cytoskeleton opposed to the plasmalemma. Here, we investigated the role of the cortical actin cytoskeleton and plasma membrane nanodomains in the control of CD36 activation in endothelial cells. Stimulation with multivalent ligands (TSP-1 and anti-CD36 IgM) resulted in the downstream phosphorylation of the Src Family kinase, Fyn. Disruption of the actin cytoskeleton or removal of cholesterol blocked this activation. To gain molecular details on the rearrangement of the receptors during TSP-1 binding, we conducted superresolution approaches (based on PhotoActivated Localisation Microscopy or PALM) and quantitative spatial distribution analysis. Endothelial cell lines stably expressing CD36-PamCherry were generated for this purpose. At steady state, CD36 receptors pre-exist in small clusters (average diameter of 100 nm), in which Fyn was also present. Upon TSP-1 binding, CD36 clusters increased in size (average diameter of 140 nm) and also became denser. The average distance between CD36 molecules in these clusters was in the range of 8 nm compare to 11 nm in the control condition. F-actin depolymerization or cholesterol depletion reduced the capacity of the ligand to induce formation of larger clusters resulting in a decreased recruitment and activation of Fyn. Our data demonstrate cooperation between cholesterol-dependent domains and the cortical actin cytoskeleton in the organization of CD36 receptors and Fyn before and during TSP-1 stimulation.

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Super-Resolution Imaging of IgE-FcεRI Stimulated with Structurally Defined Ligands
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IgE-mediated signaling via its receptor FcεRI in mast cells is of interest due to its role in allergic responses, and as a model system for immune receptor signaling. To investigate IgE-FcεRI redistribution and dynamics in response to antigen stimulation, we utilize stochastic optical reconstruction microscopy (STORM), a super-resolution imaging technique. STORM permits visualization of IgE at the membrane of live or chemically fixed cells at a resolution of about 30 nm, which allows us to examine the initial events of IgE receptor signaling at a new level of molecular detail. Physiologically, cross-linking of IgE-FcεRI by multivalent antigen initiates the transmembrane signal, and we ask how this signaling capacity relates to the cross-linked structure of the clustered IgE-FcεRI. To investigate this question, we utilize STORM imaging in conjunction with structurally defined ligands, which correspondingly cross-link IgE with a high level of definition. We use trivalent ligands based on rigid Y-shaped scaffolds of double-stranded DNA, which have one 2,4-dinitrophenyl (DNP) hapten conjugated to each arm. The development of the new platform will be exploited to address and answer surface and thereby imitate the antigen presenting cell (APC) more closely. The origami structures serve as sequence specific binding sites for a variable number of single stranded DNA strands to which antibodies against proteins important in T-cell activation can be attached. The variable linkers revealed the structural basis for experimentally demonstrated tight coupling between extra- and intra-cellular domains and the TM region, based on receptor stability and dimerization efficiency. These data improve our understanding of the assembly and signaling mechanisms in TLRs, and may facilitate design of ligands with specific immunomodulatory properties, paving the way for new therapeutic treatments of inflammatory diseases.

Exocytosis and Endocytosis

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Closure of Pre-Existing Ω Profiles Mediates Compensatory Endocytosis, Endocytosis Overshoot and Bulk Endocytosis
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In neurons and endocrine cells, depolarization induces exocytosis, whereas endocytosis retrieves exocytosed vesicles to maintain secretion. It is generally thought that after depolarization, endocytosis must involve three steps, the Ω-profile formation, vesicular membrane protein recruitment, and Ω-profile closure. Here we report a novel form of endocytosis that involves only one step after depolarization, the pore closure of pre-existing Ω-profiles, in neuro-endocrine chromaffin cells. Pre-existing Ω-profiles were fusion-generated Ω-profiles from previous rounds of exocytosis that did not collapse, but retained vesicular membrane proteins. Their pore closure was triggered by calcium influx during subsequent depolarization and was mediated by dynamin, which generated vesicles within seconds without the need for recruiting vesicular proteins. Such closures substantially mediated compensatory endocytosis, endocytosis overshoot and bulk endocytosis as widely observed in neurons and endocrine cells. We conclude that pre-existing Ω-profile closure is a major mode of endocytosis enabling efficient vesicle retrieval in secretory cells.