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Invited Review

Mechanisms of polarized cell-cell communication of T lymphocytes

D. Torralba[a,b,c], N.B. Martín-Cófreces[a,b,c], F. Sanchez-Madrid[a,b,c,*]

[a] Servicio de Inmunología, Hospital Universitario de la Princesa, UAM, IIS-IP, 28006 Madrid, Spain
[b] Area of Vascular Pathophysiology, Laboratory of Intercellular Communication Fundación Centro Nacional de Investigaciones Cardiovasculares-Carlos III, 28029 Madrid, Spain
[c] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), 28029 Madrid, Spain

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ABSTRACT

Cell-cell communication comprises a variety of molecular mechanisms that immune cells use to respond appropriately to diverse pathogenic stimuli. T lymphocytes polarize in response to different stimuli, such as cytokines, adhesion to specific ligands and cognate antigens presented in the context of MHC. Polarization takes different shapes, from migratory front-back polarization to the formation of immune synapses (IS). The formation of IS between a T cell and an antigen-presenting cell involves early events of receptor-ligand interaction leading to the reorganization of the plasma membrane and the cytoskeleton to orchestrate vesicular and endosomal traffic and directed secretion of several types of mediators, including cytokines and nanovesicles. Cell polarization involves the repositioning of many subcellular organelles, including the endosomal compartment, which becomes an effective platform for the shuttling of molecules as vesicular cargoes that lately will be secreted to transfer information to antigen-presenting cells. Overall, the polarized interaction between a T cell and APC modifies the recipient cell in different ways that are likely lineage-dependent, e.g. dendritic cells, B cells or even other T cells. In this review, we will discuss the mechanisms that mediate the polarization of different membrane receptors, cytoskeletal components and organelles in T cells in a variety of immune contexts.

1. Introduction

Cell-cell communication between the adaptive and the innate compartments enables the immune system to respond appropriately to aggression. Adaptive T cells receive input from innate cells, e.g. dendritic cells, through well-delineated cell-cell contacts, appropriately known as immune synapses (IS). The IS covers around 20% of a T cell surface when stabilized [1]. The IS determines the intensity and duration of the T cell response. Transient contacts elicit no apparent cell reorganization, and the activation of the T cell is negligible. Conversely, a stable IS implies a more profound and durable rearrangement of the T cell receptors, signaling machinery, cytoskeleton and organelles toward the synaptic contact. Recognition of an antigenic peptide-bound MHC (pMHC) by the T cell receptor (TCR) induces IS stabilization. Other adhesive contacts contribute to the stabilization of the IS, e.g. integrin adhesion receptors and co-stimulatory receptors, such as CD2 or CD28 [2].

The formation of the IS requires the close contact of the T cell membrane and the APC membrane. The TCRs exposed on the plasma membrane show a non-random distribution. TCR receptors appear in the microvilli of resting T cells, enabling them to scan the surface of the APC in rapidly occurring cell contacts. The extended microvilli from T cells can leave “messages” in the surrounding medium through the excision of the distant tips of these microvilli. The resultant “membrane patches” may circularize into a sphere-shaped form that would be found and thus transduce signals into the receptor cell [3,4] (Fig. 1A). These initial exploratory contacts are actin-independent. Once the peptide is recognized, the area of contact is immediately enlarged, allowing firm adhesion through LFA-1 and ICAM-1. ICAM-1 rapidly accumulates at the interface between the T cell and the APC upon antigen recognition [5]. The extended conformation of LFA-1 integrin, which constitutes its maximal activation status, is detected in specific areas of the T-APC contact upon stabilization, generating regions of different adhesive strength through the contact [6,7]. This conformation is relevant to organize the actin cytoskeleton underneath the plasma membrane, which sustains cell-cell contacts.

The mechanisms that trigger TCR activation and accumulation at the IS are still under study. These mechanisms include conformational changes in the αβTCR and associated CD3 complex (TCR/CD3); aggregation of receptors forming nano- and micro-clusters; and

* Corresponding author at: Laboratorio de Comunicación Intercelular, Servicio de Inmunología-Planta 1, Instituto de Investigación Sanitaria Princesa, Hospital de La Princesa, Diego de León 62, 28006 Madrid, Spain.
E-mail address: fsmadrid@salud.madrid.org (F. Sanchez-Madrid).

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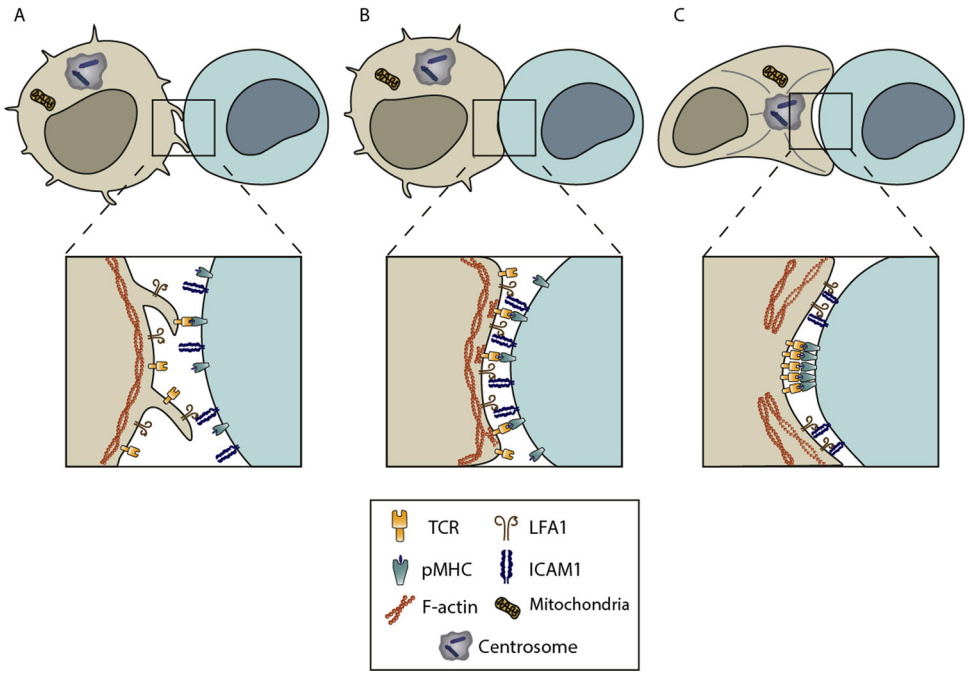

Fig. 1. Establishment of an immune synapse. (A) The initial interactions between a T cell (left) and an antigen-presenting cell (APC, right) involve the contact of microvilli or microspikes formed on the surface of the T cell with the APC surface. Integrins such as LFA-1 may contact their ligands (mainly ICAM-1) on the APC. (B) TCR complexes on the microvilli will engage with antigen-MHC complexes on the APC if the antigen is recognized, the contact will be then expanded through the surface of the T-APC. The interaction between LFA-1 and ICAM-1 will be reinforced by TCR signaling, apposing together the T cell and the APC. (C) Receptors will reorganize in different regions directed by the cytoskeleton dynamics, which also shows a polarized distribution. Actin is depicted in red.

segregation by spatial constrictions through partner receptors binding in cis and trans, cytoskeletal connections or by ectodomain-size spatial exclusion [8]. It is clear that TCR signaling precedes the formation of the IS [9]. The phosphorylation of the different ITAMs at the CD3 subunits of the TCR is essential [10,11]. Importantly, the phosphorylation of the three ITAMs in the CD3ζ subunit seems to be a sequential event, mostly dependent on the specificity rather than the affinity of the TCR for the pMHC complex [12]. The ability of the actin cytoskeleton to cluster different receptors enables T cell activation upon engagement of low affinity TCRs by a few MHC complexes [13]. The actin foci detected around the TCR microclusters are essential for downstream signaling [14] (Fig. 1B). The receptors have been proposed to move through the T cell surface by passive lateral diffusion and directional actin dynamics once the TCR recognizes the pMHC. A model proposed that TCR/CD3 is translocated into specific lipid rafts where it is activated by Lck [15]. TCR in lipid rafts engage with pMHC complexes; if these interactions are productive, TCR microclusters and their associated molecules migrate from the periphery to the center of the IS [16], shaping the so-called central supramolecular activation cluster (cSMAC). In this regard, the number of pMHC needed for TCR activation may be as small as one [17]. A relevant concept here is that both the number of pMHCs in the APC [18] and the ability of the APC to accumulate these molecules at the cell-cell contact, which is directly dependent on its cytoskeleton, control the intensity of the activation of the T cell. The formation of clusters of MHC-II and ICAM-1 on the APC helps the localization of TCR and LFA-1 at the T cell side of the IS, respectively, decreasing by 100-fold the threshold of TCR activation and shaping the IS properly [19–21]. Similar to its integrin ligand across the IS, ICAM-1 lateral mobility also depends on its interaction with the actin cytoskeleton [22,23].

The minimal duration of interactions to elicit a proper T cell response is still under debate; however, the establishment of a classical immune synapse does not seem to be essential for a durable interaction, as a dynamic kinapse would be sufficient to activate the T cell [24].

2. Dynamic organization of IS subdomains

TCR organization in microclusters has been analyzed by fluorescence and electron microscopy. Those experiments revealed that TCR assemble into clusters comprising ten to a hundred (or more) molecules upon pMHC recognition [25]. Memory T cells appear to contain preformed nanoclusters, which make them more ready to respond to their specific pMHC [26]. The clusters of TCR have been largely analyzed to study possible changes in size upon activation, as described initially. However, a clear limitation of the technique is the minimum size of clusters that can be detected with the current fluorescence-based methods, which do not detect nanoclusters (less than 20–40 molecules per cluster at 20 nm resolution) [27]. TCR microclusters are considered as the primary-signaling units within the T cell IS. The activation of TCR microclusters at the periphery of the contact and their subsequent transport to the central area of the IS by the retrograde flow of actin was observed with supported lipid bilayers and TIRFm [28] (Fig. 1B, C). In the case of co-receptor molecules as CD4, they are located at the center at the beginning of the interaction to boost ligand recognition; then they are translocated to the periphery and are not further required once T cells are activated [29]. The role of actin has been analyzed in several scenarios of T cell activation. Direct interaction of actin and TCR through CD3ζ has been described [30]. The change of conformation of the TCR/CD3 complex upon activation allows the tyrosine-kinase independent binding of Nck to specific hidden sites in CD3ε that promote actin polymerization [31]. Therefore, this event predates the phosphorylation of the ITAMs by the members of the Src tyrosine kinase Lck and Fyn. TCR engagement induces CD3 phosphorylation by Lck [32,33], which leads to the recruitment of Zap70. Nck and Vav-1 can interact independently of SLP76 [34], although the recruitment of SLP76 to LAT and phosphorylation by ZAP70 was the first described event for Vav-1 recruitment and posterior Nck binding for actin polymerization downstream the TCR [35–37]. These activities are tyrosine kinase-dependent.

CD28 is a major co-stimulating receptor that complement TCR-mediated traction forces [38]. Its ligands on the APC are CD80 and CD86. CD28 also accumulates at the cSMAC. CD28 absence causes T cell anergy [39]. CD28 regulates PKCθ function and localization at the IS to mediate activation of nuclear factors such as the NF-κB pathway [40,41]. CD28 competes for its ligands with CTLA-4, which is an important immunomodulating checkpoint that behaves as negative regulator in this context, sequestering CD80 and CD86 at the APC [42] and pushing both CD28 and PKCθ to external areas. CD28 mislocalization decreases co-stimulation and down-modulates T cell activation [43]. The trans-endocytosis of CTLA-4 coupled to CD80 and CD86, which

would decrease their concentration at the APC, has also been described in the T cell [44]. CD28 association to TCR microclusters directs CD28 to the cSMAC; however, it is not sufficient for its proper spatial segregation, and F-actin binding is crucial for its movement toward the cSMAC [45].

Classic adhesion molecules maintain the interaction between cells forming the peripheral SMAC (pSMAC), which is highly enriched in integrins such as LFA-1 on the T cell side interacting with ICAM-1 at the APC side [2,46]. LFA-1/ICAM-1 interaction is a co-stimulatory signal that induces the rearrangement and polarization of the cytoskeleton, which is reinforced by inside-out signals emanating from the TCR complex as well as chemokine receptor activation at the IS (e.g. CXCR4-dependent signals modulate the TCR-dependent organization of actin and the adhesion force of integrins [47,48]). Conversely, LFA-1 contributes to the discrimination between low and high affinity antigens by the TCR through Rac1 and Cdc42 signaling in CD8 T cells, therefore regulating actin dynamics and synaptic stability [49]. Part of the signaling molecules defined here, such as Src family members, may be shared by all these receptors. All of them control, among other processes, calcium intracellular flow at the IS, which is particularly relevant for T cell activation [50]. Formation of the SMACs is not specifically needed to observe full T cell activation, but the organized shape of the IS improves signaling by positioning properly the different components at the IS, such as receptors, cytoskeleton and organelles [50,51].

The dynamic organization of the actin cytoskeleton beneath the plasma membrane is essential for T cell activation; the different structures organized include the whole cell and not only the cortical actin beneath the membranes that form the immune synapse. Actin cytoskeleton rearranges sequentially underneath the immunological synapse upon TCR activation [52] (Fig. 1C). The use of actin depolymerizing drugs, such as Cytochalasin D, latrunculin A or jaskaplanolide, prevents the observed movement of clusters of signaling molecules and receptors such as the TCR, LFA-1, LAT and SLP76 [53–57]. These clusters act as signaling initiators, facilitating the accumulation of the different kinases and adaptor proteins needed to transduce the activation signal from the TCR to the nucleus. Also, TCRs are linked to actin foci that allow the organization of pushing and pulling forces toward the plasma membrane of the antigen-presenting cell, where the pMHCs are exposed [58] (Fig. 1B). The rapid organization of the actin cytoskeleton in different areas with distinct morphologies distributes adhesion and signaling receptors into different locations that can be observed in very stable, long-lasting IS [51,55]. In these structures, an external ring of actin exhibiting rapid retrograde flow allows T cell expansion over the APC [59]. TCRs move from the more external area onto the central zone of the integrin arrays, where receptors are internalized and recycled [28,60]. This movement is coupled to the retrograde flow of the F-actin [60], the contraction of actomyosin-II arcs [61], and the presence of myosin IIA [62]. Other signals also participate in the formation of the IS. For example, CXCL12 is a chemokine that reinforces the cytoskeletal changes that shape the IS in addition to CD3 and CD28 [48]. Its receptor, CXCR4, is localized at the immune synapse through its connection with the actin cytoskeleton [47]. CXCL12 binding to CXCR4 enables a rapid Ca^{2+} influx and subsequent activation of Rac1 [63], and its internalization seems dependent on its interaction with MIIA [64]. During the conversion of migratory polarity into the IS between cytotoxic lymphocytes and tumor cells (kinapse), the polarized delivery of CCL5 increases the local concentration of CXCR4 at the IS, which may promote T cell migration and termination of the cell-cell contact [65]. Binding of CXCL12 triggers rapid mitochondrial ATP release and T cell migration, which depends on autocrine stimulation of the P2X4 purinergic receptors [66]. This may be a relevant mechanism for IS formation and stability, since mitochondria are polarized to the IS upon TCR activation, accumulate under the F-actin ring and control phosphorylation of the myosin light chain and Myosin II activity through ATP production [67]. The accumulation of phosphatidylinositol trisphosphate (PIP_3) generated by Ras-GTPase-activated IA phosphoinositide 3-kinases associated to TCR drives the growth and maintenance of the F-actin ring below the synaptic membrane. PIP_3 recruits Dock2, a guanine-exchange factor (GEF) for Rac GTPase, to the periphery of the synapse where it drives actin polymerization through Rac GTPase [68]. An inner region of actomyosin arcs connects to the integrin receptors to fix the T cell to the APC, facilitating the movement of the TCR microclusters [59,61].

F-actin plays a role in the distribution of receptors and integrins, and it also controls the proper fusion and secretion of the vesicles at the cSMAC [69]. The dynamics of actin is crucial for organelle reorientation and polarized secretion. In CD8 T cells, lytic granules fuse with the membrane at the cSMAC, a site of reduced cortical actin and PIP2 phospholipid density [70]. These differences in composition are driven by the exclusion of PIP5 kinases from the cSMAC, which generates a perfect environment for lytic granule fusion and secretion [71]. Moreover, TCR engagement induces the formation of a dynamic network of a nuclear F-actin that ends up in differential cytokine expression patterns in CD4 T cells. This actin filament network is partly organized by Arp2/3 complex and it does neither affect immune synapse formation nor T cell proliferation [72]. Lamin A affects CD4 T cell activation, and synapse organization through the organization of the nuclear lamina underneath the nuclear membrane [73]. The association between actin cytoskeleton and the nuclear lamina regulates the strength of mechanical forces in adherent junctions and the general cell organization, and depends on nesprin 1/2 connection [74]. The rearrangement of the cytoskeleton is essential to direct the mechanical forces at the T-APC interaction. In CD8 T cells, mechanical forces directly regulate the perforin-based pore formation at the plasma membrane of the target cell and cytotoxicity, with higher forces facilitating pore opening and stability [75]. Traction forces are regulated by F-actin and Myosin IIA and by dynamic microtubules (MTs) at the interface of the T cell and the APC. Dynamic MTs suppress Rho activation and therefore interfere with non-muscle myosin II (NMII) filament assembly. In this regard, PKC activity appears to be both necessary and sufficient for NMII depletion [76].

3. Polarization of the MTOC and asymmetric division

The reorganization of the cytoskeleton is essential to organize the dynamic polarity of organelles and the cell shape at the IS. Microtubules relocate the required cellular compartments to the immune synapse, therefore guiding vesicles and organelle movement. The polarization of the T cell centrosome, a microtubule-organizing center (MTOC), to the immune synapse enables the translocation of specific organelles and the directional secretion toward the antigen-presenting cell [77–79] (Fig. 1C). Upon CD3 phosphorylation by Lck and docking of ZAP70 at the ITAMs, ZAP70 acts on LAT and Slp76, providing a signaling basis for recruitment of downstream effector enzymes that will allow MTOC polarization [80]. This is facilitated by the ability of Lck to drag LAT and Zap70 together through protein-protein interactions [81]. This complex platform recruits PLCγ1, which is localized at the cSMAC in a F-actin flow- and microcluster lateral diffusion-dependent manner [57]. PLCγ1 hydrolyzes phosphatidylinositol 4,5 bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG). DAG accumulates preferentially at the center of the immune synapse [82]. DAG foci recruit proteins containing DAG-specific C1 domains, which results in MTOC reorientation. Centrosomal movement is thus guided by a gradient of diacylglycerol at the IS [83]. Diacylglycerol kinase α (DGKα) localizes preferentially at periphery of the IS and catalyzes the conversion of DAG to phosphatidic acid. DGK-α is responsible for maintaining the DAG gradient by limiting the diffusion of this second messenger throughout the cytoplasm [84]. Losing this limiting factor results in anergy or hyperactivation of the T cell, depending on the different DGK isoforms affected, namely, DGKα or ζ [85,86]. MTOC translocation by a DAG gradient is partially explained

by the recruitment of different isoforms of PKC. Specifically, PKC-ε and PKC-η, which have redundant recruitment functions, accumulate in a broad area of the membrane. PKC-θ is also required for MTOC reorientation and is synergistically recruited with DAG [87]. In CD4+ T cells, the Lck and Itk adapter TSAd plays an important role in cell polarization and differentiation by affecting actin polymerization as well as polarization of TCR, PKCξ and MTOC at the IS [88,89]. Dynein reorients the MTOC during cognate interaction as it moves along the microtubules from the plus end to the minus end. It accumulates at the immune synapse together with ADAP, therefore connecting the motor to LFA-1 clusters [90]. Impairment of dynein-dynactin complex activity inhibits MTOC translocation after TCR antigen priming [91]. PKC isoenzymes also shape the positioning of non-muscle myosin (NMII) through direct phosphorylation of the regulatory light chain (RLC), which forms clusters behind the MTOC and probably pushes it from behind while dynein would pull from the center of the synaptic region, to move the MTOC. NMII can be phosphorylated by the Ser/Thr kinases ROCK and MLCK. ROCK inhibition impairs MTOC polarization, supporting the role of NMII in MTOC reorganization [92].

Asymmetric cell division (ACD) is observed during responses to cognate-antigenic activation and IS formation in naïve T cells, which undergo a first ACD cycle in vivo [93]. While the exact mechanism remains unclear, there are several proposed models [94,95]. One such model postulates that the contact with the APC establishes an asymmetric axis by polarizing the MTOC and orienting the mitotic axis, constituting a conserved mechanism of ACD also observed in other systems [96]. In this sense, the maintenance of the polarized state during IS formation may be an essential cue for ACD. Another model suggests that the asymmetric distribution of cell components in the mother cell determines the differences between daughter cells. For example, T-bet accumulation at the proximal pole of the APC is due to the distal accumulation of the proteasome. This mechanism can also explain the differential distribution of other proteins regulated by ubiquitination and degradation [93]. Among CD3 cells, CD8 and CD4 subsets may perform ACD to generate two different daughter cells: a pre-memory and a pre-effector cell. The mother T cell differentially polarizes specific cell components to one pole with respect to the other, such as LFA-1, CD8, IFNγR or IL2RA, which accumulate at the proximal pole of the IS. The future effector T cell emerges from the proximal pole, while the putative memory T cell will form from the distal pole, where molecules such as PKCθ or Eomesodermin (Eomes) accumulate [93]. Other works have also proved the asymmetric distribution of Numb or Par3 to the distal pole, and Scribble to the proximal pole. All these are well-known organizers of cell polarity [96].

TCR activation is essential for ACD; the observed divisions are not asymmetric in the absence of specific stimuli [93]. Indeed, TCR affinity for the ligand is a determinant of ACD; high affinity ligands trigger ACD, while low affinity ligands, such as autoantigens, fail to induce ACD [97]. Although ACD has been demonstrated in naïve CD8 and CD4 T cells, memory T cells resulting from this first division may undergo another round of ACD upon re-challenge and re-infection, assuring their proper renewal [98]. The duration of T cell-APC contact is also relevant for ACD; short-term conjugates and defects in the interaction of LFA-1 and its counterpart receptor ICAM-1 lead to impaired ACD [93,96]. From studies with naïve T cells activated on ICAM-1-covered surfaces including foci of different sizes and separation of activating signals in terms of CD3ε and CD28 stimulation, it was inferred that both TCR/co-stimulatory (anti-CD3/anti-CD28) and adhesion (ICAM-1) signals are required for ACD. A low number of DCs presenting low number of antigens led to ACD, while many DCs presenting the same antigen precluded ACD. The formation of memory T cells against this particular antigen would be prevented, acting as a negative self-regulatory mechanism to avoid the development of autoimmune syndromes [99].

Mitochondrial asymmetric distribution has been proposed as a mechanism for differential fate acquisition [100], but no differences in inheritance of mitochondrial mass or mitochondrial DNA copy number have been detected between first-division T cell daughters [101]. However, aged-mitochondria preferentially locate toward the daughter T cell that will differentiate, polarize and eventually undergo senescence. Healthy mitochondria polarize toward the daughter T cell that will be in charge of self-renewal. This event changes the metabolism of the resulting cells, which is reinforced by ROS production [102]. In CD8 T cells, asymmetric amino acid content due to asymmetric amino acid transporter distribution correlate with c-Myc expression. Asymmetric c-Myc levels in daughter T cells affect proliferation, metabolism, and their final fate [101].

4. Mitochondria reorganization at the immune synapse

Mitochondria are dynamic organelles that drive many different functions in cells. They are involved in energy production, constituting a metabolic hub that centralizes the synthesis of many intermediate metabolites. They also play a role in apoptosis, calcium homeostasis and in cell signaling, mediated by ROS production and mtDNA release [103]. In T cells, mitochondria are also involved in migration and class differentiation through asymmetric division [95]. Mitochondria also control lysosomal function during inflammatory T cell responses [104]. The tight regulation of mitochondrial distribution during IS formation facilitates their function. Mitochondria polarize toward the T cell-APC contact and happens in the T cell [105] and the APC side [106]. It is also observed in other models of immune contacts such as NK-tumor cell kinapse [107]. In T cells, mitochondria specifically localizes around the MTOC at the cSMAC/pSMAC area [67] and once there fuels the IS with local ATP and calcium for proper T cell activation [105,108,109].

In migrating T cells, microtubule dynamics are essential for transporting mitochondria to the uropod in response to CXCL12 chemotaxis [110]. Conceivably, mitochondria move in two phases when polarizing toward the IS: in a first step mitochondria get closer to the IS contact site and in a second one they get distributed and anchored. Although not experimentally proven, mitochondria may follow an initial calcium gradient in a Miro1-dynein dependent manner and accumulate around the polarized MTOC [111,112], which would be a plausible mechanism for mitochondria transport to the IS. TCR engagement is not absolutely required to observe mitochondria accumulation at the T cell-APC area; the initial LFA1-ICAM1 interaction underlies this effect, facilitated by microtubule integrity [113]. Therefore, the increase of calcium flow upon TCR engagement would increase and sustain mitochondria polarization at the IS area, as a second step. This can be achieved through proteins like Miro-1, which links the tubulin and actin cytoskeletons and fine-tunes the mitochondria movement and docking [114]. Miro-1 is a scaffold protein for Myo19, which regulates the subcellular distribution of mitochondria through regulation of long-walks on microtubules and short-walks and anchoring to actin [115]. The expression of Miro-1 and Miro-2 is required for Myo19 stability, and TRAK1/TRAK2 (adaptors for Miro proteins), compete for Miro binding [116]. In this sense, the localization of the centrosome at the IS is prior to mitochondria recruitment, which requires fragmentation by drp1 [67]. The de-localization of the centrosome from the IS through inhibition of dynein/dynactin tubulin-based molecular motor by p50-dynamitin over-expression [91], also prevents mitochondria polarization at the IS [67]. As previously mentioned, mitochondrial dynamics are crucial for proper T cell activation; drp1 has been recently shown to regulate the balance between effector and memory-like phenotype, favoring T cell exhaustion in different scenarios, such as the tumor microenvironment [117].

5. Polarization of cytokines and vesicles

The secretory machinery orientates toward the immune synapse in the T cell. Two main intracellular pathways have been described: the Golgi apparatus-endoplasmic reticulum (ER) secretory pathway and the

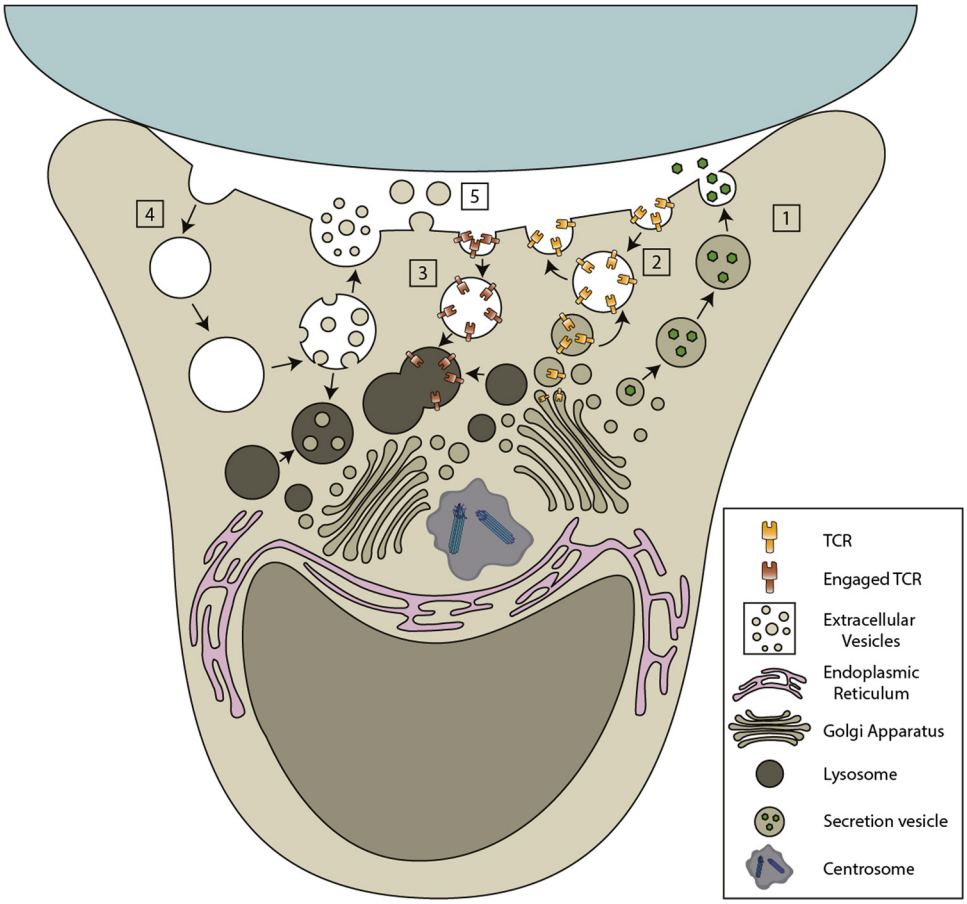

Fig. 2. The secretory and recycling pathways at the immune synapse. The secretory machinery of the T cell is actively polarized toward the T cell-APC contact by the movement of the centrosome and the coordinated action of molecular motors. (1) The ER-Golgi dependent pathways direct their vesicles toward the immune synapse to deliver newly synthetized adhesion and signaling receptors and soluble mediators such as chemokines and cytokines. (2) TCR-CD3 complexes (orange) cycle conforming a steady-state loop. Some CD3-TCR complexes are synthetized in excess and retained in the Golgi, which conforms a TCR reservoir ready to be released and engaged to induce activation. (3) T cell receptor molecules (red) are internalized at the central area of the T cell-APC contact; they can be degraded or recycled through the endolysosomal pathway. (4) Multivesicular bodies (MVB) are formed from the endosomes, through specific sorting of their components thanks to the ESCRT complexes. MVB can deliver their content into the immune synapse cleft, as extracellular vesicles (i.e., exosomes) or soluble components. The molecules at their limiting membrane will be then part of the plasma membrane. Viruses can use this pathway to spread into APCs. (5) Ectosomes can be formed, excised and released from the plasma membrane to the IS cleft.

endolysosomal system (Fig. 2). In the Golgi-ER secretory pathway, the CD3-TCR complexes are synthetized in excess and retained there. These complexes assemble and progress through the secretory pathway; the vesicles containing these complexes fuse with the plasma membrane and complexes are exposed at the T cell surface until they are recycled. When the complexes do not assemble properly, TCR and CD3 chains are degraded through the proteasome or incorporated into lysosomes [118]. Once on the plasma membrane, TCR/CD3 complexes form microclusters and traffic continuously between plasma membrane and endosomes in rapid cycles (Fig. 2-2).

The Golgi-ER secretory pathway is then guided toward the synaptic cleft [67,77,91,119–122], providing directionality to the secretion of some cytokines and signaling mediators. Specifically, IL4 is preferentially secreted by CD4 T cells toward the site of activation [123] and it accumulates at the site of T-B cell synapse together with IL5, IL2 and Interferon γ (IFNγ) after 29 h [79]. IFNγ is also secreted in a polarized manner by CD4 T cell toward the site of activation [69], although it can reach other neighboring cells [124] (Fig. 2-1). In contrast, TNFα and some chemokines are released in a non-directional manner both in CD4 and CD8 T cells [69].

In CD8 and NK cells, a rapid reorientation of Golgi-ER secretory machinery enables the organelles to become close to the contact site with the target cell [77,78]. A secretory domain near the TCR-rich signaling domain forms at the “killing synapse” of the CTLs, favoring the release of mediators for cell killing such as perforin and granzymes [125]. If translocation fails, cytotoxic activity is deficient as lytic granules are not properly secreted. Upon canonical contact with their target cells, CTLs translocate their lytic granules to the cSMAC in an actin-independent manner. This is mediated by the minus end of microtubules and the translocation of the centrosome toward the secretory domain [126]. Once at the cSMAC, granules fuse directly with the plasma membrane and granzymes and perforins are released toward the immune synapse. This is mediated by signaling endosomes. The proper fusion of cytotoxic granules and the release of their content requires the previous fusion of VAMP8 positive vesicles coming from recycling endosomes with the plasma membrane. These vesicles translocate syntaxin-11 to the plasma membrane, creating a specific exocytosis anchoring site for SNARE proteins-directed granule fusion [127]. The formation of a canonical immune synapse to secrete vesicles is not mandatory for these T cells [128]. Preformed synaptic granules can be secreted at the cytotoxic synapse before MTOC translocation [129]. TGN38/LFA-1-positive vesicle subpopulation partially co-localizes with neuroserpin, a serine protease inhibitor that is secreted axonally in neurons, contributing to the formation of the neuronal synapse and the refinement of synaptic activity. Upon TCR activation, these neuroserpin-containing vesicles translocate toward the immune synapse and release their content at the synaptic cleft and its surroundings [130]. The role of this protease inhibitor modulating the immune cell function deserves future research.

The second secretory pathway polarized toward the immune synapse is the endolysosomal system; specifically, receptors and proteins from the membrane are endocytosed and recycled. TCR signaling and function not only depend on its expression but also on its membrane localization and the dynamics of TCR-CD3 complex, including its recycling into vesicles. The degradation of the TCR is important for synapse termination and its downstream signaling. In homeostatic conditions, the TCR is continuously cycling between plasma membrane and endosomes (Fig. 2-2), and can be eventually degraded by lysosomal fusion. T cells keep a pool of CD3-TCR complexes ready to recognize antigen. T cells require vesicular traffic at the immune synapse site for activation and ending of the synapse. Upon TCR engagement, endosomal trafficking is redirected toward the immune synapse by microtubules and endosomal traffic regulators such as vesicle transport proteins, docking proteins, and fusion regulatory molecules [131–135].

TCR activation triggers its rapid uptake through a clathrin-independent pathway, and it is then incorporated into a flotilin-positive endocytic network, which is essential for the correct recycling of the TCR to the membrane again [136]. Arp2/3 controls the surface levels of TCR by regulating TCR endosome trafficking through its binding to actin filaments [137]. Moreover, Lck and LAT proteins also traffic through recycling endosomes. Nevertheless, molecules that regulate this recycling and fusion process are different. The subcellular localization and function of Lck depends on the Rab11 effector FIP3, while it only indirectly modulates CD3-TCR cell surface expression through Lck mediated TCRζ phosphorylation [138]. LAT vesicles undergo a retrograde transport to the Golgi apparatus, which increases upon TCR activation. Golgi proteins Rab6 and Syntaxin 16 are essential for the proper LAT recycling and recruitment to the immune synapse [139,140]. Therefore, a complex framework of endosomal vesicles fine-tunes the T cell response beneath the synaptic cleft membrane, by either exposing, hiding or degrading receptors and signaling molecules (Fig. 2-3).

Another level of complexity includes the role of late endosomes, also called multivesicular bodies (MVB). These organelles are enriched in specific proteins and may form part of the cell recycling system by fusing with lysosomes to degrade their content. On the other hand, they can also serve as secretory organelles, by fusing with the plasma membrane. In T cells, MVB reorient toward the immune synapse and release there their content [141,142]. Intraluminal vesicles (ILVs) form by the in-budding of the endosomal membrane. ILVs are small vesicles that are confined inside of MVB, that range between 50–100 nm of diameter and that are enriched in proteins and genetic material. The endosomal Sorting Complex proteins (ESCRT) recruit other partner proteins during this process and determine the sorting of different components to ILVs [143]. The sorting mechanisms of exosomal proteins and genetic content are still under study. We have proven the existence of sorting mechanisms that regulate the specific transport of selected RNAs and proteins into the exosomes. The process is directed by posttranslational modifications [144]. The ESCRT complex is the responsible of sorting ubiquitinated proteins to MVB [145], however, de-ubiquitination is essential for packaging the cargo into the ILV [146]. Other ubiquitin-like modifiers (UBL) have been also related with the sorting of proteins, for example ISGylation tags the proteins for lysosomal degradation and prevents them for being sorted into ILV [147]. Likewise, acetylation also impairs protein sorting into ILV [148]. Finally, some miRNA can be sorted into ILV when they are bound to post-translational modified proteins such as sumoylated hnRNPA2B1, which binds specific exo-motifs, and transport miRNA to the MVB [149]. Other sequences also target miRNA to MVB. Hence, it has been described an exo-motif that allows the binding of SYNCRIP to the miRNA and sort it to the MVB in hepatocytes [150]. Although some of the mechanisms for miRNA sorting into exosomes have been reported, this is still an area boasting many outstanding questions to be solved. This knowledge opens the way for the possible selective modification of the miRNAs exosomal cargo and increases the interest for the exosomal field for its implications in translational medicine.

At the IS, the MVB external membrane fuses with the plasma membrane to release the ILVs into the synaptic cleft, which are then called exosomes (Fig. 2-4). TCR activation can induce the secretion of TCR-TSG101-containing exosomes [151] and lead to proliferation in autologous resting CD8 T cells, acting synergistically with IL2 [152]. These extracellular vesicles (EVs) carry proteins and genetic material that are then transferred to recipient cells by using the IS as a focal point for polarized secretion. The uptake of these exosomes facilitates the release of their content into the recipient cells that may modify their behavior, consequently. Specifically, the uptake of exosomes loaded with miRNA that are secreted by CD4 T cells in the context of a synapse causes the downregulation of SOX4 in B cells [141]. The transfer of exosomes loaded with mitochondrial DNA (mtDNA) from CD4 T cells to dendritic cells (DCs) primes them, conferring antiviral resistance to these DCs [153]. Viruses (i.e. HIV) are able to hijack the polarized secretory machinery and spread across cells through immune synapses [154,155]. Apart from HIV, other viruses use the exosomal secretory machinery to disseminate [156].

The secretion of larger vesicles that bud directly from plasma membrane, usually called microvesicles or ectosomes has also been described. Upon antigen cognate recognition with the APC, membranes from CD4 T cells start to bud from the cSMAC of the synapse [157] (Fig. 2-5). TSG101, located at the cSMAC [158] is the responsible for the sorting of TCR into those EVs, while VPS4, which also belongs to the ESCRT machinery, is the responsible of the scission of the microvesicles from the plasma membrane. After activation, T cells secrete microvesicles that are loaded with transmembrane-CTLA-4 negative regulator [159]. However, the functional roles of microvesicles containing CTLA-4 needs further investigation.

6. Concluding remarks

T cell polarization is a fine-tuned, complex process that requires many steps in order to form a functional synapse and enable T cell activation. The signals sensed by receptors on the T cell surface spread to the intracellular compartment, where components that are previously located, recycled or redirected to the endosomes then participate in the translation of the signal into activating actions. Many steps of the signaling and mechanical processes remain unknown, for example, how the actin cytoskeleton directly interacts with the MTOC and microtubules and their implications. Another mainly unexplored field is the role of intermediate filaments in the engagement and signaling of the immune synapse. It is also interesting the in depth study of the molecules that are transferred through this structure and their role in the recipient cell.

The endosomes also participate in the communication with the APC, through the directed secretion of specific components that are sorted into compartments of the endolysosomal systems, such as the MVB. The control of this sorting will impact in the behavior of the antigen-presenting cells upon immune synapse completion, and it is matter of future research. The exchange of proteins and genetic material between immune cells during inflammatory processes and infectious diseases may serve to engineer different responses against threats. The elucidation of these mechanisms would open new possibilities for therapies in infectious diseases and in autoimmune conditions.

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