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Microtubule dynamics and signal transduction at the immunological synapse: new partners and new connections

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Antigen recognition induces T cells to polarize towards antigen presenting cells (APC) generating an organized cell interface named the immunological synapse. T-cell microtubules (MTs) reorient the MT-organizing centre (MTOC) to the immunological synapse central region, while MTs irradiate towards the synapse periphery. Martín-Cófreces *et al* (2012) describe in this issue that the MT plus-end-binding protein 1 (EB1) interacts with TCR cytosolic regions and mediate the organization of an immunological synapse fully functional to transduce activation signals.

The pioneer work of Kupfer and Singer (1989) established that T-cell MTs rearrange in response to specific TCR engagement by APCs, resulting in MTOC orientation to the

APC contact site in helper and cytotoxic T cells. MTOC reorientation was shown to be the result of a MT polymerization dynamic process involving MT posttranslational modifications (Kuhn and Poenie, 2002; Serrador *et al*, 2004). MT reorganization during T-cell antigen recognition is functionally linked to T-cell effector functions, like the polarized secretion of helper cytokines to B cells (Kupfer *et al*, 1991; Huse *et al*, 2006), or cytotoxic granules to target cells (Stinchcombe *et al*, 2006). MTs also transport TCR-carrying endosomes during synapse formation (Das *et al*, 2004) and TCR signalling complexes at the immunological synapse (Lasserre *et al*, 2010; Hashimoto-Tane *et al*, 2011). Altogether, these findings show that the dynamic reorganization of MTs and its related molecular

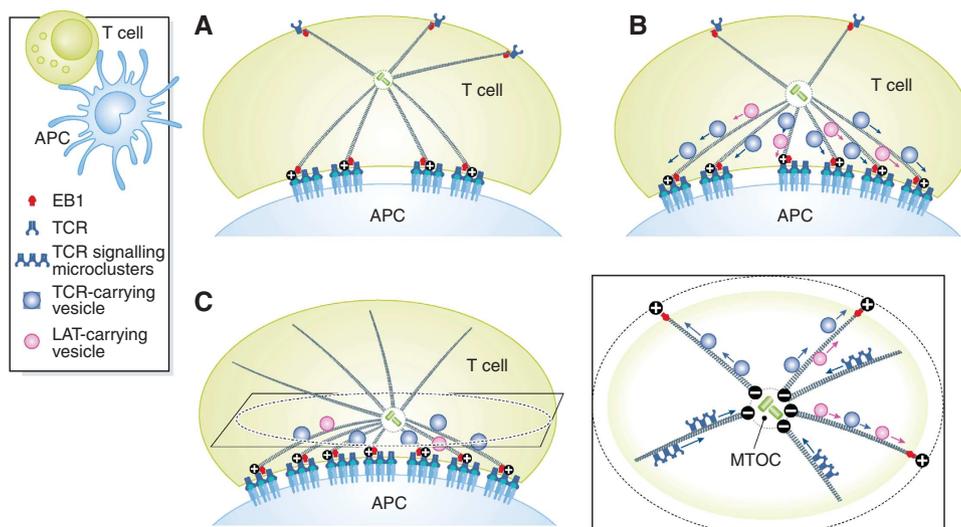


Figure 1 Model of the role of EB1 in MT dynamics and TCR signal transduction at the immunological synapse. (A) Initial T cell–APC contact. TCR initial clustering would favour the capture of EB1-containing MT plus ends at the T cell–APC contact. (B) Immune synapse formation. The increase capture of MTs plus ends by TCR clusters would promote the arrival of TCR ζ - and LAT-carrying vesicles leading to increase TCR and LAT clustering and encountering at the synapse. Alternatively, EB1 interaction with TCR could also be directly involved in TCR ζ vesicle transport to the synapse. In turn, increase TCR clustering would promote additional MT and capture, building an amplification loop for MT dynamics and vesicle transport. (C) Established immunological synapse. A structured MT network would facilitate the continuous arrival of TCR ζ - and LAT-carrying vesicles through the MT plus ends at the immunological synapse periphery. Then the centripetal movement of TCR signalling complexes towards the MT minus end at the MTOC close to the synapse centre would bring signalling complexes to signal extinction sites (i.e., endosomes). The right panel in C represents a xy section of the immunological synapse, as it is observed on stimulatory cover slips.

transport are critical for the organization and function of the immunological synapse.

Martín-Cófreces *et al* (2012) present here interesting new insights, unveiling a link between EB1 and the TCR complex. EB1 is one of a series of MT plus-end-associated proteins critical for MT polymerization dynamics (Slep, 2010). The first important finding initially issued from a two-hybrid screening was that EB1 could directly interact with TCR complex cytosolic regions. By GST pull-down and co-immunoprecipitation experiments, the authors narrowed down this interaction to two of the TCR complex subunits, ζ and ϵ , in their ITAM (immuno-receptor tyrosine-based activation motif)-containing regions, and within the C-terminal 82 amino-acid region on EB1. In T cells, EB1–TCR interaction could occur without TCR stimulation, suggesting that EB1 plays a role in TCR dynamics previous to TCR engagement. The authors then investigated EB1 localization and its involvement in synapse organization and function. Live cell imaging showed intense EB1 movement in the synapse area, with MTs growing from the MTOC to the synapse periphery, leading to an apparent concentration of EB1 at the T cell–APC interface. To analyse the relationship between MT dynamics and intracellular transport, the authors followed EB1–GFP and TCR ζ –Cherry by total internal reflection fluorescence (TIRF) microscopy in synapses formed on anti-CD3-coated cover slips. They observed transient coincident spots between EB1 and TCR ζ + vesicles, suggesting that growing MTs transport TCR ζ -carrying vesicles towards the immunological synapse. Consistently, EB1-silenced cells displayed altered TCR ζ vesicle dynamics and TCR ζ clustering at the synapse. Likewise, vesicle transport to the synapse of the signalling scaffold molecule LAT and its clustering at the synapse were altered. Finally, they observed transient encounters between TCR ζ - and LAT-carrying vesicles inhibited by EB1 silencing. These observations point out to a crucial role of EB1 and MT dynamics in the organization of the immunological synapse.

Immunological synapse organization has been related with its capacity to regulate TCR signal transduction. Therefore, Martín-Cófreces *et al* (2012) investigated how EB1 silencing impacted TCR signalling. EB1-silenced cells were indeed impaired in key TCR signalling events, like LAT tyrosine phosphorylation, which allows LAT interaction with activation effectors, like the phospholipase C (PLC) γ , promoting TCR signal propagation. Consistently, PLC γ activation was impaired in EB1-silenced cells. However, upstream activation events, like tyrosine phosphorylation of TCR ζ and of its associated protein tyrosine kinase ZAP70, were not altered. This suggests that MT-dependent LAT vesicle traffic is key for LAT phosphorylation and the generation of TCR signalling complexes.

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Altogether, Martín-Cófreces' findings reinforce the idea that polarized vesicle transport *via* organized MT networks is key to set up the immunological synapse as a signal transduction platform. EB1 interaction with two TCR subunits may link the TCR complex with MTs dynamics. It remains unanswered, however, whether EB1 also interacts with LAT, facilitating the merging at the synapse of distinct TCR ζ - and LAT-carrying vesicles.

Vesicle traffic on MTs generally occurs *via* molecular motors from the dynein and kinesin families. The former are associated with minus end-oriented transport, whereas the later mostly ensures plus-end-associated transport. The immunological synapse may use both types of transport. Thus, cytotoxic granule delivery to the synapse may mainly involve dynein-mediated vesicle traffic, since the MTOC translocates very close to the immunological synapse (Stinchcombe *et al*, 2006). Likewise, centripetal movements of signalling microclusters at the synapse involve dynein (Hashimoto-Tane *et al*, 2011). Martín-Cófreces *et al* (2012) show that TCR ζ - and LAT-carrying vesicles are transported towards MT plus ends in an EB1-dependent manner. It remains uncertain whether EB1 could play a direct transport role at the immunological synapse, helping the attachment of TCR ζ vesicles to growing MT plus ends. Alternatively, EB1 could mediate MT interactions with TCR complexes present at the plasma membrane. Initial TCR clustering at the synapse would help capturing EB1-positive MT plus ends, orienting MTs and MT-mediated traffic of TCR ζ - and LAT-carrying vesicles to the synapse by a kinesin-based transport (Figure 1), and promoting TCR ζ and LAT encountering and clustering at the synapse. EB1 silencing would perturb MT–plasma membrane interactions impairing this MT orientation and transport loop. MT polymerization kinetic studies on immunological synapses formed by EB1-silenced *versus* control T cells may help to clarify this mechanism. Although further studies will be necessary to elucidate the detailed mechanism, the work by Martín-Cófreces *et al* (2012) already highlights the importance of MT dynamics and vesicle traffic in the formation of a functional immunological synapse, raising novel and interesting questions on how the MT network helps to set up complex signal transduction machineries.

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Conflict of interest

The authors declare that they have no conflict of interest.

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