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PKC θ is a Key Regulator of T-cell Behavior and a Drug Target for T cell-mediated Diseases

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Abstract

The protein kinase C-theta (PKCθ) isoform is a member of the calcium-independent novel PKC subfamily of serine/threonine kinases. It is an essential regulatory enzyme in mature T lymphocytes, where it plays a key role in coupling the activated TCR and the CD28 costimulatory receptor to their downstream signaling pathways. TCR/CD28 engagement induces the translocation of PKCθ to the center of the immunological synapse where it undergoes posttranslational modifications and becomes fully active. The activated PKCθ then initiates signaling pathways leading to the activation of transcription factors, including NF-κB, AP-1 and NF-AT that are essential for the survival, activation and differentiation of T cells. While PKCθ ablation was found to impair a wide range of *in vitro* responses of T cells, *in vivo* studies in Prkcq$^{-/-}$ mice revealed that distinct T cell subpopulations differ in their requirements for PKCθ and that PKCθ has a selective role in different immune responses. Thus, PKCθ participates in cellular mechanisms leading to excessive inflammatory responses, autoimmunity, and graft *vs* host (GvH) disease, but is dispensable for beneficial immune responses against viruses and during graft *vs* leukemia responses. These studies suggest that PKCθ may serve as a potential drug target for catalytic and allosteric inhibitors in selected T cell-mediated diseases, and that fine-tuning of PKCθ-dependent functions may help prevent autoimmunity and GvH, without impairing the ability of T cells to eradicate viral-infected and transformed cells.

Keywords: Protein kinase C theta; PKCθ; PKC; T cell receptor; TCR; Signal transduction

Introduction

Protein phosphorylation is a ubiquitous posttranslational process mediated by kinases and serves to regulate the activation state of numerous proteins. Many kinases possess the ability to integrate signals from specific surface receptors and play important roles in regulatory networks and signal transduction pathways that control cell activation and differentiation. One important family of kinases that transduce signals from a large number of cell surface receptors is the protein kinase C (PKC), originally discovered by Nishizuka and colleagues [1]. The first PKC family members identified were found to be sensitive to diacylglycerol (DAG) and calcium (Ca^{2+}) ions, two products of phospholipase C-mediated hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5-bisphphosphate (PIP_2) [2-4]. These two second messengers transduce signals from a plethora of activated receptors where the hydrophobic DAG associates with the cell membrane, while the second product of the PIP_2 hydrolysis, the hydrophilic inositol 1,4,5-trisphosphate (IP_3) binds IP_3-receptors in the endoplasmic reticulum (ER) and triggers the release of free Ca^{2+} ions into the cytoplasm [5-7]. The utilization of phorbol esters, which mimic the activity of DAG, together with Ca^{2+} ionophores, demonstrated that PKC plays an essential role in the induction of T lymphocyte proliferation [8,9] and reactivation of effector cytotoxic T cells [10,11].

The PKC Family of Serine/Threonine Kinases

The PKC family includes nine structurally and functionally related isoforms that are encoded by distinct genes and are divided into three subfamilies based on the structure homology of their regulatory domains and their respective cofactor requirements [12,13]. Additional PKC isoforms are products of alternative splicing of the PKCβ [14] and PKCδ genes [15-17]. Members of the first subfamily, which include the conventional PKC (cPKC) isoforms, PKCα, β, and γ, are regulated via two DAG-binding C1 domains organized in tandem near the cPKC amino terminus [18-20], and an adjacent Ca^{2+} and phospholipid-binding C2 domain [19,21]. Members of the second subfamily include the novel PKC (nPKC) isoforms, PKCδ, ε, η, θ, which are DAG-dependent, but Ca^{2+}- and phospholipid-independent for their activity. Members of the third subfamily include atypical PKC (aPKC), PKC ζ and λ/ι that require neither Ca^{2+} nor DAG for their activity. The PKC enzymes are involved in metabolic processes in different cell types and are implicated in signal transduction networks that convert different environmental cues into cellular actions [22]. Individual PKC isoforms are differentially expressed in tissues and cell types, and six of these isoforms, including PKCα, δ, ε, η, θ and ζ are expressed at varying amounts in T cells [23]. Immunological studies using different genetic models and pharmacological drugs indicated that distinct PKC isoforms are required for different aspects of the activation and effector function of T cells. It is assumed therefore that distinct PKC isoforms may serve as drug targets in different T cell-mediated adaptive immune responses [24].

PKCθ

PKCθ is a member of the novel PKC subfamily, initially isolated from T cells, skeletal muscle and platelets [25-27]. Additional studies demonstrated that PKCθ exhibits a relatively restricted pattern of expression, with high levels in T lymphocytes [23,25], platelets [27-30]

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and skeletal muscle [26,27], and lower or undetectable levels in other tissues tested. For reasons that are not clear yet, it is highly expressed in gastrointestinal stromal tumors, but not in other mesenchymal or epithelial tumors [31-34].

Engagement of the TCR and the CD28 coreceptor on most T cells results in PKCθ translocation to the center of the immunological synapse (IS) [35,36]. Full activation of the enzyme requires the integration of TCR and CD28 costimulatory signals [37-39] and additional steps of posttranslational modification that increase the PKCθ catalytic activity [40]. These include the inducible phosphorylation of PKCθ at multiple sites on serine, threonine and tyrosine residues. Phosphorylation is mediated by several upstream kinases (including autophosphorylation) that affect the enzyme's topology, open the ATP binding site and activation loop, and convert PKCθ into a catalytically potent enzyme [41]. The most recent kinase described that phosphorylates the IS-residing PKCθ is the germinal center kinase (GSK)-like kinase (GLK) [42]. Similar to PKCθ, the GLK translocates to the IS of TCR-engaged T cells where it directly interacts with PKCθ and phosphorylates threonine 538 in its activation loop, thereby enabling better access of substrates to the enzyme's activation segment.

The IS-residing, enzymatically active PKCθ initiates a series of signaling events leading to activation of transcription factors, including nuclear factor-κB (NF-κB), activating protein-1 (AP-1) and nuclear factor of activated T cells (NF-AT), which are critical for T cell proliferation and differentiation [43-48]. These three types of transcription factors are primary physiological targets of PKCθ [43,49], although regulation of the NF-AT requires cooperation of PKCθ with calcineurin, a Ca^{2+}-dependent serine/threonine phosphatase that regulates the nuclear translocation of the cytoplasmic NF-AT by dephosphorylating a critical residue that masks a nuclear localization sequence [43,49]. The three PKCθ-regulated transcription factors have corresponding binding sites on the IL-2 gene promoter, and all three transcription factors are essential for the induction of an optimal IL-2 response [50].

The translocation of PKCθ to the center of the IS is not a universal phenomenon in activated T cells, as demonstrated by Dustin and colleagues using an isolated population of regulatory T cells (Treg) [51,52]. Their findings indicated that although TCR/CD28 triggering of Treg resulted in IS formation, PKCθ translocates to the opposite cell pole, away from the IS, and negatively regulates their suppressive function, although the precise mechanism of action and mode of regulation of PKCθ in Treg is not yet clear.

Studies by Rao and colleagues further demonstrated that under certain activation conditions, PKCθ has the ability to translocate to the nucleus and physically interact with chromatin. Together with other nuclear enzymes, PKCθ molecules form active complexes that bind regulatory DNA regions and control the expression of selected microRNAs and specific T cell inducible genes [53].

The exact mechanisms by which the membrane-bound PKCθ delivers signals to the nucleus have not been fully resolved, but studies provided information on a number of effector molecules that operate along these pathways in activated T cells. These studies demonstrated that the regulation of NF-κB by PKCθ involves the multisubunit inhibitor of κB (IκB) kinase (IKK) complex [44,46,47,54,55]. Another important upstream regulator of the NF-κB signaling pathway is the IκBα, which can directly associate with the cytosolic NF-κB of resting T cells, and by masking its nuclear localization signal (NLS), prevent NF-κB translocation to the nucleus [56-58]. On the other hand, IKK-mediated phosphorylation of IκBα signals the protein for degradation [59], and by exposing the NF-κB NLS, it promotes NF-κB translocation to the nucleus where transcriptional upregulation of NF-κB-dependent genes occur. The current dogma, that PKCθ regulates NF-κB activity through its effect on IKK-IκBα, is further substantiated by *in vivo* studies demonstrating that T cells from PKCθ-deficient (*Prkcq*$^{-/-}$) mice fail to respond to TCR stimulation with degradation of IκBα [45].

PKCθ Binding Proteins and Substrates

One of the most prominent target proteins that links PKCθ to IKK is the PKCθ substrate protein, caspase activation and recruitment domain (CARD) and membrane-associated guanylate kinase (MAGUK) domain-containing protein-1 (CARMA1) [60-63]. This scaffold protein is primarily expressed in lymphocytes [64,65] and its phosphorylation by PKCθ in TCR/CD28-stimulated T cells, promotes CARMA1 association with two additional effector molecules: the B-cell lymphoma/leukemia 10 (Bcl10), and the mucosa-associated lymphoid tissue 1 (MALT1) [66,67]. The resulting trimolecular complex recruits to the IS [68-70] where the three partners cooperate in delivering signals leading to maximal activation of NF-κB [61,71].

Besides its effects on transcriptional regulation, PKCθ is also involved in the regulation of many other cellular functions. Some functions require the direct or indirect association of PKCθ with upstream kinases, which may affect the enzyme's conformation, activity or subcellular distribution (i.e., Lck [72]), while other functions are dependent on PKCθ interaction with downstream kinases, which serve as intermediate PKCθ-coupled signal transduces (i.e., Ste20-related upstream mitogen-activated protein kinase (SPAK) [49]). Additional molecules that serve as substrates for PKCθ regulate distinct cellular functions, such as cytoskeletal reorganization (i.e., Cbl, 14-3-3τ and moesin [73-75]) and signal transduction (i.e., phosphoinositide-dependent-kinase-1 (PDK1) [76], insulin receptor substrate 1 (IRS-1) [77], Ras-related protein (Rap) guanine nucleotide exchange factor-2 (RapGEF2) [78], and hexamethylene bis-acetamide-inducible protein 1 (HEXIM1) [79]). Other proteins that associate with PKCθ include Fyn [80], AKT [54], PICOT [81] and the HIV nef protein [82], although the functional consequences of these interactions require further analyses.

A recent study suggests that the C2-like domain of PKCθ functions as a phosphotyrosine-binding (PTB) module that is also involved in regulating the PKCθ catalytic activity [83]. This study demonstrated that the PKCθ C2-like domain can interact with a CUB domain-containing protein 1 (CDCP1; also termed CD318)-derived tyrosine phosphorylated peptide with a relatively high affinity, and that binding increases the potency of the PKCθ catalytic activity. Furthermore, mutating the PTB sequence in a way that prevented binding of the tyrosine phosphorylated CDCP1, abrogated PKCθ activity and inhibited the TCR/CD28-mediated activation of a PKCθ reporter gene in T cells.

PKCθ in distinct T cell subsets

Following the discovery that PKCθ is expressed in T cells [50,84] studies have focused on the potential biological functions of this enzyme and provided substantial evidence to support a critical role in diverse intracellular signaling pathways that regulate T cell activation, proliferation, differentiation and apoptosis [45,48,85]. Despite its important functions in mature T cells, studies in *Prkcq*$^{-/-}$ mice demonstrated that PKCθ is dispensable for thymocyte maturation and differentiation [45], suggesting a redundancy with other thymocyte-residing PKC isoforms [86].

Additional *in vivo* studies performed in *Prkcq*$^{-/-}$ mice demonstrated that distinct T cell subpopulations differ in their requirements for PKCθ, a phenomenon that is dependent on the type of antigen and the immune response elicited. The current dogma suggests a requirement for PKCθ in Th2-type immune responses to allergens or helminth infection [87,88] and Th17-type immune responses leading to the development of experimental autoimmune encephalomyelitis (EAE), an inflammatory disease of the central nervous system that is widely used as a model of Multiple Sclerosis [85,89-92]. PKCθ is also required for the induction of experimental autoimmune myocarditis [91], Ag-induced arthritis [93], and systemic lupus erythematosus [42].

In contrast, ablation of PKCθ does not impair mouse resistance to *Leishmania major* infection, mediated primarily by Th1 cells [87,94], or CTL-mediated protection from viral infection, which may reflect compensatory activities mediated by innate immunity mechanisms [91,95-98].

Furthermore, PKCθ was found to be required for the induction of allograft rejection and graft-versus-host (GvH) and alloreactive T cell-mediated immune responses, [98,99], while being dispensable for graft-versus-leukemia responses in allogeneic bone marrow transplanted mice [98].

Although many of the effects induced by PKCθ ablation on the quality and intensity of immune responses are due to impaired TCR/CD28-coupled signaling pathways in the effector T cells (T_{eff}), some of these effects could reflect changes in the activity of regulatory T cells (T_{reg}) that under normal conditions downregulate specific functions of T_{eff}. Recent findings supported this assumption by showing that PKCθ mediates a negative feedback on T_{reg} functions [51]. In these studies activation of T_{reg} led to PKCθ sequestration away from the IS, while inhibition of PKCθ increased the suppressive activity of T_{reg} [51,52]. Furthermore, T_{reg} development in the thymus of *Prkcq*$^{-/-}$ mice was abnormal, resulting in reduced numbers of T_{reg} cells in the periphery [51,52,100], although the activity of the mature PKCθ-deficient T_{reg} was not affected [101].

The Immunological Synapse and PKCθ

The immunological synapse, also known as the supramolecular activation cluster (SMAC), is a temporally and spatially regulated plasma membrane structure formed at the interface between an antigen-presenting cell (APC) and a responding T cell, and is the site at which early T cell activation signaling events occur [102]. It is formed upon the recognition by the TCR of a cognate antigenic peptide-MHC on the surface of APC, when both cell types respond by redistributing their receptors, cytoskeletal proteins and intracellular signaling molecules to the contact area, which rearranges as a platform for effective signaling [103,104].

The IS is characterized by specific microclustering of selected receptors and effector molecules [105], and is composed of three concentric rings, each containing a relatively high concentration of a typical combination of molecules. The central-SMAC (c-SMAC) is characterized by a high content of TCR and PKCθ [35,36], in addition to costimulatory receptors (CD28, CD2, CD4, and CD8) and Src family tyrosine kinases (Lck and Fyn) [106]. Recent studies utilizing a high-resolution total internal reflection fluorescence (TIRF) microscopy observed two structurally and functionally distinct cSMAC compartments: 1. A central TCR^{high} compartment, where TCR-associated signaling complexes are internalized and degraded and the entire signaling process is being terminated [107]. 2. An outer TCR^{low} compartment enriched in PKCθ and CD28 where the two proteins are colocalized [38]. Coimmunoprecipitation studies confirmed that PKCθ and CD28 colocalization is a consequence of a physical interaction between the two proteins [38]. The second concentric ring of the IS, the peripheral-SMAC (pSMAC) possesses high concentrations of adhesion molecules (lymphocyte function-associated antigen-1 (LFA-1)) and the cytoskeletal elements (talin) [35], while the third outermost ring, the distal-SMAC (dSMAC), is characterized by its high content of CD43 and CD45 surface receptors [108,109].

Mechanism of Recruitment of PKCθ to the IS

Early investigations demonstrated that TCR engagement, which polarizes PKCθ and induces its recruitment to the IS, is greatly augmented by colligation of the CD28 stimulatory receptor [37-39]. More recent studies demonstrated a physical interaction between PKCθ and CD28 in PMA-stimulated T cells [38], findings that were further substantiated and set up the basis for the understanding of the mechanism by which the two protein interact [110]. These studies revealed that TCR/CD28 costimulation induces transient binding of PKCθ to the cytoplasmic tail of CD28.

Amino acid sequence comparison between PKCθ and PKCδ, the closest relative of PKCθ, indicated the existence of a lowest sequence homology between the two proteins in the V3 (hinge) domain (Figure 1), which corresponds to amino acids ~291-378 of human PKCθ. Since PKCδ, in contrast to PKCθ, does not translocate to the IS of TCR/CD28 activated T cells [36], the findings suggested a potential role for the PKCθ-V3 domain in targeting the enzyme to the IS.

To analyze this hypothesis, a V3-deletion mutant of PKCθ (PKCθ-ΔV3) or a PKCθ exchange mutant, in which the native V3 domain was replaced by the PKCδ V3 domain, were ectopically expressed in T cells, and tested for their ability to associate with CD28. The results demonstrated that, in contrast to the wild type PKCθ, the two mutants failed to coimmunoprecipitate with CD28 [110]. Furthermore, the two genetically modified PKCθ proteins failed to translocate to the IS and to activate PKCθ-dependent reporter genes, such as the CD28 response element (RE/AP). On the other hand, an overexpressed isolated V3 domain of PKCθ localized to the center of the IS of TCR/CD28 stimulated T cells and associated with CD28. In a complementary line of studies, T cells recovered from mouse bone marrow (BM)

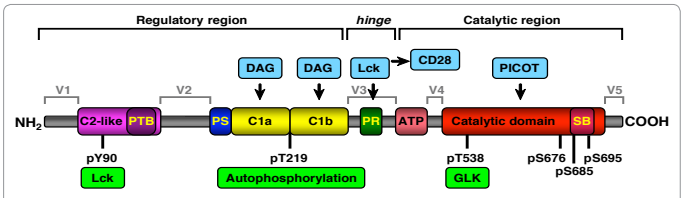

Figure 1: Structure of the human PKCθ protein.
Individual domains in the PKCθ regulatory and catalytic regions are indicated by rectangles with different colors. Molecules that bind PKCθ and are mentioned in the text are indicated above the enzyme (in light blue rectangles) and arrows point to specific PKCθ domains that mediate these interactions. PICOT is a representative of a growing number of proteins that were found to physically interact with PKCθ. Black lines represent phosphorylation sites and the type of amino acid residue that undergoes phosphorylation and its position is indicated below the black line. Kinases that are known to phosphorylate specific amino acids on PKCθ are indicated in a green box below the relevant phosphosite.

V: Variable domain; C: Constant domain; PTB: Phosphotyrosine Binding module; PS: Pseudosubstrate region; PR: Proline-Rich motif; SB: Substrate Binding region; DAG: Diacylglycerol; GLK: Germinal center kinase (GSK)-like kinase; PICOT: PKC-Interacting Cosine of Thioredoxin; Lck: Lymphoid cell kinase.

chimeras on a *Prkcq*$^{-/-}$ background that were reconstituted with the PKCθ mutants described above failed to proliferate and produce IL-2 in response to CD3/CD28 costimulation, and exhibited a diminished capacity to upregulate CD69 or CD25 expression. Furthermore, ectopic expression of the isolated PKCθ V3 domain in T cells disrupted the activation-dependent association between the endogenous PKCθ and CD28, inhibited the recruitment of PKCθ to the IS, and severely impaired PKCθ-dependent functions, including T cell proliferation, IL-2 production and CD25 and CD69 upregulation. These results indicated that the isolated PKCθ V3 domain exhibits dominant negative effects, and possesses the potential for serving as a drug target for the selective inhibition of PKCθ.

Using an array of PKCθ V3 domain mutants, Kong et al. have identified in this region an evolutionarily conserved proline-rich (PR) motif (A^{328}RPPCLPTP; corresponding to amino acid residues 328–336 of human PKCθ), which is essential and sufficient for PKCθ-CD28 association, as well as PKCθ localization to the IS, and induction of PKCθ-mediated functions. The two internal proline residues in this motif (Pro^{331} and Pro^{334}) were particularly critical in this regard [110].

Additional studies revealed that T cell activation-dependent association of PKCθ and CD28 is mediated by Lck, which operate as an intermediate protein [110]. These studies demonstrated that upon TCR/CD28 crosslinking, the Lck-SH3 domain interacts with the PR motif in the PKCθ V3 domain, while the Lck-SH2 domain interacts with phospho-Tyr^{191} in the P^{190}YAP motif in the CD28 cytoplasmic tail.

Taken together, the above findings demonstrate a unique signaling mode of CD28 and establish the molecular basis for the specialized localization and function of PKCθ in antigen-stimulated T cells.

PKCθ as a Drug Target

Studies over the past two decades have demonstrated the critical role of PKCθ in the regulation of T cell proliferation and differentiation, and in the induction of specific types of T cell-mediated adaptive immune responses. While individual cPKC isoforms have been targeted for drug discovery for over 20 years [111], the interest in PKCθ as a potential drug target has become a focus of interest following the discoveries of its fundamental roles in the induction of harmful inflammatory responses mediated by Th2 (allergies) and Th17 (autoimmunity) cells, as well as in GvH and allograft rejection. The fact that PKCθ is dispensable for beneficial adaptive immune responses against virally infected cells and graft versus leukemia response following allogeneic bone marrow transplantation, supported the assumption that PKCθ may serve as a potential drug target, and that inhibitors of PKCθ may selectively suppress autoimmunity and allograft rejection, without affecting anti-viral and anti-cancer immunity.

The need for immunosuppressants that block T-cell activation has led pharmaceutical companies to develop new small molecules capable of modulating the expression or biological activity of PKC-θ. Among the most promising drugs, AEB071 (sotrastaurin), is currently in the early phase of clinical trials [112] and although it inhibits several different PKC isoforms, in addition to PKCθ, this drug was found to downregulate NF-κB and NF-AT, but not AP-1, at nanomolar concentrations, and to inhibit IL-2 production and CD25 expression on the surface of primary human and mouse T cells. The mechanism by which AEB071 affect PKC and inhibits T cell activation is different from that of the cyclosporine A (an inhibitor of the calcineurin protein phosphatase) and therefore the two drugs exhibit complementary inhibitory effects on T-cell signaling pathways.

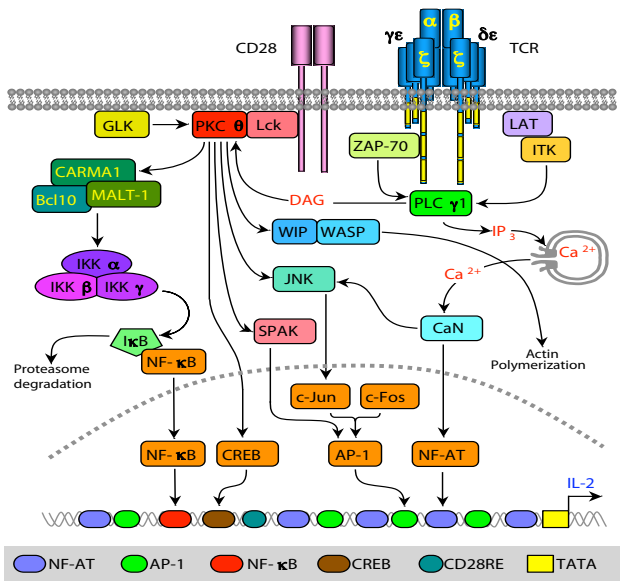

Figure 2: PKCθ-mediated regulation of signal transduction from activated TCR/CD28 receptors.
TCR/CD28 engagement triggers the activation of protein tyrosine kinases (PTKs) and phosphorylation of multiple substrates, including the cytoplasmic tail of the CD28. As a result, the phosphorylated CD28 becomes a docking site for the cytoplasmic Lck PTK, which is tethered to the plasma membrane via its constitutive association with the cytoplasmic tail of CD4 or CD8 (not show) and its N-terminal fatty acid residues. Simultaneous activation of phospholipase C γ1 (PLCγ1), which hydrolyzes the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3), enables PKCθ anchoring to the plasma membrane and the release of Ca^{2+} ions from intracellular stores, respectively. Colocalization of PKCθ and CD28 in the immunological synapse is regulated by PKCθ interaction with Lck and formation of a trimolecular complex comprising of CD28-Lck-PKCθ.
DAG anchoring of PKCθ to the plasma membrane, in addition to the indirect association of PKCθ with CD28, the phosphorylation of PKCθ by Lck and the germinal center kinase (GCK)-like kinase (GLK), as well as autophosphorylation result in activation of the PKCθ catalytic domain. As a result, PKCθ phosphorylates several substrates, including CARD (caspase-associated recruitment domain)-containing MAGUK (membrane-associated guanylate kinase) protein 1 (CARMA1), cAMP responsive element binding protein (CREB), STE20/SPS1-related proline/alanine-rich kinase (SPAK), and c-Jun N-terminal kinase (JNK), which are directly or indirectly involved in signaling pathways regulating the interleukin-2 (IL-2 gene) transcription.
Phosphorylation of the scaffold protein, CARMA1, results in a conformational change that allows CARMA1 interaction with B-cell lymphoma/leukemia 10 (Bcl-10) and mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1), forming a trimolecular complex that activates the IκB kinases (IKKs). Phosphorylation of IκB, which detaches from the p65 and p50 subunits of nuclear factor-κB (NF-κB), and is degraded by the S26 proteasome, enables the p65 and p50 heterodimer enter the nucleus and target the IL-2 promoter NF-κB sites.
Phosphorylation and activation of JNK leads to phosphorylation of c-Jun and its dimerization with a newly synthesized c-Fos, forming an active AP-1 transcription factor that enters the nucleus and targets AP-1 binding sites. In addition, PKCθ-mediated phosphorylation and activation of SPAK increases AP-1 activity via an unknown mechanism. PKCθ also phosphorylates the CREB transcription factor and promotes its binding to the CREB site in the IL-2 promoter, although the effect on gene transcription appears to be regulated by the relative activity of phosphorylated CREB and cAMP-responsive element modulator (CREM).
Phosphorylation of the Wiskott-Aldrich Syndrome Protein (WASP)-interacting (WIP) by PKCθ disengages it from WASP and releases WASP from WIP inhibition. This promotes WASP interaction and activation of the Arp2/3 complex that regulate actin polymerization. Calcineurin (CaN), which is activated by Ca^{2+} ions, dephosphorylates cytoplasmic NFAT proteins, exposing their nuclear localization sequences and inducing their translocation into the nucleus where they bind NFAT sites on the IL-2 gene promoter together with the c-Jun/c-Fos dimers.

Most of the existing small molecule PKC inhibitors have toxic side effects because of their lack of absolute specificity, which reflects the relatively high conservation of catalytic domains within PKC family members. Furthermore, since most catalytic kinase inhibitors are ATP competitors, they need to be used at relatively high and potentially toxic concentrations in order to effectively compete with ATP, whose intracellular concentration is ~1 mM. As a result, current studies are aimed at the development of allosteric inhibitors that interact with regions on the kinase molecule, which are outside of the catalytic site, and are therefore likely to be more selective to individual PKC isoforms and exhibit less non-specific toxic effects [113]. The studies by Kong et al., revealed a new potential approach for attenuating PKCθ-dependent functions utilizing allosteric compounds based on the critical PR motif in the PKCθ-V3 domain that block PKCθ binding to CD28 [110]. Since this interaction is essential for PKCθ recruitment to the IS and for the induction of PKCθ-dependent downstream signaling, this new approach could serve as a basis for the development of new therapeutic agents that would selectively suppress undesired T cell-mediated inflammation and autoimmunity or prevent allograft rejection, while preserving desired immunity, such as antiviral responses.

Concluding Remarks

Past studies have established the requirement for PKCθ in the regulation of many fundamental processes in T cell biology (Figure 2). More recent findings indicated that pharmacological inhibitors of PKCθ might represent beneficial therapeutic modalities for blocking pathological T cell mediated immune responses, without interfering with anti-viral and anti-cancer immunity. Furthermore, since PKCθ is a positive regulator of T cell surveillance, its selective inhibition may help eradicate leukemic T cells by increasing their sensitivity to apoptosis. The development of new small molecule inhibitors and allosteric inhibitors of PKCθ provides promises for future ability to manipulate PKCθ in different disease conditions. Precautions must be taken prior to the clinical application of such inhibitors, in order to evaluate their potential *in vivo* effects on other PKCθ-positive cell types, and potential iatrogenic effects on PKCθ-negative tissues.

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References

1. Inoue M, Kishimoto A, Takai Y, 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.
2. Kishimoto A, Takai Y, Mori T, Kikkawa U, Nishizuka Y (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. J Biol Chem 255: 2273-2276.
3. Nishizuka Y (1984) Turnover of inositol phospholipids and signal transduction. Science 225: 1365-1370.
4. Berridge MJ, Irvine RF (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312: 315-321.
5. Khan AA, Steiner JP, Klein MG, Schneider MF, Snyder SH (1992) IP3 receptor: localization to plasma membrane of T cells and cocapping with the T cell receptor. Science 257: 815-818.
6. Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T, et al. (1979) Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J Biol Chem 254: 3692-3695.
7. Bourguignon LY, Iida N, Sobrin L, Bourguignon GJ (1994) Identification of an IP3 receptor in endothelial cells. J Cell Physiol 159: 29-34.
8. Truneh A, Albert F, Golstein P, Schmitt-Verhulst AM (1985) Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. Nature 313: 318-320.
9. Isakov N, Altman A (1987) Human T lymphocyte activation by tumor promoters: role of protein kinase C. J Immunol 138: 3100-3107.
10. Isakov N, 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.
11. Isakov N, Mally MI, Scholz W, Altman A (1987) T-lymphocyte activation: the role of protein kinase C and the bifurcating inositol phospholipid signal transduction pathway. Immunol Rev 95: 89-111.
12. Mellor H, Parker PJ (1998) The extended protein kinase C superfamily. Biochem J 332 : 281-292.
13. Newton AC (1995) Protein kinase C: structure, function, and regulation. J Biol Chem 270: 28495-28498.
14. Ono Y, Kurokawa T, Fujii T, Kawahara K, Igarashi K, et al. (1986) Two types of complementary DNAs of rat brain protein kinase C. Heterogeneity determined by alternative splicing. FEBS Lett 206: 347-352.
15. Sakurai Y, Onishi Y, Tanimoto Y, Kizaki H (2001) Novel protein kinase C delta isoform insensitive to caspase-3. Biol Pharm Bull 24: 973-977.
16. Kawaguchi T, Niino Y, Ohtaki H, Kikuyama S, Shioda S (2006) New PKCdelta family members, PKCdeltaIV, deltaV, deltaVI, and deltaVII are specifically expressed in mouse testis. FEBS Lett 580: 2458-2464.
17. Jiang K, Apostolatos AH, Ghansah T, Watson JE, Vickers T, et al. (2008) Identification of a novel antiapoptotic human protein kinase C delta isoform, PKCdeltaVIII in NT2 cells. Biochemistry 47: 787-797.
18. Hurley JH, Newton AC, Parker PJ, Blumberg PM, Nishizuka Y (1997) Taxonomy and function of C1 protein kinase C homology domains. Protein Sci 6: 477-480.
19. Johnson JE, Giorgione J, Newton AC (2000) The C1 and C2 domains of protein kinase C are independent membrane targeting modules, with specificity for phosphatidylserine conferred by the C1 domain. Biochemistry 39: 11360-11369.
20. Ho C, Slater SJ, Stagliano B, Stubbs CD (2001) The C1 domain of protein kinase C as a lipid bilayer surface sensing module. Biochemistry 40: 10334-10341.
21. Nalefski EA, Falke JJ (1996) The C2 domain calcium-binding motif: structural and functional diversity. Protein Sci 5: 2375-2390.
22. Rosse C, Linch M, Kermorgant S, Cameron AJ, Boeckeler K, et al. (2010) PKC and the control of localized signal dynamics. Nat Rev Mol Cell Biol 11: 103-112.
23. Meller N, Elitzur Y, Isakov N (1999) Protein kinase C-theta (PKCtheta) distribution analysis in hematopoietic cells: proliferating T cells exhibit high proportions of PKCtheta in the particulate fraction. Cell Immunol 193: 185-193.
24. Baier G, Wagner J (2009) PKC inhibitors: potential in T cell-dependent immune diseases. Curr Opin Cell Biol 21: 262-267.
25. Baier G, Telford D, Giampa L, Coggeshall KM, Baier-Bitterlich G, et al. (1993) Molecular cloning and characterization of PKC theta, a novel member of the protein kinase C (PKC) gene family expressed predominantly in hematopoietic cells. J Biol Chem 268: 4997-5004.
26. Osada S, Mizuno K, Saido TC, Suzuki K, Kuroki T, et al. (1992) A new member of the protein kinase C family, nPKC theta, predominantly expressed in skeletal muscle. Mol Cell Biol 12: 3930-3938.
27. Chang JD, Xu Y, Raychowdhury MK, Ware JA (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.
28. Meller N, Altman A, Isakov N (1998) New perspectives on PKCtheta, a member of the novel subfamily of protein kinase C. Stem Cells 16: 178-192.
29. Cohen S, Braiman A, Shubinsky G, Ohayon A, Altman A, et al. (2009) PKCtheta is required for hemostasis and positive regulation of thrombin-induced platelet aggregation and alpha-granule secretion. Biochem Biophys Res Commun 385: 22-27.
30. Cohen S, Braiman A, Shubinsky G, Isakov N (2011) Protein kinase C-theta in platelet activation. FEBS Lett 585: 3208-3215.

31. Blay P, Astudillo A, Buesa JM, Campo E, Abad M, et al. (2004) Protein kinase C theta is highly expressed in gastrointestinal stromal tumors but not in other mesenchymal neoplasias. Clin Cancer Res 10: 4089-4095.

32. Motegi A, Sakurai S, Nakayama H, Sano T, Oyama T, et al. (2005) PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. Pathol Int 55: 106-112.

33. Ou WB, Zhu MJ, Demetri GD, Fletcher CD, Fletcher JA (2008) Protein kinase C-theta regulates KIT expression and proliferation in gastrointestinal stromal tumors. Oncogene 27: 5624-5634.

34. Kim KH, Nelson SD, Kim DH, Choi KU, Kim SJ, et al. (2012) Diagnostic relevance of overexpressions of PKC-θ and DOG-1 and KIT/PDGFRA gene mutations in extragastrointestinal stromal tumors: a Korean six-centers study of 28 cases. Anticancer Res 32: 923-937.

35. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A (1998) Three-dimensional segregation of supramolecular activation clusters in T cells. Nature 395: 82-86.

36. Monks CR, Kupfer H, Tamir I, Barlow A, Kupfer A (1997) Selective modulation of protein kinase C-theta during T-cell activation. Nature 385: 83-86.

37. Huang J, Lo PF, Zal T, Gascoigne NR, Smith BA, et al. (2002) CD28 plays a critical role in the segregation of PKC theta within the immunologic synapse. Proc Natl Acad Sci U S A 99: 9369-9373.

38. Yokosuka T, Kobayashi W, Sakata-Sogawa K, Takamatsu M, Hashimoto-Tane A, et al. (2008) Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters and protein kinase C theta translocation. Immunity 29: 589-601.

39. Tseng SY, Waite JC, Liu M, Vardhana S, Dustin ML (2008) T cell-dendritic cell immunological synapses contain TCR-dependent CD28-CD80 clusters that recruit protein kinase C theta. J Immunol 181: 4852-4863.

40. Wang X, Chuang HC, Li JP, Tan TH (2012) Regulation of PKC-θ function by phosphorylation in T cell receptor signaling. Front Immunol 3: 197.

41. Seco J, Ferrer-Costa C, Campanera JM, Soliva R, Barril X (2012) Allosteric regulation of PKCθ: understanding multistep phosphorylation and priming by ligands in AGC kinases. Proteins 80: 269-280.

42. Chuang HC, Lan JL, Chen DY, Yang CY, Chen YM, et al. (2011) The kinase GLK controls autoimmunity and NF-κB signaling by activating the kinase PKC-θ in T cells. Nat Immunol 12: 1113-1118.

43. Baier-Bitterlich G, Uberall F, Bauer B, Fresser F, Wachter H, et al. (1996) Protein kinase C-theta isoenzyme selective stimulation of the transcription factor complex AP-1 in T lymphocytes. Mol Cell Biol 16: 1842-1850.

44. Coudronniere N, Villalba M, Englund N, Altman A (2000) NF-kappa B activation induced by T cell receptor/CD28 costimulation is mediated by protein kinase C-theta. Proc Natl Acad Sci U S A 97: 3394-3399.

45. Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, et al. (2000) PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. Nature 404: 402-407.

46. Lin X, O'Mahony A, Mu Y, Geleziunas R, Greene WC (2000) Protein kinase C-theta participates in NF-kappaB activation induced by CD3-CD28 costimulation through selective activation of IkappaB kinase beta. Mol Cell Biol 20: 2933-2940.

47. Dienz O, Hehner SP, Droge W, Schmitz ML (2000) Synergistic activation of NF-kappa B by functional cooperation between vav and PKCtheta in T lymphocytes. J Biol Chem 275: 24547-24551.

48. Pfeifhofer C, Kofler K, Gruber T, Tabrizi NG, Lutz C, et al. (2003) Protein kinase C theta affects Ca2+ mobilization and NFAT cell activation in primary mouse T cells. J Exp Med 197: 1525-1535.

49. Li Y, Hu J, Vita R, Sun B, Tabata H, et al. (2004) SPAK kinase is a substrate and target of PKCtheta in T-cell receptor-induced AP-1 activation pathway. EMBO J 23: 1112-1122.

50. Isakov N, Altman A (2002) Protein kinase C(theta) in T cell activation. Annu Rev Immunol 20: 761-794.

51. Zanin-Zhorov A, Ding Y, Kumari S, Attur M, Hippen KL, et al. (2010) Protein kinase C-theta mediates negative feedback on regulatory T cell function. Science 328: 372-376.

52. Zanin-Zhorov A, Dustin ML, Blazar BR (2011) PKC-θ function at the immunological synapse: prospects for therapeutic targeting. Trends Immunol 32: 358-363.

53. Sutcliffe EL, Bunting KL, He YQ, Li J, Phetsouphanh C, et al. (2011) Chromatin-associated protein kinase C-Î¸ regulates an inducible gene expression program and microRNAs in human T lymphocytes. Mol Cell 41: 704-719.

54. Bauer B, Krumböck N, Fresser F, Hochholdinger F, Spitaler M, et al. (2001) Complex formation and cooperation of protein kinase C theta and Akt1/protein kinase B alpha in the NF-kappa B transactivation cascade in Jurkat T cells. J Biol Chem 276: 31627-31634.

55. Khoshnan A, Bae D, Tindell CA, Nel AE (2000) The physical association of protein kinase C theta with a lipid raft-associated inhibitor of kappa B factor kinase (IKK) complex plays a role in the activation of the NF-kappa B cascade by TCR and CD28. J Immunol 165: 6933-6940.

56. Jacobs MD, Harrison SC (1998) Structure of an IkappaBalpha/NF-kappaB complex. Cell 95: 749-758.

57. Régnier CH, Song HY, Gao X, Goeddel DV, Cao Z, et al. (1997) Identification and characterization of an IkappaB kinase. Cell 90: 373-383.

58. Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, et al. (1997) IKK-1 and IKK-2: cytokine-activated IkappaB kinases essential for NF-kappaB activation. Science 278: 860-866.

59. Karin M (1999) How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. Oncogene 18: 6867-6874.

60. Ruefli-Brasse AA, French DM, Dixit VM (2003) Regulation of NF-kappaB-dependent lymphocyte activation and development by paracaspase. Science 302: 1581-1584.

61. Ruland J, Duncan GS, Elia A, del Barco Barrantes I, Nguyen L, et al. (2001) Bcl10 is a positive regulator of antigen receptor-induced activation of NF-kappaB and neural tube closure. Cell 104: 33-42.

62. Ruland J, Duncan GS, Wakeham A, Mak TW (2003) Differential requirement for Malt1 in T and B cell antigen receptor signaling. Immunity 19: 749-758.

63. Xue L, Morris SW, Orihuela C, Tuomanen E, Cui X, et al. (2003) Defective development and function of Bcl10-deficient follicular, marginal zone and B1 B cells. Nat Immunol 4: 857-865.

64. Bertin J, Wang L, Guo Y, Jacobson MD, Poyet JL, et al. (2001) CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF-kappa B. J Biol Chem 276: 11877-11882.

65. Hara H, Wada T, Bakal C, Kozieradzki I, Suzuki S, et al. (2003) The MAGUK family protein CARD11 is essential for lymphocyte activation. Immunity 18: 763-775.

66. Matsumoto R, Wang D, Blonska M, Li H, Kobayashi M, et al. (2005) Phosphorylation of CARMA1 plays a critical role in T Cell receptor-mediated NF-kappaB activation. Immunity 23: 575-585.

67. Sommer K, Guo B, Pomerantz JL, Bandaranayake AD, Moreno-García ME, et al. (2005) Phosphorylation of the CARMA1 linker controls NF-kappaB activation. Immunity 23: 561-574.

68. Che T, You Y, Wang D, Tanner MJ, Dixit VM, et al. (2004) MALT1/paracaspase is a signaling component downstream of CARMA1 and mediates T cell receptor-induced NF-kappaB activation. J Biol Chem 279: 15870-15876.

69. Gaide O, Favier B, Legler DF, Bonnet D, Brissoni B, et al. (2002) CARMA1 is a critical lipid raft-associated regulator of TCR-induced NF-kappa B activation. Nat Immunol 3: 836-843.

70. Hara H, Bakal C, Wada T, Bouchard D, Rottapel R, et al. (2004) The molecular adapter Carma1 controls entry of IkappaB kinase into the central immune synapse. J Exp Med 200: 1167-1177.

71. McAllister-Lucas LM, Inohara N, Lucas PC, Ruland J, Benito A, et al. (2001) Bimp1, a MAGUK family member linking protein kinase C activation to Bcl10-mediated NF-kappaB induction. J Biol Chem 276: 30589-30597.

72. Liu Y, Witte S, Liu YC, Doyle M, Elly C, et al. (2000) Regulation of protein kinase Ctheta function during T cell activation by Lck-mediated tyrosine phosphorylation. J Biol Chem 275: 3603-3609.

73. Liu Y, Liu YC, Meller N, Giampa L, Elly C, et al. (1999) Protein kinase C activation inhibits tyrosine phosphorylation of Cbl and its recruitment of Src homology 2 domain-containing proteins. J Immunol 162: 7095-7101.

74. Meller N, Liu YC, Collins TL, Bonnefoy-Bérard N, Baier G, et al. (1996) Direct interaction between protein kinase C theta (PKC theta) and 14-3-3 tau in T

cells: 14-3-3 overexpression results in inhibition of PKC theta translocation and function. Mol Cell Biol 16: 5782-5791.

75. Pietromonaco SF, Simons PC, Altman A, Elias L (1998) Protein kinase C-theta phosphorylation of moesin in the actin-binding sequence. J Biol Chem 273: 7594-7603.

76. Wang C, Liu M, Riojas RA, Xin X, Gao Z, et al. (2009) Protein kinase C theta (PKCtheta)-dependent phosphorylation of PDK1 at Ser504 and Ser532 contributes to palmitate-induced insulin resistance. J Biol Chem 284: 2038-2044.

77. Li Y, Soos TJ, Li X, Wu J, Degennaro M, et al. (2004) Protein kinase C Theta inhibits insulin signaling by phosphorylating IRS1 at Ser(1101). J Biol Chem 279: 45304-45307.

78. Letschka T, Kollmann V, Pfeifhofer-Obermair C, Lutz-Nicoladoni C, Obermair GJ, et al. (2008) PKC-theta selectively controls the adhesion-stimulating molecule Rap1. Blood 112: 4617-4627.

79. Fujinaga K, Barboric M, Li Q, Luo Z, Price DH, et al. (2012) PKC phosphorylates HEXIM1 and regulates P-TEFb activity. Nucleic Acids Res 40: 9160-9170.

80. Ron D, Napolitano EW, Voronova A, Vasquez NJ, Roberts DN, et al. (1999) Direct interaction in T-cells between thetaPKC and the tyrosine kinase p59fyn. J Biol Chem 274: 19003-19010.

81. Witte S, Villalba M, Bi K, Liu Y, Isakov N, et al. (2000) Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-kappaB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. J Biol Chem 275: 1902-1909.

82. Smith BL, Krushelnycky BW, Mochly-Rosen D, Berg P (1996) The HIV nef protein associates with protein kinase C theta. J Biol Chem 271: 16753-16757.

83. Stahelin RV, Kong KF, Raha S, Tian W, Melowic HR, et al. (2012) Protein kinase Cθ C2 domain is a phosphotyrosine binding module that plays a key role in its activation. J Biol Chem 287: 30518-30528.

84. Arendt CW, Albrecht B, Soos TJ, Littman DR (2002) Protein kinase C-theta;: signaling from the center of the T-cell synapse. Curr Opin Immunol 14: 323-330.

85. Anderson K, Fitzgerald M, Dupont M, Wang T, Paz N, et al. (2006) Mice deficient in PKC theta demonstrate impaired in vivo T cell activation and protection from T cell-mediated inflammatory diseases. Autoimmunity 39: 469-478.

86. Fu G, Hu J, Niederberger-Magnenat N, Rybakin V, Casas J, et al. (2011) Protein kinase C η is required for T cell activation and homeostatic proliferation. Sci Signal 4: ra84.

87. Marsland BJ, Soos TJ, Späth G, Littman DR, Kopf M (2004) Protein kinase C theta is critical for the development of in vivo T helper (Th)2 cell but not Th1 cell responses. J Exp Med 200: 181-189.

88. Salek-Ardakani S, So T, Halteman BS, Altman A, Croft M (2004) Differential regulation of Th2 and Th1 lung inflammatory responses by protein kinase C theta. J Immunol 173: 6440-6447.

89. Salek-Ardakani S, So T, Halteman BS, Altman A, Croft M (2005) Protein kinase Ctheta controls Th1 cells in experimental autoimmune encephalomyelitis. J Immunol 175: 7635-7641.

90. Tan SL, Zhao J, Bi C, Chen XC, Hepburn DL, et al. (2006) Resistance to experimental autoimmune encephalomyelitis and impaired IL-17 production in protein kinase C theta-deficient mice. J Immunol 176: 2872-2879.

91. Marsland BJ, Nembrini C, Grün K, Reissmann R, Kurrer M, et al. (2007) TLR ligands act directly upon T cells to restore proliferation in the absence of protein kinase C-theta signaling and promote autoimmune myocarditis. J Immunol 178: 3466-3473.

92. Kwon MJ, Ma J, Ding Y, Wang R, Sun Z (2012) Protein kinase C-θ promotes Th17 differentiation via upregulation of Stat3. J Immunol 188: 5887-5897.

93. Healy AM, Izmailova E, Fitzgerald M, Walker R, Hattersley M, et al. (2006) PKC-theta-deficient mice are protected from Th1-dependent antigen-induced arthritis. J Immunol 177: 1886-1893.

94. Ohayon A, Dong G, Isakov N (2007) Involvement of PKCθ in CD4+ T cell polarization and mouse resistance to cutaneous Leishmaniasis. In: Isakov N (Ed) Lymphocyte Activation and Signal Transduction. Transworld Research Network, Trivandrum, Kerala, pp: 221-238.

95. Berg-Brown NN, Gronski MA, Jones RG, Elford AR, Deenick EK, et al. (2004) PKCtheta signals activation versus tolerance in vivo. J Exp Med 199: 743-752.

96. Marsland BJ, Nembrini C, Schmitz N, Abel B, Krautwald S, et al. (2005) Innate signals compensate for the absence of PKC-{theta} during in vivo CD8(+) T cell effector and memory responses. Proc Natl Acad Sci U S A 102: 14374-14379.

97. Giannoni F, Lyon AB, Wareing MD, Dias PB, Sarawar SR (2005) Protein kinase C theta is not essential for T-cell-mediated clearance of murine gammaherpesvirus 68. J Virol 79: 6808-6813.

98. Valenzuela JO, Iclozan C, Hossain MS, Prlic M, Hopewell E, et al. (2009) PKCtheta is required for alloreactivity and GVHD but not for immune responses toward leukemia and infection in mice. J Clin Invest 119: 3774-3786.

99. Manicassamy S, Yin D, Zhang Z, Molinero LL, Alegre ML, et al. (2008) A critical role for protein kinase C-theta-mediated T cell survival in cardiac allograft rejection. J Immunol 181: 513-520.

100. Schmidt-Supprian M, Tian J, Grant EP, Pasparakis M, Maehr R, et al. (2004) Differential dependence of CD4+CD25+ regulatory and natural killer-like T cells on signals leading to NF-kappaB activation. Proc Natl Acad Sci U S A 101: 4566-4571.

101. Gupta S, Manicassamy S, Vasu C, Kumar A, Shang W, et al. (2008) Differential requirement of PKC-theta in the development and function of natural regulatory T cells. Mol Immunol 46: 213-224.

102. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, et al. (1999) The immunological synapse: a molecular machine controlling T cell activation. Science 285: 221-227.

103. Dustin ML, Zhu C (2006) T cells like a firm molecular handshake. Proc Natl Acad Sci U S A 103: 4335-4336.

104. Dustin ML, Chakraborty AK, Shaw AS (2010) Understanding the structure and function of the immunological synapse. Cold Spring Harb Perspect Biol 2: a002311.

105. Yokosuka T, Sakata-Sogawa K, Kobayashi W, Hiroshima M, Hashimoto-Tane A, et al. (2005) Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. Nat Immunol 6: 1253-1262.

106. Lee KH, Holdorf AD, Dustin ML, Chan AC, Allen PM, et al. (2002) T cell receptor signaling precedes immunological synapse formation. Science 295: 1539-1542.

107. Vardhana S, Choudhuri K, Varma R, Dustin ML (2010) Essential role of ubiquitin and TSG101 protein in formation and function of the central supramolecular activation cluster. Immunity 32: 531-540.

108. Delon J, Kaibuchi K, Germain RN (2001) Exclusion of CD43 from the immunological synapse is mediated by phosphorylation-regulated relocation of the cytoskeletal adaptor moesin. Immunity 15: 691-701.

109. Freiberg BA, Kupfer H, Maslanik W, Delli J, Kappler J, et al. (2002) Staging and resetting T cell activation in SMACs. Nat Immunol 3: 911-917.

110. Kong KF, Yokosuka T, Canonigo-Balancio AJ, Isakov N, Saito T, et al. (2011) A motif in the V3 domain of the kinase PKC-θ determines its localization in the immunological synapse and functions in T cells via association with CD28. Nat Immunol 12: 1105-1112.

111. Boschelli DH (2009) Small molecule inhibitors of PKCTheta as potential antiinflammatory therapeutics. Curr Top Med Chem 9: 640-654.

112. Evenou JP, Wagner J, Zenke G, Brinkmann V, Wagner K, et al. (2009) The potent protein kinase C-selective inhibitor AEB071 (sotrastaurin) represents a new class of immunosuppressive agents affecting early T-cell activation. J Pharmacol Exp Ther 330: 792-801.

113. Lamba V, Ghosh I (2012) New directions in targeting protein kinases: focusing upon true allosteric and bivalent inhibitors. Curr Pharm Des 18: 2936-2945.

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