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Engagement of CD28 Outside of the Immunological Synapse Results in Up-Regulation of IL-2 mRNA Stability but Not IL-2 Transcription

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During T cell activation by APC, CD28 is colocalized with TCR in the central supramolecular activation cluster (cSMAC) region of the immunological synapse. CD28 signaling through PI3K results in the recruitment of protein kinase C (PKC)θ to the cSMAC, activation of NF-κB, and induction of IL-2 transcription. These results suggest that localized engagement of CD28 within the cSMAC may be required for CD28 activation and/or signal integration with TCR signals. To test this model we have examined the mechanism of CD28-mediated induction of IL-2 secretion when CD28 is engaged outside of the immunological synapse. CD4 T cells were stimulated with Ag presented by B7-negative APC and CD28 costimulation was provided in trans by anti-CD28-coated beads or by class II-negative, B7-positive cells. We show that induction of IL-2 secretion under these conditions did not require expression of PKCθ and did not induce NF-κB activation or IL-2 transduction. In contrast, CD28 costimulation in trans did induce IL-2 mRNA stability, accounting for the up-regulation of IL-2 secretion. These results indicate that the ability of CD28 to up-regulate IL-2 transcription requires colocalization of TCR and CD28 at the plasma membrane, possibly within the cSMAC of the immunological synapse. In contrast, the ability of CD28 to promote IL-2 mRNA stability can be transduced from a distal site from the TCR, suggesting that signal integration occurs downstream from the plasma membrane. These data support the potential role of trans costimulation in tumor and allograft rejection, but limit the potential functional impact that trans costimulation may have on T cell activation. The Journal of Immunology, 2006, 176: 4778–4784.

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t has been shown that T cell costimulation through CD28 can have a dramatic impact on T cell activation, differentiation, and tolerance (1–4). T cell stimulation in the absence of CD28 leads to T cell anergy rather than activation and only costimulation through CD28, and not other costimulatory molecules, can protect against anergy induction (5, 6). CD28 costimulation leads to a dramatic up-regulation in IL-2 expression mediated by both enhanced transcription and induction of mRNA stabilization (7, 8). CD28 costimulation also plays an important role in T cell survival, inducing expression of the antiapoptotic protein Bcl-xL (9) and regulating the metabolic activity of T cells (10). Finally, CD28 plays a key role on the generation of Th2 responses (11). In combination, these effects resulted in a dramatic loss in T cell expansion and effective immune responses in CD28-deficient mice.

Despite the well-recognized functional importance of CD28, the biochemical signaling pathways induced downstream of CD28 are still not completely understood (1, 4). This result may be due, in part, to the ability of CD28 to amplify TCR-initiated signaling events (1). CD28 has been shown to lower the threshold of TCR engagement (12) and T cell responses are diminished, but not absent, in CD28-deficient T cells. Both protein profiling of signaling intermediates (13) and genetic profiling of changes in gene expression (14, 15) have suggested that CD28 costimulation functions primarily to modify those signaling pathways that can be induced by the TCR itself and it has been difficult to identify a unique contribution of CD28.

One potential site where CD28 could impact on TCR signaling is within the central supramolecular activation cluster (cSMAC)3 of the immunological synapse (16–19). Although T cells express a number of protein kinase C (PKC) isoforms, PKCθ is selectively activated and recruited to the immunological synapse, where it is colocalized with TCR and CD28 in the cSMAC (20, 21). PKCθ plays an essential role in transducing TCR-mediated activation of NF-κB (22–24). Expression of CD28 is required for the targeting of PKCθ to the cSMAC. In the absence of CD28, PKCθ is recruited to the immunological synapse, but it is diffusely distributed across the synapse and is not focused into the cSMAC (25, 26). This disruption in PKCθ localization in the absence of CD28 correlates with a loss in PKCθ-dependent induction of NF-κB and IL-2 transcription. Interestingly, all of these functions of CD28 (recruitment of PKCθ to the cSMAC, activation of NF-κB, and up-regulation of IL-2 transcription) are lost by a single amino acid mutation of the PI3K interaction site in the cytosolic tail of CD28 (26). These results suggest that CD28-mediated activation of PI3K leads to a localized concentration of phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the synapse that results in the recruitment and activation of PKCθ and subsequent induction of IL-2 transcription. Recent data on the recruitment of a GFP-PH domain fusion protein to the synapse (27–29) and the possible role for phosphoinositide-dependent protein kinase PDK1 in PKCθ activation (30) support this model. Taken together, these results suggest

3 Abbreviations used in this paper: cSMAC, central supramolecular activation cluster; PKCθ, protein kinase Cθ; WT, wild type; ARE, AU-rich element; UTR, untranslated region; TTP, tristetraprolin.
that signal integration between TCR and CD28 may occur within and through the spatial organization of proteins in the immunological synapse.

In addition, CD28 has been shown to transduce costimulatory signals in trans, i.e., from a separate site on the cell surface from TCR engagement. This was first shown by the ability of MHC-disparate APC to provide T cell costimulation and protect against clonal anergy (30). Later, trans costimulation was shown to be mediated through CD28 (31–33). In vivo, trans costimulation may contribute to T cell activation to Ags expressed by nonhemopoietic cells, such as in tumor or allograft rejection (34). These functional studies suggested that CD28 did not have to be localized to the immunological synapse to provide costimulatory signals to T cells. In this study we show that when CD28 is provided in trans, the induction of IL-2 expression is not mediated through PKCθ activation and induction of IL-2 transcription. Rather, CD28 costimulation in trans effectively enhances T cell activation through the induction of IL-2 mRNA stability.

Materials and Methods

Cell lines and T cell stimulation

6132 Pro cell transfectants expressing class II (I-Ad) in combination with ICAM-1 (ProAd-ICAM) or with ICAM-1 and B7-1 (ProAd-ICAM-B7) (35) and the class II-negative fibroblast L cell line (DAP-3), which constitutively expresses B7 (36), were maintained in DMEM (Invitrogen Life Technologies) supplemented with 10% FCS, 2 mM glutamine, 0.1 mM nonessential amino acids, 40 μg/ml gentamicin, and 50 μM 2-ME. To maintain selection of transfected genes, G418 (200 μg/ml) and/or MXH (6 μg/ml mycophenolic acid, 250 μg/ml xanthine, 15 μg/ml hypoxanthine) were added as appropriate. CD4-positive lymph node T cells were purified as previously described (35) from wild-type (WT), CD28-deficient, PKCθ-deficient (24), and IL-2–luciferase reporter transgenic (26, 37, 38) DO11.10 TCR transgenic mice. Freshly isolated DO11.10 T cells were assayed directly or after in vitro activation with Ag presented by irradiated BALB/c splenocytes under neutral conditions. Previously activated T cells are essential 100% CD4 and KJ1.26-positive and contain a mixture of IL-4 and IFN-γ-secreting cells (typically 10–20% IL-4 and 40–60% IFN-γ-secreting). Previously activated T cells were rested for 7–14 days before use in experiments. For retroviral transduction, freshly isolated DO11.10 T cells were stimulated with Ag as shown and 1 day later were transduced with a retrovirus encoding an NF-κB p65-GFP fusion protein (39). For trans costimulation with anti-CD28-coated beads, surfactant-free surface latex beads were first coated with goat anti-Syrian hamster Ab at room temperature overnight, and after blocking with BSA, anti-CD28 mAb (37.51) was captured at room temperature for 4 h. T cell to bead ratio was 1:3 in all experiments performed.

IL-2 ELISA and luciferase assay

T cells were stimulated with Ag in the absence of CD28 costimulation (ProAd-ICAM), in the presence of CD28 in cis (ProAd-ICAM-B7), or by providing CD28 in trans (ProAd-ICAM with anti-CD28-coated beads or B7-positive L cells). Supernatants were collected at 24 or 48 h and IL-2 was measured by a capture ELISA. For luciferase activity, T cells were stimulated under the same conditions, but after 24 h of stimulation, T cells were lysed and luciferase activity was determined according to the manufacturer’s instructions (Promega).

Immunofluorescence microscopy

T cells were centrifuged with peptide-pulsed APC with and without anti-CD28-coated beads or B7-positive L cells for 20 s at relative centrifugal force of 2000. The cell pellet was incubated for 5 min at 37°C, resuspended in DMEM, and plated on poly-L-lysine-coated coverslips for 10 min at 37°C. Cells were fixed in 3% (w/v) paraformaldehyde, and stained with rabbit anti-PKCθ (C-18; Santa Cruz Biotechnology) and anti-rabbit-FITC or anti-rabbit-Cy3 (Jackson ImmunoResearch Laboratories). For NF-κB nuclear localization, the incubation time on poly-L-lysine-coated coverslips was increased to 25 min and cells were fixed in 3% (w/v) paraformaldehyde and 0.3% Triton X-100. Localization of NF-κB was detected with rabbit anti-p65 (SC-109; Santa Cruz Biotechnology) or in retrovirally infected cells by detection of the p65-GFP fusion protein. After staining, nuclei were labeled with Hoechst dye. Samples were analyzed on a Zeiss Axiovert microscope controlled by SlideBook software (Intelligent Imaging Innovations).

Real-time RT-PCR

Activated T cells (1 × 10⁶) were stimulated with Ag presented by ProAd-ICAM alone, ProAd-ICAM, and anti-CD28-coated beads (trans costimulation) or ProAd-ICAM-B7 (cis costimulation) for 4 h and further IL-2 transcription was inhibited by the addition of 10 μg/ml cyclosporin A (Calbiochem). Total RNA was isolated using TRIzol (Invitrogen Life Technologies) and reverse transcribed into cDNA. The level of IL-2 mRNA was determined by real-time PCR following normalization to a T cell-specific gene, CD3δ, using the ΔΔCt method for relative quantitation with IL-2 mRNA levels in the absence of Ag as the calibrator. TaqMan probe and primers for IL-2 and CD3δ were obtained from Applied Biosystems.

Results

CD28 costimulation in trans is not mediated through PKCθ activation

CD28 has the unique ability among potential costimulatory molecules to function when it is engaged on the T cell at a site that is distal to the TCR (in trans). CD28 costimulation in trans can lead to the up-regulation of IL-2 secretion (Fig. 1), but it is not clear how signals from CD28 in trans are integrated with TCR-derived signals. We have recently found that there are two independent pathways for CD28 costimulation (26). One pathway is mediated through PI3K and leads to the cSMAC localization of PKCθ, activation of NF-κB, and the transcriptional up-regulation of IL-2. The second pathway is not dependent on CD28-mediated activation of PKCθ and results in the up-regulation of IL-2 secretion through the induction of IL-2 mRNA stability. To determine whether CD28 in trans can induce one or both of these pathways, we initially examined the localization of PKCθ within the immunological synapse (Fig. 2). WT DO11.10 T cells were stimulated with Ag presented by ProAd-ICAM. LFA-1 costimulation in the absence of CD28 costimulation can induce PKCθ recruitment to the immunological synapse, but not to the cSMAC (26). To determine whether CD28 must be engaged within the immunological synapse to impart this effect, PKCθ localization was assayed after providing CD28 costimulation in trans. When anti-CD28-coated beads were added to allow the formation of T cell:APC:bead conjugates, the level of IL-2 secretion was increased (Fig. 1), but PKCθ remained diffusely localized to the synapse and was not focused into the cSMAC (Fig. 2C). Instead, recruitment of PKCθ...
cells providing CD28 costimulation in contrast image. Note that there are two T cells in the example with L gates. The position of T cells (T), ProAd-ICAM cells (APC), L cells (L), or with L cells. Both T cells display a diffuse pattern of PKC

4-chloromethylcoumarin (CMAC; blue) to distinguish them from the by immunofluorescent microscopy. L cells were prelabeled with 7-amino-

L cells (L cells), or with ProAd-ICAM-B7 to provide CD28 costimulation by natural ligand, B7-positive, class II-negative L cells were used mediated by anti-CD28-coated beads mimicked CD28 engagement, whether the T cells were in contact with beads alone or with both APC and beads. To confirm that trans costimulation mediated by anti-CD28-coated beads mimicked CD28 engagement by natural ligand, B7-positive, class II-negative L cells were used to provide CD28 costimulation in trans. CD4+ T cells costimulated in trans by L cells displayed the same phenotype, as was observed when CD28 costimulation was provided by anti-CD28-coated beads (Fig. 2). Thus, unlike when CD28 costimulation was provided at the same site as TCR engagement, CD28 costimulation in trans did not direct the localization of PKCθ to the cSMAC.

These results suggested that the ability of CD28 to costimulate IL-2 expression in trans was not mediated through activation of PKCθ. To test this possibility directly, we compared the ability of CD28 in trans to costimulate CD4 T cells from WT and PKCθ-deficient DO11.10 mice (Fig. 3A). PKCθ was required for optimal IL-2 secretion when CD28 was provided in cis, as predicted from previous studies (24). However, T cells from WT and PKCθ-deficient mice induced similar levels of IL-2 secretion when CD28 costimulation was only provided by anti-CD28-coated beads (Fig. 2). Thus, unlike when CD28 costimulation was provided at the same site as TCR engagement, CD28 costimulation in trans did not direct the localization of PKCθ to the cSMAC.

These results suggested that the ability of CD28 to costimulate IL-2 expression in trans was not mediated through activation of PKCθ. To test this possibility directly, we compared the ability of CD28 in trans to costimulate CD4 T cells from WT and PKCθ-deficient DO11.10 mice (Fig. 3A). PKCθ was required for optimal IL-2 secretion when CD28 was provided in cis, as predicted from previous studies (24). However, T cells from WT and PKCθ-deficient mice induced similar levels of IL-2 secretion when CD28 was provided in trans. Similar results were obtained when CD28 costimulation in trans was provided by anti-CD28-coated beads or

L cells, n = 87; and cis, n = 156. CD28 costimulation in trans significantly inhibits PKCθ recruitment to the immunological synapse (p = 0.001). In contrast, costimulation in cis enhances both recruitment of PKCθ to the synapse and localization to the cSMAC (p < 0.001).
with cells expressing the natural ligand for CD28 (B7-positive L
cells). CD28-deficient T cells were included as a negative control,
as these cells are unable to respond to either cis or trans CD28 costimulation. Similar results were obtained when we tested pre-
viously activated and freshly isolated lymph node T cells (Fig. 3B).
Interestingly, the greater potency of cis costimulation compared with trans costimulation was lost in the PKC-θ-deficient T cells,
suggesting that this increased potency of cis costimulation may be
dependent on CD28-mediated recruitment of PKC-θ to the cSMAC.
Together, these results indicate that CD28 costimulation in cis is mediated through a PKC-θ-independent pathway.

CD28 costimulation in trans does not induce NF-κB nuclear translocation or IL-2 transcription

As discussed, CD28 costimulation through PI3K and the recruit-
ment of PKC-θ to the cSMAC has been implicated in the ability of
CD28 to up-regulate IL-2 secretion. Because CD28 costimulation
in trans is independent of PKC-θ, it suggests that the ability of
CD28 in trans to up-regulate IL-2 secretion is not mediated
through the up-regulation of IL-2 transcription. To test the possi-
bility directly, we first assessed nuclear localization of NF-κB
(Fig. 4). CD28 costimulation in cis resulted in the induction of
nuclear localization of NF-κB. In contrast, CD28 in trans had little

effect on NF-κB activation. Similar results were obtained when
nuclear localization of endogenous NF-κB was monitored by im-
munofluorescent staining (Fig. 4, A and B) or when an NF-κB
p65-GFP fusion protein was retrovirally transduced to follow
NF-κB activation (Fig. 4C). To assay, IL-2 transcription directly
we used DO11.10 T cells that contain a transgenic IL-2-luciferase
reporter construct (38). We have previously established that this
transgene is a good indicator of endogenous IL-2 transcriptional
activity (37). As expected from previous studies, cis costimulation
resulted in transcriptional activation of the IL-2 enhancer (26). In
contrast, only a modest increase in IL-2 transcriptional activation
was detected with trans costimulation (Fig. 5). Collectively, these
data indicate that CD28 engagement outside of the immunological
synapse does not result in PKC-θ-dependent activation of NF-κB
and subsequent up-regulation of IL-2 transcription.

CD28 costimulation in trans can increase the stability of IL-2
mRNA

The ability of CD28 costimulation in trans to up-regulate IL-2
secretion, but not IL-2 transcription, indicates that trans costimu-
lation must be mediated through posttranscriptional mechanisms.
IL-2 can be regulated at several posttranscriptional events, includ-
ing mRNA elongation, mRNA stability, translation, and secretion
(7, 8, 40–42), but the best-described effect of CD28 costimulation
is on mRNA stability. To determine the half-life of IL-2 mRNA, T
cells were stimulated for 4 h, transcription was then blocked by
addition of cyclosporin A, and IL-2 mRNA levels were determined
by real-time PCR at different time points (Fig. 6). In the absence
of CD28 costimulation IL-2 mRNA rapidly decays with a half-life
of ~30 min. When costimulation is provided by CD28 in cis the
half-life increases to ~90 min. Importantly, the increase in the
half-life of IL-2 mRNA is similar when CD28 costimulation is
provided in cis or in trans. Thus, engagement of CD28 outside of
the immunological synapse is not sufficient to induce up-regulation
of IL-2 transcription, but is able to transduce signals necessary for
induction of IL-2 mRNA stability.
FIGURE 6. Costimulation by CD28 in trans is mediated through IL-2 mRNA stability. Previously activated T cells were stimulated with Ag presented by ProAd-ICAM (no CD28), by ProAd-ICAM with anti-CD28-coated beads (trans), or by ProAd-ICAM-B7 (cis) for 4 h. IL-2 transcription was blocked by the addition of cyclosporin A. Cyclosporin A rapidly inhibits NFAT-dependent IL-2 transcription without affecting IL-2 mRNA stability (37, 62, 74). Levels of IL-2 mRNA were measured every hour by real-time PCR and shown as a percentage of IL-2 mRNA before the addition of cyclosporin A. The fold induction of IL-2 mRNA after the initial 4 h of activation was 3200 (no CD28), 5600 (trans), and 9800 (cis). This experiment is one representative experiment of two completed.

Discussion

We have recently proposed that there are two distinct pathways for CD28-mediated costimulation (26). The first is mediated through recruitment of P3K to the cSMAC of the immunological synapse and results in the activation of PKCα, nuclear translocation of NF-κB, and up-regulation of IL-2 transcription. Costimulation through this pathway may reflect integration of plasma membrane-proximal signals mediated through TCR/C/28 within the context of the immunological synapse. The second pathway of CD28 costimulation is not affected by mutation of the P3K interaction site in CD28 and drives IL-2 production through stabilization of IL-2 mRNA. In this study, we show that when CD28 is engaged outside of the immunological synapse, only the second pathway of CD28 costimulation is induced. Thus, CD28-mediated up-regulation of IL-2 transcription requires membrane colocalization with TCR, possibly within the cSMAC of the immunological synapse. In contrast, CD28 signals that mediate IL-2 mRNA stability do not need to be colocalized with TCR, supporting the model that costimulation through this pathway may reflect signal integration downstream of the plasma membrane.

In addition to providing costimulation, there is evidence that CD28 can activate T cells in the absence of TCR engagement. Early studies showed that anti-CD28 cross-linking could induce a calcium response and anti-CD28 in combination with phorbol esters could induce IL-2 secretion and T cell proliferation (43, 44). More recently, it has been shown that CD28 cross-linking, in the absence of TCR engagement, can be sufficient to activate Vav and SLP-76 (45). However, these effects of anti-CD28 cross-linking can be restricted to certain cells and mAb epitopes. Recent data has provided some insight into this issue (46, 47). There are at least two major mAb epitopes expressed on CD28. Conventional Ab epitopes are localized near the B7 binding site and are thought to mimic natural ligand binding. The 37.51 mAb used in the present studies is a conventional Ab. Superagonists Ab epitopes have been mapped to a site that is proximal to the membrane and are functionally mitogenic, inducing IL-2 secretion and T cell proliferation. Interestingly, T cell activation by superagonist Abs can be mediated through PKCθ and NF-κB and up-regulation of IL-2 transcription (48). This suggests that CD28 is capable of independently activating T cells, but it is not clear whether these same signaling pathways mediate CD28 costimulation of TCR-derived signals. Furthermore, the relationship between CD28 engagement by superagonist Abs and by natural ligand binding is not understood. In the experiments reported, we show that trans costimulation using a conventional mAb or by natural ligand is not mediated through PKCθ and NF-κB, but rather enhances IL-2 secretion through mRNA stability.

CD28 has been shown to function as a signal amplifier for TCR-transduced signals (1). Thus, the current model would suggest that colocalization of TCR and CD28 during cis costimulation would facilitate CD28 amplification of proximal TCR signals. The requirement for CD28-mediated activation of P3K is consistent with this model because the product of PI3K, PIP3, would enhance the recruitment of PH domain-containing proteins, such as VAV, Itk, Akt, and PKD1, to the site of activation. In this case, colocalization of TCR and CD28 would create a signaling subdomain within the immunological synapse. However, Hüning and colleagues (47–49) have suggested an additional role for TCR/CD28 colocalization. They have shown that expression of the superagonist epitope on CD28 is enhanced following T cell activation, and activated T cells are more responsive to activation by superagonist anti-CD28 mAbs. Thus, TCR signaling may induce a conformational change in CD28, which reveals the mitogenic Ab epitope and potentiates CD28 signaling. In this case, localization of CD28 to the site of TCR engagement may promote CD28 activation. This activity could be mediated by CD28 association with lipid raft domains and/or by access of CD28 to Lck. Lck binding to the polyproline rich region has been proposed to be the first step in CD28 signaling. Lck is then thought to phosphorylate Y170 on the CD28 cytosolic tail creating the binding site for the Src homology 2 domain of P3K (50, 51). Thus, TCR/CD28 colocalization within the cSMAC may have a dual function, first in the activation of CD28 and subsequently in the signal integration of TCR- and CD28-derived signals.

In addition to enhancing proximal TCR-derived signals, CD28 signaling can also induce mRNA stability. The regulation of mRNA stability is largely controlled by AU-rich elements (ARE) within the 3′ untranslated region (UTR). ARE-mediated mRNA degradation plays an important role in regulation of many genes (52, 53), including cytokines (8). The current model for regulated mRNA stability is that AU-binding proteins that induce mRNA instability, such as tristetraprolin (TTP), bind to the 3′ UTR in unstimulated cells. TTP recruits the multicomponent exosome, allowing for deadenylation and 3′ exonuclease digestion of the mRNA (54, 55). In the absence of ARE-mediated mRNA degradation, either by genetic disruption of TTP expression (56) or the deletion of the ARE from TNF (57), overexpression of TNF results in the induction of autoimmune inflammatory diseases. The stability of ARE-containing mRNAs can also be enhanced during cell activation events, although the mechanisms that mediate this stabilization are not well understood. One model that has been proposed is that cell signaling induces the recruitment of different AU-binding proteins, such as HuR, that may compete with TTP for binding to the 3′ UTR and, thus, interfere with TTP-dependent recruitment of the exosome. T cell activation leads to an increase in expression of TTP and HuR, and TTP can bind to the AU-rich region in the IL-2 3′ UTR and drive IL-2 mRNA degradation (58, 59). However, HuR does not recognize the specific AU-rich region in the IL-2 mRNA and another AU-binding protein, NF90, that can compete with TTP binding, has been implicated in signal-dependent IL-2 mRNA stabilization (60). Access of HuR and NF90 to target mRNA may be regulated by shuttling these nuclear proteins to the cytosol, and this process could be mediated by signal-dependent association of HuR with nuclear shuttle proteins (60, 61).
MAPK activation has been implicated in the induction of mRNA stability. JNK can induce IL-2 and IL-3 mRNA stability (62–64); p38 has been implicated in the stabilization of IL-2, IL-6, IL-8, and TNF-α mRNA (65–67); and ERK can stabilize COX-2 mRNA in smooth muscle cells. Although there is some evidence of signal-dependent phosphorylation of AU-binding proteins, the exact role for these phosphorylation events in mediating mRNA stability is not clear (65, 68). Importantly, the proximal signals that are induced uniquely by CD28 to up-regulate mRNA stability have not been elucidated.

Trans costimulation has been proposed to play a role in immune responses to nonhemopoietic cells, such as in tumor and allograft rejection and autoimmune. Using genetically deficient cells that eliminate the possibility of cis costimulation, it was directly demonstrated that trans costimulation could result in rejection of cardiac allografts (34). Nevertheless, any function of trans costimulation in vivo would be limited by two key factors. First, it requires diac allografts (34). Nevertheless, any function of trans costimulation or are mediated through the CD28 signaling pathway. In vivo reconstitution of CD28−deficient mice with CD28 mutants that cannot activate the PKCθ pathway (Y170F) restores many, but not all, CD28−dependent functions. There are notable defects in up-regulation of Bcl-xL, radiation resistance, and glagversus-host disease; however, T cell activation, IL-2 production, and proliferation are largely intact in mice expressing the Y170F mutation (71–73). In addition these mice generate WT levels of Th2 and T cell-dependent B cell responses, and normal numbers of CD25+ regulatory T cells (69, 70). Whether all of these functions can be induced by trans costimulation or are mediated through the regulation of mRNA stability will require additional investigation.

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Disclosures

The authors have no financial conflict of interest.

References

The document is a list of references, primarily focusing on the role of CD28 in immune cell function. Key points include: