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Spatial Regulation of IgE Receptor Signaling: Generating Quantitative Data for Modeling through High Resolution Microscopy

Oliver, J.M.[1], B.S. Wilson[1], S. Steinberg[2], J.S. Edwards[3], K. Lidke[4], and D.S. Lidke[1]

Departments of [1]Pathology, [2]Mathematics and Statistics,. [3]Molecular Genetics and Chemical Engineering. and [4]Physics, University of New Mexico, Albuquerque NM USA.
Email: joliver@salud.unm.edu

Abstract: Cell signaling pathways are triggered by changes in the spatial proximity, dynamics, interactions and biochemical modifications of membrane receptors and signaling proteins. Most current cell signaling models focus on the biochemical events and fail to consider the importance of topographical relationships between receptors and signaling proteins. We have established a range of complementary high resolution microscopy approaches to study the membrane organization and dynamics of the high affinity IgE receptor (FcεRI) and its signaling partners on basophils and mast cells. The FcεRI binds IgE with sub-nanomolar affinity. Signaling is initiated by the addition of bi- or multivalent allergen to crosslink receptors, leading to the release of inflammatory mediators by degranulation. Using immunogold labeling and high resolution transmission electron microscopy (TEM) imaging of fixed membrane sheets, we have shown that receptors are inherently distributed in singlets and small clusters. After crosslinking, clusters increase dramatically in size and recruit specific downstream signaling molecules to form primary and secondary signaling domains. In complementary studies, we have used atomic force microscopy (AFM) to map the topographical features of the cytoplasmic face of the membrane and to demonstrate the importance of cholesterol to the height of these features. Additionally, we have generated two novel quantum dot (QD) based probes, monovalent QD-IgE (binds FcεRI without crosslinking) and polyvalent 2,4-dinitrophenol (DNP)-QD (mimics multivalent crosslinking allergen), to study the dynamics of early events in FcεRI signaling in real time using confocal and total internal reflectance (TIRF) microscopy. Simultaneous observations of quantum dot-labeled FcεRI motion and GFP-tagged actin dynamics has provided direct evidence that membrane-proximal actin filament bundles form “corrals” that restrict long-range receptor motion. Within the corrals, multiple FcεRI often maintain extended close proximity without protein-protein interactions, suggesting that they are co-confined within membrane domains. Receptors become immobilized within seconds of cross-linking by moderate to high doses (0.1-1 μg/ml) of multivalent antigen. However, immobilization does not occur at lower antigen concentrations that nevertheless induce calcium mobilization and degranulation, indicating that receptor immobilization is a feature of highly-aggregated receptors and more likely involved in signal termination than activation. Our quantitative data on receptor diffusion, clustering and oligomerization in the plasma membrane are being used, in combination with quantitative measurements of protein biochemical modifications, to test and refine new 3D stochastic spatial models of cell signaling. This work was supported in part by NIH grants R01 GM49814, R01 AI051575 and P20 GM 67594.

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Abstract only