the C terminus appears to regulate trafficking of this highly toxic protein. Perhaps the most compelling questions remain, however. What mechanisms restrain this toxic protein as it traverses organelles so rich in phospholipids that would support its binding and oligomerization? Are there specific chaperones unique to PFN that hasten the monomer through the ER and TGN? Is the phospholipid composition of the organelles less desirable than the content of the external leaflet of the plasma membrane dissuading the monomer from enforcing its destructive tendencies (Yang et al., 2010)? And, would the protective mechanisms differ for PFN that is subjected to regulated versus constitutive secretion? Although these questions loom large, it would at least appear that the endoplasmic reticulum regards nascently synthesized perforin as an unwelcome occupant and is prepared to evict this tenant posthaste.

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Lymphocyte Signaling Converges on Microtubules

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Movement of immunoreceptor microclusters tunes lymphocyte activation, but the underlying mechanisms are incompletely understood. In this issue of *Immunity*, Schnyder et al. (2011) and Hashimoto-Tane et al. (2011) show that cytoplasmic dynein drives microcluster centralization along microtubules.

Lymphocyte activation involves a high degree of spatiotemporal control. Initial T cell receptor (TCR) signaling drives remodeling of cytoskeletal elements at the T cell–antigen-presenting cell contact site, forming the scaffold for the "immunological synapse" (IS) (Burkhardt et al., 2008). This polarized cortical domain sustains cell-cell adhesion and promotes subsequent signaling events needed for full T cell activation. Recently, it has become clear that B cell recognition of surface-bound antigens involves a similar cytoarchitecture, which in this case serves to facilitate B cell receptor (BCR) signaling and antigen internalization (Harwood and Batista, 2010).

As early as the 1980s, several groups studying the T cell response observed polarization of the actin cytoskeleton as well as the microtubule-organizing center (MTOC) and associated secretory organelles toward the T cell–antigen-presenting cell binding site (Kupfer and Singer, 1989). Initially, the field focused on the role of cytoskeletal elements in promoting polarized effector function. In particular, it was noted that lytic granules and cytokine-containing secretory vesicles associate with the MTOC, such that polarization of the microtubule array sets the stage for delivery of cytotoxins or cytokines to the intended target cell. Recent research in this arena has focused on defining the signaling events that control the polarization of the MTOC and associated secretory organelles (Huse et al., 2008; Lasserre and Alcover, 2010). Available evidence points to a tension-based mechanism driven by the minus-end-directed microtubule motor protein cytoplasmic dynein, anchored to the T cell cortex at sites of T cell signaling. Numerous other proteins have also been implicated in MTOC reorientation, including LFA-1 and the adaptor protein ADAP, the formins diaphanous and FLH1, the actin-binding protein ezrin and associated PDZ-domain protein hDLG1, diacylglycerol and PKC, and the tubulin regulatory histone deacetylase HDAC6. The mechanisms through which these diverse molecules coordinate MTOC reorientation remain to be elucidated.

In addition to organizing effector function, the T cell cytoskeleton serves as a dynamic scaffold for TCR signaling. Actin polymerization is needed to initiate and sustain TCR signals, and high-resolution imaging techniques show that actin

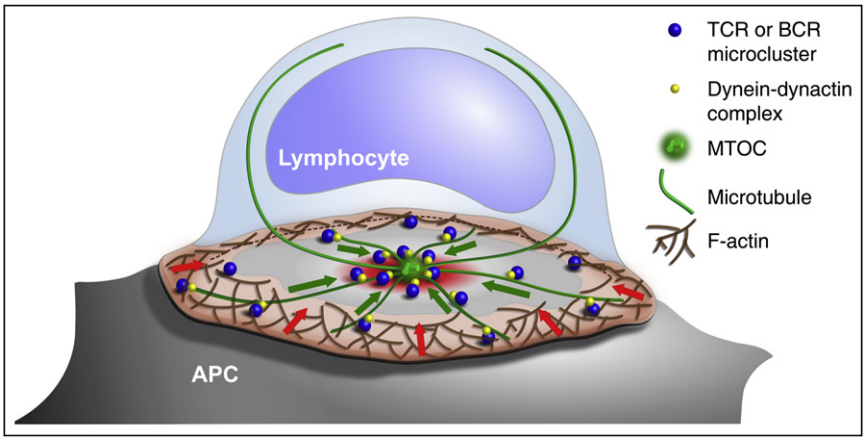

Figure 1. Cytoplasmic Dynein Mediates Microtubule-Dependent Trafficking of Immunoreceptor Microclusters, Resulting in Signal Extinction in T Cells and Enhanced Antigen Gathering in B Cells

B and T lymphocytes spreading on stimulatory planar lipid bilayers exhibit assembly of immunoreceptors and associated proteins into membrane-bound microclusters that translocate continuously toward the center of the immunological synapse. F-actin retrograde flow drives microcluster formation and initial peripheral movement (red arrows). Hashimoto-Tane et al. (2011) and Schnyder et al. (2011) now show that cytoplasmic dynein drives microcluster movement along microtubules and plays an essential role in microcluster centralization (green arrows). The delivery of signaling molecules to the central zone (red gradient) is associated with downmodulation of signaling in T cells and with efficient antigen uptake in B cells.

polymerization promotes the assembly of signaling microclusters at the periphery of the IS and drives their centripetal flow toward the central region where signal termination takes place (Burkhardt et al., 2008). Parallel events occur in B cells, where BCR engagement of surface-associated antigen initiates actin-dependent spreading and contraction (Harwood and Batista, 2010), in conjunction with centripetal movement of microclusters containing BCR, bound antigen, and specific signalosome components. During the contraction phase, this centripetal movement leads to the gathering of antigen into the central region of the IS, where it is internalized for degradation and presentation to T cells.

Although substantial progress has been made in defining actin-dependent signaling mechanisms, much less is known about the role of the microtubule cytoskeleton as an organizer of lymphocyte signaling. Microtubules direct the recycling of membrane-associated signaling components to the IS. Moreover, microtubules are known to serve as a reservoir for certain signaling molecules, and MTOC reorientation could bring these proteins into proximity with the IS. Recent studies, however, point to a more direct role for microtubules in shaping events at the IS. For example, TCR signaling induces transient deacetylation and reacetylation of tubulin, and overexpression of the relevant deacetylase, HDAC6, leads to disorganization of signaling microclusters at the IS and diminished production of IL-2 (Serrador et al., 2004). Similarly, suppression of the actin-binding protein ezrin results in perturbation of the MTOC array at the IS, an alteration that is correlated with disorganization of microcluster dynamics and perturbation of signaling (Lasserre et al., 2010).

In T cells and B cells that have undergone immunoreceptor-induced cytoskeletal polarization, the MTOC lies at the convergence point for centripetal microcluster movement. Thus, it has been appealing to posit that microclusters move along the radial microtubule array. However, direct evidence supporting this idea has been lacking. In this issue, Hashimoto-Tane et al. (2011) provide evidence that TCR signaling microclusters move along microtubules in a dynein-dependent fashion, and Schnyder et al. (2011) demonstrate a parallel process for BCR-dependent antigen gathering. Cytoplasmic dynein is a large, multichain molecule that typically associates with cargo via a cofactor termed the dynactin complex. Hashimoto-Tane et al. use a combination of biochemical and microscopy-based approaches to show that the CD3ε associates with cytoplasmic dynein and the dynactin complex. The authors show that some microtubules are closely apposed to the T cell plasma membrane, and they have captured images of TCR microclusters moving along these tracks toward the central supramolecular activation cluster (cSMAC), the central region of the IS. Interestingly, although cytoplasmic dynein function is typically associated with movement of intracellular organelles, the authors show that the dynein-driven TCR complexes remain in the plasma membrane. Thus, this process may bring together cell-surface molecules with intracellular vesicles containing signaling molecules such as LAT. Finally, and most importantly, the authors show that suppression of cytoplasmic dynein expression perturbs cSMAC formation. Consistent with the idea that the cSMAC functions as a site for signal termination, dynein suppression is also associated with prolonged phosphorylation of signaling molecules.

The paper by Schnyder et al. reports parallel findings in the B cell system and provides additional information about the signaling molecules that link BCR microclusters to the microtubule cytoskeleton. Like the T cell study, this paper shows that components of the BCR signaling complex interact with components of the cytoplasmic dynein-dynactin complex. Moreover, this study shows that perturbation of dynein-mediated motility (either by suppression of cytoplasmic dynein expression or by disruption of the dynactin complex) inhibits centripetal microcluster movement and antigen gathering. Importantly, B cell spreading and initial microcluster formation, processes that depend on the actin cytoskeleton, are intact in these cells. Finally, the authors take advantage of the powerful genetics provided by the chicken DT40 system in conjunction with quantitative mass spectrometry to identify molecules that link microclusters to the dynein motor complex.

Together, these two papers provide long-needed evidence that microtubules play an active role in lymphocyte signaling at the level of microcluster dynamics, leading to a model in which a circumferential actomyosin network intersects with a radial microtubule array (Figure 1). Now, important new questions arise. First, of course, it will be interesting to ask whether the signaling intermediates

identified by Schnyder et al. in B cells play an analogous role in T cells. And in both systems, it will be important to continue to define the molecular mechanisms that control microcluster movement along the microtubule network. But there are also broader questions. The implication from these papers is that signaling microclusters generated in an actin-dependent fashion in the periphery of the IS and moved initially by actomyosin-dependent forces transition to dynein-dependent movement for final delivery into the center of the IS. If so, how does this "hand-off" take place without an obvious change in microcluster velocity or trajectory, and why does depolymerization of actin filaments result in loss of microcluster movement, rather than enhanced dynein-dependent movement? How does the microtubule network contribute to the supramolecular segregation of signaling components? Clearly, current models invoking differential actin binding or actin-dependent clustering (Hartman et al., 2009) must be revised. How do the two filament systems work with respect to plasma membrane-associated proteins versus vesicle-associated molecules? And finally, how are the actin and microtubule networks coordinated? Based on studies in nonhematopoietic cells (Etienne-Manneville, 2004), it seems likely that regulation of these two scaffolding systems is intertwined, and that understanding this crosstalk will be essential for understanding cytoskeletal control of lymphocyte function.

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A Fine Romance: T Follicular Helper Cells and B Cells

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T follicular helper (Tfh) cells help B cells to generate affinity-matured antibodies. Three papers in this issue of *Immunity* (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011) provide information about the reciprocal relationship between B cells and Tfh cells.

It was reported more than two decades ago that T cell clonal expansion in lymph nodes (LN) was impaired in mice made deficient in B cells by continuous injections of antibodies directed against the heavy chain from birth (Ron and Sprent, 1987). Like other T cells, B cells are crucial for the development of a specialized subset of $CD4^+$ T helper cells known as T follicular helper (Tfh) cells (Haynes et al., 2007). The relationship between Tfh cells and B cells is thought to be especially important because of a reciprocal dependency played out during the generation of affinity-matured antibody. This cognate interaction occurs in specialized, temporary structures, termed germinal center (GC) reactions, that form within B cell follicles of secondary lymphoid organs after infection or immunization with nonreplicating T cell-dependent antigen.

GC B cells require T cell help to produce affinity-matured antibody. More recently, however, evidence has been presented to show that this dependence is not reciprocal because Tfh cells can develop without B cells, provided that the T cells get adequate stimulation from peptide antigen-MHCII complexes displayed on other antigen-presenting cells (APCs) (Deenick et al., 2010). This finding indicated that the role of B cells may reflect their ability to provide an ample source of antigen to Tfh cells and questioned whether B cells provide any unique signals. Which antigen-presenting cells Tfh cells interact with at different points during their activation is explored in detail in three papers in the current issue of *Immunity* (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). One clear