalian diaphanous-related formins mDia (61). All these proteins have well established roles in actin polymerization, which further supports the role of the actin cytoskeleton in synapse formation. Similar to T cells (19, 22, 26), the activation of LFA-1 on the DCs stabilizes the immunological synapse and promotes T cell priming (62).

Nevertheless, it is still controversial whether DCs prevent the formation of a monocentric synapse, and some reports show that DCs and T cells form a monocentric synapse containing MHC molecules and Notch ligands in the cSMAC at the DC side (63). This controversy likely relates to the precise type of immunological synapse formed. Antigen recognition from fully activated DCs by a primary naïve T cell likely results in a different type of synapse than observed with commonly used model systems that rely on immortalized cell lines, exogenously added antigenic peptides, allogenic interactions (T cells and DCs from two different donors) or superantigens (Figure 1). The activation state of the DC is also very important for both the structure and duration of the immunological synapse (14). The type of T cell matters as well, as we observed differences in synapse formation between DCs with a Jurkat T cell line compared to primary CD8⁺ T lymphocytes. Both these T cells formed antigen specific synapses with DCs, as both were transfected with a recombinant TCR recognizing tumor antigen gp100 residues 280–288 (64). The Jurkat cells first spread on the surface of the DC followed by cellular contraction and remained stably attached (>1 h; Figure 1A), which is reminiscent of

the formation of a monocentric synapse (19). Similar morphological steps of spreading followed by contraction of the T cells are frequently observed and well understood at the molecular level [reviewed in (32)]. In contrast, the behavior was completely different with primary T lymphocytes. There the DCs actively moved around the T cell indicating large structural rearrangements within the DCs (Figure 1B). This is in line with the finding that DCs can rearrange their actin cytoskeleton toward T cells in case of antigen recognition (51), but also shows that the type of T cell is a critical factor for eliciting such a rearrangement.

Interestingly, immunological synapses can have a three-dimensional structure. T cells can form pseudopodia that penetrate but do not disrupt the APC, a mechanism that was suggested to extend the contact area to facilitate screening for antigen in MHC molecules (12, 65). In this respect, it was noted that the T cell side of the immunological synapse morphologically and functionally resembles cilia formation [reviewed in ref. (4, 66)]. Similarly, DCs can form extensions called microvilli at the immunological synapse which contain a high density of co-stimulatory molecules and peptide loaded MHC complexes and this may also facilitate T cell activation (67).

Local membrane trafficking at the immunological synapse

Polarized trafficking at the T cell side of the immunological synapse

Membrane trafficking plays an important role in T cell effector functions, because it leads to surface display of TCRs and other membrane proteins, recycling of exhausted receptors, as well as to release of cytokines and chemokines at the immunological synapse. Most studies that deal with the immunological synapse looked at signaling and trafficking of molecules within the T cell. The best understood form of exocytosis at the immunological synapse is the release of cytolytic granules from CD8⁺ T-cells and NKs. However, other types of cargo also undergo polarized membrane trafficking at the T cell side of the immunological synapse. For example, cytokines (e.g. IFN γ) and membrane receptors (TCR, ICAM-1) are delivered and/or recycled at the synapse (4, 6, 12, 26, 66, 68, 69). The polarized delivery of these molecules to the immunological synapse allows a more sensitive antigen presentation and/or promotes T cell effector functions, while preventing unwanted activation of other (immune) cells nearby. Membrane trafficking is well studied for the

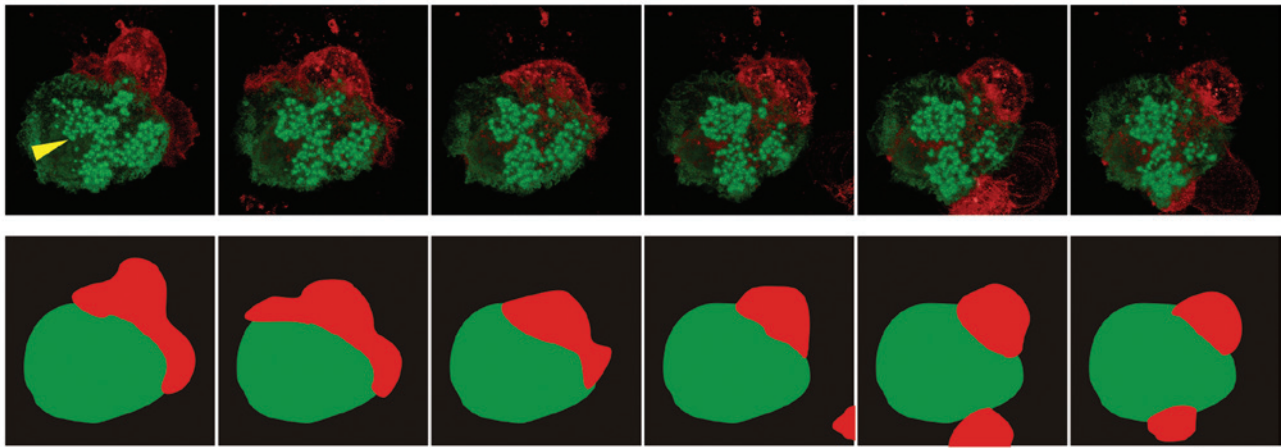
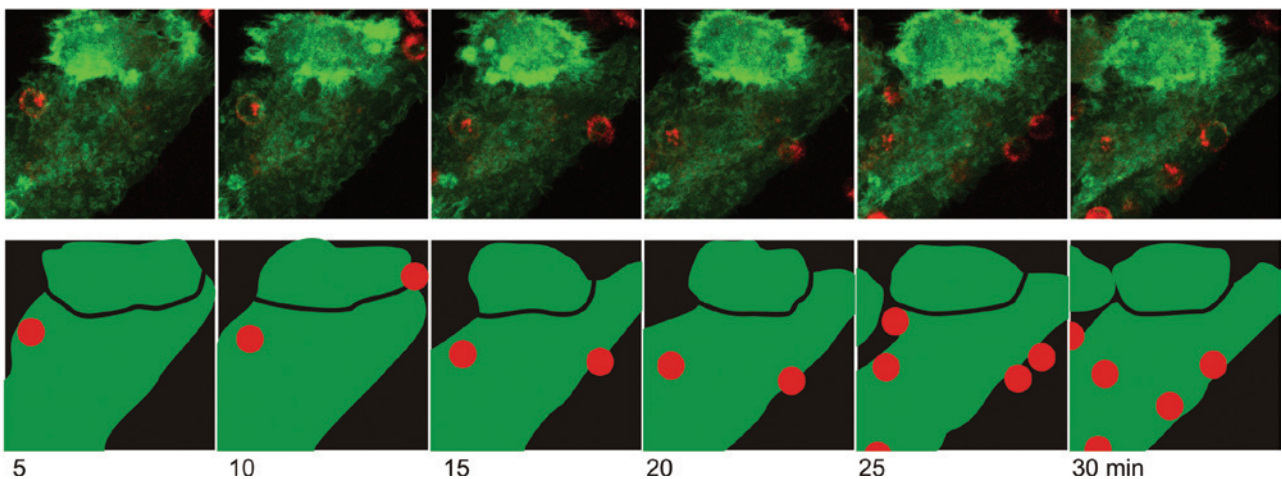
A DC–Jurkat T cell**B** DC–CTL

Figure 1: Structural differences in DC immunological synapses with Jurkat or primary T cells.

(A) An antigen specific synapse between a Jurkat T cell (red) and a DC differentiated from peripheral blood mononuclear cells (green). Jurkat T cells were heterologously expressing a TCR recognizing a tumor antigenic peptide (gp100 residues 280–288) (64). DCs were expressing the actin binding protein LifeAct fused to GFP (green), as described (108), and were loaded with an excess of antigenic gp100 peptide. The Jurkat T cells were labeled with a far-red fluorescent membrane marker (red). The bright F-actin rich structures in the DC are podosomes (marked with a yellow arrowhead for $t=5$ min) (108). The upper row shows z-projections of confocal stacks recorded at the indicated time points after T cell addition. The lower row shows the schematic outline of the cells. Note that the Jurkat T cell first spreads out covering a large area of the DC surface and this is followed by cellular contraction. (B) Same as panel A, but now with primary CD8⁺ T cells transfected with the same TCR recognizing tumor antigen from gp100 (64). Primary T cells are much smaller (~5–10 μm diameter) than Jurkat T cells (20–50 μm diameter). Note that with primary T cells, the DCs (but not the T cells) show large structural deformations and cover large areas of the surface of the T cells. Scale bar: 20 μm .

classical ‘bull’s eye’ immunological synapse, where the TCR reaches the plasma membrane at the dSMAC and gets internalized for degradation and recycling at the cSMAC (23–25, 30). At the secretory synapse of CD8⁺ T-cells and NKs, cytolytic granules are released at the cSMAC [reviewed in (4, 10, 32, 70)]. Thus, endocytosis and exocytosis in T cells can be spatially restrained to discrete areas of the immunological synapse.

The mechanism of release of cytotoxic granules at the immunological synapse from CD8⁺ killer T cells and NKs

towards the target cell is now well understood. Secretory vesicles are transported via the microtubular network by motor proteins towards the cSMAC of the cell membrane (33). This is followed by fusion of the vesicles with the plasma membrane, a process which is remarkably similar to neurotransmitter release from neurons and neuroendocrine cells [reviewed in ref. (5)]. Indeed, several studies have identified proteins participating in lytic granule release with functional and structural homology to those involved in neurotransmitter release. Examples include

the C2-type calcium sensor synaptotagmin-7 (71), which is a close homolog of the main calcium-trigger for neurotransmitter release synaptotagmin-1 (72), and the docking/tethering proteins Munc18-2, Munc13-1 and Munc13-4 (73–76), which are homologous or identical to the tethering/docking factors of synaptic vesicles Munc18-1 and Munc13-1 (72). The similarity of lytic granule release with neurotransmitter release is even more apparent from the recent finding that the final membrane fusion step of lytic granule release is catalyzed by the SNARE protein VAMP2 (77). This is remarkable, because VAMP2, also called synaptobrevin-2, is very well known as the R-SNARE for neurotransmitter release (72). The role of VAMP2 in lytic granule release was previously missed because of the embryonically lethal phenotype of the VAMP2 knockout mouse (due to neurological defects) and because of the very low and transient expression of VAMP2 in cytotoxic T cells. This finding answers the long standing question of which R-SNARE catalyzes lytic granule release (5, 77, 78).

It is also known how the TCR reaches the dSMAC of the immunological synapse. The TCR traffics via recycling endosomal compartments that polarize towards the immunological synapse (66, 68). Membrane fusion is catalyzed by the SNARE proteins SNAP-23 and syntaxin-4 which cluster in the plasma membrane and likely interact with the SNARE protein VAMP3 in the TCR-containing trafficking vesicle (68, 79). Similar to VAMP2 described above, these SNAREs also have well-known roles outside the immune system. Syntaxin-4 and SNAP-23 catalyze constitutive exocytosis in many different cell types, but also catalyze forms of evoked release such as insulin secretion by β -cells and surface display of GLUT4 by adipocytes (80). Moreover, release of vesicular compartments containing the TCR subunit ζ and the adapter protein LAT at the immunological synapse depends on synaptotagmin-7 (69), which also plays a role in the delivery of lytic granules at the synapse (71). Thus, immunological synapses not only resemble neurological synapses morphologically (3), but also contain a similar or even identical protein machinery. Additionally, the formation of immunological synapses (and of neurological synapses) structurally and morphologically resembles ciliogenesis (4, 66), with the small GTPase Rab8 being involved in both processes (79).

Polarized trafficking at the DC side of the immunological synapse

Although much less studied than the T cell side of the immunological synapse, polarized membrane trafficking occurs at the APC side as well. In DCs, MHC class I and II

(81–84) and the costimulatory molecule CD40 (85) can be locally trafficked to and presented at the immunological synapse. The local release of these molecules improves the efficiency of T cell activation and helps to explain how T-cells can detect as few as a handful [or even a single (86)] of MHC ligands among an abundance of endogenous peptide-bound MHC [reviewed in ref. (12)]. In addition, IL-12 is also locally released by the DC at the immunological synapse with T cells (87). IL-12 promotes a T helper 1 response, enhances the cytolytic activity of CD8⁺ T cells and induces production of IFN- γ by T-cells. The polarized release of IL-12 was also observed at the immunological synapse between DCs and NKs (52, 53).

The intracellular sorting and trafficking of MHC class II in DCs is well understood [reviewed in (88–90)]. After assembly in the ER and Golgi, MHC class II bound to the inactivating Li fragment traffics to the plasma membrane. It then reaches the antigen processing compartment by endocytosis. In this compartment, which is of endosomal/lysosomal nature and is called MIIC (MHC class II-containing compartment), MHC class II is activated by proteolytic degradation of the Li fragment and exchange with an antigenic peptide. In immature (i.e. inactivated) DCs from mice, a large fraction of MHC class II remains in MIIC. Upon DC maturation by cytokines and/or recognition of pathogens, tubular vesicles extend from MIIC towards the plasma membrane (83, 91–93). Vesicles bud off from these tubules for membrane fusion with the plasma membrane (91). Pathogen recognition by Toll-like receptors, such as TLR4, promotes tubulation and this process may (83) or may not (94) depend on the adapter protein MyD88. TLR-stimulated tubulation further requires the small GTPases Rab7 and Arl8b, as well as the effector proteins RILP, FYCO1 and SKIP, at least in macrophages (95). TLR induced tubulation of late endosomal compartments can also be observed in human DCs derived from blood monocytes (i.e. a commonly used DC model system) (84), but here a large fraction of peptide loaded MHC class II resides on the plasma membrane already in the immature state (Figure 2). Upon formation of an antigen-specific immunological synapse, the MIIC tubules can orientate selectively towards the immunological synapse in mouse DCs (81–83). This polarized MIIC tubulation is dependent on the clustering of signaling proteins, such as TCR and CD4, on the T cell surface and MHC class II on the DC surface, but also on the engagement of adhesion molecules such as LFA-1 with ICAMs (82).

Similar to the T cell side of the immunological synapse, the cytoskeleton plays a major role in the polarized trafficking of molecules to the DC side. Upon formation of an immunological synapse, the MTOC in DCs can reorient

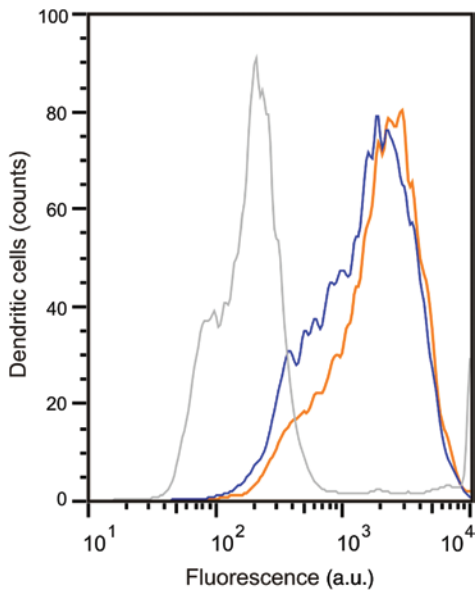


Figure 2: Surface display of peptide loaded MHC class II by both immature and mature human monocyte-derived DCs. Histogram with FACS data of MHC class II surface staining of DCs differentiated from peripheral blood mononuclear cells (with PE labeled antibody L243). Blue curve: inactivated DCs (immature). Orange curve: DCs stimulated overnight with $1 \mu\text{g ml}^{-1}$ of the TLR4 ligand LPS (mature DCs). Gray curve: isotype control (PE labeled mouse IgG2a). L243 does not bind to MHC class II with the invariant Li chain associated (109).

towards the T cell and this remodeling is mediated by the GTPase Cdc42 (87). MIIC tubules and other compartments of endosomal nature move over microtubules (84, 93, 94, 96), although MHC class II recruitment via actin has also been reported (97). The transport of IL-12, CD86 and ICAM-1 also depends on the microtubular cytoskeleton (26, 87, 98). As described above, the actin cytoskeleton of DCs rearranges upon antigen recognition in MHC class II and this is required for formation of a T cell activating synapse (50, 51). This actin reorganization is induced by LFA-1 interactions with ICAMs, which also induces MHC class II redistribution to the immunological synapse (27).

Recycling endosomes carrying MHC class I in human DCs were also shown to tubulate and to extend towards CD8^+ T cells upon formation of an antigen specific synapse and this was dependent on ICAM-1 engagement with LFA-1 (84). As mentioned above, co-stimulatory molecules, such as CD40 (85) and IL-12 (87), can also be recruited to the immunological synapse between DCs and T cells. Some co-stimulatory molecules may share their intracellular trafficking pathway with MHC, such as ICAM-1 and CD70, that may traffic via tubular MIIC to the plasma membrane (26, 96, 99). Other molecules do not traffic via MIIC to the plasma membrane such as the co-stimulatory receptor

CD86 (98). It remains an interesting possibility that IL-12 shares the same transport route with MHC class II and traffics to the immunological synapse in MIIC. How MHC class I and II, IL-12, CD40 and other stimulatory factors and adhesion molecules are finally released in a polarized fashion at the immunological synapse is still unknown. At the plasma membrane, peptide loaded MHC class II complexes are clustered in cholesterol dependent membrane domains, often referred to as lipid rafts (100–102). Clustering of MHC class II promotes screening for antigen peptides by TCR and thereby facilitates antigen presentation (100, 103–105). These membrane domains are already formed prior to the arrival of MHC class II at the immunological synapse (103, 105). MHC class II containing membrane domains are enriched in tetraspanins (CD63, CD82) that also traffic via tubular membrane structures (94, 104).

Expert opinion and outlook

In this review we discussed polarized trafficking of cargo molecules to both the T cell (e.g. TCR, lytic granules) and DC (MHC class I and II, IL-12, CD40) sides of the immunological synapse (Figure 3). The T cell side of the immunological synapse is extensively studied and the mechanisms that underlie polarized trafficking are increasingly well understood. In contrast, how polarized trafficking to the DC side of the immunological synapse is achieved is still almost completely unknown. Studying polarized trafficking to the immunological synapse in DCs is technically challenging, because of the clear dependency on the precise types and activation states of the DC as well as of the T cell. Priming of naïve T cells by DCs requires a multitude of factors and, as we discussed, different cells or activation states can lead to a completely different structure of the synapse. This makes it imperative to work with primary cells, which are, however, very heterogeneous and difficult to study with conventional low-throughput microscopy based techniques. New high-throughput based techniques are clearly required to cope with this challenge such as the recently developed flow cytometry based technique that allows fast imaging of large numbers of immunological synapses (106).

As we discussed above, polarized release at the T cell side of the immunological synapse mechanistically resembles other forms of evoked release such as neurotransmitter release. Indeed, it is increasingly clear that all forms of evoked release in mammalian cells have a common evolutionary origin and consequently share many mechanistic similarities. It therefore seems reasonable to hypothesize

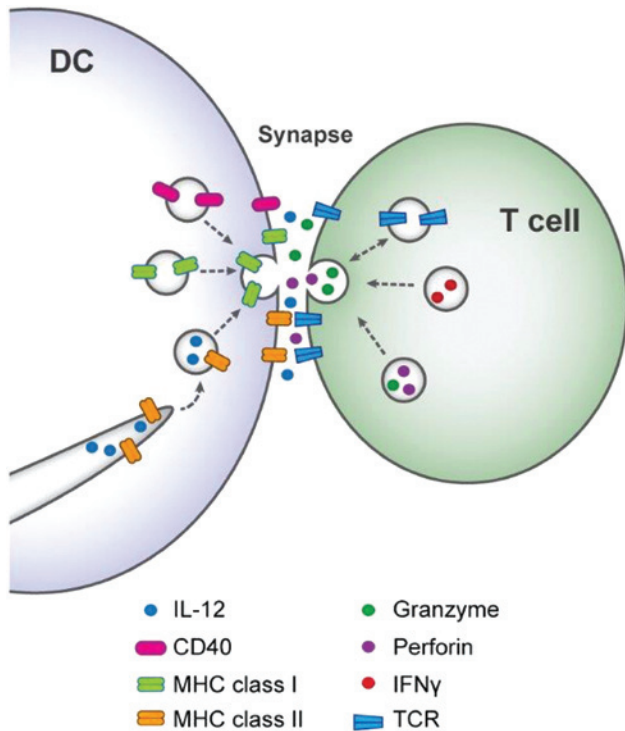


Figure 3: Scheme of membrane trafficking to the DC and T cell sides of the immunological synapse.

At the T cell side, lytic granules containing granzyme and perforin, but also vesicles containing cargo, such as TCR and IFN γ , traffic to the synapse in a highly polarized fashion. Similarly, vesicles and/or tubulovesicular structures carrying molecular cargo, such as MHC class I and II, CD40 and IL-12, specifically traffic to the DC side of the immunological synapse as well. We hypothesize IL-12 traffics in MIIC, but this is still unknown; see text for details.

that polarized release at the DC side of the immunological synapse will also share many structural and mechanistic similarities to polarized release at the T cell side. In this respect, it would be interesting to more systematically study the role in DCs of signaling molecules and metabolites with well-known roles at the T cell side of the immunological synapse, such as the small GTPases RhoA, Rac and Rap1, kinases such as PKC, and metabolites such as cAMP and calcium which can all be measured with FRET probes (107). As we discussed above, our understanding of release at the T cell side was propelled by the development of supported bilayers as surrogate APC. This system, with the artificial bilayers carrying MHC (or antibodies against TCR) and the adhesion molecule ICAM-1, allowed not only to study synapse formation in well-defined conditions, but also to address the structure of the synapse with high resolution microscopy and without interference from the APC. For these same reasons, reversing this model, thus reconstituting TCR and LFA-1 in planar model membranes

for synapse formation with DCs, would be a very powerful tool for elucidating the mechanisms of polarized trafficking to the immunological synapse in DCs.

It bears no question that a better understanding of the mechanism of polarized trafficking to the immunological synapse in DCs is not only of fundamental importance for the field of immunology, but also has large therapeutic potential. A functional immunological synapse between DCs and naïve T cells is essential to mount functional T cell responses, and is thereby vital for the induction of an adaptive immune response and for homeostasis of self-tolerance. Any mechanistic insight in this process will therefore uncover important potential targets for the development of new immunostimulatory or immunosuppressive drugs to combat a wide variety of diseases such as infection, cancer and auto-immune diseases.

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References

1. Chaplin DD. Overview of the immune response. *J Allergy Clin Immunol* 2010; 125: S3–23.
2. Norcross MA. A synaptic basis for T-lymphocyte activation. *Ann Immunol (Paris)* 1984; 135D: 113–34.
3. Dustin ML. Signaling at neuro/immune synapses. *J Clin Invest* 2012; 122: 1149–55.
4. Griffiths GM, Tsun A, Stinchcombe JC. The immunological synapse: a focal point for endocytosis and exocytosis. *J Cell Biol* 2010; 189: 397–406.
5. Becherer U, Medart MR, Schirra C, Krause E, Stevens D, Rettig J. Regulated exocytosis in chromaffin cells and cytotoxic T lymphocytes: how similar are they? *Cell Calcium* 2012; 52: 303–12.
6. Angus KL, Griffiths GM. Cell polarisation and the immunological synapse. *Curr Opin Cell Biol* 2013; 25: 85–91.
7. Ritter AT, Angus KL, Griffiths GM. The role of the cytoskeleton at the immunological synapse. *Immunol Rev* 2013; 256: 107–17.
8. Rodríguez-Fernández JL, Riol-Blanco L, Delgado-Martín C. What is the function of the dendritic cell side of the immunological synapse? *Sci Signal* 2010; 3: re2.
9. Guermónprez P, Valladeau J, Zitvogel L, Théry C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 2002; 20: 621–67.
10. Friedl P, den Boer AT, Gunzer M. Tuning immune responses: diversity and adaptation of the immunological synapse. *Nat Rev Immunol* 2005; 5: 53.

11. Thauland TJ, Parker DC. Diversity in immunological synapse structure. *Immunology* 2010; 131: 466–72.
12. Xie J, Tato CM, Davis MM. How the immune system talks to itself: the varied role of synapses. *Immunol Rev* 2013; 251: 65–79.
13. Gérard A, Beemiller P, Friedman RS, Jacobelli J, Krummel MF. Evolving immune circuits are generated by flexible, motile, and sequential immunological synapses. *Immunol Rev* 2013; 251: 80–96.
14. Benvenuti F, Lagaudrière-Gesbert C, Grandjean I, Jancic C, Hivroz C, Trautmann A, Lantz O, Amigorena S. Dendritic cell maturation controls adhesion, synapse formation, and the duration of the interactions with naive T lymphocytes. *J Immunol* 2004; 172: 292–301.
15. Guy CS, Vignali KM, Temirov J, Bettini ML, Overacre AE, Smeltzer M, Zhang H, Huppa JB, Tsai YH, Lobry C, Xie J, Dempsey PJ, Crawford HC, Aifantis I, Davis MM, Vignali DA. Distinct TCR signaling pathways drive proliferation and cytokine production in T cells. *Nat Immunol* 2013; 14: 262–70.
16. Stinchcombe JC, Bossi G, Booth S, Griffiths GM. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* 2001; 15: 751–61.
17. Azar GA, Lemaître F, Robey EA, Bousso P. Subcellular dynamics of T cell immunological synapses and kinapses in lymph nodes. *Proc Natl Acad Sci USA* 2010; 107: 3675–80.
18. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* 2004; 427: 154–9.
19. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse: a molecular machine controlling T cell activation. *Science* 1999; 285: 221–7.
20. Dustin ML. Supported bilayers at the vanguard of immune cell activation studies. *J Struct Biol* 2009; 168: 152–60.
21. Rossy J, Pigeon SV, Davis DM, Gaus K. Super-resolution microscopy of the immunological synapse. *Curr Opin Immunol* 2013; 25: 307–12.
22. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 1998; 395: 82–6.
23. Varma R, Campi G, Yokosuka T, Saito T, Dustin ML. T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity* 2006; 25: 117–27.
24. Saito T, Yokosuka T. Immunological synapse and microclusters: the site for recognition and activation of T cells. *Curr Opin Immunol* 2006; 18: 305–13.
25. Yokosuka T, Sakata-Sogawa K, Kobayashi W, Hiroshima M, Hashimoto-Tane A, Tokunaga M, Dustin ML, Saito T. Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. *Nat Immunol* 2005; 6: 1253–62.
26. Jo JH, Kwon MS, Choi HO, Oh HM, Kim HJ, Jun CD. Recycling and LFA-1-dependent trafficking of ICAM-1 to the immunological synapse. *J Cell Biochem* 2010; 111: 1125–37.
27. de la Fuente H, Mittelbrunn M, Sanchez-Martin L, Vicente-Manzanares M, Lamana A, Pardi R, Cabañas C, Sánchez-Madrid F. Synaptic clusters of MHC class II molecules induced on DCs by adhesion molecule-mediated initial T-cell scanning. *Mol Biol Cell* 2005; 16: 3314–22.
28. Allenspach EJ, Cullinan P, Tong J, Tang Q, Tesciuba AG, Cannon JL, Takahashi SM, Morgan R, Burkhardt JK, Sperling AI. ERM-dependent movement of CD43 defines a novel protein complex distal to the immunological synapse. *Immunity* 2001; 15: 739–50.
29. Freiberg BA, Kupfer H, Maslanik W, Delli J, Kappler J, Zaller DM, Kupfer A. Staging and resetting T cell activation in SMACs. *Nat Immunol* 2002; 3: 911–7.
30. Campi G, Varma R, Dustin ML. Actin and agonist MHC-peptide complex-dependent T cell receptor microclusters as scaffolds for signaling. *J Exp Med* 2005; 202: 1031–6.
31. Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM. Centrosome polarization delivers secretory granules to the immunological synapse. *Nature* 2006; 443: 462–5.
32. Soares H, Lasserre R, Alcover A. Orchestrating cytoskeleton and intracellular vesicle traffic to build functional immunological synapses. *Immunol Rev* 2013; 256: 118–32.
33. Kurowska M, Goudin N, Nehme NT, Court M, Garin J, Fischer A, de Saint Basile G, Ménasché G. Terminal transport of lytic granules to the immune synapse is mediated by the kinesin-1/Slp3/Rab27a complex. *Blood* 2012; 119: 3879–89.
34. Brown AC, Oddos S, Dobbie IM, Alakoskela JM, Parton RM, Eissmann P, Neil MA, Dunsby C, French PM, Davis I, Davis DM. Remodelling of cortical actin where lytic granules dock at natural killer cell immune synapses revealed by super-resolution microscopy. *PLoS Biol* 2011; 9: e1001152.
35. Rak GD, Mace EM, Banerjee PP, Svitkina T, Orange JS. Natural killer cell lytic granule secretion occurs through a pervasive actin network at the immune synapse. *PLoS Biol* 2011; 9: e1001151.
36. Benvenuti F, Hugues S, Walmsley M, Ruf S, Fetler L, Popoff M, Tybulewicz VL, Amigorena S. Requirement of Rac1 and Rac2 expression by mature dendritic cells for T cell priming. *Science* 2004; 305: 1150–3.
37. Miller MJ, Safrina O, Parker I, Cahalan MD. Imaging the single cell dynamics of CD4+ T cell activation by dendritic cells in lymph nodes. *J Exp Med* 2004; 200: 847–56.
38. Shakhbar G, Lindquist RL, Skokos D, Dudziak D, Huang JH, Nussenzweig MC, Dustin ML. Stable T cell-dendritic cell interactions precede the development of both tolerance and immunity *in vivo*. *Nat Immunol* 2005; 6: 707–14.
39. Katzman SD, O’Gorman WE, Villarino AV, Gallo E, Friedman RS, Krummel MF, Nolan GP, Abbas AK. Duration of antigen receptor signaling determines T-cell tolerance or activation. *Proc Natl Acad Sci USA* 2010; 107: 18085–90.
40. Henrickson SE, Perro M, Loughhead SM, Senman B, Stutte S, Quigley M, Alexe G, Iannaccone M, Flynn MP, Omid S, Jesneck JL, Imam S, Mempel TR, Mazo IB, Haining WN, von Andrian UH. Antigen availability determines CD8+ T cell-dendritic cell interaction kinetics and memory fate decisions. *Immunity* 2013; 39: 496–507.
41. Cahalan MD, Parker I. Close encounters of the first and second kind: T-DC and T-B interactions in the lymph node. *Semin Immunol* 2005; 17: 442–51.
42. Germain RN, Robey EA, Cahalan MD. A decade of imaging cellular motility and interaction dynamics in the immune system. *Science* 2012; 336: 1676–81.
43. Miller MJ, Hejazi AS, Wei SH, Cahalan MD, Parker I. T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. *Proc Natl Acad Sci USA* 2004; 101: 998–1003.

44. Storim J, Bröcker EB, Friedl P. A dynamic immunological synapse mediates homeostatic TCR-dependent and -independent signaling. *Eur J Immunol* 2010; 40: 2741–50.
45. Hommel M, Kyewski B. Dynamic changes during the immune response in T cell-antigen-presenting cell clusters isolated from lymph nodes. *J Exp Med* 2003; 197: 269–80.
46. Beltman JB, Henrickson SE, von Andrian UH, de Boer RJ, Marée AF. Towards estimating the true duration of dendritic cell interactions with T cells. *J Immunol Methods* 2009; 347: 54–69.
47. Benson RA, MacLeod MK, Hale BG, Patakas A, Garside P, Brewer JM. Antigen presentation kinetics control T cell/dendritic cell interactions and follicular helper T cell generation *in vivo*. *eLife* 2015; 10: 4.
48. Gunzer M, Schäfer A, Borgmann S, Grabbe S, Zänker KS, Bröcker EB, Kämpgen E, Friedl P. Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. *Immunity* 2000; 13: 323–32.
49. Brossard C, Feuillet V, Schmitt A, Randriamampita C, Romao M, Raposo G, Trautmann A. Multifocal structure of the T cell – dendritic cell synapse. *Eur J Immunol* 2005; 35: 1741–53.
50. Al-Alwan M, Rowden G, Colp P, Bunker J, West K. The dendritic cell cytoskeleton is critical for the formation of the immunological synapse. *J Invest Dermatol* 2001; 117: 1452–6.
51. Al-Alwan MM, Liwski RS, Haeryfar SMM, Baldrige WH, Hoskin DW. Cutting edge: dendritic cell actin cytoskeletal polarization during immunological synapse formation is highly antigen-dependent. *J Immunol* 2003; 171: 4479–83.
52. Borg C, Jalil A, Laderach D, Maruyama K, Wakasugi H, Charrier S, Ryffel B, Cambi A, Figdor C, Vainchenker W, Galy A, Caignard A, Zitvogel L. NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood* 2004; 104: 3267–75.
53. Barreira da Silva R, Graf C, Münz C. Cytoskeletal stabilization of inhibitory interactions in immunologic synapses of mature human dendritic cells with natural killer cells. *Blood* 2011; 118: 6487–98.
54. Comrie WA, Li S, Boyle S, Burkhardt JK. The dendritic cell cytoskeleton promotes T cell adhesion and activation by constraining ICAM-1 mobility. *J Cell Biol* 2015; 208: 457–73.
55. Hsu CJ, Hsieh WT, Waldman A, Clarke F, Huseby ES, Burkhardt JK, Baumgart T. Ligand mobility modulates immunological synapse formation and T cell activation. *PLoS One* 2012; 7: e32398.
56. Riol-Blanco L, Delgado-Martín C, Sánchez-Sánchez N, Alonso-C LM, Gutiérrez-López MD, Del Hoyo GM, Navarro J, Sánchez-Madrid F, Cabañas C, Sánchez-Mateos P, Rodríguez-Fernández JL. Immunological synapse formation inhibits, via NF- κ B and FOXO1, the apoptosis of dendritic cells. *Nat Immunol* 2009; 10: 753–60.
57. Fooksman DR, Shaikh SR, Boyle S, Edidin M. Cutting edge: phosphatidylinositol 4,5-bisphosphate concentration at the APC side of the immunological synapse is required for effector T cell function. *J Immunol* 2009; 182: 5179–82.
58. Van den Bogaart G, Ter Beest M. Domains of phosphoinositides in the plasma membrane. In: Cambi A, Lidke, DS, editors. *Cell membrane nanodomains: from biochemistry to nanoscopy*. Boca Raton, USA: CRC Press, Taylor & Francis Group, 2015: 173–98.
59. Bouma G, Burns S, Thrasher AJ. Impaired T-cell priming *in vivo* resulting from dysfunction of WASp-deficient dendritic cells. *Blood* 2007; 110: 4278–84.
60. Pulecio J, Tagliani E, Scholer A, Prete F, Fetler L, Burrone OR, Benvenuti F. Expression of Wiskott-Aldrich syndrome protein in dendritic cells regulates synapse formation and activation of naive CD8⁺ T cells. *J Immunol* 2008; 181: 1135–42.
61. Tanizaki H, Egawa G, Inaba K, Honda T, Nakajima S, Moniaga CS, Otsuka A, Ishizaki T, Tomura M, Watanabe T, Miyachi Y, Narumiya S, Okada T, Kabashima K. Rho-mDia1 pathway is required for adhesion, migration, and T-cell stimulation in dendritic cells. *Blood* 2010; 116: 5875–84.
62. Balkow S, Heinz S, Schmidbauer P, Kolanus W, Holzmann B, Grabbe S, Laschinger M. LFA-1 activity state on dendritic cells regulates contact duration with T cells and promotes T-cell priming. *Blood* 2010; 116: 1885–94.
63. Luty WH, Rodeberg D, Parness J, Vyas YM. Antiparallel segregation of notch components in the immunological synapse directs reciprocal signaling in allogeneic Th:DC conjugates. *J Immunol* 2007; 179: 819–29.
64. Schaft N, Lankiewicz B, Gratama JW, Bolhuis RL, Debets R. Flexible and sensitive method to functionally validate tumor-specific receptors via activation of NFAT. *J Immunol Methods* 2003; 280: 13–24.
65. Ueda H, Morphew MK, McIntosh JR, Davis MM. CD4⁺ T-cell synapses involve multiple distinct stages. *Proc Natl Acad Sci USA* 2011; 108: 17099–104.
66. Finetti F, Baldari CT. Compartmentalization of signaling by vesicular trafficking: a shared building design for the immune synapse and the primary cilium. *Immunol Rev* 2013; 251: 97–112.
67. Fisher PJ, Bulur PA, Vuk-Pavlovic S, Prendergast FG, Dietz AB. Dendritic cell microvilli: a novel membrane structure associated with the multifocal synapse and T-cell clustering. *Blood* 2008; 112: 5037–45.
68. Das V, Nal B, Dujecourt A, Thoulouze MI, Galli T, Roux P, Dautry-Varsat A, Alcover A. Activation-induced polarized recycling targets T cell antigen receptors to the immunological synapse: involvement of SNARE complexes. *Immunity* 2004; 20: 577–88.
69. Soares H, Henriques R, Sachse M, Ventimiglia L, Alonso MA, Zimmer C, Thoulouze MI, Alcover A. Regulated vesicle fusion generates signaling nanoterritories that control T cell activation at the immunological synapse. *J Exp Med* 2013; 210: 2415–33.
70. Bossi G, Trambas C, Booth S, Clark R, Stinchcombe J, Griffiths GM. The secretory synapse: the secrets of a serial killer. *Immunol Rev* 2002; 189: 152–60.
71. Fowler KT, Andrews NW, Huleatt JW. Expression and function of synaptotagmin VII in CTLs. *J Immunol* 2007; 178: 1498–504.
72. Jahn R, Fasshauer D. Molecular machines governing exocytosis of synaptic vesicles. *Nature* 2012; 490: 201–7.
73. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, Lambert N, Ouachée-Chardin M, Chedeville G, Tamary H, Minard-Colin V, Vilmer E, Blanche S, Le Deist F, Fischer A, de Saint Basile G. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell* 2003; 115: 461–73.
74. Ménager MM, Ménasché G, Romao M, Knapnougol P, Ho CH, Garfa M, Raposo G, Feldmann J, Fischer A, de Saint Basile G. Secretory cytotoxic granule maturation and exocytosis require the effector protein hMunc13-4. *Nat Immunol* 2007; 8: 257–67.

75. Côte M, Ménager MM, Burgess A, Mahlaoui N, Picard C, Schaffner C, Al-Manjomi F, Al-Harbi M, Alangari A, Le Deist F, Gennery AR, Prince N, Cariou A, Nitschke P, Blank U, El-Ghazali G, Ménasché G, Latour S, Fischer A, de Saint Basile G. Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. *J Clin Invest* 2009; 119: 3765–73.
76. Dudenhöffer-Pfeifer M, Schirra C, Pattu V, Halimani M, Maier-Peuschel M, Marshall MR, Matti U, Becherer U, Dirks J, Jung M, Lipp P, Hoth M, Sester M, Krause E, Rettig J. Different Munc13 isoforms function as priming factors in lytic granule release from murine cytotoxic T lymphocytes. *Traffic* 2013; 14: 798–809.
77. Matti U, Pattu V, Halimani M, Schirra C, Krause E, Liu Y, Weins L, Chang HF, Guzman R, Olausson J, Freichel M, Schmitz F, Pasche M, Becherer U, Bruns D, Rettig J. Synaptobrevin 2 is the v-SNARE required for cytotoxic T-lymphocyte lytic granule fusion. *Nat Comm* 2013; 4: 1439.
78. Pattu V, Qu B, Schwarz EC, Strauss B, Weins L, Bhat SS, Halimani M, Marshall M, Rettig J, Hoth M. SNARE protein expression and localization in human cytotoxic T lymphocytes. *Eur J Immunol* 2012; 42: 470–5.
79. Finetti F, Patrussi L, Galgano D, Cassioli C, Perinetti G, Pazour GJ, Baldari CT. The small GTPase Rab8 interacts with VAMP-3 to regulate the delivery of recycling TCRs to the immune synapse. *J Cell Sci* 2015; 128: 2541–52.
80. Jewell JL, Oh E, Thurmond DC. Exocytosis mechanisms underlying insulin release and glucose uptake: conserved roles for Munc18c and syntaxin 4. *Am J Physiol Regul Integr Comp Physiol* 2010; 298: R517–31.
81. Boes M, Cerny J, Massol R, Op den Brouw M, Kirchhausen T, Chen J, Ploegh HL. T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. *Nature* 2002; 418: 983–8.
82. Bertho N, Cerny J, Kim YM, Fiebiger E, Ploegh H, Boes M. Requirements for T cell-polarized tubulation of class II+ compartments in dendritic cells. *J Immunol* 2003; 171: 5689–96.
83. Boes M, Bertho N, Cerny J, den Brouw MO, Kirchhausen T, Ploegh H. T cells induce extended class II MHC compartments in dendritic cells in a toll-like receptor-dependent manner. *J Immunol* 2003; 171: 4081–8.
84. Compeer EB, Flinsenberg TW, Boon L, Hoekstra ME, Boes M. Tubulation of endosomal structures in human dendritic cells by Toll-like receptor ligation and lymphocyte contact accompanies antigen cross-presentation. *J Biol Chem* 2014; 289: 520–8.
85. Foster N, Turnbull EL, Macpherson G. Migrating lymph dendritic cells contain intracellular CD40 that is mobilized to the immunological synapse during interactions with antigen-specific T lymphocytes. *J Immunol* 2012; 189: 5632–7.
86. Huang J, Brameshuber M, Zeng X, Xie J, Li QJ, Chien YH, Valitutti S, Davis MM. A single peptide-major histocompatibility complex ligand triggers digital cytokine secretion in CD4⁺ T cells. *Immunity* 2013; 39: 846–57.
87. Pulecio J, Petrovic J, Prete F, Chiaruttini G, Lennon-Dumenil A-M, Desdouets C, Gasman S, Burrone OR, Benvenuti F. Cdc42-mediated MTOC polarization in dendritic cells controls targeted delivery of cytokines at the immune synapse. *J Exp Med* 2010; 207: 2719–32.
88. Rocha N, Neeffjes J. MHC class II molecules on the move for successful antigen presentation. *EMBO J* 2008; 27: 1–5.
89. Berger AC, Roche PA. MHC class II transport at a glance. *J Cell Sci* 2009; 122: 1–4.
90. Neeffjes J, Jongsma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol* 2011; 11: 823–36.
91. Kleijmeer M, Ramm G, Schuurhuis D, Griffith J, Rescigno M, Ricciardi-Castagnoli P, Rudensky AY, Ossendorp F, Melief CJ, Stoorvogel W, Geuze HJ. Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. *J Cell Biol* 2001; 155: 53–63.
92. Chow A, Toomre D, Garrett W, Mellman I. Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. *Nature* 2002; 418: 988–94.
93. van Nispen tot Pannerden HE, Geerts WJ, Kleijmeer MJ, Heijnen HF. Spatial organization of the transforming MHC class II compartment. *Biol Cell* 2010; 102: 581–91.
94. Vyas JM, Kim Y-M, Artavanis-Tsakonas K, Love JC, Van der Veen AG, Ploegh HL. Tubulation of class II MHC compartments is microtubule dependent and involves multiple endolysosomal membrane proteins in primary dendritic cells. *J Immunol* 2007; 178: 7199–210.
95. Mrakovic A, Kay JG, Furuya W, Brumell JH, Botelho RJ. Rab7 and Arl8 GTPases are necessary for lysosome tubulation in macrophages. *Traffic* 2012; 13: 1667–79.
96. Keller AM, Groothuis TA, Veraar EAM, Marsman M, Maillette de Buy Wenniger L, Janssen H, Neeffjes J, Borst J. Costimulatory ligand CD70 is delivered to the immunological synapse by shared intracellular trafficking with MHC class II molecules. *Proc Natl Acad Sci USA* 2007; 104: 5989–94.
97. Turley SJ, Inaba K, Garrett WS, Ebersold M, Untermaier J, Steinman RM, Mellman I. Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 2000; 288: 522–7.
98. Smyth CM, Logan G, Boadle R, Rowe PB, Smythe JA, Alexander IE. Differential subcellular localization of CD86 in human PBMC-derived macrophages and DCs, and ultrastructural characterization by immuno-electron microscopy. *Int Immunol* 2005; 17: 123–32.
99. Zwart W, Peperzak V, de Vries E, Keller AM, van der Horst G, Veraar EA, Geumann U, Janssen H, Janssen L, Naik SH, Neeffjes J, Borst J. The invariant chain transports TNF family member CD70 to MHC class II compartments in dendritic cells. *J Cell Sci* 2010; 123: 3817–27.
100. Hiltbold EM, Poloso NJ, Roche PA. MHC class II-peptide complexes and APC lipid rafts accumulate at the immunological synapse. *J Immunol* 2003; 170: 1329–38.
101. Poloso NJ, Muntasell A, Roche PA. MHC class II molecules traffic into lipid rafts during intracellular transport. *J Immunol* 2004; 173: 4539–46.
102. Khandelwal S, Roche PA. Distinct MHC class II molecules are associated on the dendritic cell surface in cholesterol-dependent membrane microdomains. *J Biol Chem* 2010; 285: 35303–10.
103. Anderson HA, Hiltbold EM, Roche PA. Concentration of MHC class II molecules in lipid rafts facilitates antigen presentation. *Nat Immunol* 2000; 1: 156–62.
104. Vogt AB, Spindeldreher S, Kropshofer H. Clustering of MHC-peptide complexes prior to their engagement in the immunological synapse: lipid raft and tetraspan microdomains. *Immunol Rev* 2002; 189: 136–51.
105. Bosch B, Heipertz EL, Drake JR, Roche PA. Major histocompatibility complex (MHC) class II-peptide complexes arrive at the

- plasma membrane in cholesterol-rich microclusters. *J Biol Chem* 2013; 288: 13236–42.
106. Markey KA, Gartlan KH, Kuns RD, MacDonald KP, Hill GR. Imaging the immunological synapse between dendritic cells and T cells. *J Immunol Methods* 2015; 423: 40–4.
107. Randriamampita C, Lellouch AC. Imaging early signaling events in T lymphocytes with fluorescent biosensors. *Biotechnol J* 2014; 9: 203–12.
108. Baranov MV, Ter Beest M, Reinieren-Beeren I, Cambi A, Figdor CG, van den Bogaart G. Podosomes of dendritic cells facilitate antigen sampling. *J Cell Sci* 2014; 127: 1052–64.
109. Bijlmakers MJ, Benaroch P, Ploegh HL. Assembly of HLA DR1 molecules translated *in vitro*: binding of peptide in the endoplasmic reticulum precludes association with invariant chain. *EMBO J* 1994; 13: 2699–707.