Actin organization in T cells on clustered ligands imaged by STORM

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Abstract

Spreading of T cells on antigen presenting cells (APC) is a crucial initial step in immune response. T cells are a class of lymphocytes that carry distinctive receptors called TCR (T cell receptor), which are responsible for specific recognition of foreign peptides in the body. Molecular interactions between the TCR complex and its ligand on APC is followed by cell adhesion and spreading accompanied by changes at molecular and cellular scale. TCR ligands are now believed to be clustered on the surface of APCs [1]. Such clustering is known to impact the adhesion [2, 3] and organization of the membrane [3] of the T cell. Here we report the impact of ligand clustering on the actin organization. We prepare APC mimetic substrates bearing patterns of sub-micron sized cluster of ligands (α-CD3) against the TCR-CD3 complex separated by a polymer (PEG) mimicking the glycocalyx. Cell spreading is seen to be influenced by the amount of PEG on the surface, spreading being higher on low-PEG surfaces. We also varied the pattern size: either 700 nm diameter separated by 2 microns or 300 nm separated by 1 micron. The cells spread more on the larger dots. In all the cases, the actin organization shows one of the two following morphologies: in the vast majority of cells the actin is homogeneously distributed in the form of a network. In a handful of cells, in total internal reflection fluorescence microscopy (TIRF) actin appears as dots that co-localize with the ligand clusters. Detailed observation using stochastic optical reconstruction microscopy (STORM) however indicates that these dots may in fact be sites where actin bundles cross each other forming nodes that appear as dots at a lower resolution.

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