Application Note
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Effect of CD48 elongation on the Immunological Synapse

Professor Michael Dustin and colleagues at the NYU School of Medicine are investigating the importance of the Immunological Synapse (IS) for T-cell activation in health and disease. The IS is the interface between an antigen-presenting cell (APC) and a lymphocyte. The structure of the IS comprises a central, peripheral and distal supramolecular activation complex (SMAC), arranged in concentric rings.

T-cell activation is dependent upon an interaction between the T-cell receptor (TCR) with a peptide-presenting major histocompatability complex (MHC). This interaction is facilitated by adhesion molecules, including the small immunoglobulin superfamily molecule CD2 on T-cells, which interacts with CD48 on the surface of the antigen presenting cell. The wild type CD48 molecule comprises 2 Ig-like domains. As part of this study, the researchers used 3D imaging to examine the effect of elongating the CD48 molecule (via the incorporation of additional Ig-like domains) on the pattern of accumulation of CD2 within the synapse, and on the co-localization of CD2 and TCR.

Naive T-cells were seeded on monolayers of CHO cells, transfected with CD48-WT (2 Ig-like domains) or CD48-CD22 (5 Ig-like domains) and imaged using a confocal microscope. Image stacks of 8-22 planes were acquired with a Z step of 0.48 µm. The image stacks were rendered in 3D using Volocity Visualization, to allow visualisation of the entire IS from different points of view. Rendered 3D data sets were rotated into the en face and side orientations and 2D projections were generated in Volocity. The results showed that in T-cells conjugated to CHO cells transfected with CD48-WT (Figure 1a), the CD2 aggregated centrally, however in T-cells conjugated to CHO cells transfected with CD48-CD22 (Figure 1b), the IS was re-organized, and CD2 tended to aggregate peripherally. This demonstrates that the elongated form of CD48 induces peripheral clustering.

These central and peripheral CD2 clusters were then evaluated for their colocalization with TCR, using the colocalization feature of Volocity Quantitation. The results showed that when CD48-WT was expressed in CHO cells, TCR co-localized with 100 % of the IS with central CD2 clustering (Figure 2a). When CD48-CD22 was expressed in CHO cells, TCR colocalized with peripheral CD2 clusters in 65 % of synapses (Figure 2b). This showed that TCR association with CD2 was maintained even though CD2 was present in the periphery when elongated CD48 was used.

The results suggested that the reorganization of the IS by elongated forms of CD48 sequesters TCR in a location where it cannot interact with its ligand, thereby dramatically reducing T-cell sensitivity. Using Volocity to render the IS in 3D gave a more biologically relevant perspective to this work.

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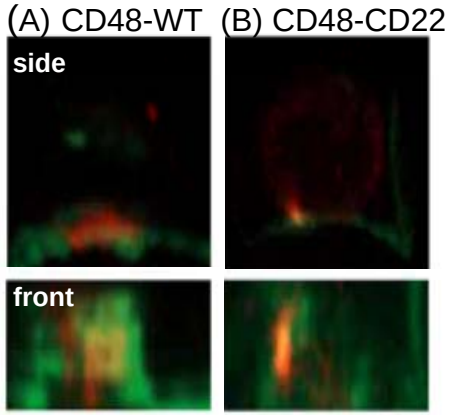

Fig. 1 CD2 is excluded from the central SMAC by elongated CD48. CD2 appears in red and CD48 in green. Side and front (en face) views are displayed

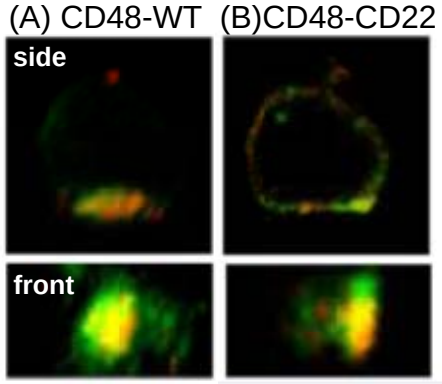

Fig. 2 Sequestration of the TCR in peripheral clusters induced by elongated CD48. CD2 appears in red and TCR in green.