T-cell synaptic ectosomes relay signals through microcluster transfer

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Background: Extracellular vesicles (EV) are proposed to transfer information between cells. In the immunological synapse T cell receptor (TCR) interaction with pMHC drives microcluster formation and signalling that is terminated in parts through sorting of TCR into EVs that bud into the synapse, synaptic ectosomes (SE). Previously, we used correlative light and electron microscopy to characterize SEs. However, this approach has some limitations such as the poor resolution of fluorescent signals and the lack of information on receptor organization in individual SE.
Methods: SE released by CD4 T cells were captured on planar supported lipid bilayer (PSLB) containing either ICAM1, ICAM1 and aCD3 or ICAM1, aCD3, CD40 and ICOSL. SEs were stained with WGA to visualize the membrane and with directly conjugated antibodies against TCR, CD40L, ICOS, BST2 and imaged by multicolour dSTORM. To assess functionality of released SEs, DCs maturation after incubation with SEs was measured by cytokine array.
Results: SEs released onto PSLB containing ICAM1, CD40, ICOSL and aCD3 were ~80 nm in size and about 40 SEs were released per cell. Three subsets of SEs were observed, one having only TCR, another having only CD40L, ICOS or BST2, and the majority double positive for TCR and CD40L, ICOS or BST2. TCR microclusters colocalized with ICOS and BST2, but segregated from CD40L within single SEs. This is consistent with TCR, BST2 and ICOS occupying overlapping microclusters, whereas TCR and CD40L occupy spatially distinct microclusters. Distinct TCR and CD40L microclusters on SEs transferred to PSLB can stimulate DCs in an antigen independent manner. Our data suggest that T cells release different subsets of SEs with different protein composition that constitute functional units to activate DCs.
Summary/Conclusion: We propose that SE transfer perpetuates the biological impact of receptors and ligands coclustering in interfaces beyond the separation of the interacting cells. This enables T cells to distribute information in cellular networks within tissues. Linkage of TCR and CD40L clusters in single SE offers additional opportunities for specificity and synergy. SEs provide a general strategy to perpetuate signals initiated in cell–cell interfaces beyond the period of synapsis.
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