Archivum Immunologiae et Therapiae Experimentalis, 2002, **50**, 169–177
PL ISSN 0004-069X

Review

CD28 Costimulatory Molecule – Expression, Structure and Function

DOROTA BOĆKO[1], AGATA KOSMACZEWSKA[1], LIDIA CISZAK[1], RENATA TEODOROWSKA[1] and IRENA FRYDECKA[1, 2]*

[1] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland, [2] Department of Hematology, Medical University, Wrocław, Poland

Abstract. T cell activation is a key event in triggering an antigen specific immune response of the organism. The process is induced primarily by the signal generated by direct interaction of a T cell receptor with an antigen bound to the major histocompatibile complex on an antigen-presenting cell (APC). Although the signal is critical in exciting immune response, an additional, costimulating signal is required. The major second signal is generated by interaction of the CD28 molecule expressed on most T lymphocytes with its natural ligands CD80 and CD86 located on APCs. The signal excited by CD28 triggering involves multiple second-messenger cascades, leading to the activation of transcription factors and finally results in cell proliferation, cytokine production, and the generation of effector functions. The importance of CD28-delivered costimulatory signals was proven in experiments with CD28-deficient mice. T cells from these mice exhibited an impaired pattern of cytokine secretion and defects in T cell-dependent antibody production. Certain forms of immunopathology might result from the aberrant regulation of CD28 expression.

Key words: T cell activation; CD28 molecule; costimulation.

CD28 Expression and Structure

In 1970 a two-signal model of lymphocyte activation was proposed by BRETSCHER and COHN[10], who suggested that T cell receptor (TCR) occupancy alone is an insufficient stimulus to excite T cell differentiation and effector functions. T cell activation requires the recognition of an antigen/major histocompatibile complex (MHC) located on antigen-presenting cells (APCs) by TCR, although an additional, costimulatory signal is required to excite complete T cell activation, resulting in proliferation and cytokine secretion (Fig. 1). Triggering of TCR/CD3 alone in the absence of a costimulatory signal not only fails to induce immune response, but may also lead to a state of hyporesponsiveness or anergy[36, 61].

CD28 is known to be the major costimulatory molecule, and it has been identified as a 44 kDa homodimeric or monomeric[5] transmembrane glycoprotein expressed on $CD3^+$ immature thymocytes, all $CD3^+$ thymocytes, 95% of $CD4^+$ and approximately 50% $CD8^+$ T cells[43, 44], on γδ T cells[53, 84], plasma cells[50], and on human foetal, but not adult, peripheral blood natural killer (NK) cells[62]. A variant of the CD28 molecule was recently reported on a subpopulation of human peripheral blood NK cells[31]. Ligation of CD28 costimulates

*Correspondence to: Prof. Irena Frydecka, M.D. Ph.D., Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland, tel.: +48 71 373 22 74 ext. 273, fax: +48 71 373 25 87, e-mail: frydecka@hemat.am.wroc.pl

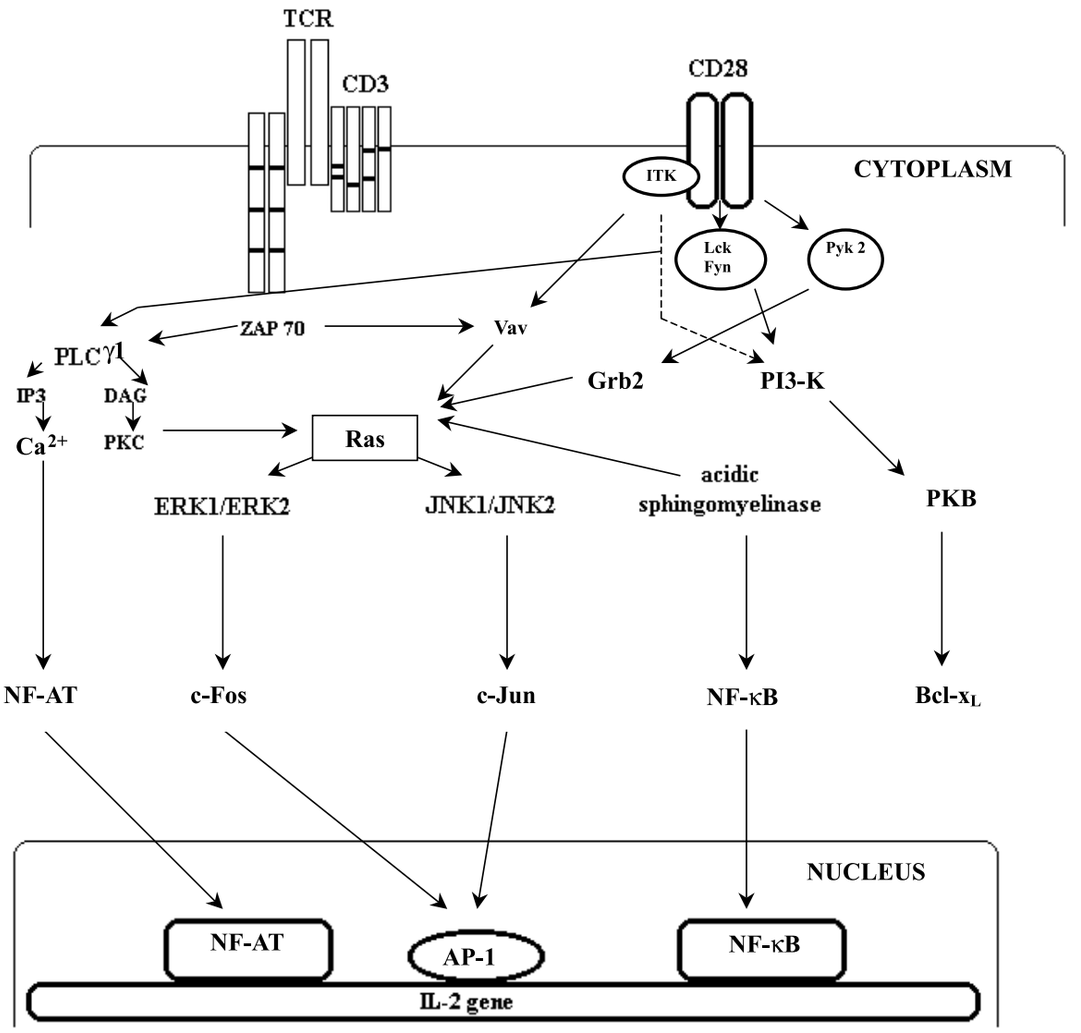

Fig. 1. Molecules involved in signal transduction in activated T cells (modified from ALEGRE[1])

T cell proliferation, generation of cytotoxic lymphocytes, and cytokine production (IL-1α[16], IL-2[26], IL-3[34], IL-4[89], IL-5[28], IL-6[57], IL-10[94], IL-13[59], TNF-α, GM-CSF, LT[85], IFN-γ[63], CSF-1[17]). In addition, costimulation through the CD28 molecule improves T cell survival, both through the increased IL-2 secretion and the ability to up-regulate Bcl-x_L[7, 71].

CD28 expression on cell surface was described to be constant, although LINSLEY et al.[56] showed that CD28 stimulation with phytohemaglutinin (PHA), monoclonal antibody (mAb) 9.3 (anty-CD28 mAb) or B7.1-tranfected Chinese hamster ovary cells caused a marked reduction of CD28 mRNA levels within 4 h and by 24 h after stimulation had returned to initial levels. Also, the addition of $B7^+$ cells to cultured T cells resulted in a decrease of CD28 expression after 12 to 24 h of stimulation, returning to the starting level after 48 h. The transient down-regulation of CD28 expression described above could be associated with ligation-stimulated CD28 receptor endocytosis, occurring via clathrin coated pits, which are directed to lysosomes for proteolytic degradation or recycling to the cell surface. There is evidence that phosphatidylinositol 3-kinase (PI3-K) is implicated in ligation-stimulated CD28 endocytosis, since point mutation studies at tyrosine[173] (Tyr) site of the CD28 molecule, known as the region for PI3-K binding, negatively affected the ability of endocytosis[15].

The mature CD28 molecule consists of 202 amino-acids and its 134 amino-acid extracellular region contains a single disulphide-linked IgV-like domain. The extracellular tail of the CD28 receptor possesses the

hexapeptide motif MYPPPY, essential for its counter-receptor binding[68]. The CD28 41-amino-acid cytoplasmic tail contains four tyrosine residues, which can be phosphorylated upon activation. Of special interest is the Tyr[173] included within a consensus sequence motif YMNM which when phoshorylated, forms a binding site for the SH2 domain of PI3-K[94] and Grb 2 adaptor protein[76].

Counterreceptors

At present two natural ligands for CD28 on APCs, B7.1 and B7.2, have been identified, although recent studies indicate the existence of another molecule, B7h, being the member of the B7 family of molecules[18]. The genes for B7 family members are localized to 3q13.3--3q21[30]. The structure of the B7 molecule family comprises a single IgV-like domain and a single IgC2-like domain within their extracellular parts. Studies on the amino-acid sequence revealed the presence of SQDXXXELY domain within B7.1, and B7.2, but not in the B7h molecule.

B7.1 is expressed on activated B cells, T cells, macrophages and dendritic cells, whereas B7.2, also expressed on activated T and B cells, is constitutively present on resting monocytes[4]. These studies could indicate that B7.2 is probably the major initial ligand for CD28 during T cell activation. However, functional data has indicated that B7.1 seems to be the more potent ligand for the CD28 molecule in terms of activation[75].

B7.1 (CD80)

B7.1 (CD80) was first described in 1981 by YOKOCHI et al.[93] as a B cell activation molecule recognized by anti-BB-1 mAb and identified as a natural ligand for the CD28 molecule[33]. The mature form of B7.1 was found to exist as a transmembrane monomeric glycoprotein consisting of 262 amino-acids with a molecular weight of 60 kDa. The molecule was identified to consist of two extracellular disulphide-linked Ig-like domains, a transmembrane domain and a short, 19-amino-acid cytoplasmic tail. PEACH et al.[68] identified by site-directed mutagenesis within the IgV-like domain of B7.1 11-amino-acids responsible for receptor binding. Furthermore, similar corresponding residues were established in the B7.2 structure. Additionally, mutagenesis of several conserved ABED β-sheet face in the IgC-like domain of B7.1 completely stopped receptor binding[69].

B7.2 (CD86)

Structurally, B7.2, of 70 kDa weight shares homology to B7.1. Its cytoplasmic tail contains three putative residues for protein kinase C (PKC), indicating that B7.2 is involved in signaling function in APCs. Recently HÖLLSBERG et al.[41] provided evidence, that human T lymphocytes may also express a hypoglycolysed form of B7.2. The hypoglycosylated form of B7.2 expressed on T lymphocyte could be responsible for providing a negative regulatory signal and, thus, down-regulating immune response.

B7 homologue

Although this molecule shares similarity with other members of the B7 family, it does not contain the unique sequence SQDXXXELY possessed by B7.1 and B7.2. Moreover, B7 homologue (B7h) does not bind to CD28 nor CTLA-4, but is identified as a ligand for the novel costimulatory molecule ICOS.

The B7h expression pattern differs from that of B7.1 and B7.2. B7h was established to be expressed constitutivelly on B cells and its expression is up-regulated upon activation in B cells, monocytes, dendritic cells as well as on lung and heart[13, 18]. B7h seems to provide costimulatory signals to T cells previously activated through the CD28/B7.1-B7.2 pathway in the context of professional APCs[13].

$CD28^-$ T lymphocytes

The importance of CD28-delivered costimulatory signals was proven in experiments with CD28 deficient mice. $CD28^{-/-}$ mice have demonstrated pronounced immune deficiency, and while T cell development and cytotoxic T lymphocyte generation is normal in these mice[78], their peripheral T cells display impaired lymphokine secretion after stimulation with lectin concanavalin A. Further studies have extended these results by demonstrating that superantigen responses are impaired in CD28 knock-out mice[60]. These findings were consistent with previous studies that indicated a requirement for the CD28 : B7 interaction to achieve maximal mitogen-initiated activation[85].

Additionally, studies performed by AZUMA et al.[5] demonstrated that, upon optimal stimulation conditions, the $CD8^+/CD28^-$ cells showed poor proliferative response, which was not reversible by exogenous IL-2. The humoral immune responses in these CD28 deficient mice were also defective. FERGUSON et al.[25] demonstrated that CD28-deficient mice exhibited lower le-

vels of certain isotypes of immunoglobulins and, furthermore, germinal centres were not formed in response to immunization.

Protein Tyrosine Kinases Involvment in the CD28 Signaling Pathway

The signal derived after CD28 stimulation induces recruitment of several cytoplasmic protein tyrosine kinases (PTKs) including the Src family kinases $p56^{lck}$ and $p59^{fyn}$ [2, 42], the Tec family kinase Itk/EMT[44, 48], and the proline-rich tyrosine kinase 2 (Pyk2)[86]. Tyrosine phosphorylation represents one of the earliest events in CD28 signaling.

The $p56^{lck}$-dependent pathway leads to the phospholipase Cγ (PLCγ) activation and a further increase in calcium concentration, whereas the $p56^{lck}$ kinase-independent cascade leads to substrate p110 phosphorylation, which corresponds to the p110 catalytic subunit of PI3-K[58].

The Tec family protein kinase Itk/EMT is associated with the cytoplasmic domain of CD28 and becomes phosphorylated upon CD28 ligation. Tyrosine phosphorylation of Itk/EMT occurs in the absence of Lck activation and, downstream, leads to phosphorylation of guanine nucleotide exchange factor Vav[3].

It is plausible that Itk/EMT could be involved in the $p56^{lck}$-independnet cascade because Itk/EMT becomes activated in the absence of $p56^{lck}$ and may cause PI3-K activation[48]. The consequence of the PTK induction mentioned above is phosphorylation of the tyrosine residues within the CD28 cytoplasmic domain, critical for interaction with PI3-K. Studies performed *in vitro* conditions revealed that $p56^{lck}$ is involved only in Tyr^{173} phosphorylation. Itk/EMT phosphorylates all 4 tyrosine residues (Tyr^{173}, Tyr^{188}, Tyr^{193}, and Tyr^{200})[48]. TSUCHIDA et al.[86] recently reported that the member of the focal tyrosine kinase family Pyk2 kinase also became tyrosine-phosphorylated upon CD28 and TCR ligation. The phosphorylation of the kinase at its Tyr^{402} has been established as a critical event for the further association with other signaling molecules, such Grb2/Sos complex, or Src kinases[22], thus linking the kinase to the GTPase Rho signaling pathway[72].

PI3-K – the Role in the CD28 Signaling Pathway

PI3-K exists as a heterodimer of 85 and 110 kDa polypeptides[39] with dual specificity both as a lipid and a protein serine kinase[21]. The mentioned enzyme is known to play a pivotal role in the alternative inositol phospholipid metabolic pathway, and as a consequence of its activity. Phosphorylation of membrane phosphatidylinositol lipids results in the generation of D-3 phosphoinositide lipids:

- phosphatidylinositol phosphate (PtdIns(3) P),
- phosphatidylinositol bisphosphate (PtdIns(3,4) P_2),
- phosphatidylinositol trisphosphate (PtdIns(3,4,5) P_3)[88].

It has been proven, that CD28 couples to PI3-K by studies demonstrating that CD28 ligation with B7.1 resulted in an increased concentration of PtdIns (3, 4, 5) P_3 in Jurkat cells[90, 91]. Later UEDA et al.[87] observed a similar effect after B7.2 or anti-CD28 mAb ligation in these cells. The functional importance of PI3-K recruitment for CD28-dependet costimulation is still unclear, although the functional significance of the association was proven in studies with murine T cell hybridomas with point mutation site in Tyr^{173}, which were not able to produce IL-2 after CD28 ligation[66]. Additionally, there are reports that PI3-K, indirectly via D-3 phosphoinositides, activates protein kinase B (PKB) after CD28 ligation[67]. This protein plays a pivotal role in cell-death prevention by the phosphorylation of the death-promoting protein BAD, resulting in survival factor – Bcl-x_L activation[20].

Adaptor Proteins Involved in the CD28-Dependent $p21^{ras}$ Cascade

Grb2 molecule

Phosphorylated form of CD28 acts as a target for the p85 regulatory subunit of PI3-kinase binding, but it also uses the same pYMNM motif to recruit a second intracellular protein, Grb2. Mutation of Tyr^{191} within the pYMNM motif of CD28 markedly disrupts Grb2 binding to the CD28 cytoplasmic domain[76]. However, the Grb2 SH2 domain binds to CD28 with 10–100-fold less intensity than PI3-K, suggesting the importance of the latter enzyme in the CD28 signaling pathway[70]. CD28 cross-linking with anti-CD28 mAb, but not with natural ligand, could induce association between the phosphorylated form of the molecule with Grb2 and tyrosine phosphorylation of p36 (Grb2-associated molecule)[76]. Grb2-complexed guanine nucleotide exchange factor, activated by Pyk2 kinase upon CD28 ligation[83], is responsible for converting $p21^{ras}$ to active stage and, thus, acts as an important link of CD28 through the $p21^{ras}$-dependent signaling pathway to MAP kinase activation[76].

Studies performed by NUNES et al.[64] showed that CD28 ligation with its counterreceptors additionally resulted in tyrosine phosphorylation of p62, the unique protein for the CD28 signaling pathway, which is able to interact with the Grb2 SH2 domain only under *in vitro* conditions. The p62 GAP-associated protein phosphorylation was found to be regulated by residues 182–202 in the CD28 cytoplasmic domain, but not upon TCR/CD3 stimulation[49]. The above-mentioned domain in the CD28 cytoplasmic compartment is involved in IL-2 production, because deletion of amino-acids 193–202 in the CD28 C-terminal tail resulted in the lack of IL-2 production in murine T cell hybridomas[65]. Therefore, this could suggest a close correlation between p62 activation and IL-2 production upon CD28 stimulation.

Vav

Stimulation via CD28 antigen results in guanine nucleotide exchange factor Vav phosphorylation and this process is regulated by residues 173–181 within the cytoplasmic domain of CD28[51]. Studies performed by NUNES et al.[64] indicated that anti-CD28 mAb was able to excite Vav and p36 phosphorylation and further $p21^{ras}$ cascade activation. Guanine nucleotide exchange factor Vav is known to be involved in c-Jun amino-terminal kinase (JNK) activation[55] and, moreover, the phosphorylated form of Vav possesses the ability to create a complex with SLP-76 molecule by its Tyr^{113} and Tyr^{128} residue binding. Vav overexpression enhances nuclear factor activity in an activated T cell and induces CD28-mediated IL-2 promoter activity, instead SLP-76 leads to activation of AP-1 transcription factors and enhances nuclear factor activity. The synergy between these molecules may be the integration signal point derived from TCR and CD28 during the regulation of IL-2 gene expression[23].

CD28-Induced Sphingomyelinase-Ceramide Pathway

CHAN and OCHI[19] provided evidence that CD28 costimulatory molecule is also coupled to the sphingomyelin-ceramide pathway by demonstrating, that anti-CD28 mAb-treated T cells were able to generate ceramide after sphingomyelin hydrolysis. Moreover additional incubation with C6 ceramide analogue resulted in rapid (within 30 min) IL-2 mRNA stabilization.

Further studies on the molecular mechanisms of the above-mentioned reaction revealed that CD28 as well as cell-permeable ceramide analogue can activate a serone/threonine p21-activated kinase (PAK), which was established to be up-stream activated by GTP-bound Rac or CDC42. Subsequently, it appeared that MEKK1 is the substrate for PAK in CD28-stimulated T cells, which upon CD28 engagement becomes phosphorylated at its N-terminal domain[8, 45]. Activation of the PAK/MEKK1 signaling cascade resulting in augmentation of JNK activity, is followed by its translocation to the nucleus and subsequent activation of c-Jun transcription factor[40].

A number of reports indicate that ceramide could be a mediator of CD28-induced costimulatory signals, regulating programmed cell death[35]. It seems to be plausible that three G-protein-coupled MAP kinase cascades result in activation of JNK, ERK and p38. The integration of the signals is required for the determination of death or proliferation of the cells[24]. Indeed, studies performed by XIA et al.[92] on PC12 pheochromocytoma cells showed that parallel JNK and p38 kinases with ERK kinase inhibition appears to be critical for apoptosis induction.

Activation of the PLCγ Pathway

PLCγ cleaves a membrane phosphoinositide to active second messengers: diacyloglycerol (DAG) and 1,4,5-trisphosphate inositol (IP_3). Increase of IP_3 concentration within the cellular compartment results in a rise in intracellular calcium, while DAG activates PKC. These two signals synergize to induce IL-2 gene transcription. Another three phosphoinositides, namely PtdIns, PtdIns(4)P and PtdIns $(4,5)P_2$, after PI3-K triggering become phosphorylated in the 3rd position of the inositol ring, but they are not the substrates for PLC[77]. Further mobilization of intracellular calcium activates calmodulin-dependent phosphatase calcineurin. The latter may lead to an alteration of the cytoplasmic component of nuclear factor-activated T cells ($NF\text{-}AT^C$), allowing it to move into the nucleus, where it combines with nuclear component ($NF\text{-}AT^N$) and two proteins: Jun and Fos. This NF-AT complex binds to a response element of IL-2 enhancer.

The way of CD28 coupling to PLCγ seems to be distinct from that related with TCR because of the inositol phospholipid (InsP) production and increase in Ca^{2+} concentration after CD28 engagement, since it was enhanced by pretreatment with phorbol myristate acetate (PMA), while InsP levels and Ca^{2+} mobilization signals after TCR crosslinking were suppressed by PMA[52].

Nuclear Events Mediated by CD28 Molecule

Su et al.[82] investigated phorbol ester, Ca^{2+} ionophore on TCR and CD28 stimulation on JNK1 and JNK2 activation. They found that integration of the two pathways occurs at the level of JNK activation and that CD28 costimulation significantly increased JNK activation (approximately 28-fold).

More recently, Shin and Han[80] demonstrated on leukemic Jurkat T cells that the c-Jun promoter contains binding sites for trancritption factors, e.g. MEF2 and AFT. Point mutation at these binding sites resulted in strongly reduced CD28 induction of c-Jun promoter in Jurkat cells. Moreover, additional pretreatment with acidic sphingomyelinase inhibitor prompted completely inhibited c-Jun promoter activation, whereas wortmannin, the PI3-K inhibitor, did not influence promoter induction. The above report indicates that CD28 signaling leads to c-Jun promoter induction and the activation of factors binding to MEF2 and ATF sites utilizing the acid sphingomyelinase pathway, but not PI3-K.

CD28-Mediated Cytokine Production

There is a large body of data showing that CD28 signaling pathway is involved in IL-2 gene transcription. CD28 stimulation induces activation of NF-κB transcription factor, which constitutively exists as an inactive dimmeric complex anchored in the cytoplasm by two inhibitory proteins, IκBα and IκBβ[81]. Activation of NF-κB is controlled by the targeted phosphorylation and subsequent degradation of IκB. Studies performed on a mutated form of IκBα indicated the probable sites of phosphorylation at serine-32 or serine-36 within the inhibitory protein[11]. The active NF-κB dimmers move to the nucleus, bind to target DNA elements, and activate the transcription of genes encoding proteins involved in immune responses[6]. Accessible reports indicate that the CD28 signaling pathway induces down-regulation of IκB inhibitory proteins and enhances c-Rel[51], NF-κB1, and Rel A translocation into the nucleus[12]. Additionally, the CD28 signal mediates rapid degradation particularly of IκBβ, rather than IκBα[37, 38], which both need to be previously phosphorylated by mitogen-activated kinases[38, 47]. Shapiro et al.[79] showed that CD28 responsiveness is also influenced by a composite element containing CD28 responsive element (CD28RE) and NF-IL2B AP-1 within the IL-2 promoter. The latter was found as the integration point of two separate signaling pathways activated upon TCR and CD28 stimulation. Moreover, the NF-IL2B AP-1 site was shown to bind c-Rel transcription factor within CD28RE after CD28 activation.

Aberrant expression of CD28 costimulatory molecule contributes to pathophysiology in several neoplastic[73], autoimmune[46], and infections diseases[14], as well as in patients after liver transplantation[32]. Further phenotypical and function studies on the CD28 costimulatory pathway might contribute to the development of new prophylactic and therapeutic approaches[74].

Acknowledgment. This work was supported by the State Committee for Scientific Research (KBN, Poland, grant no. 6PO5B 120 21). We are grateful to Prof. Janina Kwiatkowska-Korczak for comments on the manuscript.

References

1. Alegre M. L. (1999): Costimulatory molecules as targets for the induction of transplantation tolerance. Nephrol. Dial. Transplant., **14**, 322–332.
2. August A. and Dupont B. (1994): Activation of src family kinase lck following CD28 crosslinking in the Jurkat leukemic cell line. Biochem. Biophys. Res. Commun., **199**, 1466–1473.
3. August A., Gibson S., Kawakami Y., Kawakami T., Mills G. B. and Dupont B. (1994): CD28 is associated with and induces the immediate tyrosine phosphorylation and activation of the Tec family kinase ITK/EMT in the human Jurkat leukemic T-cell line. Proc. Natl. Acad. Sci. USA, **91**, 9347–9351.
4. Azuma M., Ito D., Yagita H., Okumura K., Phillips J. H., Lanier L. L. and Somoza C. (1993): B70 antigen is a second ligand for CTLA-4 and CD28. Nature, **366**, 76–79.
5. Azuma M., Phillips J. H. and Lanier L. L. (1993): $CD28^-$ T lymphocytes. Antigenic and functional properities. J. Immunol., **150**, 1147–1159.
6. Baldwin A. S. jr. (1996): The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu. Rev. Immunol., **14**, 649–683.
7. Boise L., Minn A. J. and Noel P. J. (1995): CD28 costimulation can promote T cell survival by enhancing the expression of BCL-xl. Immunity, **3**, 87–98.
8. Boucher L. M., Wiegmann K., Fütterer A., Pfeffer K., Machleidt T., Schütze S., Mak T. W. and Krönke M. (1995): CD28 signals through acidic sphingomyelinase. J. Exp. Med., **181**, 2059–2068.
9. Boussiotis V. A., Freeman G. J., Gribben J. G., Daley J., Gray G. and Nadler L. M. (1993): Activated human B lymphocytes express three CTLA-4 counterreceptors that costimulate T-cell activation. Proc. Natl. Acad. Sci. USA, **90**, 11059–11063.
10. Bretscher P. and Cohn M. (1970): A theory of self-nonself discrimination. Science, **169**, 1042–1049.
11. Brown K., Gerstberger S., Carlson L., Franzoso G. and Siebenlist U. (1995): Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. Science, **267**, 1485–1488.
12. Bryan R. G., Li Y., Lai J. H., Van M., Rice N. R., Rich

R. R. and TAN T. H. (1994): Effect of CD28 signal transduction on c-Rel in human peripheral blood T cells. Mol. Cell. Biol., **14**, 7933–7942.
13. BUGEON L. and DALLMAN M. J. (2000): Costimulation of T cells. Am. J. Respir. Crit. Care Med., **162**, 164–168.
14. CARUSO A., CANTALAMESSA A., LICENZIATI S., PERONI L., PRATI E., MARTINELLI F., CANARIS A. D., FOLGHERA S., GORLA R., BALSARI A., CATTANEO R. and TURANO A. (1994): Expression of CD28 on $CD8^+$ and $CD4^+$ lymphocytes during HIV infection. Scand. J. Immunol., **40**, 485–490.
15. CÉFAÏ D., SCHNEIDER H., MATANGKASOMBUT O., KANG H., BRODY J. and RUDD C. E. (1998): CD28 receptor endocytosis is targeted by mutations that disrupt phosphatidylinositol 3-kinase binding and costimulation. J. Immunol., **160**, 2223–2230.
16. CERDAN C., MARTIN Y., BRAILLY H., COURCOUL M., FLAVETTA S., COSTELLO R., MAWAS C., BRIG F. and OLIVE D. (1991): IL-1 alpha is produced by T lymphocytes activated via CD2 plus CD28 pathways. J. Immunol., **146**, 560–564.
17. CERDAN C., RAZANAJOANA D., MARTIN Y., COURCOUL M. and PAVON C. (1992): Contributions of the CD2 and CD28 T lymphocyte activation pathways to the regulation of the expression of the colony-stimulating factor (CSF-1) gene. J. Immunol., **149**, 373–379.
18. CHAMBERS C. A. (2001): The expanding world of co-stimulation: the two-signal model revisited. Trends Immunol., **22**, 217–223.
19. CHAN G. and OCHI A. (1995): Sphingomyelin-ceramide turnover in CD28 costimulatory signaling. Eur. J. Immunol., **25**, 1999–2004.
20. DATTA S. R., DUDEK H., TAO X., MASTERS S., FU H., GOTOH Y. and GREENBERG M. E. (1997): Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell, **91**, 231–241.
21. DHAND R., HILES I., PANAYOTOU G., ROCHE S., FRY M. J., GOUT I., TOTTY N. F., TRUONG O., VICENDO P. and YONEZAWA K. (1994): PI3-kinase is a dual specificity enzyme: autoregulation by an intrinsic protein-serine kinase activity. EMBO J., **13**, 522–533.
22. DIKIC I., TOKIWA G., LEV S., COURTNEIDGE S. A. and SCHLESSINGER J. (1996): A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. Nature, **383**, 547–550.
23. FANG N. and KORETZKY G. A. (1999): SLP-76 and Vav function in separate, but overlapping pathways to augment interleukin-2 promoter activity. J. Biol. Chem., **274**, 16206–16212.
24. FANGER G. R., GERWINS P., WIDMANN C., JARPE M. B. and JOHNSON G. L. (1997): MEKKs, GCKs, MLKs, PAKs, TAKs, and tpls: upstream regulators of the c-Jun amino-terminal kinases? Curr. Opin. Genet. Dev., **7**, 67–74.
25. FERGUSON S. E., HAN S., KELSOE G. and THOMPSON C. B. (1996): CD28 is required for germinal center formation. J. Immunol., **156**, 4576–4581.
26. FRASER J. D., IRVING B. A., CRABTREE G. R. and WEISS A. (1991): Regulation of interleukin-2 gene enhancer activity by the T cell accessory molecule CD28. Science, **251**, 313–316
27. FRASER J. D., STRAUS D. and WEISS A. (1993): Signal transduction events leading to T-cell lymphokine gene expression. Immunol. Today, **14**, 357–362.
28. FRASER J. D. and WEISS A. (1992): Regulation of T-cell lymphokine gene transcription by accessory molecule CD28. Mol. Cell. Biol., **12**, 4754–4765.
29. FREEMAN G. J., CARDOSO A. A., BOUSSIOTIS V. A., ANUMANTHAN A., GROVES R. W., KUPPER T. S., CLARK E. A. and NADLER L. M. (1998): The BB1 monoclonal antibody recognizes both cell surface CD74 (MHC class II-associated invariant chain) as well as B7-1 (CD80), resolving the question regarding a third CD28/CTLA-4 counterreceptor. J. Immunol., **161**, 2708–2715.
30. FREEMAN G. J., DISTECHE C. M., GRIBBEN J. G., ADLER D. A., FREEDMAN A. S., DOUGERY J. and NADLER L. M. (1992): The gene for B7, a costimulatory signal for T-cell activation, MAPs to chromosomal region 3q13.3-3q21. Blood, **79**, 489–494.
31. GALEA-LAURI J., DARLING D., GAN S. U., KRIVOCHTCHAPOV L., KUIPER M., GÄKEN J., SOUBERBIELLE B. and FARZANEH F. (1999): Expression of a variant of CD28 on a subpopulation of human NK cells: implications for B7-mediated stimulation of NK cells. J. Immunol., **163**, 62–70.
32. GARCIA-ALONSO A. M., MINGUELA A., MURO M., ONTAÑON J., TORIO A., MARIN L., LOPEZ-SEGURA J., MIRAS M. and ALVAREZ-LOPEZ M. R. (1997): CD28 expression on peripheral blood T lymphocytes after orthotopic liver transplant: upregulation in acute rejection. Hum. Immunol., **53**, 64–72.
33. GIMMI C. D., FREEMAN G. J., GRIBBEN J. G., SUGITA K. and FREEDMAN A. S. (1991): B-cell surface antigen B7 provides a costimulatory signal that induces T cells to proliferate and secrete interleukin 2. Proc. Natl. Acad. Sci. USA, **88**, 6575–6579.
34. GUBA S. C., STELLA G., TURKA L. A., JUNE C. H. THOMPSON C. B. and EMERSON S. G. (1989): Regulation of interleukin 3 gene induction in normal human T cells. J. Clin. Invest., **84**, 1701–1706.
35. HANNUN Y. A. and OBEID L. M. (1995): Ceramide: an intracellular signal for apoptosis. Trends. Biochem. Sci., **20**, 73–77.
36. HARDING F. A., MCARTHUR J. G., RAULET D. H. and ALLISON J. P. (1992): CD28-mediated signaling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature, **356**, 607–609.
37. HARHAJ E. W., MAGGIRWAR S. B., GOOD L. and SUN S. C. (1996): CD28 mediates a potent costimulatory signal for rapid degradation of I kappa B beta which is associated with accelerated activation of various NF-kappaB/Rel heterodimers. Mol. Cell. Biol., **16**, 6736–6743.
38. HARHAJ E. W. and SUN S. C. (1998): I kappa B kinases serve as a target of CD28 signaling. J. Biol. Chem., **273**, 25185–25190.
39. HILES I. D., OTSU M., VOLINIA S., FRY M. J., GOUT I., DHAND R., PANAYOTOU G., RUIZ-LARREA F., THOMPSON A. and TOTTY N. F. (1992): Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. Cell, **70**, 419–429.
40. HILL C. S. and TREISMAN R. (1995): Transcriptional regulation by extracellular signals: mechanisms and specificity. Cell, **80**, 199–211.
41. HÖLLSBERG P., SCHOLZ C., ANDERSON D. E., GREENFIELD E. A., KUCHROO V. K., FREEMAN G. J. and HAFLER D. A. (1997): Expression of a hypoglycosylated form of CD86 (B7-2) on human T cells with altered binding properities to CD28 and CTLA-4. J. Immunol., **159**, 4799–4805.
42. HUTCHCROFT J. E. and BIERER B. E. (1994): Activation-dependent phosphorylation of the T-lymphocyte surface receptor CD28 and associated proteins. Proc. Natl. Acad. Sci. USA, **91**, 3260–3264.
43. JUNE C. H., BLUESTONE J. A. and NADLER L. M. (1994): Thompson CB The B7 and CD28 receptor families. Immunol. Today, **15**, 321–331.

44. JUNE C. H., LEDBETTER J. A., LINSLEY P. S. and THOMPSON C. B. (1990): Role of the CD28 receptor in T-cell activation. Immunol. Today, **11**, 211–216.
45. KAGA S., RAGG S., ROGERS K. A. and OCHI A. (1998): Activation of p21-CDC42/Rac activated kinases by CD28 signaling: p21-activated kinase (PAK) and MEK kinase 1 may mediate the interplay between CD3 and CD28 signals. J. Immunol., **160**, 4182–4189.
46. KANEKO H., SAITO K., HASHIMOTO H., YAGITA H., OKUMURA K. and AZUMA M. (1996): Preferential elimination of $CD28^+$ T cells in systemic lupus erythematosus (SLE) and the relation with activation-induced apoptosis. Clin. Exp. Immunol., **106**, 218–229.
47. KEMPIAK S. J., HIURA T. S. and NEL A. E. (1999): The Jun kinase cascade is responsible for activating the CD28 response element of IL-2 promoter: proof of cross-talk with the I kappa B kinase cascade. J. Immunol., **162**, 3176–3182.
48. KING P. D., SADRA A., TENG J. M. C., LIU X.-R., HAN A., SELVAKUMAR A., AUGUST A. and DUPONT B. (1997): Analysis of CD28 cytoplasmic tail tyrosine residues as regulators and substrates for protein kinanses EMT and LCK. J. Immunol., **158**, 580–590.
49. KLASEN S., PAGES F., PEYRON J. F., CANTRELL D. A. and OLIVE D. (1998): Two distinct regions of CD28 intracytoplasmic domain are involved in the tyrosine phosphorylation of Vav and GTPase activating protein-associated p62 protein. Int. Immunol., **10**, 481–489.
50. KOZBOR D., MORETTA A., MESSNER H. A., MORETTA L. and CROCE C. M. (1987): Tp44 molecules involved in antigen-independent T cell activation are expressed on human plasma cells. J. Immunol., **138**, 4128–4132.
51. LAI J. H. and TAN T. H. (1994): CD28 signaling causes a sustained down-regulation of I kappa B alpha which can be prevented by the immunosuppressant rapamycin. J. Biol. Chem., **269**, 30077–30080.
52. LEDBETTER J. A., IMBODEN J. B., SHIEVEN G. L., GROSMAIRE L. S., RABINOVITCH P. S., LINDSTEN T., THOMPSON C. B. and JUNE C. H. (1990): CD28 ligation in T-cell activation: evidence for two signal transduction pathways. Blood, **75**, 1531–1539.
53. LEE K. P., TAYLOR C., PETRYNIAK B., TURKA L. A., JUNE C. H., and THOMPSON C. B. (1990): The genomic organization of the CD28 gene. Implications for the regulation of CD28 mRNA expression and heterogeneity. J. Immunol., **145**, 344–352.
54. LESSLAUER W. and GMÜNDER H. (1986): Biochemical characterization of the 9.3 antigens of human T-cells: simultaneous expression of disulfide-bonded 90-kilodalton dimers and free subunits at the cell surface. Mol. Immunol., **23**, 271–278.
55. LI Y. S., SHYY J. Y., LI S., LEE J., SU B., KARIN M. and CHIEN S. (1996): The Ras-JNK pathway is involved in shear-induced gene expression. Mol. Cell. Biol., **16**, 5947–5954.
56. LINSLEY P. S., BRADSHAW J., URNES M., GROSMAIRE L. and LEDBETTER J. A. (1993): CD28 engagement by B7/BB-1 induces transient down-regulation of CD28 synthesis and prolonged unresponsiveness to CD28 signaling. J. Immunol., **150**, 3161–3169.
57. LORRE K., KASRAN A., VAN VAECK F., DE BOER M. and CEUPPENS J. L. (1994): Interleukin-1 and B7/CD28 interaction regulate interleukin-6 production by human T cells. Clin. Immunol. Immunopathol., **70**, 81–90.
58. LU Y., PHILIPS C. A., BJORNHALD J. M. and TREVILLYAN J. M. (1994): CD28 signal transduction: tyrosine phosphorylation and receptor association of phosphoinositide 3-kinase correlates with Ca^{2+}-independent costimulatory activity. Eur. J. Immunol., **24**, 2732–2739.
59. MINTY A., CHALON P., DEROCQ J. M., DUMONT X., GUILLEMOT J. C., KAGHAD M., LABIT C., LEPLATOIS P., LIAUZUN P., MILOUX B., MINTY C., CASELLAS P., LOISON G., LUPKER J., SHIRE D., FERRARA P. and CAPUT D. (1993): Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. Nature, **362**, 248–250.
60. MITTRUCKER H. W., SHAHINIAN A., BOUCHARD D., KUNDIG T. M. and MAK T. W. (1996): Introduction of unresponsiveness and impaired T cell expansion by staphylococcal enterotoxin B in CD28-deficient mice. J. Exp. Med., **183**, 2481–2488.
61. MUELLER D. L., JENKINS M. K. and SWARTZ R. H. (1989): An accessory cell-derived costimulatory signal acts independently of protein kinase C activation to allow T cell proliferation and prevent the induction of unresponsiveness. J. Immunol., **142**, 2617–2628.
62. NAGLER A., LANIER L. L., CWIRLA S. and PHILIPS J. H. (1989): Comparative studies of human FcRII I-positive and negative natural killer cells. J. Immunol., **143**, 3183–3191.
63. NANDI D., GROSS J. A. and ALLISON J. P. (1994): CD28-mediated costimulation is necessary for optimal proliferation of murine NK cells. J. Immunol., **152**, 3361–3369.
64. NUNES J. A., TRUNEH A., OLIVE D. and CANTRELL D. A. (1996): Signal transduction by CD28 costimulatory receptor on T cells. B7-1 and B7-2 regulation of tyrosine kinase adaptor molecules. J. Biol. Chem., **271**, 1591–1598.
65. PAGÈS F., RAGUENEAU M., KLASEN S., BATTIFORA M., COUEZ D., SWEET R., TRUNEH A. and WARD S. G. (1996): Two distinct intracytoplasmic regions of T-cell adhesion molecule CD28 participate in phosphatidylinositol 3-kinase association. J. Biol. Chem., **271**, 9403–9409.
66. PAGÈS F., RAGUENEAU M., ROTTAPEL R., TRUNEH A., NUNES J., IMBERT J. and OLIVE D. (1994): Binding of phosphatidylinositol-3-OH kinase to CD28 is required for T-cell signaling. Nature, **369**, 327–329.
67. PARRY R. V., REIF K., SMITH G., SANSOM D. M., HEMMINGS B. A. and WARD S. G. (1997): Ligation of the T cell co-stimulatory receptor CD28 activates the serine-threonine protein kinase protein kinase B. Eur. J. Immunol., **27**, 2495–2501.
68. PEACH R. J., BAJORATH J., BRADY W., LEYTZE G., GREENE J., NAEMURA J. and LINSLEY P. S. (1994): Complementarity determining region 1 (CDR1)- and CDR3-analogous regions in CTLA-4 and CD28 determine the binding to B7-1. J. Exp. Med., **180**, 2049–2058.
69. PEACH R. J., BAJORATH J., NAEMURA J., LEYTZE G., GREENE J., ARUFFO A. and LINSLEY P. S. (1995): Both extracellular immunoglobin-like domains of CD80 contain residues critical for binding T cell surface receptors CTLA-4 and CD28. J. Biol. Chem., **270**, 21181–21187.
70. PRASAD K. V., CAI Y. C., RAAB M., DUCKWORTH B., CANTLEY L., SHOELSON S. E. and RUDD C. E. (1994): T-cell antigen CD28 interacts with the lipid kinase phosphatidylinositol 3-kinase by a cytoplasmic Tyr(P)-Met-Xaa-Met motif. Proc. Natl. Acad. Sci. USA, **91**, 2834–2838.
71. RADVANYI L. G., SHI Y. and VIZIRI H. (1996): CD28 costimulation inhibits TCR-induced apoptosis during a primary T cell response. J. Immunol., **156**, 1788–1798.
72. REIF K. and CANTRELL D. A. (1998): Networking Rho family GTPases in lymphocytes. Immunity, **8**, 395–401.

73. ROSSI E., MATUTES E., MORILLA R., OWUSU-ANKOMAH K., HEFFERNAN A. M. and CATOVSKY D. (1996): Zeta chain and CD28 are poorly expressed on T lymphocytes from chronic lymphocytic leukemia. Leukemia, **10**, 494–497.
74. SALOMON B. and BLUESTONE J. A. (2001): Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation Annu. Rev. Immunol., **19**, 225–252.
75. SANSOM D. M. (2000): CD28, CTLA-4 and their ligands: who does what and to whom? Immunology, **101**, 169–177.
76. SCHNEIDER H., CAI Y. C., PRASAD K. V., SHOELSON S. E. and RUDD C. E. (1995): T-cell antigen CD28 binds to the Grb2/SOS complex, regulators of $p21^{ras}$. Eur. J. Immunol., **25**, 1044–1050.
77. SERUNIAN L. A., HABER M. T., FUKUI T., KIM J. W., RHEE S. G., LOWENSTEIN J. M. and CANTLEY L. C. (1989): Polyphosphoinositides produced by phosphatidylinositol 3-kinase are poor substrates for phospholipases C from rat liver and bovine brain. J. Biol. Chem., **264**, 17809–17815.
78. SHAHINIAN A., PFEFFER K., LEE K. P., KUNDIG T. M., KISIHARA K., WAKEHAM A., KAWAI K., OHASHI P. S., THOMPSON C. B. and MAK T. W. (1993): Differential T cell costimulatory requirements in CD28-deficient mice. Science, **261**, 609–612.
79. SHAPIRO V. S., TRUITT K. E., IMBODEN J. B. and WEISS A. (1997): CD28 mediates transcriptional upregulation of the interleukin-2 (IL-2) promoter through a composite element containing the CD28RE and NF-IL2B AP-1 sites. Mol. Cell Biol., **17**, 4051–4058.
80. SHIN H. M. and HAN T. H. (1999): CD28-mediated regulation of the c-Jun promoter involves the MEF2 transcription factor in Jurkat T cells. Mol. Immunol., **36**, 197–203.
81. SIEBENLIST U., FRANZOSO G. and BROWN K. (1994): Structure, regulation and function of NF-kappa B. Annu. Rev. Cell Biol., **10**, 405–455.
82. SU B., JACINTO E., HIBI M., KALLUNKI T., KARIN M. and BEN-NERIAH Y. (1994): JNK is involved in signal integration during costimulation of T lymphocytes. Cell, **77**, 727–736.
83. SU B. and KARIN M. (1996): Mitogen-activated protein kinase cascades and regulation of gene expression. Curr. Opin. Immunol., **8**, 402–411.
84. TESTI R. and LANIER L. L. (1989): Functional expression of CD28 on T cell antigen receptor gamma/delta-bearing T lymphocytes. Eur. J. Immunol., **19**, 185–188.
85. THOMPSON C. B., LINDSTEN T., LEDBETTER J. A., KUNKEL S. L., YOUNG H. A., EMERSON S. G., LEIDEN J. M. and JUNE C. H. (1989): CD28 activation pathway regulates the production of multiple T-cell-derived lymphokines/cytokines. Proc. Natl. Acad. Sci. USA, **86**, 1333–1337.
86. TSUCHIDA M., KNECHTLE S. J. and HAMAWY M. M. (1999): CD28 ligation induces tyrosine phosphorylation of Pyk2 but not Fak in Jurkat T cells. J. Biol. Chem., **274**, 6735–6740.
87. UEDA Y., LEVINE B. L., HUANG M. L., FREEMAN G. J., NADLER L. M., JUNE C. H. and WARD S. G. (1995): Both CD28 ligands CD80 (B7-1) and CD86 (B7-2) activate phosphatidylinositol 3-kinase, and wortmannin reveals heterogeneity in the regulation of T cell IL-2 secretion Int. Immunol., **7**, 957–966.
88. VANHAESEBROECK B., LEEVERS S. A., PANAYOTOU G. and WATERFIELD M. D. (1997): Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends Biochem. Sci., **22**, 267–272.
89. WALTER H., SCHEPENSN S., VAN WAUWE J. and BOER M. (1994): Ligation of CD28 on resting T cells by its ligand B7 results in the induction of both Th1 and Th2-type cytokines. Eur. Cytokine Netw., **5**, 13–21.
90. WARD S. G., JUNE C. H. and OLIVE D. (1996): PI3-kinase: a pivotal pathway in T cell activation. Immunol. Today, **17**, 187–197.
91. WARD S. G., WESTWICK J., HALL N. D. and SANSOM D. M. (1993): Ligation of CD28 receptor by B7 induces formation of D-3 phosphoinositides in T lymphocytes independently of T cell receptor/CD3 activation. Eur. J. Immunol., **23**, 2572–2577.
92. XIA Z., DICKENS M., RAINGEAUD J., DAVIS R. J. and GREENBERG M. E. (1995): Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science, **270**, 1326–1331.
93. YOKOCHI T., HOLLY R. D. and CLARK E. A. (1981): B lymphoblast antigen BB-1 expressed on Epstein-Barr virus activated B cell blasts, B lymphoblastoid cell lines and Burkitt's lymphomas. J. Immunol., **128**, 823–827.
94. ZHOU S. Y., SHOELSON S. E., CHAUDHURI M., GISH G., PAWSON T., HASER W. G., KING F., ROBERTS T., RATNOFSKY S., LECHLEIDER R. J., NEEL B. G., BIRGE R. B., FAJARDO J. E., CHOU M. M., HANAFUSA H., SCHAFFHAUSEN B. and CANTLEY L. (1993): SH2 domains recognise specific phosphopeptide sequences. Cell, **72**, 767–778.

Received in October 2001
Accepted in January 2002