Microvilli set the stage for T-cell activation

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T cells are a major cellular component of the adaptive immune system. In particular, the recent wave of cancer immunotherapies that target the patient's own T cells to attack malignant tumors have put the spotlight on T-cell antigen receptors (TCRs). T-cell activation begins when the T-cell receptor engages an antigenic peptide bound to a major histocompatibility complex-encoded molecule (pMHC) on the surface of an antigen-presenting cell (APC). This interaction catalyzes a number of signaling cascades downstream of the TCR, leading to T-cell differentiation, cytokine production, and targeted cell killing (1).

In addition to the TCR, a number of key enzymes and adapter molecules involved in T-cell activation, as well as the biochemical processes underlying their interactions, have been identified in the past few decades (2). Advances in microscopy have enabled a number of these discoveries, leading to a more detailed and comprehensive understanding of the T-cell activation process. In the study by Jung et al. (3), a novel combination of superresolution microscopy techniques is used to visualize the 3D distribution of TCRs and other molecules at the T-cell surface, leading to the discovery of a new role for microvilli in priming T cells for activation.

Receptors involved in antigen recognition (e.g., TCR), adhesion (e.g., integrins), and down-regulation of T-cell activation (e.g., CD45) have been shown to segregate into distinct, concentric regions at the activated T-cell membrane, forming a bull's-eye-patterned structure called the immunological synapse (4). The immunological synapse is thought to mediate the sustained TCR signaling required for full T-cell activation and is formed via actin-dependent transport of heterogeneous, multiprotein complexes called microclusters (5–7). Previous studies have shown that microclusters are composed of molecules involved in the TCR signaling cascade and, consistent with the results in Jung et al., appear rapidly at the first points of contact between the T-cell membrane and an activating surface (5). In addition, superresolution imaging of activated T cells has shown that distinct patterns of molecular localization can be observed within individual

Fig. 1. (A) Scanning EM image of an effector T cell by Jung et al. (3). (Scale bar: 1 μm .) (B) Representative 3D surface reconstruction map of a human resting T-cell membrane by Jung et al. (3), obtained from VA-TIRFM, an imaging method that measures the distance of the cell membrane from the glass surface, as indicated by δz (in nanometers). (Scale bar: 0.5 μm .) (C) Localization map (red dots) of $\alpha\beta$ TCR molecules on the cell from B, overlaid with the 3D surface reconstruction map (gray scale) by Jung et al. (3). (Scale bar: 0.5 μm .)

microclusters [e.g., TCR ζ and linker for activation of T cells (LAT)] (8, 9).

Despite these observations, the basis for the formation of spatially segregated microclusters and the mechanism of their rapid recruitment to the contact site still remain unknown. One model has proposed that the major TCR signaling molecules, TCR ζ and LAT, exist in segregated domains called “protein islands” before activation and concatenate into the bigger microcluster structure upon activation (8). Another study has proposed that the TCR ζ chain and LAT exist predominantly as complexes of two to three molecules that nucleate larger microclusters upon activation (9). However, these superresolution studies were performed at a single plane of illumination and lacked information about their location within the 3D topography of the T-cell membrane.

Scanning EM images show that a dominant feature of the surface of resting T cells are finger-like protrusions called microvilli (Fig. 1A). Microvilli are formed by polymerized F-actin filaments and characterized by clusters of adhesion proteins (e.g., L-Selectin) at their tips (10). To visualize the distribution of receptors at the surface of resting T cells, Jung et al. used a combination of variable-angle-total internal reflection fluorescence microscopy (VA-TIRFM) to visualize the 3D topography of the cell surface (Fig. 1B), and stochastic localization nanoscopy to locate single molecules within the

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VA-TIRFM image (Fig. 1C). Surprisingly, in addition to the microvilli marker L-Selectin, Jung et al. observed clusters of TCR molecules at the microvilli tips in resting T cells (Fig. 1C). As the TCR clusters were observed before T-cell activation and were microvilli dependent, these results suggest that microvilli serve as the structural scaffold for clustering of TCRs and for the recruitment of microclusters upon TCR engagement. Moreover, these results are consistent with the observation of preformed TCR clusters in unactivated T cells (8, 9) and mirror the selective localization of MHC molecules at the tips of membrane protrusions in APCs (11).

This novel role of microvilli in positioning preformed TCR clusters has multiple implications regarding the spatial and dynamic regulation of T-cell activation. First, previous models of microcluster formation have assumed that microclusters arise from stochastic interactions between freely exchanging membrane proteins. Instead, microcluster formation may be limited to areas of membrane that

initiate mutual contact between protrusions of T cells and APCs. Second, microcluster size may be limited to the physical dimensions of microvilli tips, unlike the large variations in size that are permitted in both the protein island and oligomer nucleation models. Third, the involvement of microvilli would add an important structural element to the kinetic segregation model of immunological synapse formation, which predicts that activated surface receptors segregate into distinct, concentric regions based on the relative sizes of their extracellular domains (12). Last, the connection between microclusters and the actin cytoskeleton may be at the tips of bundled actin filaments present in microvilli and provide the mechanism by which microclusters become transported by cortical actin flow. As the implications of microvilli's involvement in microcluster formation and T-cell activation are wide-ranging and important, further experiments will be needed to validate and extend the observations made by Jung et al.

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