Review

Intracellular Signals of T Cell Costimulation

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Ligation of T cell receptor (TCR) alone is insufficient to induce full activation of T lymphocytes. Additional ligand-receptor interactions (costimulation) on antigen presenting cells (APCs) and T cells are required. T cell costimulation has been shown to be essential for eliciting efficient T cell responses, involving all phases during T cell development. However, the mechanisms by which costimulation affects the function of T cells still need to be elucidated. In recent years, advances have been made in studies of costimulation as potential therapies in cancer, infectious disease as well as autoimmune disease. In this review, we discussed intracellular costimulation signals that regulate T cell proliferation, cell cycle progression, cytokine production, survival, and memory development. In general, the pathway of phosphoinositide-3 kinase (PI3K)/protein kinase B (PKB, also known as Akt)/nuclear factor κB (NF-κB) might be central to many costimulatory effects. Through these pathways, costimulation controls T-cell expansion and proliferation by maintenance of survivin and aurora B expression, and sustains long-term T-cell survival and memory development by regulating the expression of bcl-2 family members. *Cellular & Molecular Immunology*. 2008;5(4):239-247.

Key Words: costimulation, signal transduction, T-cell development

Introduction

Two signals for T cell activation have been described for many years: the first signal is specific requiring TCR recognizing and binding to antigen bond major histocompatibility complex (MHC) presented by APCs; the second one is nonspecific, resulting from the binding of APC expressed costimulation ligands to its receptor on the T cell. If T cells receive these two signals they will undergo proliferation, differentiation, and then acquire effector functions. In contrast, if lacking costimulatory signals, either receptors on T cells or ligands on APCs, T cells will undergo apoptosis or become anergic (1, 2). Costimulatory signals play crucial roles in all phases of T cell response, not only in activation phase, but also in effector phase, expansion phase, and memory phase (Table 1).

Since the CD28/B7 interaction was identified as a prominent costimulatory signal for T cells, many other costimulatory molecules have also been identified. The majority of costimulatory molecules belong to the immunoglobulin superfamily (IgSF), the tumor necrosis factor receptor (TNFR) family, and the integrin family. The IgSF, also called B7 family, has six members with defined costimulatory activity, including CD80 (B7-1), CD86 (B7-2), B7-H1/PDL1, B7-DC/PDL2, B7-RP-1 and B7-H3. Several ligands or receptors of B7 costimulatory molecules have been identified such as CD28, cytotoxic T-lymphocyte antigen 4 (CTLA-4) for B7-1/B7-2, inducible costimulator (ICOS) for B7-H2 and programmed death 1 (PD-1) for B7-H1/B7-DC. Several TNF family members expressed by APCs can costimulate T cell activation by binding to specific TNFR family members expressed on T cells. 4-1BB (CD137) ligand and OX40 (CD134) ligand, two costimulatory receptors of the TNF family, are expressed on APCs and their ligand-receptor pairs could provide costimulatory signals from APCs to T cells (3). CD27 and CD30 are two additional TNFR family members expressed by T cells that might be interesting targets for the activation or inhibition of antigen-specific responses. Integrin molecules are a family of hetero-dimer formed by two covalently linked α and β glycoprotein chains, including lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18), and very late activation antigen-4 (VLA-4, CD49d/CD29). They also act as costimulatory molecules involved in the bi-directional signaling in and out of T cells. Costimulatory signals may perform several functions, such as augmenting production of interleukin (IL)-2 or the archetypal growth factor, promoting cell cycle progression, inducing effector cytokine production, such as those of the Th1 and Th2 type, suppressing cell death by

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Table 1. Functional comparison of CD28 and OX40

Costimulatory molecule	Expression pattern	Family	T-cell modulation	T-cell differentiation	T-cell function	Location of action
CD28	Constitutively	IgSF	Positive	Th1 and Th2	Effector and memory	Central
OX40	Inducible	TNFR	Positive	Th2	Memory	Central and peripheral

IgSF, immunoglobulin superfamily; TNFR, tumor necrosis factor receptor; Th1, T helper 1; Th2, T helper 2.

altering bcl-2 and caspase protein function, and enhancing memory T cell development (4, 5). Advances in our understanding of T cell costimulatory molecules have provided a vast array of novel approaches in preventing autoimmune, infectious, inflammatory, and tumorous diseases (6-9).

CD28 is an IgSF family member and constitutively expressed on T cells, and CD28 ligand--B7 family members are also IgSF family members but expressed similarly on APCs. It has been shown that T cell costimulation through CD28 has a dramatic impact on T cell activation, differentiation, tolerance, and memory (10). T cell stimulation in the absence of CD28 as well as other costimulatory molecules leads to T cell anergy rather than activation, only costimulation through CD28 can protect against anergy induction (11). CD28 costimulation leads to a dramatic up-regulation of IL-2 expression through enhanced transcription and mRNA stabilization (12). In addition, CD28 costimulation also plays an important role in T cell survival, by inducing the expression of antiapoptotic protein bcl-xL and regulating the metabolic activity of T cells (13). Finally, CD28 plays a crucial role on the generation of Th2 responses (14). OX40 is a TNFR family member and expressed from 12 to 24 h after naïve T cell activation. Similarly, OX40 ligand (OX40L) is another TNF family member, which is expressed on APCs hours or days after activation. T cells lacking OX40 keep dividing initially but their ability of dividing diminishes over time and eventually die (15-17) .

The integration of costimulatory receptor signaling with TCR signaling is still not clear. Although data already show costimulatory signals are necessary for T cells to obtain optimal activation, cytokine production, survival, and memory generation, how these signals function still needs to be unraveled. Elucidating molecular targets of costimulation will provide new insight towards understanding of the importance of these molecules in antigen-reactive T cells, and may define novel targets for augmenting T-cell immunity against diseases. In this review, we will focus on CD28 and OX40, and discuss their intracellular signals during T cell responses to antigen stimulation (Table 2).

Common PI3K/PKB/NF-κB pathways

The PI3K family enzymes are recruited upon stimulation and produce 3' phosphoinositide lipids. The lipid products of PI3K act as second messengers by binding to and activating the serine/threonine protein kinase PKB, the latter activates IκB kinase (IKK) complex and ultimately leads to NF-κB activation. These events constitute the start of a complex signaling cascade, which ultimately results in the conducting of cellular activities such as proliferation, differentiation, chemotaxis, survival, trafficking, and glucose homeostasis (18).

CD28 has several Src homology (SH)-2 binding domains (e.g., YMNM) which are able to bind p85, the regulatory subunit of PI3K, and is a potent activator of PI3K (19). CD28 has a cytoplasmic domain of approximately 40 amino acid residues that is highly conserved across species and that contains four tyrosine residues (Y170, 185, 188, 197), potentially mediating protein-protein interactions. Several lines of evidence suggest that phosphorylation of one or more of these cytoplasmic tyrosine residues is important for the delivery of the CD28 costimulus. More importantly, the phosphorylation of Tyr188 plays a critical role in signal transduction through CD28 (20). To determine the role of PI3K activation in the context of CD28 signaling, transgenic mouse that harbors a mutant form of CD28 (Y170F) that is unable to activate PI3K showed a retained capacity to undergo CD28-dependent proliferation, IL-2 secretion, and B cell development assistance, but failed to up-regulate bcl-xL protein and showed a reduced capacity to resist stress-induced apoptosis (21). These observations demonstrated that a single amino acid substitution within the cytoplasmic domain of CD28 is able to uncouple signals required for proliferation and survival, possibly through inhibiting the recruitment of PI3K, and linkage with protein kinase C-theta (PKCθ) (22). T cell activation is associated with the formation of the supramolecular activation complex (SMAC) or as known as the immunological synapse, as well, the colocalization of TCR, CD28, and other molecules are effective in coordinating optimal T cell activation. Importantly, PKCθ is the only PKC isoform that has been detected in the SMAC after antigen-specific T cell engaged with APCs and the translocation of PKCθ from the cytosol to the membrane is an important step that regulates its activation. It has been shown that engagement of the TCR and CD28 leads to the translocation and activation of PKCθ to the lipid rafts in the SMAC (22). This process also involves other signaling molecules, including leukocyte-specific protein tyrosine kinase lck, Vav, Zap-70, and PI3K (23, 24).

Although OX40 does not have conserved residues directly bind to PI3K, and the relationship between OX40 and PI3K-dependent PKB activation is not clear, OX40 ligation on pre-activated T cells leads to rapid activation of PKB suggesting a relatively direct effect of PI3K/PKB. Several reports have shown that OX40 recruits tumor necrosis factor receptor-associated factors (TRAFs) 2, 3, and

Table 2. Comparison of intracellular signaling of CD28 and OX40

Costimulatory molecule	PI3K/NF-κB activation pattern	Cytokine production	Cell proliferation	Cell cycle progression	Cell survival	Memory
CD28	SH-2 binding domains	NF-AT, AP1, SLP-76, PKC, etc.	PTEN and aurora B	p27, mTOR	Bcl-xL, bcl-2, bfl-1 and mcl-1	Bcl-xL and bcl-xγ
OX40	TRAFs	GATA-3	Survivin	Survivin	Bcl-xL, bcl-2 and bfl-1	Bcl-xL

SH-2, Src homology 2; TRAF, TNFR-associated factor; NF-AT, nuclear factor of activated T-cell; AP1, activator protein 1; SLP-76, SH2-domain-containing leukocyte protein of 76 kDa; PKC, protein kinase C; GATA-3, nuclear GATA binding protein 3; PTEN, phosphatase and tensin homolog; mTOR, mammalian target of rapamycin; bcl, B-cell CLL/lymphoma 2.

5 to its cytoplasmic tail, and in a number of mammalian cell transfection systems, the OX40 interaction with TRAFs 2 and 5 can lead to activation of the NF-κB1 pathway (25, 26). TRAFs were initially discovered through their ability to bind to the p75 TNFR and were classified as a gene family based on a conserved domain on the C terminus, i.e., the TRAF domain and they had been characterized as TRAFs 1 to 6 (25). The TRAF domain enables TRAFs to interact with various receptors, including members of TNFRSF and multiple intracellular signaling molecules (27). OX40 can function with TRAF2 and mediated downstream signaling pathways (28), whereas TRAF1 acts as a modulator of TRAF2 signaling, TRAF3 serves as an inhibitor of TRAF2/5-mediated NF-κB activation (29, 30). TRAFs mediate OX40 signals *via* NF-κB-inducing kinase (NIK) that activate noncanical NF-κB activation, a PI3K-independent pathway (31). In addition, several groups reported that OX40 signals could sustain PI3K/PKB activities, which may also impact on canonical NF-κB activation (15, 16). More importantly, recent data directly showed that OX40 signaling could target the NF-κB1 pathway in peripheral antigen-responding CD4 T cells. Phosphorylation of IκBα, nuclear translocations of NF-κB1/p50 and RelA, and activation of NF-κB1, are impaired in OX40-deficient T cells. Retroviral transduction of active IKKβ that constitutively activates NF-κB1 rescues the poor proliferation and survival of OX40-deficient T cells, directly correlating with increased expression and activity of survivin, aurora B, and bcl-2 family members. Furthermore, effective IKKβ expression alone is sufficient to restore the defective proliferation and survival of OX40-deficient T cells *in vivo* when responding to antigen. Thus, OX40 signals regulate T cell number and viability through the NF-κB1 pathway that controls the expression and activity of intracellular targets for proliferation and survival (32). Collectively, mediated by PI3K/PKB/NF-κB pathways, CD28 and OX40 regulate expression of bcl-2 family members and survivin/aurora B that control T cell proliferation, cell cycle progression, and longevity (16, 33).

Intracellular signals of costimulation in T cell response

Cytokine production

Costimulation is essential for optimal cytokine production. In many situations during the absence of costimulation, cytokine secretion will be affected, and T cell expansion, proliferation, survival, and memory development will be affected consequently. In the presence of costimulation, T cells would secrete large amounts of IL-2, IL-4, interferon (IFN)-γ, etc., and undergo differentiation into Th1/Th2/Th17 (34-37).

CD28 engagement induces massive IL-2 secretion, which is dependent upon the activation of mitogen-activated protein (MAP) kinase (19, 38). Using yeast-hybrid technique, a novel CD28 cytoplasmic tail (CD28 CYT) interacting protein, the MAP kinase phosphatase-6 (MKP6) was isolated, which showed to inactivate MAP kinases, so it is associated with cytokine secretion (39). Besides MKP6 protein, the YMNM motif on the CD28 cytoplasmic domain is also known as a binding site for PI3K and growth factor receptor-bound protein 2 (Grb-2) hence is considered to be important for CD28-induced IL-2 secretion (40, 41). Several groups have reported that CD28 engagement also interacts with Tec, a prototypical member of the protein tyrosine kinase family, which mediates cytokine secretion, at the contact zone between T cells and APCs after relocalization. Upon CD28 ligation with specific antibodies or natural ligands, Tec translocates to the plasma membrane where it colocalizes with the CD28 molecule (42). In this process, the Src-homology 3 (SH3) domain of Tec and the two proline-rich motifs of CD28 are involved. Furthermore, CD28 signaling requires the SH3 domain of Tec as well as proline residues present in the intracytoplasmic tail of CD28 (42). Since Vav proteins are known to play a critical role in T cell activation and proliferation *via* promoting cytoskeleton reorganization, transcription factor activation, and cytokine production, so people are interested to investigate the relationship between Vav proteins and CD28 signals (43). CD28 can cooperate with the guanine nucleotide exchange factor VAV/SLP-76 adaptors to upregulate IL-2/4 transcriptions independently of TCR ligation, and CD28 signaling is dependent on the formation of VAV/SH2-domain-containing leukocyte protein of 76 kDa (SLP-76) complex and induction of these complexes to locate on membrane localization (44). These complexes cooperate with ribosomes assembly chaperone (Rac) signaling pathway downstream effector of Vav and the mitogen-activated kinase kinase 1 (MEKK1), a kinase known to regulate the c-jun N-terminal kinase (JNK) pathway, regulates both NF-κB and the activator protein 1 (AP-1),

which controls cytokine secretion (43, 45, 46). Moreover, Vav-1 plays a key role in the control of NF-κB pathway by targeting IKKα in the T cell membrane and favoring its activation in response to CD28 stimulation (47), which increases protein arginine methyltransferase activity and subsequently promotes the methylation on arginine residues of Vav1 (48). It is also shown that PKC isotypes are not dispensable in the signaling pathway of cytokine production induced by CD28, which recruits PKCθ in immunological synapse and enhances nuclear factor of activated T cells (NF-AT), AP1 translocation that mediates IL-2 secretion (49). During T cell activation by APC, CD28 is colocalized with TCR in the cSMAC region of the immunological synapse. CD28 signaling through PI3K results in the recruitment of PKCθ to the cSMAC, and then, activation of NF-κB, as well as induction of IL-2 transcription (50). Besides this, PKCα is also necessary for T cell-dependent IFN-γ production, because CD3/CD28 antibodies and MHC alloantigen induced PKCα-deficient T cells were severely impaired in IFN-γ production (51). Similarly to PKC isotypes, CD28 binding Grb2-related adaptor protein 2 (Gads) is also essential for CD28-mediated NF-κB activation, and its binding requires the whole CD28 cytoplasmic domain in addition to the YMNM motif. The mutagenesis experiments have indicated that mutations in the N-terminal and/or C-terminal PXXP motif(s) of CD28 significantly reduced their association with Gads and induced strong activity of the NF-AT/AP-1 reporter comparably, but weak activity of the NF-κB reporter, which was consistent with the weak activation of the IL-2 promoter (19). Consistent with this result, a mutation in the C-terminal proline-rich region of the cytoplasmic tail of CD28 showed that this motif is essential for CD28-dependent regulation of IL-2 secretion and T cell proliferation. *In vivo* analysis revealed that mutation of this motif dissociated the CD28-dependent regulation of cellular and humoral responses in an allergic airway inflammation model (52). From the transcription level, the NIK regulates IL-2 synthesis by activating the CD28 responsive element (CD28RE) of the IL-2 promoter and strongly synergizes withc-Rel in this reaction, by interacting with the N-terminal domain of c-Rel through phosphorylating the C-terminal transactivation domain (TAD) of c-Rel and inducing Gal4-c-Rel-transactivating activity (53). In conclusion, CD28 signals recruit PKC isotypes and VAV/SLP-76 complex to active canonical, nocanonical NF-κB pathways, and MAP/JNK kinase pathways that regulate cytokine production.

In contrast, blockade of OX40 signals reduces IFN-γ, IL-2, IL-4 and IL-13 secretion and inhibits T cell proliferation, implying OX40 signals may mediate both Th1 and Th2 subtype differentiation (54). It has been suggested a number of years ago that B cell costimulation *via* the OX40/OX40L pathway was required for T cell expansion, survival, and Th2 development. Recent data proved that Th2 but not Th1 responses were impaired in OX40L-deficient recipients and normal responses were restored in OX40L sufficient B cells. The results suggest that without engagement of OX40L on B cells, $CD4^+$ T cell responses to many protein antigens would be dominated to Th1 cytokines (55). Similarly, Sendai virus stimulation-induced down-regulation of IFN-α showed plasmacytoid dendritic cells (DCs) also regulated Th2 cell responses through OX40 ligand (56). Moreover, OX40 signals subsequently enhance nuclear GATA binding protein (GATA)-3 accumulations through an IL-4R-dependent pattern, leading to Th2 differentiation. Collectively, in the absence of an exogenous source of IL-4, OX40 provides a critical temporal but synergistic signal with other noncytokine ligands to modulate NK-AT c1 and to promote optimal Th2 differentiation (57).

Proliferation

T cells proliferate properly in the presence of costimulatory signals. However, the mechanism of how costimulation regulates T cell proliferation is still unclear. The enhanced proliferation mediated by costimulation is strongly correlated with the increased cytokine secretion, i.e., IL-2 and IL-12, which promote T cell proliferation (12, 15, 58).

Previous studies have linked CD28 and PKCθ as a potential signaling pathway that influences T cell activation, however, by comparing the responses of CD28 and PKCθ-deficient T cells *in vivo* and *in vitro*, Berg-Brown et al. demonstrate that CD28 signals that augment T cell proliferation is independent of CD28-PKC pathways (22). Recent data indicated that a target of CD28, phosphatase and tensin homolog (PTEN), might mediate the CD28 signals in T cell proliferation. PTEN is dual-specific phosphatase acting on phospho amino acids but also on three kinds of phosphorylated inositol phospholipids. CD28 signals can directly inhibit PTEN, which will result in massively increased proliferation. Furthermore, this inhibition was sensitive to the PI3K inhibitor wortmannin (59), suggesting that PTEN is a target of PI3K, and suppression of PTEN by CD28 could control T cell proliferation. In addition, bcl-xγ, a member of the bcl-x family whose expression is restricted to activated T cells also regulates T cell proliferation mediated by CD28 signals. The cytosolic protein of bcl-xγ is an essential downstream link in the CD28-dependent signaling pathway that underlies T cell costimulation (60). It has been shown that bcl-xγ did not sustain cell survival, but involved in proliferation, cytokine secretion and memory development of T cells (61). Our recent paper reported that CD28 controlled aurora B (AIM1, aurora-1), described as a serine/threonine kinase and a chromosome passenger involved in cytokinesis and chromosome architecture, which mediated T cell proliferation and expansion (33). Expression of aurora B in CD28-deficient T cells augmented phosphorylation of mammalian target of rapamycin (mTOR) substrates, expression of cyclin A, hyperphosphorylation of retinoblastoma tumor suppressor gene (Rb) and activation of cyclin-dependent kinases (CDKs) 1 and 2 with a promoted pattern of cell cycle progression. IL-2 enhanced aurora B activity, and inactivated aurora B prevented IL-2-induced proliferation. Moreover, expression of aurora B restored CD28-deficient T cell proliferation and promoted inflammation *in vivo*, indicating that aurora B, along with mTOR, was a CD28 mediated G1-S checkpoint regulator in T cells (33).

OX40 can promote the expression of survivin, a member of the apoptosis inhibitor family, which is regulated most likely by PI3K/PKB pathway products to control T cell proliferation (16). Survivin is induced by OX40-independent mitotic progression in late G1, and blocking survivin suppresses S-phase transition then division of T cells and leads to apoptosis. In addition, survivin expression alone is sufficient to restore proliferation and antagonize apoptosis in costimulation-deficient T cells, and rescue T cell expansion *in vivo*, suggesting that sustained survivin expression from OX40 costimulatory signals drives T cell proliferation (16).

Cell cycle progression

During cell cycle procession in T cells, several molecules control the G1-S transition of the cell cycle. In partcular, cyclins and CDKs formed phosphorylated complexes could phosphorylate retinoblastoma protein (Rb) and initiate gene expression involved in S phase activities. These complexes can be regulated by p21, p27, p15, p16, or PI3K/mTOR (62, 63).

A critical role of cyclin-dependent kinase inhibitor p27 (kip1), a major CDK binding protein expressed by T cells, has been demonstrated to control CD28 induced cell cycle progression. CD28 costimulation can directly regulate cell cycle progression by inducing transcription of the substrate recognition components of Skp1/Cul1/F-box (SCF)-skp2 ubiquitin ligase that targets p27 (kip1) for degradation (64). Using p27 (kip1) gene dosage, it has been demonstrated that early after activation, p27 (kip1) acts to promote, rather than inhibit, G1 to S phase progression in the first division cycle. However, throughout subsequent cell divisions p27 (kip1) behaves as a negative regulator, directly reducing the threshold amount of growth factor signals required to support continued cell division. During this phase, signals from CD28 and IL-2R cooperate with the TCR to "tune" this threshold by inducing the degradation of p27 (kip1) protein. In addition, agents that block these pathways require elevated p27 (kip1) levels for their full anti-proliferative activity, and p27 (kip1) opposes the development of $CD4^+$ T cell effector function, and is required for the full development of anergy in response to a tolerizing stimulus (65). These suggest that p27 (kip1) plays a complex and important role in the regulation of cell division and effector function development in $CD4^+$ T cells (65-67). Besides this, it has been shown that cell cycle re-entry of quiescent T cells is dependent upon CDK2, and inhibition of CDK2 by p27 (kip1) is the principal constraint on S-phase entry in T cells. In addition, the deficiency for p27 (kip1) has a more pronounced effect on the expansion of murine naïve $CD8^+$ T cells and this disparity is caused by a reduced requirement for CD28-mediated costimulation in p27 (kip1) lacking $CD8^+$ but not $CD4^+$ equivalents. These data highlight a previously unappreciated difference in the way CD28 signaling is coupled to the core cell cycle machinery in these two T cell subsets (68). Furthermore, cell cycle progression of primary T lymphocytes requires simultaneous activation of PI3K- and MEK1/2-dependent pathways (66). Ligation of CD28 alone is sufficient for activation of PI3K target PKB and VAV1 (69). In addition, CD28 ligation alone is also sufficient to mediate phosphorylation of the Forkhead family transcription factor FOXO1, and decreasing phosphorylation of PKB target glycogen synthase kinase-3 (GSK-3), which also resulted in down-regulation of p27 (kip1) (66, 69). Interestingly, the inhibitors of CDK4 (INK4) bind CDK4/6 to prevent their association with D-cyclins and inhibit G1 cell cycle initiation and progression. Among the seven CDK inhibitors, p18 (INK4c) plays an important role in modulating TCR-mediated T cell proliferation, where CD28 costimulation is to counteract the p18 (INK4c) inhibitory activity on CDK6-cyclin D complexes, and then regulates T cell cycle progression (70). It has also been reported that CD28 mediated G1-S transition of the cell cycle is controlled by aurora B, survivin and mTOR. More importantly, expression of aurora B in CD28-deficient T cells augments phosphorylation of mTOR substrates, expression of cyclin A, hyperphosphorylation of Rb, and activation of CDK1 and 2, and promotes cell cycle progression (33).

Sustained PI3K and PKB activation by OX40 controls survivin expression, which is independent of mitotic progression in late G1 phase. Blocking survivin suppresses S-phase transition and division of T cells mediated by OX40 signals and leads to apoptosis, implying that sustained survivin expression from OX40 costimulatory signals controls T cell cycle progression (16).

Cell survival

Two main pathways of cell death have been described for many years. One involves signals from TNFR family members called active death possessing death domain, the second is governed by a number of bcl-2 family members. Both CD28 and OX40 signals promote T cell survival by augmenting expression of bcl-2 family members.

CD28 promotes T cell survival by enhancing the expression of bcl-xL *via* a specific recruitment of RelA and p52 NF-κB subunits (71). The molecular basis of how CD28 regulates the expression of bcl-xL has been defined as upon interacting with its ligand, B7-1 or B7-2 and CD28 gets phosphorylated on tyrosine residues. The CD28 cytoplasmic domain that binds with a cytosolic protein called translationally controlled tumor protein (TCTP), a novel multifunctional antiapoptotic bcl-xL-interacting protein to regulate cell survival (72, 73). In particular, one tyrosine (Y170 in mouse CD28, Y173 in human CD28) that is important in PI3K activation permits CD28 to recruit SH2-containing signaling molecules, including PI3K, Grb2, and Gads that control the regulation of bcl-xL (21, 74). However, whether or not this regulation was dependent on mammalian target of rapamycin pathway is still controversial (13, 75). CD28 also promotes expression of bcl-2, bfl-1 (A1), and myeloid cell leukemia sequence 1 (mcl-1) to regulate T cell survival (76-78).

OX40 regulates T cell survival by sustaining PKB mediated expression of bcl-2 and bcl-xL (15, 76). Upon interaction with its ligand--OX40L, the cytoplasmic tail of OX40 associates with an adapter protein TRAF2 (79) to mediate signal transduction, probably through PI3K/PKB/

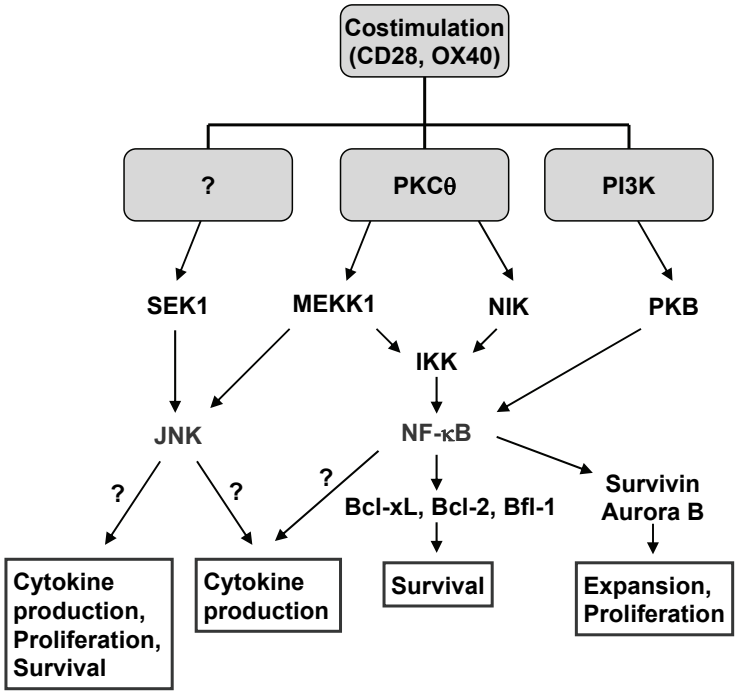

Figure 1. Molecular basis of T cell costimulation. Costimulation regulates PI3K/PKB/NF-κB to control expression of bcl-2 family members that sustain T cell survival and expression of survivin and aurora B that control T cell expansion and proliferation. In addition, costimulation might also through PKCθ or unknown molecules regulate JNK or NF-κB that controls cytokine production, survival and proliferation.

NF-κB pathway then regulates expression of bcl-xL, A1, and bcl-2 that controls T cell longevity (4).

Memory T cell development

Memory T cell development is enhanced with costimulation engagement, which is defective in CD28 or OX40-deficent T cells. During development of memory T cell, CD28 and herpesvirus entry mediator (HVEM) are constitutively expressed on naïve T cells, which may control initial activation and division. CD27 is upregulated quickly; they can regulate clonal expansion (80). OX40 and 4-1BB are induced later, which prevent apoptosis and regulate the persistence of effector cells. Without costimulation, fewer effector T cells will be generated and survive as functional memory T cells.

Although CD28 promotes memory T cell development, the mechanism how CD28 mediates the signal still needs to be elucidated (34, 81, 82). Previous data suggested that the antiapoptotic protein bcl-xL plays an essential role in the generation of effector and memory T lymphocytes. This is correlated with the fact that bcl-xL is induced in activated T lymphocytes upon costimulation through CD28, and bcl-xL is also highly enriched in memory T lymphocytes, which sustained T cell survival as functional memory T cells. However, mice with a conditional deletion of bcl-xL in T lymphocytes develop a normal $CD8^+$ T cell response to Listeria monocytogenes infection. Furthermore, conditional bcl-xL knockout mice exhibit normal T-dependent humoral immune responses. These results indicate that bcl-xL is dispensable for the generation of effector and memory T lymphocytes (83). In addition, bcl-xγ-deficient (bcl-x$\gamma^{-/-}$) T cells display defective proliferation and cytokine responses to CD28-dependent costimulatory signals, impaired memory responses to proteolipid protein peptide (PLP), and do not develop PLP-induced experimental autoimmune encephalomyelitis (EAE). Collectively, these results imply that bcl-xγ might be a target of CD28 that regulated memory T cell development (61).

OX40 engagement can boost the generation of antigen-specific T cell memory (84, 85), particularly control memory T helper 2 cells that drive lung inflammation (86, 87). The molecular targets of OX40 that regulate memory development is still unclear, but OX40 sustains expression of bcl-xL, which controls long-term T cell survival, suggesting that bcl-xL might also mediate memory T cell development (76). To confirm this, retroviral expression of bcl-xL in tumor-reactive $CD8^+$ T cells conferred greatly enhanced tumor protection following adoptive transfer, indicating bcl-xL would be the target of OX40, which involves memory T cell development (88, 89).

Summary

Costimulation controls cytokine production, proliferation, cell cycle survival, and memory development by regulating the intracellular PI3K/PKB/NF-κB signal transduction pathway. By this signaling pathway, costimulation either regulates expression of bcl-2 family members that sustain T

cell survival, and memory development, or sustains expression of survivin and aurora B that control T cell proliferation and expansion. In addition, bcl-2 family members and survivin/aurora B also involve in cell cycle progression and memory development. Besides these, costimulation might also cooperate with PKC family members through PI3K/PKB/NF-κB pathway to mediate cytokine production (Figure 1). The mechanisms how costimulation regulates cytokine secretion and memory are largely unknown, especially in some areas, are still controversial. For instance, whether or not bcl-xL is a main target of CD28 that controls central T-cell memory. However, targeting the T-cell costimulation (e.g., CD28, OX40, 4-1BB) is a promising strategy to overcome T-cell-mediated immune disorders including cancer, infectious disease, and autoimmune disease, by regulating the T cells that cause disease. In these cases, CD28 blockade mainly suppresses the generation of effector T cells from naïve T cells, whereas OX40 blockade inhibits the survival and activation of effector T cells generated from either naïve or memory T cells.

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