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Translocation of the microtubule organizing center: How localized signaling leads to localized secretion in T cells.

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When T cells make contact with cells displaying antigen, signaling through the T cell receptor leads to rapid formation of a specialized contact site known as the immunological synapse. The synapse represents the localized assembly of signaling, cytoskeletal, and adhesion complexes that orchestrate the T cell effector response, which at its heart, entails secretion. This secretory response depends on translocation of the microtubule organizing center (MTOC) up to the synapse bringing with it attached secretory vesicles[1, 2] and spatially directing subsequent vesicle movements[3].

In order to better understand how the MTOC translocated to the synapse we developed a new type of microscopy called modulated polarization microscopy that allowed us to monitor individual microtubules and the MTOC in living cytotoxic T cells[4]. Modulated polarization microscopy works by modulating the plane of polarized light presented to the specimen and then demodulating the light after passing through the specimen. This causes birefringent elements to exhibit a sinusoidal optical signal against a dark background that can be isolated and quantified using a single frequency Fourier filtering algorithm. Using this instrument, we were able to follow movements of the MTOC and showed that these movements correlated with tensioning of microtubules anchored to the cortex. Real-time studies of microtubule and MTOC movements suggested that motor proteins (presumably dynein) formed a circular array at the synapse[5]. Using computerized 3D reconstructions of antibody-labeled microtubules together with enhancement using 3D identification and filtering algorithms we obtained clear pictures of the microtubule array that showed that that there was a circular zone where microtubules came in contact with the cortex and this zone corresponded to regions where the integrin LFA-1 was clustered at the synapse, a region known as the pSMAC.

Subsequent studies using Jurkat cells showed that certain antibodies against the dynein intermediate chain (DIC) labeled a circular ring-like structure at the synapse whereas other anti-DIC antibodies labeled only microtubules and the MTOC. The dynein ring colocalized with ADAP, a scaffold protein that is part of the T cell signaling cascade as well as some known dynein binding proteins including β-catenin and PLAC-24[6]. Interestingly, it did not correlate with the localization of dynactin, which was more clearly associated with microtubules and the MTOC. When ADAP expression was reduced, dynein failed to accumulate at the synapse and MTOC translocation was blocked. Although ADAP seems to be essential for MTOC translocation it is not required in mouse T cells making it likely that other conserved molecules are involved. One of these appears to be LIS-1 which also part of the dynein complex at the synapse.

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