Protein Kinase Cθ (PKCθ): A Key Enzyme in T Cell Life and Death[1]

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The novel protein kinase C (PKC) isoform, PKCθ, is expressed in a relatively selective manner in T lymphocytes (and muscle). Recent analysis of this PKC isotype in T cells and the characterization of PKCθ-deficient mice revealed important clues about its function and regulation. PKCθ does not have an obvious role in T cell development, but it is essential for the activation of mature T cells. The requirement of PKCθ for T cell activation, proliferation and cytokine production reflects the essential role of this isotype in inducing signaling pathways leading to the activation of the transcription factors AP-1 and NF-κB in a T cell–specific manner. A unique feature of PKCθ is its highly selective translocation to the central region of the immunological synapse (IS) in antigen-stimulated T cells, a property apparently important for its proper signaling functions. This localization implies unique pathway(s) that regulate the translocation and/or activation of this enzyme. Our work suggests that sustained PKCθ membrane translocation and phosphorylation are relatively independent of phospholipase C (PLC) activation and diacylglycerol (DAG) production. Instead, a pathway that requires Vav, phosphatidylinositol 3-kinase (PI3-K), Rac1 and actin cytoskeleton reorganization mediates these events. Additionally, PKCθ provides an important survival signal to T cells. Nevertheless, several questions regarding the function and regulation of PKCθ and the identity of its immediate targets/substrates remain open. Resolution of these questions could open the way to the development of selective PKCθ inhibitors, which may have therapeutic potential in immunological diseases and in cancer.

Key words: AP-1, immunological synapse, NF-κB, PKCθ, T cell.

1. Introduction

Much has been learned in recent years about the complex signaling pathways that are set in motion by triggering of the antigen-specific T cell receptor (TCR) and accessory/costimulatory receptors such as CD28. Historically, research on T cell signaling pathways began in the early 1980s with key studies that illuminated the important role of inositol phospholipid turnover in T cell activation (reviewed in Ref. *1*). Key among these were the findings that a combination of phorbol ester and Ca^{2+} ionophore mimicked the TCR signals required for complete T cell activation as measured by interleukin-2 (IL-2) production and proliferation (*2, 3*). Coming on the heels of the discovery of protein kinase C (PKC) (*4, 5*), and findings that PKC is a cellular receptor and activation target for tumor-promoting phorbol esters (*6–8*), these findings implicated a role for PKC in T cell activation. Thus, it was suggested that lymphocyte mitogens may stimulate inositol phospholipid hydrolysis and production of diacylglycerol (DAG), which acts as a PKC-activating second messenger (*9*). Subsequent studies employing PKC depletion or pharmacological inhibition indicated that PKC activity is required for T cell activation and proliferation (reviewed in Ref. *1*).

At the same time, the PKC family grew with the isolation of new members in different laboratories (*10*). However, for a long time the contribution of distinct PKC isoforms to TCR-mediated activation events was not clearly understood. Recent studies have begun to fill this gap by providing new information on PKCθ, which is now known to selectively mediate several essential functions in TCR-linked signaling leading to cell activation, differentiation, and survival (*11, 12*).

2. Discovery, structure, and expression of PKCθ

The finding that PKC activity is essential for TCR/CD3-induced T cell activation (*1*) led us to initiate a search for PKC isoforms that may play a specific role in T cell development and/or activation, resulting in the identification of a new member of the Ca^{2+}-independent novel PKC subfamily termed PKCθ by us (*13*) and others (*14, 15*). The human *PKCθ* gene maps to the short arm of chromosome 10 (10p15), a region prone to mutations leading to T-cell leukemia and lymphomas and T-cell immunodeficiencies (*16*),

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Abbreviations: AP-1, activating protein-1; APC, antigen-presenting cell; Cn, calcineurin; DAG, diacylglycerol; IKK, IκB kinase; IL-2, interleukin-2; IS, immunological synapse; PI3-K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLC, phospholipase C; SMAC, supramolecular activation cluster; TCR, T cell receptor.

and to mouse chromosome 2 (*17*). The predicted structure of PKCθ displays the highest homology with members of the Ca^{2+}-independent novel PKC subfamily, including PKCδ, ε, and η, and a functional assay confirmed dependency of its catalytic activity on phospholipids, but not on Ca^{2+} ions (*18*). Within this subfamily, PKCθ is most closely related to PKCδ, since the V1 domains of these two enzymes share 49% homology (*13*).

PKCθ displays a relatively restricted pattern of expression in mouse and rat tissues (reviewed in Refs. *11* and *12*). High levels of PKCθ are found in skeletal muscle and lymphoid tissues, predominantly in the thymus and lymph nodes, with lower levels in spleen, and undetectable expression in the bone marrow. Expression in the muscle is restricted to the plasma membrane, and immunochemical analysis demonstrated an association of PKCθ with the sarcolemma of skeletal muscle and its localization in the neuromuscular junction (*19*). Analysis of PKCθ mRNA expression during mouse development by *in situ* hybridization revealed expression in yolk sac blood islands and in the liver, and later in the thymus and skeletal muscle. In addition, high expression was detected in the embryonic nervous system, including spinal ganglia, spinal cord, trigeminal and facial ganglia, and a subsection of the thalamus (*20*). The highest expression of PKCθ mRNA in the adult thymus was found in the cortex (*20*).

3. Functions and regulation of PKCθ in T cells

Soon after the discovery of PKCθ and the realization that it is selectively expressed in T lymphocytes, we and others began to search for unique functions of this PKC isotype in signaling pathways associated with T cell development and/or activation. These studies yielded a substantial amount of information that clearly establishes a critical role for PKCθ in several aspects of T cell life and death.

a) AP-1 and the c-Jun N-terminal kinase (JNK) pathways. The first clue for a selective function of PKCθ in T cell activation came when we found that it activates activating protein-1 (AP-1) (*21*). AP-1, a transcription factor composed of a dimer of Jun and/or Fos proteins, plays an important role in the transcriptional activation of many genes (*22*). Using transient transfection assays with constitutively active or dominant negative mutants of PKC, we demonstrated that PKCθ, but not other PKC isoforms, activates AP-1 and, furthermore, that this PKCθ activity is dependent on intact Ras function (*21*). Selective AP-1 activation by PKCθ most likely accounts for the positive effects of PKCθ, in synergy with Ca^{2+} signals, on the activation of

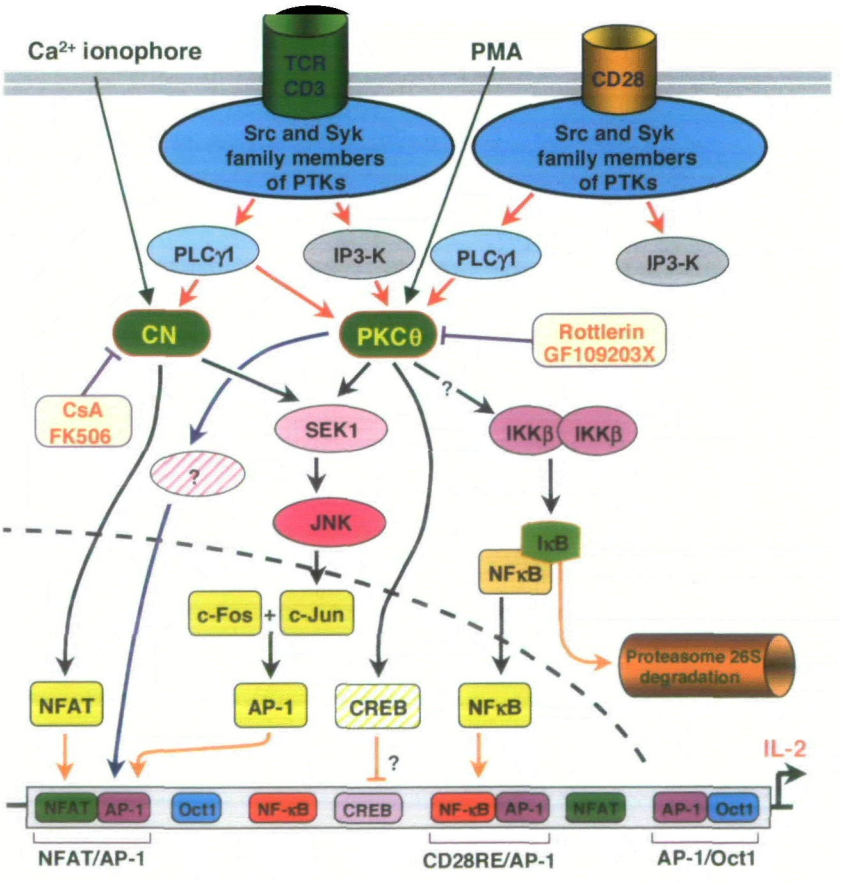

Fig. 1. **The central role of PKCθ in TCR/CD28 signaling.** Costimulation induces the activation of Src-, Syk-, and Tec-family PTK, leading to stimulation and membrane recruitment of PLCγ1, PI3K, and Vav (red arrows). A Vav-mediated pathway, which depends on Rac and actin cytoskeleton reorganization (*27*) as well as on PI3K (*42*), is most likely responsible for the selective recruitment of PKCθ to the cSMAC (*36*), possibly via some undefined, cytoskeleton-associated scaffold. These events appear to be relatively independent of PLCγ1-generated DAG (*42*), although a role for DAG in the initial membrane recruitment of PKCθ cannot be ruled out. PLCγ1-generated Ca^{2+} signals also activate calcineurin (CN) via a pathway that is relatively CD28-indpendent (green arrow) and sensitive to immunosuppressive drugs (CsA, FK506). The receptor signals leading to the activation of PKCθ and CN can be mimicked by phorbol ester (PMA) and Ca^{2+} ionophore, respectively (blue arrows). The two major targets of activated PKCθ are NF-κB and AP-1. PKCθ activates IKKβ *via* unknown intermediates, resulting in the stimulation and nuclear translocation of NF-κB. Activation of AP-1 may proceed through SEK1 and JNK, which also require Ca^{2+}/CN signals (*23, 25*). However, the finding that JNK activation is intact in PKCθ-deficient T cells (*28*) implicates an alternative JNK-independent pathway for AP-1 activation in primary T cells (orange arrow). JNK may even inhibit IL-2 production by primary T cells, perhaps *via* negative regulation of NFAT, but the role of PKCθ-dependent JNK activation in IL-2 induction by memory or effector T cells is unresolved. Activated NF-κB and AP-1 bind to their cognate sites in the *IL-2* promoter, including the CD28RE, which is a major target of the PKCθ pathway (yellow arrows). In addition, PKCθ may phosphorylate and induce the binding of CREB to a cAMP-response element in the IL-2 promoter (*34*). The functional outcome of this event is undear, but it may be associated with the termination of IL-2 production and/or T cell anergy (*35*). Yellow rectangles represent active transcription factors, purple lines correspond to inhibitory pathways, and question marks denote potential and/or unresolved pathways. The dashed line indicates the border between the cytoplasm and the nucleus. The structure of the IL-2 promoter is based on Ref. *32*.

the distal NFAT site in the *IL-2* gene promoter (*23*), since this site is known to bind a combinatorial complex of AP-1/NFAT (*24*).

Since JNK contributes to AP-1 activation by phosphorylating two positive regulatory serine residues in the activation domain of c-Jun (*22*), it was reasonable to implicate JNK as a potential PKCθ target. Several studies demonstrated that in transfected Jurkat T cells, PKCθ (but not other PKC isotypes) activates JNK and its upstream activating kinase, SEK1/MKK4, in a T cell–specific manner, and that this activity is synergistically enhanced by calcineurin (Cn) (*23, 25–27*). However, the immediate target for PKCθ-mediated phosphorylation in the SEK1/JNK pathway is unknown. The physiological relevance of JNK activation by PKCθ remains unclear since peripheral T cells from PKCθ-deficient mice display intact TCR/CD28-induced JNK activation in the face of defective AP-1 activation (*28*).

b) The NF-κB pathway. Recent studies highlighted the essential role of PKCθ in activating the NF-κB signaling pathway in mature T cells. In transfected Jurkat T cells, PKCθ, but not other PKCs, mediates the activation of the NF-κB complex induced by TCR/CD28 costimulation *via* selective activation of IκB kinase β (IKKβ) (*29, 30*). This effect is T cell–specific since PKCθ is relatively inefficient in activating NF-κB in 293T cells (*29*). PKCθ appears to integrate signals from TCR and CD28 (*29*). This notion is supported by the findings that CD28 costimulation enhances and stabilizes the TCR-induced membrane translocation and enzymatic activity of PKCθ (*29*), as well as its localization to membrane lipid rafts (*31*). Thus, the NF-κB cascade, together with AP-1, appears to represent the major and physiologically most critical target of PKCθ in the TCR/CD28 costimulatory pathway leading to IL-2 production.

c) PKCθ and IL-2 production. The critical role of AP-1 and NF-κB in inducing transcription of the *IL-2* gene (*32*) implicates PKCθ as an essential signal for the production of IL-2, a major T cell growth factor. Indeed, PKCθ synergizes with an active Cn mutant to effectively activate NFAT and induce the *IL-2* gene in transfected cells (*23, 26*). These two signaling molecules may represent primary targets for two pharmacophores, *i.e.*, phorbol aster and Ca^{2+} ionophore, which have long been known to mimic physiological receptor signals leading to T cell proliferation (*3*). This notion does not rule out a role for another important phorbol ester target in T cells, *i.e.*, the Ras-activating exchange factor Ras-GRP (*33*), in IL-2 induction . In fact, a functional link, the molecular details of which remain to be defined, seems to exist between PKCθ and the Ras signaling pathway in T cells (*11, 21*).

Surprisingly, a recent study suggested that PKCθ-dependent phosphorylation of cAMP response element-binding protein (CREB) negatively regulates IL-2 transcription (*34*). Thus, T cell activation was found to induce a PKCθ-dependent phosphorylation of CREB, an event associated with T cell anergy (*35*). However, it is unknown whether CREB is a direct substrate of PKCθ. It is difficult to reconcile such a negative regulatory role of PKCθ with the findings that PKCθ is required for mature T cell activation (*28*). It is possible that this represents a feedback regulatory mechanism to terminate IL-2 production by activated T cells. Mapping of the phosphorylation sites in CREB and identification of the relevant phosphorylating kinases should resolve this question.

d) Phenotype of PKCθ-deficient mice. The generation and characterization of PKCθ-null mice (*28*) revealed phenotypic manifestations that are generally consistent with the biochemical studies implicating a critical role for this isotype in mature T cell activation. The mature T cells of these mice produce very little IL-2 and undergo minimal proliferation in response to TCR/CD28 costimulation (*28*). These deficiencies could be traced to a selective defect in the receptor-induced activation of NF-κB and AP-1. Other early activation events, such as inducible tyrosine phosphorylation and activation of MAP kinases (JNK or ERK), as well as T-cell development and the numbers and phenotypes of T cells in the periphery, are normal, indicating that PKCθ is not required for T cell development and thymic selection (*28*). Beyond this, the effect of the *PKCθ* mutation on a variety of *in vivo* immune responses has not been analyzed in detail.

e) PKCθ, the immunological synapse and lipid rafts. Contact between antigen-specific T cells and antigen-presenting cells (APCs) induces the formation of a highly ordered structure, termed the supramolecular activation complex (SMAC; *36*) or the immunological synapse (IS; *37*), at the contact site. This process involves the highly ordered and directional assembly of productive signaling complexes, which include the TCR and costimulatory receptors, intracellular signaling enzymes and adapters, actin cytoskeleton and associated proteins, and specialized membrane microdomains known as lipid rafts (*38, 39*). The discovery that PKCθ selectively colocalizes to the T cell synapse (*40*) represents an important step forward in our understanding of the unique function of this enzyme in T cells. Subsequently, the mature SMAC/IS was resolved into a central SMAC (cSMAC) where the TCR and PKCθ colocalize, and a peripheral SMAC (pSMAC), where other signaling components such as LFA-1 and talin are found (*36*).

T cell activation is also accompanied by the clustering of lipid rafts to the site of T cell engagement (*i.e.*, the SMAC/IS) and the recruitment of different membrane or intracellular signaling proteins into these rafts (*31, 39, 41*). We found recently that antigen stimulation also induces PKCθ translocation into lipid rafts (*31*). This translocation is mediated by the regulatory domain of the enzyme and is dependent on the T cell–specific tyrosine kinase Lck. Lipid raft recruitment is required in order for PKCθ to activate NF-κB (*31*).

The highly selective translocation of PKCθ to the IS/SMAC suggests that some unique mechanism(s) in addition to, or in lieu of, the conventional mechanism for membrane recruitment of PKC, *i.e.*, DAG formation by activated phospholipase C (PLC), regulates the specific localization, and perhaps activation, of PKCθ. Indeed, we found that unlike other PKC isotypes, the membrane recruitment and phosphorylation (activation?) of PKCθ remain relatively intact when several independent strategies are used to inhibit cellular PLC activity (*42*). Instead, intact phosphatidylinositol 3-kinase (PI3-K), Vav1, Rac activity, and actin cytoskeleton reorganization are essential for this selective process (*27, 42*). In fact, only PKCθ, and not other T cell-expressed PKC isotypes, associates with the actin cytoskeleton in activated T cells (*43, 44*). These findings do not rule out a requirement for DAG binding to the PKCθ C1 domain as an important step in its membrane binding and activa-

tion. It is possible that a cooperative dual mechanism regulates the localization and activation of PKCθ. Initially, DAG could weakly recruit PKCθ to the membrane, and this event may facilitate interaction of the enzyme with a membrane or cytoskeletal component(s) required for its proper translocation to the IS/SMAC and its full activation. It is tempting to speculate that some scaffold protein serves to link PKCθ to the cytoskeleton and recruit it to the IS/SMAC during antigen stimulation.

f) The role of PKCθ in T cell survival. PKC-activating phorbol esters have long been known to protect T cells from apoptosis, and PKC inhibitors can sensitize T cells to Fas-mediated apoptosis. Recent studies demonstrated that PKCθ and, to a lesser extent, PKCε (but not Ca^{2+}-dependent PKCs), promote T cell survival by protecting the cells from Fas-induced apoptosis and, furthermore, that this protective effect is mediated, at least in part, through the RSK-dependent phosphorylation and inactivation of the proapoptotic protein BAD (*45, 46*). Fas triggering leads to a selective and transient activation of PKCθ, which is followed later by caspase-dependent cleavage of the enzyme and proteasome-mediated degradation and inactivation of its catalytic fragment, events that precede the onset of cell apoptosis (*46*). This anti-apoptotic function implicates PKCθ as a potential drug target for therapeutic intervention to facilitate the apoptosis of leukemic T cells (reviewed in Ref. *47*).

In the early steps of activation-induced cell death (AICD) of T lymphocytes, PKCθ (in synergy with Cn) has been found to play a positive role in upregulating the expression of the Fas ligand (FasL) on the T cell surface (*48, 49*), a prerequisite for the subsequent Fas-induced death of activated T cells.

4. PKCθ-interacting proteins and substrates

a) Substrates. Progress in identifying physiological PKCθ substrates has been slow in coming, but with ongoing improvements in phosphoproteomics and other technologies, progress will likely be made in the near future. To date, only moesin, a member of the ezrin-radixin-moesin (ERM) protein family, which serves to crosslink the plasma membrane to the actin cytoskeleton, has been identified as a specific PKCθ substrate *in vitro* (*50*). This phosphorylation occurs on Thr^{558}, a conserved residue in ERM proteins that regulates their interaction with actin. Interestingly, PKCθ minimally phosphorylates moesin under standard assay conditions using phosphatidylserine and DAG as lipid cofactors. Instead, phosphorylation is much more effective when phosphatidylglycerol is used (*50*), suggesting that PKCθ may have unique cofactor requirements toward its physiological substrates. It is not known, however, whether moesin (or other ERM proteins) are specifically phosphorylated *in vivo* by PKCθ. The potential ability of PKCθ to phosphorylate and regulate ERM proteins is of interest, since these proteins were recently found to become phosphorylated and segregated from the IS into the rear end (uropod) of the T cell early after T cell activation (*51, 52*).

b) Interacting proteins. recent studies have identified several PKCθ-interacting proteins but, for the most part, these interactions are not specific for the θ isotype since they can be observed with some other isotypes. Proteins that associate with PKCθ but also with other isotypes include members of the 14-3-3 family (*43*), Cbl (*53*), and PICOT, a novel thioredoxin-homology domain-containing protein (*54*). 14-3-3 retains PKCθ in the cytosol and inhibits its membrane translocation, thereby inhibiting PKCθ-dependent functions such as IL-2 production by T cells (*43*). PKC-mediated phosphorylation of Cbl enhances its association with 14-3-3 (*55*). Conversely, it inhibits the TCR-induced tyrosine phosphorylation of Cbl and, consequently, its interaction with SH2 domain–containing proteins (*53*). PICOT, on the other hand inhibits the PKCθ-induced activation of the JNK/AP-1 and NF-κB pathways, but is not a substrate for PKCθ (*54*).

Interactions of some other proteins with PKCθ are apparently more specific. PKCθ interacts with two Src-family tyrosine kinases, Fyn and Lck. Fyn has been implicated as a PKCθ-specific receptor for activated C kinase (RACK), which phosphorylates PKCθ and enhances its catalytic activity, thereby possibly contributing to its selective IS/SMAC translocation (*56*). PKCθ also associates with, and is phosphorylated at, a defined site (Tyr^{90}) by Lck (*57*). Mutation of this site reduces the ability of PKCθ to activate NFAT and NF-κB or promote the growth of Jurkat T cells (*31, 57*). In fact, the PKCθ-Lck complex associates preferentially with the lipid raft fraction (*31*).

Akt/PKB, a Ser/Thr kinase that lies at the crossroads of multiple cellular signaling pathways and acts as a transducer of many functions initiated by receptors that activate PI3-K, is constitutively and directly associated with PKCθ in T cells. However, the two kinases do not phosphorylate each other (*58*). This interaction may be functionally significant since PKCθ and Akt synergize to activate NF-κB (*58, 59*), but the mechanism of this cooperation is not fully understood.

Finally, PKCθ has been found (*60*) to constitutively associate with Nef, an HIV protein that is expressed early after viral infection and is essential for efficient viral infectivity. The *in vitro* interaction is enhanced by phosphatidylserine and DAG. Indirect support for the possible modulation of PKCθ by Nef was obtained by the finding that Nef expression results in a rapid loss of membrane-residing immunoreactive PKCθ, but not PKCβ, -ε, or -ζ (*60*). These results suggest that Nef-induced changes in the content and distribution of PKCθ could partially account for some functional alterations in HIV-infected T cells.

5. Functions of PKCθ in other tissues

As is evident from the above discussion, the overwhelming majority of studies on PKCθ were conducted using T cells. Another potential function of PKCθ in T cells is its positive modulation of retinoid X receptor α-dependent transcription in activated T cells (*61*).

Little is known about the function of PKCθ in other tissues, but its expression in skeletal muscle suggests some important role(s) in this tissue. One such role may be the regulation of insulin sensitivity, where DAG production and the resulting PKC activation are thought to terminate insulin receptor signaling and, thus, induce insulin resistance. Several studies have reported a selective increase in the membrane translocation and/or activity of PKCθ in several experimental models of muscle insulin resistance (*62, 63*). In vascular endothelial cells, PKCθ is required for cell cycle progression and the formation of actin stress fibers and filopodia (*64*). Similarly, inhibition of fetal myoblast dif-

ferentiation by transforming growth factor β was found to depend upon the selective expression of PKCθ (*65*). It stands to reason that future studies on the role of PKCθ in other tissues, particularly skeletal muscle and the nervous system, may reveal novel functions of this PKC isotype in non-T cells.

REFERENCES

1. Altman, A., Coggeshall, K.M., and Mustelin, T. (1990) Molecular events mediating T cell activation. *Adv. Immunol* **48**, 227–360
2. Isakov, N. and Altman, A. (1985) Tumor promoters in conjunction with calcium ionophores mimic antigenic stimulation by reactivation of alloantigen-primed murine T lymphocytes. *J. Immunol.* **135**, 3674–3680
3. Truneh, A., Albert, F., Golstein, P., and Schmitt-Verhulst, A.M. (1985) Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. *Nature* **313**, 318–320
4. Inoue, M., Kishimoto, A., Takai, Y., and Nishizuka, Y. (1977) Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. II. Proenzyme and its activation by calcium-dependent protease from rat brain. *J. Biol. Chem.* **252**, 7610–7616
5. Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T., and Nishizuka, Y. (1979) Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J. Biol. Chem.* **254**, 3692–3695
6. Kikkawa, U., Takai, Y., Tanaka, Y., Miyake, R., and Nishizuka, Y. (1983) Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters. *J. Biol. Chem.* **258**, 11442–11445
7. Niedel, J.E., Kuhn, L.J., and Vandenbark, G.R. (1983) Phorbol diester receptor copurifies with protein kinase C. *Proc. Natl. Acad. Sci. USA* **80**, 36–40
8. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., and Nishizuka, Y. (1982) Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.* **257**, 7847–7851
9. Ku, Y., Kishimoto, A., Takai, Y., Ogawa, Y., Kimura, S., and Nishizuka, Y. (1981) A new possible regulatory system for protein phosphorylation in human peripheral lymphocytes. II. Possible relation to phosphatidylinositol turnover induced by mitogens. *J. Immunol.* **127**, 1375–1379
10. Mellor, H. and Parker, P.J. (1998) The extended protein kinase C superfamily. *Biochem. J.* **332** (Pt 2), 281–292
11. Altman, A., Isakov, N., and Baier, G. (2000) PKCθ: a new essential superstar on the T cell stage. *Immunol. Today* **21**, 567–573
12. Isakov, N. and Altman, A. (2002) Protein kinase C-θ in T cell activation. *Annu. Rev. Immunol.* **20**, 761–794
13. Baier, G., Telford, D., Giampa, L., Coggeshall, K.M., Baier-Bitterlich, G., Isakov, N., and Altman, A. (1993) Molecular cloning and characterization of PKCθ, a human novel member of the protein kinase C (PKC) gene family expressed predominantly in hematopoietic cells. *J. Biol. Chem.* **268**, 4997–5004
14. Osada, S., Mizuno, K., Saido, T.C., Suzuki, K., Kuroki, T., and Ohno, S. (1992) A new member of the protein kinase C family, nPKCθ, predominantly expressed in skeletal muscle. *Mol. Cell. Biol.* **12**, 3930–3938
15. Chang, J.D., Xu, Y., Raychowdhury, M.K., and Ware, J.A. (1993) Molecular cloning and expression of a cDNA encoding a novel isoenzyme of protein kinase C (nPKC). A new member of the nPKC family expressed in skeletal muscle, megakaryoblastic cells, and platelets. *J. Biol. Chem.* **268**, 14208–14214
16. Erdel, M., Baier-Bitterlich, G., Duba, C., Isakov, N., Altman, A., Utermann, G., and Baier, G. (1995) Mapping of the human protein kinase Cθ gene locus to the short arm of chromosome 10 (10p15) by FISH. *Genomics* **25**, 595–597
17. Blanco, G. and Brown, S.D. (1997) Genetic mapping of protein kinase C θ (Pkcθ) to mouse chromosome 2. *Mamm. Genome* **8**, 70–71
18. Baier, G., Baier-Bitterlich, G., Meller, N., Coggeshall, K.M., Telford, D., Giampa, L., Isakov, N., and Altman, A. (1994) Expression and biochemical characterization of human protein kinase C-θ. *Eur. J. Biochem.* **225**, 195–203
19. Hilgenberg, L. and Miles, K. (1995) Developmental regulation of a protein kinase C isoform localized in the neuromuscular junction. *J. Cell Sci.* **108** (Pt 1), 51–61
20. Bauer, B., Krumböck, N., Ghaffari-Tabrizi, N., Kampfer, S., Villunger, A., Wilda, M., Hameister, H., Utermann, G., Leitges, M., Überall, F., and Baier, G. (2000) T cell expressed PKCθ demonstrates cell-type selective function. *Eur. J. Immunol.* **30**, 3645–3654
21. Baier-Bitterlich, G., Überall, F., Bauer, B., Fresser, F., Wachter, H., Grünicke, H., Utermann, G., Altman, A., and Baier, G. (1996) PKCθ isoenzyme selective stimulation of the transcription factor complex AP-1 in T lymphocytes. *Mol. Cell. Biol.* **16**, 1842–1850
22. Karin, M., Liu, Z., and Zandi, E. (1997) AP-1 function and regulation. *Curr. Opin. Cell. Biol.* **9**, 240–246
23. Werlen, G., Jacinto, E., Xia, Y., and Karin, M. (1998) Calcineurin preferentially synergizes with PKC-θ to activate JNK and IL-2 promoter in T lymphocytes. *EMBO J.* **17**, 3101–3111
24. Rao, A., Luo, C., and Hogan, P.G. (1997) Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* **15**, 707–747
25. Avraham, A., Jung, S., Samuels, Y., Seger, R., and Nen-Neriah, Y. (1998) Co-stimulation-dependent activation of a JNK-kinase in T lymphocytes. *Eur. J. Immunol.* **28**, 2320–2330
26. Ghaffari-Tabrizi, N., Bauer, B., Altman, A., Utermann, G., Uberall, F., and Baier, G. (1999) Protein kinase Cθ, a selective upstream regulator of JNK/SAPK and IL-2 promoter activation in Jurkat T-cells. *Eur. J. Immunol.* **29**, 132–142
27. Villalba, M., Coudronniere, N., Deckert, M., Teixeiro, E., Mas, P., and Altman, A. (2000) Functional interactions between Vav and PKCθ are required for TCR-induced T cell activation. *Immunity* **12**, 151–160
28. Sun, Z., Arendt, C.W., Ellmeier, W., Schaeffer, E.M., Sunshine, M.J., Gandhi, L., Annes, J., Petrzilka, D., Kupfer, A., Schwartzberg, P.L., and Littman, D.R. (2000) PKC-θ is required for TCR-induced NF-κB activation in mature but not immature T lymphocytes. *Nature* **404**, 402–407
29. Coudronniere, N., Villalba, M., Englund, N., and Altman, A. (2000) NF-κB activation induced by CD28 costimulation is mediated by PKCθ. *Proc. Natl. Acad. Sci. USA* **97**, 3394–3399
30. Lin, X., O'Mahony, A., Geleziunas, R., and Greene, W.C. (2000) Protein kinase C θ participates in NF-κB/Rel activation induced by CD3/CD28 costimulation through selective activation of IκB β (IKKβ). *Mol. Cell. Biol.* **20**, 2933–2940
31. Bi, K., Tanaka, Y., Coudronniere, N., Hong, S., Sugie, K., van Stipdonk, M.J.B., and Altman, A. (2001) Antigen-induced translocation of PKC-θ to membrane rafts is required for T cell activation. *Nat. Immunol.* **2**, 556–563
32. Jain, J., Loh, C., and Rao, A. (1995) Transcriptional regulation of the IL-2 gene. *Curr. Opin. Immunol.* **7**, 333–342
33. Ebinu, J.O., Bottorff, D.A., Chan, E.Y., Stang, S.L., Dunn, R.J., and Stone, J.C. (1998) RasGRP, a Ras guanyl nucleotide- releasing protein with calcium- and diacylglycerol-binding motifs. *Science* **280**, 1082–1086
34. Solomou, E.E., Juang, Y.T., and Tsokos, G.C. (2001) Protein kinase C-θ participates in the activation of cyclic AMP-responsive element-binding protein and its subsequent binding to the -180 site of the IL-2 promoter in normal human T lymphocytes. *J. Immunol.* **166**, 5665–5674
35. Powell, J.D., Lerner, C.G., Ewoldt, G.R., and Schwartz, R.H. (1999) The -180 site of the IL-2 promoter is the target for CREB/CREM binding in T cell anergy. *J. Immunol.* **163**, 6631–6639
36. Monks, C.R., Freiberg, B.A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998) Three-dimensional segregation of supramolecular clusters in T cells. *Nature* **395**, 82–86

37. Grakoui, A., Bromley, S.K., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M., and Dustin, M.L. (1999) The immunological synapse: a molecular machine controlling T cell activation. *Science* **285**, 221–227
38. Bromley, S.K., Burack, W.R., Johnson, K.G., Somersalo, K., Sims, T.N., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M., and Dustin, M.L. (2001) The immunological synapse. *Annu. Rev. Immunol.* **19**, 375–396
39. Bi, K. and Altman, A. (2001) Lipid microdomains and the role of PKCθ in T cell activation. *Semin. Immunol.* **13**, 139–146
40. Monks, C.R.F., Kupfer, H., Tamir, I., Barlow, A., and Kupfer, A. (1997) Selective modulation of protein kinase C-θ during T-cell activation. *Nature* **385**, 83–86
41. Xavier, R., Brennan, T., Li, Q., McCormack, C., and Seed, B. (1998) Membrane compartmentation is required for efficient T cell activation. *Immunity* **8**, 723–732
42. Villalba, M., Bi, K., Hu, J., Altman, Y., Bushway, P., Reits, E., Neefjes, J., Baier, G., Abraham, R.T., and Altman, A. (2002) Translocation of PKCθ in T cells is mediated by a nonconventional, PI3-K- and Vav-dependent pathway, but does not absolutely require phospholipase C. *J. Cell Biol.* **157**, 253–263
43. Meller, N., Liu, Y.C., Collins, T.L., N., B.-B., Baier, G., Isakov, N., and Altman, A. (1996) Direct interaction between protein kinase C θ (PKC-θ) and 14-3- 3τ in T cells: 14-3-3 overexpression results in inhibition of PKC-θ translocation and function. *Mol. Cell. Biol.* **16**, 5782–5791
44. Villalba, M., Bi, K., Rodriguez, F., Tanaka, Y., Schoenberger, S., and Altman, A. (2001) Vav1/Rac-dependent actin cytoskeleton reorganization is required for lipid raft clustering in T cells. *J. Cell Biol.* **155**, 331–338
45. Bertolotto, C., Maulon, L., Filippa, N., Baier, G., and Auberger, P. (2000) PKC-θ and ε promote T cell survival by a Rsk-dependent phosphorylation and inactivation of BAD. *J. Biol. Chem.* **275**, 37246–37250
46. Villalba, M., Bushway, P., and Altman, A. (2001) PKCθ mediates a selective T cell survival signal via phosphorylation of BAD. *J. Immunol.* **166**, 5955–5963
47. Villalba, M. and Altman, A. (2002) Protein kinase C-θ (PKCθ), an example of a drug target for therapeutic intervention with human T cell leukemias. *Curr. Cancer Drug Targets* **2**, 125–134
48. Villalba, M., Kasibhatla, S., Genestier, L., Mahboubi, A., Green, D.R., and Altman, A. (1999) PKCθ is a necessary component, and cooperates with calcineurin, to induce FasL expression during activation-induced T cell death. *J. Immunol.* **163**, 5813–5819
49. Villunger, A., Ghaffari-Tabrizi, N., Tinhofer, I., Krumbök, N., Bauer, B., Schneider, T., Kasibhatla, S., Greil, R., Baier-Bitterlich, G., Überall, F., Green, D.R., and Baier, G. (1999) Synergistic action of protein kinase C θ and calcineurin is sufficient for Fas ligand expression and induction of a CrmA-sensitive apoptosis pathway in Jurkat T cells. *Eur. J. Immunol.* **29**, 3549–3561
50. Pietromonaco, S.F., Simons, P.C., Altman, A., and Elias, L. (1998) Protein kinase C-θ phosphorylation of moesin in the actin-binding sequence. *J. Biol. Chem.* **273**, 7594–7603
51. Allenspach, E.J., Cullinan, P., Tong, J., Tang, Q., Tesciuba, A.G., Cannon, J.L., Takahashi, S.M., Morgan, R., Burkhardt, J.K., and Sperling, A.I. (2001) ERM-dependent movement of CD43 defines a novel protein complex distal to the immunological synapse. *Immunity* **15**, 739–750
52. Delon, J., Kaibuchi, K., and Germain, R.N. (2001) Exclusion of CD43 from the immunological synapse is mediated by phosphorylation-regulated relocation of the cytoskeletal adaptor moesin. *Immunity* **15**, 691–701
53. Liu, Y., Liu, Y.-C., Meller, N., Giampa, L., Elly, C., Doyle, M., and Altman, A. (1999) PKC activation inhibits tyrosine phosphorylation of Cbl and its recruitment of SH2 domain-containing proteins. *J. Immunol.* **162**, 7095–7101
54. Witte, S., Villalba, M., Liu, Y., Hernandez, J., Isakov, N., and Altman, A. (2000) Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-κB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin-homology domain. *J. Biol. Chem.* **275**, 1902–1909
55. Liu, Y.C., Liu, Y., Elly, C., Yoshida, H., Lipkowitz, S., and Altman, A. (1997) Serine phosphorylation of Cbl induced by phorbol ester enhances its association with 14-3-3 proteins in T cells via a novel serine-rich 14-3-3-binding motif. *J. Biol. Chem.* **272**, 9979–9985
56. Ron, D., Napolitano, E.W., Voronova, A., Vasquez, N.J., Roberts, D.N., Calio, B.L., Caothien, R.H., Pettiford, S.M., Wellik, S., Mandac, J.B., and Kauvar, L.M. (1999) Direct interaction in T-cells between θ-PKC and the tyrosine kinase $p59^{fyn}$. *J. Biol. Chem.* **274**, 19003–19010
57. Liu, Y., Witte, S., Liu, Y.-C., Doyle, M., Elly, C., and Altman, A. (2000) Regulation of PKCθ function during T cell activation by Lck-mediated tyrosine phosphorylation. *J. Biol. Chem.* **275**, 3603–3609
58. Bauer, B., Krumbök, N., Fresser, F., Hochholdinger, F., Spitaler, M., Schwaiger, W., Simm, A., Überall, F., Schraven, B., and Baier, G. (2001) Complex formation and cooperation of PKCθ and Akt1/PKBα in the transactivation of the IKKβ/I-κBα-/NF-κB signaling cascade in T cells. *J. Biol. Chem.* **276**, 31627–31634
59. Kane, L.P., Andres, P.G., Howland, K.C., Abbas, A.K., and Weiss, A. (2001) Akt provides the CD28 costimulatory signal for up-regulation of IL-2 and IFN-γ but not T_H2 cytokines. Nat. *Immunol.* **2**, 37–44
60. Smith, B.L., Krushelnycky, B.W., Mochly Rosen, D., and Berg, P. (1996) The HIV nef protein associates with protein kinase C θ. *J. Biol. Chem.* **271**, 16753–16757
61. Ishaq, M., Fan, M., Wigmore, K., Gaddam, A., and Natarajan, V. (2002) Regulation of retinoid X receptor responsive element-dependent transcription in T lymphocytes by Ser/Thr phosphatases: Functional divergence of protein kinase C (PKC)θ; and PKCα in mediating calcineurin-induced transactivation. *J. Immunol.* **169**, 732–738
62. Griffin, M.E., Marcucci, M.J., Cline, G.W., Bell, K., Barucci, N., Lee, D., Goodyear, L.J., Kraegen, E.W., White, M.F., and Shulman, G.I. (1999) Free fatty acid-induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. *Diabetes* **48**, 1270–1274
63. Bell, K.S., Schmitz-Peiffer, C., Lim-Fraser, M., Biden, T.J., Cooney, G.J., and Kraegen, E.W. (2000) Acute reversal of lipid-induced muscle insulin resistance is associated with rapid alteration in PKC-θ localization. *Am. J. Physiol. Endocrinol. Metab.* **279**, E1196–E1201
64. Tang, S., Morgan, K.G., Parker, C., and Ware, J.A. (1997) Requirement for Protein kinase C θ for cell cycle progression and formation of actin stress fibers and filopodia in vascular endothelial cells. *J. Biol. Chem.* **272**, 28704–28711
65. Zappelli, F., Willems, D., Osada, S., Ohno, S., Wetsel, W.C., Molinaro, M., Cossu, G., and Bouche, M. (1996) The inhibition of differentiation caused by TGFβ in fetal myoblasts is dependent upon selective expression of PKCθ: a possible molecular basis for myoblast diversification during limb histogenesis. *Dev. Biol.* **180**, 156–164