Effect of MHC I expression and the cytoskeleton on clustering and mobility of interleukin-2 and -15 receptors in T cells studied by STED, FCS and FRET

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Interleukin-2 and -15 receptors play important roles in regulating the homeostasis and function of T cells. They form supramolecular clusters with MHC I and II glycoproteins in lipid rafts of T cells. The role of highly expressed MHC I in maintaining these clusters is unknown. We knocked down MHC I in FT7.10 human T cells, and studied protein clustering at two hierarchic levels: molecular aggregations and mobility by FRET and fluorescence correlation spectroscopy, and segregation into larger domains or superclusters by superresolution STED microscopy. FCS based molecular brightness analysis revealed that the studied molecules diffused as tight aggregates of several proteins of a kind. MHC I knockdown increased the mobility of not only MHC I but also that of IL-2Rα/IL-15Rα, indicating the general size decrease of tight aggregates. Analysis of STED images revealed that the diameter of MHC I superclusters diminished from 400-600 to 200-300 nm, whereas those of IL-2Rα/IL-15Rα hardly changed. Our results prove that changes in expression level may significantly alter the organization and mobility of interacting membrane proteins.
We were interested whether the higher order organization of the cell membrane and the cytoskeleton were necessary for maintaining protein-protein interactions at the nm-scale. Large blebs formed during apoptosis/necrosis do not possess cytoskeletal connections, and there is no sign of any higher order (microdomain) organization at the resolution of STED. Such blebs can thus be considered as almost planar bilayers having a relatively homogeneous distribution. Whereas MHC glycoproteins and IL-2/15R formed largely overlapping patches in the intact membrane of FT7.10 T lymphoma cells, they were evenly distributed in blebs according to STED analysis. The characteristic FRET efficiencies measured between the appropriate epitopes were equal within experimental error when measured in intact membranes or in apoptotic blebs, i.e. small scale molecular clusters remained stable. These results were corroborated by line-scanning FCCS experiments indicating stable codiffusion of the studied molecular complexes in blebs and in intact membranes. Our experiments suggest that the interactions stabilizing the studied small-scale molecular clusters persist even after the disappearance of the higher order organization of the T cell plasma membrane, and that these interactions do not depend critically on the cytoskeleton.