

CD28 regulates proximal signaling events at the immunological synapse.

Kimberly Howland, Pietro Andres, Abul Abbas, Mathew Krummel, Department of Pathology, University of California, San Francisco

The costimulatory molecule CD28 is a key regulator of CD4⁺ T cells. However, when and how this molecule affects the T cell-antigen presenting cell (APC) interaction is unclear. We used real-time molecular imaging to determine the role of CD28 in proximal T cell signaling events. **METHODS:** To visualize CD28 at the immunological synapse we transfected a GFP-tagged CD28 construct into the D10 T cell line, which was then imaged interacting with the B cell lymphoma line CH27 loaded with cognate antigen. To determine the role of CD28 in driving T cells to flux calcium, we loaded CD4⁺ T cells from wild-type and CD28^{-/-} DO11.10 mice with the calcium dye Fura-2, and imaged them while they were interacting with I-A^d + B7-transfected, OVA-pulsed CHO cells. To determine the effects of costimulation in cis versus trans, CD28-deficient T cells were reconstituted with CD28 Y170F mutants and restimulated with CHO cells transfected with I-A^d with or without CHO cells transfected with B7 or with CHO cells transfected with both I-A^d and B7. T cell proliferation and IL-2 production were used as read-outs. **RESULTS:** Prior to contact with an APC, CD28 is distributed evenly across the surface of the T cell. Afterwards, CD28 rapidly moves to the point of contact between T cell and APC. This movement precedes calcium flux. Compared to wild-type cells, calcium flux is 3-fold reduced in CD28^{-/-} cells, reflecting a defect in recruiting extracellular calcium stores. Given the rapid clustering of CD28 in the T cell-APC contact zone, we reasoned that one function of CD28 is to recruit signaling molecules to the synapse. To test this hypothesis, we examined the ability of CD28 cytoplasmic domain mutants to function independently of the T cell receptor. We have previously shown that a Y170F mutant of CD28, which cannot bind PI3K, still drives T cell activation. We thus asked if the Y170F construct could signal when ligated in trans or in cis with the TCR. Compared to a wild-type CD28 construct, the Y170F mutant was unable to restore IL-2 production when stimulated in trans, but functioned normally in cis. **CONCLUSIONS:** CD28 migrates rapidly to the immunological synapse and functions early in the T cell-APC interaction. While CD28 may initiate some signals independently of the T cell receptor, its principal role may be to provide a site, its cytoplasmic tail, for co-localization of signaling molecules.