7. Abstract

Resting T cells need two signals to become fully activated: one delivered via the T cell receptor (TCR) and a second one provided by costimulatory receptors, with CD28 being the most important one. Whereas for a long time the ligation of two types of receptors was thought to elicit two distinct signals (two-signal-model) that are integrated at the level of signal cascades, especially the JNK pathway, and the induction of transcription factors, recent findings have led to the proposal that costimulation rather than eliciting a distinct signal enhances and supports TCR mediated signalling. It does so by triggering transport processes that lead to redistribution of membrane microdomains or rafts to the contact area between T cell and antigen-presenting cell (APC), thus aiding in the formation of the immunological synapse. This accumulation of signalling competent molecules results in enhanced phosphorylation and sustained signalling.

The mitogenic activity of some rat CD28-specific mAb which trigger all primary rat T cells to proliferate and produce cytokines without TCR engagement seemed to contradict the dual signal hypothesis. The signalling pathways elicited by one of these mitogenic CD28-specific mAb were analyzed and compared to the signals given in classical costimulation with TCR contribution. This should aid in clarifying the role of CD28 in generating mitogenic signals that are specific for CD28 and distinguishable from TCR derived signals.

Proliferation assays showed that costimulation as well as direct CD28 stimulation depend on src kinases and the MAPK ERK. The two modes of stimulation revealed a different dependence on PI3-K for induction of proliferation, since costimulation was resistant to inhibition of PI3-K whereas direct CD28 stimulation was not.

Biochemical analysis confirmed the importance of src kinases for T-cell stimulation as the src kinase lck was activated upon both, costimulation and direct CD28 stimulation. Lack of ZAP-70 phosphorylation, a central element of the TCR`s signalling pathway, and only weak and late phosphorylation of LAT and SLP-76, two adapter proteins crucial for TCR signalling, seem to prove that direct CD28 signalling does not simply ”hijack” the TCR signal transduction machinery.

This is supported by the observation that direct CD28 stimulation only induces weak ERK phosphorylation, a MAPK that is mainly controlled by the TCR and thus independent of costimulation, which could be confirmed for the rat system. The signal-integrating JNK and p38 cascades are activated to a similar extent in costimulation and direct CD28 stimulation. Thus for the first time it was proven that CD28 can address JNK directly.

JNK activity induced by costimulation and by mitogenic CD28 stimulation is dependent on src kinases and a functional cytoskeleton. In contrast, PI3-K or PKC inhibition have no or only a minor effect on JNK activity upon direct CD28 stimulation, wheras costimulation induced JNK activity is sensitive to inhibition of these enzymes.

These results suggest that direct CD28 stimulation does not mimic TCR signals but elicits distinct, CD28-specific signals. CD28 seems only to be capable of generating mitogenic signals if mobilized into a signalling-competent form which is hardly available on resting T cells. The exact mechanism of CD28 mobilization remains to be clarified but possibly requires cytoskeletal-dependent restructuring of CD28`s molecular environment. Thus, direct CD28 stimulation does not contradict the dual signal hypothesis as mitogenic CD28-specific mAb trigger CD28 mobilization, whereas under physiological conditions CD28 is mobilized by TCR engagement. We propose a two-step-activation-model of costimulation according to which the TCR first mobilizes CD28 into a signalling-competent form which then upon binding its natural ligands executes the mitogenic signal.

Thus, CD28 as a costimulatory molecule not only supports TCR mediated signalling but functions as the generator of mitogenic signals. This could be relevant to the type of immune reaction triggered since the relative contribution of TCR and CD28 signals to T cell activation has been shown to influence the functional differentiation of T cells towards the Th1 or Th2 subset.