NIH Public Access
Author Manuscript
Adv Pharmacol. Author manuscript; available in PMC 2014 January 27.

Published in final edited form as:
Adv Pharmacol. 2013 ; 66: 267–312. doi:10.1016/B978-0-12-404717-4.00006-8.

The Yin and Yang of Protein Kinase C-theta (PKCθ): A Novel Drug Target for Selective Immunosuppression

Elizabeth Yan Zhang[*], **Kok-Fai Kong**[*], and **Amnon Altman**[†]
Division of Cell Biology, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA

Abstract

Protein kinase C-θ (PKCθ) is a PKC family member expressed predominantly in T lymphocytes, and extensive studies addressing its function have been conducted. PKCθ is the only T cell-expressed PKC that localizes selectively to the center of the immunological synapse (IS) following conventional T cell antigen stimulation, and this unique localization is essential for PKCθ-mediated downstream signaling. While playing a minor role in T cell development, early *in vitro* studies relying, among others, on the use of PKCθ-deficient (*Prkcq*$^{-/-}$) T cells revealed that PKCθ is required for the activation and proliferation of mature T cells, reflecting its importance in activating the transcription factors NF-κB, AP-1 and NFAT, as well as for the survival of activated T cells. Upon subsequent analysis of *in vivo* immune responses in *Prkcq*$^{-/-}$ mice, it became clear that PKCθ has a selective role in the immune system: It is required for experimental Th2 and Th17-mediated allergic and autoimmune diseases, respectively, and for alloimmune responses, but is dispensable for protective responses against pathogens and for graft-*vs*.-leukemia responses. Surprisingly, PKCθ was recently found to be excluded from the IS of regulatory T cells (Tregs) and to negatively regulate their suppressive function. These attributes of PKCθ make it an attractive target for catalytic or allosteric inhibitors that are expected to selectively suppress harmful inflammatory and alloimmune responses without interfering with beneficial immunity to infections. Early progress in developing such drugs is being made, but additional studies on the role of PKCθ in the human immune system are urgently needed.

Keywords

PKCθ; TCR; signaling; T cell activation; autoimmunity; Treg

I. Introduction

The immune system is an immensely complex array of different cell types and soluble products – antibodies, cytokines, chemokines, growth factors and other mediators - that have evolved in order to protect us against the many pathogens that we face throughout our life. Because we face a multitude of danger signals presented by bacteria, viruses, fungi, parasites and growing tumors that can potentially harm us by acting on different cell types and tissues in the body via a large number of distinct mechanisms of action, the immune system has to be equally diverse and multifaceted in order to effectively protect us against diseases and ensure our health and survival. Thus, many layers of regulation exist in the immune system to maximize the probability that immune responses are sufficient to afford

[†]To whom correspondence should be addressed at: Division of Cell Biology, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037, Tel.: (858)752-6808, Fax: (858) 752-6986, amnon@liai.org.
[*]These authors share first authorship

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

protection, but are not excessive so as to provoke harmful tissue inflammation. Thus, for almost every action of the immune system, there is a reaction. A prime example of this sophisticated regulation is represented by regulatory T cells (Tregs), which function to maintain immune homeostasis and dampen excessive immune responses (Josefowicz, Lu, & Rudensky, 2012; Rudensky, 2011; Sakaguchi, Yamaguchi, Nomura, & Ono, 2008). However, this regulatory complexity of the immune system comes at a potentially heavy price, namely, when genetic predisposition or environmental factors disturb and alter the fine balance between beneficial and harmful immunity, the outcome, in the form of inflammation and autoimmunity, can result in debilitating, and sometimes fatal diseases. For example, humans and mice lacking functional Tregs due to mutations in the *Foxp3* gene succumb to a severe lymphoproliferative and inflammatory disease (Bennett, et al., 2001; Brunkow, et al., 2001; Gambineri, Torgerson, & Ochs, 2003). Hence, a major goal of immunology research has been to understand the regulatory mechanisms that operate in the immune system, with the ultimate goal of developing therapeutic strategies for diseases and conditions that result from altered and/or undesired immune responses, be it therapies designed to dampen undesired immune responses such as autoimmune diseases, inflammation and transplant rejection, or immune interventions aimed at boosting desired responses such as anti-tumor immunity or viral clearance in immunosuppressed individuals (*e.g*., HIV infection and AIDS).

Given the critical role of T lymphocytes in controlling and mediating various types of immune responses, it is not surprising that T cells have served, and continue to serve, as logical and major drug targets for treating immunological diseases and cancer. Various treatment modalities that consist of T cell depletion, alteration of T cell adhesion and trafficking, potentiation or inhibition of costimulatory receptors, modulation of cytokines and their signaling pathways, and intervention in T cell receptor (TCR) signaling pathways have been devised and applied clinically with different degrees of success (Steward-Tharp, Song, Siegel, & O'Shea, 2010). However, most of these drugs and treatments are not sufficiently specific and, as a result, have undesirable toxic side effects. This is the case with the major component of immunosuppressive drug combinations, *i.e*., calcineurin (CN) inhibitors such as cyclosporine A (tacrolimus), or with the use of anti-CD3 antibodies to deplete T cells - treatments which prevent organ transplant rejection, graft *vs*. host disease (GvHD) and other undesired immune responses but, at the same time, also render treated patients susceptible to infection due to their immunosuppressed status (Riminton et al., 2011). Hence, a major effort in recent years has gone toward the rational development of more effective immune therapies, which display increased selectivity and reduced toxicity. An emerging promising drug target that falls into this category is protein kinase C-theta (PKCθ), an enzyme that is predominantly expressed in T cells, where it plays critical roles in TCR signaling pathways. Of particular importance, recent evidence, mostly based on animal studies, indicates that the requirement for PKCθ is quiet selective - deleterious immune responses such as autoimmunity strongly depend on it, while it is dispensable for other, beneficial forms of immunity such as protection against viral infections. Hence, there is currently substantial interest in targeting PKCθ as a means of selectively modulating immunity in favor of the patient. Several relatively recent articles have reviewed the history, functions and regulation of this enzyme (Hayashi & Altman, 2007; Isakov & Altman, 2012; Sun, 2012; Zanin-Zhorov, Dustin, & Blazar, 2011). Therefore, this communication is not meant to provide a comprehensive review of everything that is known about PKCθ but, rather, serve as a compact summary of current knowledge about this enzyme and, in particular, highlight those of its known functions in T cell biology that make it an increasingly attractive immunomodulatory drug target. In addition, we also define important open questions, and provide future perspectives related to PKCθ.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

II. History, Structure and Expression of PKCθ

The protein kinase C (PKC) family consists of serine/threonine kinases that mediate a wide variety of cellular processes (Baier, 2003; Hug & Sarre, 1993; Newton, 1997 {Baier, 2003 #703)(Baier, 2003){Mellor, 1998 #249; Mellor & Parker, 1998; Newton, 1997; Nishizuka, 1995). PKC, which was initially purified from brain extract, was defined as a novel cyclic nucleotide-independent, lipid and Ca^{2+}-dependent enzyme (Inoue, Kishimoto, Takai, & Nishizuka, 1977; Takai, et al., 1979), and later found to serve as the cellular receptor for tumor-promoting phorbol esters (Castagna, et al., 1982; Kikkawa, Takai, Tanaka, Miyake, & Nishizuka, 1983; Niedel, Kuhn, & Vandenbark, 1983). Initially considered to be a single entity, it later became clear, with the molecular cloning of the cDNAs encoding the first three members of the PKC family, PKCα, β and γ (Coussens, et al., 1986; Parker, et al., 1986), soon to be followed by the isolation of additional related enzymes, that PKC constitutes a new family of enzymes, which now contains ten defined members.

All PKCs are composed of an N-terminal regulatory domain and a C-terminal catalytic domain that are separated by a flexible hinge region, also known as the V3 domain. Based on structural differences in the regulatory domain and cofactor requirements for activation, PKCs are subdivided into three subfamilies: conventional (or classical) PKCs (cPKC; α, βI, βII, and γ), novel PKCs (nPKC; δ, ε, η, and θ) and atypical PKCs (aPKC; ζ and ι/λ). The regulatory region of cPKCs contains three lipid-binding domains; two contiguous cysteine-rich C1 domains (C1A and C1B; ~50 residues each) involved in binding of the second messenger diacylglycerol (DAG) or phorbol esters, followed by an N-terminal C2 domain (~130 residues) that is responsible for Ca^{2+}-dependent binding to membrane-localized phosphatidylserine or other phospholipids. Hence, binding of both DAG and Ca^{2+} to the C2 and C1 domain, respectively, is required for the activation of cPKCs. The nPKCs also have two tandem DAG-binding C1 domains and an N-terminal C2 domain, which, however, does not bind Ca^{2+} but may bind phospholipids. DAG binding to the tandem C1 domains activates nPKC subfamily members. The positioning of the C2 and C1 domains in nPKCs is reversed relative to the cPKCs, such that the tandem C1 domains follow the N-terminal C2 domain. Finally, the aPKCs have only one C1 domain that does not bind DAG, and the cofactors or mechanisms that activate aPKCs are less well understood. In addition, the regulatory region of all PKCs contains a short conserved pseudosubstrate sequence corresponding to an ideal PKC substrate recognition site, with the exception that a Ser/Thr residue, which would normally be phosphorylated, is replaced by an Ala residue (Baier, 2003; Pfeifhofer-Obermair, Thuille, & Baier, 2012).

The discovery of PKC enzymes as cellular receptors for phorbol esters provided a potential explanation for the T cell mitogenic and activating properties of phorbol esters (Abb, Bayliss, & Deinhardt, 1979; Touraine, et al., 1977). A separate group of studies at about the same time documented the ability of a combination of phorbol esters and Ca^{2+} ionophores to mimic TCR and costimulatory signals (such as those provided by CD28, the prototypical T cell costimulatory receptor) leading to T cell activation and proliferation (Isakov & Altman, 1985; Kaibuchi, Takai, & Nishizuka, 1985; Truneh, Albert, Golstein, & Schmitt-Verhulst, 1985a, 1985b). Together, these two sets of independent findings implicated PKCs as potentially important players in T cell activation. Subsequently, three independent groups, including ours, cloned human and mouse cDNAs encoding a new member of the PKC family, termed PKCθ (Baier, et al., 1993; Chang, Xu, Raychowdhury, & Ware, 1993; Osada, et al., 1992). As it turned out later, the discovery of PKCθ was only the “opening shot” to an extensive series of studies by many groups, which have revealed (and continue to do so) the importance of this unique PKC family member in several cell types, but particularly in T lymphocytes.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Chromosomal mapping located the human PKCθ gene (*Prkcq*) to the short arm of chromosome 10 (10p15) (Erdel, et al., 1995), a region prone to mutations that lead to T cell leukemias, lymphomas and T cell immunodeficiencies (Monaco, et al., 1991; Verma, et al., 1987). The *Prkcq* gene has an open reading frame corresponding to a protein with 706 amino acid residues having a molecular weight of ~79–81 kD, which consists of an amino-terminal regulatory domain (amino acids ~1-378) and a carboxy-terminal catalytic domain (amino acids ~379–706). The hinge/V3 domain, representing a part of the regulatory domain, consists of residues ~291–378 (Baier, et al., 1993; Chang, et al., 1993; Xu, et al., 2004). The crystal structure of the PKCθ catalytic domain has been solved (Xu, et al., 2004), revealing that PKCθ displays two main conformational states, *i.e*., an "open/active" and a "closed/inactive" state (Seco, Ferrer-Costa, Campanera, Soliva, & Barril, 2012; Xu, et al., 2004). The allosteric change of PKCθ from a "closed" to an "open" state involves two important mechanisms: DAG binding to the C1 domains and phosphorylation of Thr-538 (T538) in the activation loop (Budde, et al., 2010; Seco, et al., 2012), which is most likely constitutively phosphorylated, resulting in a constitutively competent, but not fully active, kinase (Liu, Graham, Li, Fisher, & Shaw, 2002). The interface of the regulatory and catalytic domains constitutes the active site cleft, which is responsible for the substrate binding and phosphate delivery from the active catalytic site to the substrate. In addition to Thr-538, whose phosphorylation is essential for kinase activation, there are several other phosphorylation sites in PKCθ (Freeley, Volkov, Kelleher, & Long, 2005; Liu, et al., 2002; Liu, et al., 2000; Thuille, et al., 2005; X. Wang, Chuang, Li, & Tan, 2012), some of which are shared by other PKCs (Ser-676 and -695 in PKCθ), and others being unique to PKCθ (Tyr-90 and Thr-219). These phosphorylation sites play distinct role in controlling the activity and/or cellular localization of PKCθ (X. Wang, et al., 2012).

PKCθ is most abundant in hematopoietic cells, especially T cells (Baier, et al., 1993). The high expression level of PKCθ in T cells accounts for the abundance of this enzyme in the thymus and lymph nodes, with lower levels in spleen, and undetectable expression in the bone marrow (Meller, Altman, & Isakov, 1998; Meller, Elitzur, & Isakov, 1999). In addition to T cells, PKCθ is readily detected in mast cells, natural killer cells and platelets, but not in B cells, erythrocytes, neutrophils, monocytes, or macrophages (Liu, et al., 2001; Meller, et al., 1998; Meller, et al., 1999; Vyas, et al., 2001). High expression level of PKCθ is also observed in skeletal muscle (Baier, et al., 1993; Chang, et al., 1993; Meller, et al., 1998; Osada, et al., 1992), where PKCθ has been implicated in mediating insulin resistance associated with type 2 diabetes (Griffin, et al., 1999; Itani, Zhou, Pories, MacDonald, & Dohm, 2000; Kim, et al., 2004; Serra, et al., 2003). Analysis of PKCθ mRNA expression during mouse development 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 (Bauer, et al., 2000).

III. Specialized Functions of PKCθ in Conventional T Cells: The Yin

Given the important role of PKCθ in TCR-mediated T cell activation, it is worthwhile to briefly review the major features of TCR signaling. TCR ligation by a peptide antigen-major histocompatibility complex (MHC) complexes together with the engagement of CD28 by its ligand, B7, leads to the activation of Src-family tyrosine kinases (Lck and Fyn), which phosphorylate the immunoreceptor tyrosine-based activation motifs (ITAMs) in the TCR-ζ chains and CD3 subunits (Chan & Shaw, 1996). Recruitment and activation of ZAP-70 and Tec-family tyrosine kinases follow, resulting in the phosphorylation and activation of additional enzymes and adaptor proteins and formation of multi-component signaling complexes that ultimately activate various downstream signaling pathways. These events culminate in the activation of key transcription factors (AP-1, NF-κB and NFAT), which

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

induce gene expression programs leading to T cell activation and proliferation (Kane, Lin, & Weiss, 2000; Samelson, 2002). Two key early events in TCR signaling are the tyrosine phosphorylation-dependent activation of the adaptor protein, LAT, which serves as a scaffold for the recruitment of signaling proteins and assembly of a TCR signalosome (Wange, 2000), and the activation of phospholipase C-γ1 (PLC-γ1), which hydrolizes membrane inositol phospholipids to generate two second messengers that activate two bifurcating signaling pathways: IP_3 initiates Ca^{2+} signaling pathways, and DAG activates PKC and some other targets, including Ras signaling (Kane, et al., 2000; Samelson, 2002). Thus, the activation of cPKCs and nPKCs in T cells lies on the pathway initiated by DAG.

T cells express at various levels up to eight distinct members of the PKC family, *i.e*., PKCα, β, δ, ε, η, θ, ζ and ι (Baier, 2003; Hug & Sarre, 1993; Pfeifhofer-Obermair, et al., 2012). The expression of multiple PKC isoforms in T cells suggests functional redundancy and possible specialization. Indeed, T cell-expressed PKCs other than PKCθ have been reported to control various functions in T cells (Baier, 2003; Pfeifhofer-Obermair, et al., 2012). Nevertheless, the predominantly high expression of PKCθ in T cells has suggested that it might play potentially unique and non-redundant functions in T cell biology. The first clue pointing in this direction was a report that PKCθ, but not PKCα, activated the transcription factor AP-1, which is required for productive T cell activation. However, it was not until *Prkcq*$^{-/-}$ mice were generated that the tools required to directly address the question of redundancy became available (Pfeifhofer, et al., 2003; Sun, et al., 2000).

Prkcq$^{-/-}$ mice are generally healthy and fertile, and early studies indicated that T cell development was intact in these mice (Pfeifhofer, et al., 2003; Sun, et al., 2000). A later study using TCR-transgenic mice expressing a lower avidity TCR revealed a substantial, albeit not absolute, role for PKCθ in mediating positive selection in the thymus (Morley, Weber, Kao, & Allen, 2008). In this regard, PKCθ and another nPKC, PKCη, seem to behave in a partially redundant manner (Fu, et al., 2011). The most prominent defect in *Prkcq*$^{-/-}$ mice was, however, the severely impaired TCR-mediated activation of mature, peripheral T cells. In both *Prkcq*$^{-/-}$ mouse models, impaired responses to TCR/CD28-induced stimulation were observed in proliferation, IL-2 production, NF-κB and AP-1 activation. The original finding of impaired NF-κB activation in *Prkcq*$^{-/-}$ T cells coincided with *in vitro* biochemical studies that similarly established NF-κB as being a major target of PKCθ, reflecting the PKCθ-dependent activation of IκB kinase-β (IKKβ), but not IKKα (Coudronniere, Villalba, Englund, & Altman, 2000; Lin, O'Mahony, Mu, Geleziunas, & Greene, 2000). However, there were some notable differences between the two *Prkcq*$^{-/-}$ mouse models: Whereas *Prkcq*$^{-/-}$ T cells displayed impaired TCR/CD28-induced CD25 and CD69 upregulation and phorbol ester- plus ionomycin-induced proliferation but intact NFAT activation in one study (Sun, et al., 2000), the same responses were normal in the other report (Pfeifhofer, et al., 2003). Conversely, NFAT activation was found to be impaired (Pfeifhofer, et al., 2003) or intact (Sun, et al., 2000). Some of these differences may be related to the different strategies used by the two groups to generate *Prkcq*$^{-/-}$ mice. Thus, Littman *et al*. inactivated the *Prkcq* gene by homologous recombination in embryonic stem cells via replacement of the exon encoding the ATP-binding site of the kinase with a neomycin resistance gene (Sun, et al., 2000), potentially resulting in residual expression of the N-terminal regulatory region. Baier *et al*. generated a null *Prkcq* allele by using the Cre/LoxP system to delete exons 3 and 4 encoding amino acid residues 10–87, resulted in a frame shift after amino acid residue 9 of mouse PKCθ and essentially a complete deletion of the corresponding protein (Pfeifhofer, et al., 2003). Nevertheless, later studies using *Prkcq*$^{-/-}$ mice generated by Littman *et al*. (Sun, et al., 2000) demonstrated that, in fact, Ca^{2+} signaling and NFAT activation are impaired in T cells from these mice (Altman, et al., 2004; Manicassamy, Sadim, Ye, & Sun, 2006), raising the possibility that the use of saturating, non-physiological anti-CD3/CD28 antibody concentrations in the original study (Sun, et al.,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2000) likely masked the more subtle effects of *Prkcq* deletion on Ca^{2+} signaling. Hence, PKCθ regulates to various degrees all three transcription factors required for productive T cell activation, *i.e*., NF-κB, AP-1, and NFAT, accounting for the impaired proliferation and cytokine production by $Prkcq^{-/-}$ T cells.

Among the three transcription factors that are regulated by PKCθ and are known to be important for productive T cell activation, the pathway leading from PKCθ to NF-κB activation has been analyzed most extensively. Several enzymes and adaptor proteins play a role in TCR-mediated activation of the canonical NF-κB (NF-κB1) pathway. These include caspase recruitment domain (CARD), membrane-associated guanylate kinase (MAGUK) protein-1 (CARMA1, also termed CARD11), B-cell lymphoma-10 (Bcl10), mucosa-associated lymphoid tissue-1 (MALT1), and the IKK complex (Lin & Wang, 2004; Weil & Israel, 2004). The latter consists of two enzymatic components, IKKα and IKKβ, and a regulatory subunit, IKKγ (also known as NEMO). CARMA1 is constitutively associated with lipid rafts, and becomes further enriched in these rafts after TCR stimulation. Following T cell activation, PKCθ phosphorylates CARMA1 on several serine residues, a modification essential for the ability of CARMA1 to activate NF-κB (D. Wang, et al., 2002). PKCθ-phosphorylated CARMA1 recruits the Bcl10-MALT1 complex, which then activates IKK by inducing ubiquitination and degradation of IKKγ, allowing activated IKKβ (and perhaps IKKα) to phosphorylate the inhibitory IκB proteins. This, in turn, results in IκB degradation and, consequently, in NF-κB1 nuclear translocation and activation (Ghosh & Karin, 2002).

The pathway leading from PKCθ to AP-1 activation is less clearly understood. AP-1 activation depends on several mitogen-activated protein (MAP) kinases (Shaulian & Karin, 2002). Despite early findings that the TCR/CD28-induced activation of MAP kinases in $Prkcq^{-/-}$ T cells is intact (Pfeifhofer, et al., 2003; Sun, et al., 2000), we found more recently that $Prkcq^{-/-}$ $CD8^+$ T cells displayed impaired ERK and JNK activation in response to specific antigen stimulation (Barouch-Bentov, et al., 2005). Thus, the importance of PKCθ in MAP kinase activation may have been masked by costimulation with saturating concentrations of anti-CD3/CD28 antibodies (Pfeifhofer, et al., 2003; Sun, et al., 2000). The PKCθ-mediated AP-1 activation is dependent on Ras (Baier-Bitterlich, et al., 1996), and we have found that SPAK, a Ste20-related MAP kinase, is a direct interactor and substrate of PKCθ in the pathway leading to AP-1, but not NF-κB, activation (Li, et al., 2004). Least understood of all is the mechanism that links PKCθ to the activation of NFAT, but it may involve Tec-family tyrosine kinases such as Itk and Tec as PKCθ interactors that link it to PLC-γ1 activation (Altman, et al., 2004).

In addition to its importance in T cell activation, PKCθ has been shown to play an important role in the survival of T cells. This effect may involve several distinct mechanisms. The first mechanism is related to the process of activation-induced cell death in T cells, whereby binding of FasL, the ligand for the major death receptor Fas (CD95) expressed on activated T cells, triggers the death of these cells via an extrinsic apoptotic pathway. Thus, PKCθ and CN cooperated to induce expression of FasL (Villalba, et al., 1999; Villunger, et al., 1999), and full activation of the *Fasl* gene promoter required binding sites for the three major transcription factors positively regulated by PKCθ, namely, AP-1, NF-κB and NFAT (Villalba, et al., 1999), the latter being a prominent target of CN. Along the same line, the Fas-mediated lytic activity of cytotoxic T lymphocytes (CTLs) was also found to involve a PKCθ-dependent pathway of FasL upregulation (Pardo, et al., 2003). Second, PKCθ (but also another nPKC, PKCε) were found to rescue T lymphocytes from Fas-mediated apoptosis via phosphorylation and inactivation of Bcl2-associated death promoter (BAD) (Bertolotto, Maulon, Filippa, Baier, & Auberger, 2000; Villalba, Bushway, & Altman, 2001), a Bcl2 family member that antagonizes the effect of the pro-survival proteins Bcl2

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and Bcl_{xL}, by physically associating with them. Similarly, PKCθ was required for the survival of both activated $CD4^+$(Manicassamy, Gupta, Huang, & Sun, 2006; Saibil, Jones, et al., 2007) and $CD8^+$ T cells (Barouch-Bentov, et al., 2005; Saibil, Jones, et al., 2007) by regulating the expression of Bcl2 family proteins, *i.e*., increasing the expression of the anti-apoptotic proteins mentioned above (Bcl2 and Bcl_{xL}) and, conversely, suppressing expression of the related proapoptotic protein Bim_{EL}. c-Rel, a component of the NF-κB1 transactivating complex, seems to link PKCθ to this survival signal (Saibil, Jones, et al., 2007).

In addition to its function as a signal transducer from the TCR and CD28 on the T cell surface, PKCθ most likely also has biologically relevant nuclear functions. The first clue for such a function came from a report that PKCθ, but not other tested PKCs, associates with centrosomes and kinetochore structures of the mitotic spindle within the nucleus of murine erythroleukemia cells, suggesting a role in cell proliferation (Passalacqua, Patrone, Sparatore, Melloni, & Pontremoli, 1999). More recently, it was found that PKCθ physically associates with the proximal promoter and coding regions of inducible immune response genes in human T cells (Sutcliffe, et al., 2011). Chromatin-tethered PKCθ formed an active nuclear complex by associating with RNA polymerase II, the histone kinase MSK-1, and the adaptor protein 14-3-3ζ. Furthermore, a chromatin immunoprecipitation (ChIP)-on-ChIP assay demonstrated that PKCθ also localizes to the regulatory regions of a distinct cluster of micro-RNA promoters and negatively regulates their transcription (Sutcliffe, et al., 2011).

IV. The differential Role of PKCθ in Immune Responses

The severe defects observed early on in the *in vitro* activation, proliferation and IL-2 production by *Prkcq*$^{-/-}$ T cells (Pfeifhofer, et al., 2003; Sun, et al., 2000) generally led to the notion that PKCθ is globally required for all T cell-mediated immune responses, raising doubts about its utility as a drug target for immunosuppression. The concern was that, similar to the widely used immunosuppressive drugs such as CN inhibitors (*e.g*., tacrolimus), inhibition of PKCθ would non-selectively suppress desired immune responses and render patients susceptible to infections. It was not until 2004, namely four years after the generation of the first *Prkcq*$^{-/-}$ mouse model (Sun, et al., 2000), that the first report analyzing *in vivo* immune function of *Prkcq*$^{-/-}$ mice has appeared, documenting the somewhat surprising finding that CTL and antibody response against vesicular stomatitis virus (VSV) infection were intact in these mice (Berg-Brown, et al., 2004). This study was soon followed by additional reports by many other groups, leading to the now widely accepted view that the requirement of PKCθ for immune responses is, in fact, quiet selective (Table I), providing one of the strongest arguments for the promise of this enzyme as a useful drug target.

The first report that PKCθ is dispensable for antiviral responses mediated by CTLs was confirmed by several other groups in the context of several different virus or bacteria infection models (Giannoni, Lyon, Wareing, Dias, & Sarawar, 2005; Marsland, et al., 2005; Valenzuela, et al., 2009), although one of these studies reported reduced antiviral antibody and type 1 helper T (Th1) responses (Giannoni, et al., 2005). The relative importance of PKCθ in protective immunity against pathogen infection is likely determined in part by the pathogen load, as indicated by finding that *Prkcq*$^{-/-}$ mice can clear *Listeria monocytogenes* infection when inocculated with 2 x10^3 colony-forming units of bacteria (Valenzuela et al., 2009), but not when a 25-fold higher bacterial load is used (Sakowicz-Burkiewicz et al., 2008). These findings suggest that alternative signals such as innate immunity provided by infection with live pathogens can compensate for the lack of PKCθ *in vivo* and allow an adequate protective response. Indeed, more recent studies demonstrated that increased activation signals delivered *in vivo* by highly activated dendritic cells (Marsland, et al.,

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

2005) or by a toll-like receptor (TLR) ligand (Marsland, et al., 2007), as present during viral infections, overcome the requirement for PKCθ during $CD8^+$ T cell antiviral responses. Consistent with these findings, mouse T cell responses triggered by immunization with a protein antigen plus an LPS adjuvant (a TLR4 agonist) were relatively well preserved in the absence of PKCθ (Valenzuela, et al., 2009). The *in vitro* differentiation of *Prkcq*$^{-/-}$ Th1 cells is moderately impaired (Marsland, Soos, Spath, Littman, & Kopf, 2004; Salek-Ardakani, So, Halteman, Altman, & Croft, 2004), most likely due to the lack of such innate immunity signals in culture. However, the ability of innate immunity signals to bypass the requirement for protective responses to pathogens may not be generalizable or absolute. In fact, studies demonstrated that the immune response against two unicellular protozoan parasites, *Toxoplasma gondii* (Nishanth, et al., 2010) or *Plasmodium berghei* (Ohayon, et al., 2010) was impaired in *Prkcq*$^{-/-}$ mice, reflecting defects in both Th1 and Th2 cytokines. The primary defect in these infections may lie in impaired activation of innate TLR signaling pathways (Griffith, et al., 2007; Yarovinsky, et al., 2005), with the subsequent adaptive immunity defect representing a secondary phenomenon. Therefore, it remains to be determined whether unicellular parasites represent a special case where immunity and subsequent pathology require PKCθ and/or whether some ligands that stimulate the innate immune system are incapable of rescuing its deletion.

Using several models of Th2-mediated immune responses such as allergic lung inflammation and immunity to parasites, it has been clearly demonstrated that *in vivo* Th2 responses as well as Th2 differentiation *in vitro* are critically dependent on PKCθ (Marsland, et al., 2004; Salek-Ardakani, et al., 2004). This dependence almost certainly reflects the importance of PKCθ in upregulating the expression of GATA-3, the master transcription factor for Th2 development (Stevens, et al., 2006). Although several studies demonstrated that PKCθ plays a less important role in Th1 responses (Marsland, et al., 2004; Salek-Ardakani, et al., 2004), Several recent studies demonstrated that *Prkcq*$^{-/-}$ mice were resistant to the development of several experimental autoimmune diseases that are generally considered to represent reliable models of their human counterparts, including experimental autoimmune encephalomyelitis (EAE), adjuvant-induced arthritis, and colitis (Anderson, et al., 2006; Healy, et al., 2006; Salek-Ardakani, So, Halteman, Altman, & Croft, 2005; Tan, et al., 2006). These autoimmune disease models, which were once considered to be induced by Th1 cells, are now known to be largely mediated by pathogenic Th17 cells. Indeed, *Prkcq*$^{-/-}$ $CD4^+$ T cells display impaired *in vitro* differentiation into Th17 cells (Anderson, et al., 2006; Kwon, Ma, Ding, Wang, & Sun, 2012; Salek-Ardakani, et al., 2005). The mechanistic basis for the regulation of Th17 differentiation by PKCθ appears to involve the upregulation of Stat3 expression, which, in turn, is dependent on NF-κB and AP-1 (Kwon, et al., 2012), the two transcription factors that are well known to represent PKCθ targets.

More recent studies expanded the list of *in vivo* immune responses that are differentially regulated by PKCθ. Analysis of cardiac allograft rejection revealed that *Prkcq*$^{-/-}$ mice showed a mildly prolonged allograft survival (Gruber, et al., 2009) or a severe defect in their ability to reject such allografts (Manicassamy, et al., 2008). The difference between the two studies can most likely be explained by the different strategies used to generate the corresponding *Prkcq*$^{-/-}$ mice (Pfeifhofer, et al., 2003; Sun, et al., 2000), as discussed earlier. Of interest, combined deletion of *Prkcq* and *Prkca* genes in double knockout mice resulted in a more severe delay in allograft rejection, suggesting a cooperation between these two PKC isoforms, most likely operating at the level of NFAT activation (Gruber, et al., 2009). The other study (Manicassamy, et al., 2008) additionally demonstrated that the defect in allograft rejection mediated by *Prkcq*$^{-/-}$ T cells can be rescued by transgenic expression of the anti-apoptotic protein Bcl_{xL} indicating that this defect is due, at least in part to the poor survival of *Prkcq*$^{-/-}$ T cells. This conclusion is consistent with the well-established role of PKCθ as a T cell survival factor (Barouch-Bentov, et al., 2005; Manicassamy, Gupta, et al.,

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

2006; Saibil, Jones, et al., 2007) that regulates pro- and anti-apoptotic Bcl2 family members in opposite ways, respectively. Of interest, a blocking (antagonistic) anti-CD28 antibody was found to enable long-term survival of heart allografts across a complete MHC mismatch, and this effect was associated with impaired early TCR signaling events, including PKCθ activation (Jang, et al., 2008).

Allogeneic bone marrow transplantation (BMT) is commonly used as therapy for hematopoietic malignancies, and it relies on the T cell-dependent graft-*versus*-leukemia (GvL) response to eradicate residual tumor cells. However, GvHD elicited by alloreactive donor T cells that recognize mismatched recipient's histocompatibility antigens can cause severe damage to hematopoietic and epithelial tissues, and is often a potentially lethal complication of allogeneic BMT. Hence, strategies to eliminate the deleterious effects of GvHD and preserve the beneficial GvL response are highly desirable, but have been proven extremely difficult to achieve. A recent interesting publication reported that PKCθ was required for alloreactivity and GvHD induction, but was dispensable for the induction of a GvL response after BMT in mice (Valenzuela, et al., 2009). In contrast, another study demonstrated an important role for PKCθ in the immune response to *de novo* arising leukemias induced by Moloney murine leukemia virus in mice (Garaude, et al., 2008). This role reflected the importance of PKCθ in both the activation of tumor-specific T cells as well as in the fitness of the growing leukemic cells. The latter effect is consistent with the critical role of PKCθ in T cell survival, including in T cell leukemias (Villalba & Altman, 2002). Thus, unlike pathogen infections, where PKCθ is dispensable for immune pathogen clearance, elimination of danger signals represented by growing tumors appears to require PKCθ, potentially due to the absence of TLR ligands, which can compensate for the lack of PKCθ, on tumor cells.

Although the function of PKCθ was studied nearly exclusively in T cells, some studies also addressed its role in natural killer (NK) and natural killer T (NKT) cells, two T cell-related innate immune cells that are activated rapidly in response to danger signals such as those presented by tunor cells or virus-infected cells. PKCθ is expressed in NK cells (Balogh, de Boland, Boland, & Barja, 1999; Vyas, et al., 2001) and, similar to T cells (see below), it translocates to the NK cell immunological synpase (IS) upon activation by MHC class I-deficient target cells (Davis, et al., 1999). PKCθ was found to be important for NK cell-mediated surveillance of tumor cells and virus-infected cells via several potential mechanisms that may reflect its requirement for NK production of cytokines such as IFNγ and TNFα (Page, Chaudhary, Goldman, & Kasaian, 2008; Tassi, et al., 2008), NK cell degranulation (Aguilo, Garaude, Pardo, Villalba, & Anel, 2009), or induction of FasL (Pardo, et al., 2003; Villalba, et al., 1999; Villunger, et al., 1999). A potential mechanism for a role of PKCθ in NK (and CTL) degranulation could involve the phosphorylation of WASP-interacting protein (WIP) during NK cell activation (Krzewski, Chen, Orange, & Strominger, 2006), since WIP and WASP are components of the cellular machinery that regulates the actin cytoskeleton, a process important for cell polarization and directional cytokine and lytic granules secretion (Krzewski, et al., 2006). Other hypothetical mechanisms that may underlie the importance of PKCθ in tumor surveillance are plausible as well. The role of PKCθ in NK cell function has recently been reviewed in detail (Anel, et al., 2012).

Prkcq$^{-/-}$ mice also display defects in the development of function of NKT cells (Fang, et al., 2012; Schmidt-Supprian, et al., 2004). The requirement of PKCθ for the thymic development of NKT cells most likely reflects the critical role of NF-κB, a known PKCθ target, in NKT development (Schmidt-Supprian, et al., 2004). Fang et al. used an *in vivo* model of concanavalin A (ConA)-induced acute hepatitis, an inflammatory response known to be mediated by rapidly activated NKT cells, to study the role of PKCθ. They found that

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Prkcq$^{-/-}$ mice were resistant to acute hepatitis, reflecting a requirement of PKCθ in both NK cell development (resulting in reduced NK cell numbers in the periphery) and activation (Fang, et al., 2012). Thus, ConA- or NKT-specific lipid ligand-stimulated *Prkcq*$^{-/-}$ NKT cells displayed impaired IFNγ, TNFα and IL-6 production, and this defect was most likely intrinsic to the NK cells.

V. PKCθ and the Immunological Synapse

When the TCR on CD4^{+} or CD8^{+} T cells is engaged by antigen-presenting cells (APCs) that present a complex of peptide and MHC class II molecule, or by target cells displaying a peptide antigen bound to MHC class I molecule, respectively, the T cells go through a complex and dynamic process, whereby its surface proteins, plasma membrane (PM) lipids, the actin cytoskeleton, and intracellular signal mediators undergo spatial and temporal reorganization to form an IS in the contact area between the T cells and the APCs (or target cells). This IS acts as platform for signal initiation and termination (Bromley, et al., 2001; Dustin, 1997; Dustin, Allen, & Shaw, 2001; Dustin & Cooper, 2000; Grakoui, et al., 1999; K. H. Lee, et al., 2003; Vardhana, Choudhuri, Varma, & Dustin), where proteins and lipids segregate into distinct IS subdomains known as the central supramolecular activation cluster (cSMAC), peripheral SMAC (pSMAC), and distal SMAC (dSMAC) (Monks, Freiberg, Kupfer, Sciaky, & Kupfer, 1998). The use of high-resolution imaging techniques such as total internal reflection fluorescence (TIRF) microscopy, which allows imaging of protein localization in live cells and in real time, revealed that upon T cell engagement by APCs and recognition of antigen, microclusters containing TCRs and additional signaling molecules continuously form at the periphery of the IS, whereupon they migrate centripetally into the center of the IS (Campi, Varma, & Dustin, 2005; Saito & Yokosuka, 2006; Varma, Campi, Yokosuka, Saito, & Dustin, 2006; Yokosuka, et al., 2005). Formation of these microclusters precedes the organization of a mature IS, and they represent a site for antigen recognition and T cell activation (Saito & Yokosuka, 2006). One of the most prominent discoveries about PKCθ was the finding that upon antigen stimulation, PKCθ translocates at a high stoichiometry into the cSMAC in the IS and, furthermore, that the level of its translocation positively correlated with the strength of the TCR signal (Monks, et al., 1998; Monks, Kupfer, Tamir, Barlow, & Kupfer, 1997). At the time of this discovery, it was thought that PKCθ was the only T cell-expressed PKC family member to translocate to the IS, but very recent studies revealed that two other nPKCs, PKCε and η, also translocate to the IS, albeit not specifically to the cSMAC but, rather, in a diffuse pattern all over the IS (Quann, Liu, Altan-Bonnet, & Huse, 2011; Singleton, et al., 2011).

As a result of DAG formation in the PM following receptor-induced, PLCγ-mediated hydrolysis of membrane inositol phospholipids, most cPKCs and nPKCs are recruited to the PM via their tandem C1 domains (Newton, 1997; Nishizuka, 1995). However, PKCθ is unique in its highly selective localization to the cSMAC, suggesting that, in addition to the high local concentration of DAG at the T cell IS (Spitaler, Emslie, Wood, & Cantrell, 2006), which mediates PKCθ (and perhaps other PKCs) recruitment to the IS in general, but not to the cSMAC specifically (Carrasco & Merida, 2004; Spitaler, et al., 2006), another mechanism exists to direct PKCθ specifically to the cSMAC. This hypothetical mechanism remained an enigma for a long time, as did the apparent paradox that PKCθ, which is thought to sustain TCR signaling in the IS for hours, is localized in an IS subdomain (the cSMAC), where TCR signaling complexes are degraded and signaling is terminated (K. H. Lee, et al., 2003; Vardhana, et al.). Findings that CD28, but not other costimulatory receptors, is essential for PKCθ localization at the cSMAC (Huang, et al., 2002; Sedwick, et al., 1999), and that PKCθ colocalizes with CD28 in TCR-dependent microclusters and, later, in a cSMAC subregion distinct from the TCR-high subregion (Tseng, Liu, & Dustin, 2005; Tseng, Waite, Liu, Vardhana, & Dustin, 2008; Yokosuka, et al., 2008) provided a potential

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

resolution to these unresolved question. Yokosuka *et al*. were the first to demonstrate a physical association (revealed by coimmunoprecipitation) between PKCθ and CD28 (Yokosuka, et al., 2008), and this finding was extended and further explored by us under conditions of specific antigen stimulation (Kong, et al., 2011). We demonstrated that the V3 (hinge) domain of PKCθ is required and sufficient for the recruitment of PKCθ to the cSMAC, reflecting an indirect physical association between the V3 domain and the cytoplasmic tail of CD28. The intermediate protein in this trimolecular complex is the Lck tyrosine kinase, which associates via its SH2 and SH3 domains with a distal tyrosine-phoshorylated motif in the CD28 tail and with an evolutionary conserved proline-rich motif in the PKCθ V3 domain (which is not found in other PKCs), respectively (Kong, et al., 2011). The PKCθ-CD28 association was essential for downstream PKCθ-dependent functions as V3 mutations that abolished this interaction, or ectopic expression of the isolated PKCθ V3 domain, which functioned in a dominant negative manner to disrupt the endogenous PKCθ-CD28 association, disrupted the activation of NF-κB and the differentiation of naïve T cells into Th2 or Th17, but not Th1 cells (Kong, et al., 2011). This effect on Th differentiation is fully consistent with the studies described earlier, which documented the requirement, or lack thereof, of PKCθ for the differentiation of these Th subsets.

Other mechanisms may also contribute to the IS and cSMAC localization of PKCθ. First, TCR/CD28-induced autohosphorylation of Thr-219 in the regulatory domain of PKCθ was important for IS localization as well as for NF-κB activation (Thuille, et al., 2005). Our recent study (Kong, et al., 2011) is consistent with this correlation between the IS localization and function of PKCθ. Second, intact catalytic activity was also important since deletion of the catalytic domain or mutation of several PKCθ phosphorylation sites, including Thr-538, which is essential for the catalytic competence of the kinase, abolished or greatly reduced its IS recruitment following antigen stimulation (Cartwright, Kashyap, & Schaefer, 2011). Third, a very recent study demonstrated that PKCε and PKCη are also recruited to the IS in a diffuse manner. Interestingly, the recruitment of these two nPKCs preceded that of PKCθ to the cSMAC and, in fact, seemed to be obligatory since RNA-mediated knockdown of these two PKCs reduced the subsequent IS/cSMAC localization and function of PKCθ (Quann, et al., 2011). Furthermore, PKCθ was found to be important for the organization of the microtubule organizing complex (MTOC) under the IS in this study. Thus, a PKC cascade may operate in T cells to promote the unique localization and function of PKCθ, at least in T effector (Teff) cells. Additional signaling proteins that appear to participate in the regulation of PKCθ cellular localization and function include the ERK-activating MEK kinase (Praveen, Zheng, Rivas, & Gajewski, 2009), phosphatidylinositol 3-kinase (PI3K) (Praveen, et al., 2009; Villalba, et al., 2002) and Vav (Dienz, Hehner, Droge, & Schmitz, 2000; Dienz, et al., 2003; Villalba, et al., 2002; Villalba, et al., 2000).

The stable IS is a symmetrical ("bull's eye") structure that forms upon T cells contact with APCs. However, T cells can undergo transient interactions with APCs, in which disengagement from the APC and the subsequent T cell motility result in breaking of the IS symmetry and formation of an unstable, non-symmetrical synapse termed kinapse (Dustin, 2008). The kinapse would then reassemble into a stable, symmetrical synapse when the T cell serially engages a new APC. Naive T cells encountering APCs were found to undergo cycles of stable IS formation and autonomous T cell migration associated with kinaspe formation, which was driven by PKCθ (Sims, et al., 2007). In these motile T cells, PKCθ was localized to the F-actin-dependent peripheral pSMAC. Consistent with an important role of PKCθ in promoting destabilization of the IS and formation of a kinase, *Prkcq*$^{-/-}$ T cells formed hyperstable IS *in vitro* and *in vivo*; conversely, the Wiscott Aldrich Syndrome protein (WASp) promoted the formation of a stable IS (Sims, et al., 2007). Thus, opposing

NIH-PA Author Manuscript

effects of PKCθ and WASp control IS stability through pSMAC symmetry breaking and reformation.

Along the same line, PKCθ was reported to destabilize the IS in $CD4^+$ CTLs since a selective PKCθ inhibitor increased IS stability and sensitivity of specific target cell lysis (Beal, et al., 2008). Conversely, disruption of the pSMAC by treatment with anti-LFA-1 antibody destabilized the $CD8^+$ CTL IS and decreased target cell sensitivity to lysis. This study also demonstrated that $CD4^+$ CTLs form a less stable IS with target cells than their $CD8^+$ counterparts, which correlates with relatively reduced lytic efficiency. These results suggest that formation of a stable pSMAC, which is inhibited by PKCθ, functions to confine the released lytic molecules at the synaptic interface and to enhance the effectiveness of target cell lysis. However, evidence also exists indicating that PKCθ promotes IS stability. Thus, PKCθ was reported to activate the β2 integrin LFA-1 (*i.e*., increase its avidity for its ligand ICAM-1) downstream of the TCR by phosphorylating the guanine nucleotide exchange factor Rap-GEF2, an activator of the small GTPase Rap1 (Letschka, et al., 2008). Additional studies will be required to settle this apparent discrepancy.

As discussed earlier, T cell activation leads to a segregation of PM domains to form TCR signaling clusters and eventually the IS. At these T cell activation sites, protein networks reside in PM regions that contain highly ordered lipids such as cholesterol and sphingomyelin in subdomains broadly referred to as lipid rafts. These lipid rafts are implicated in signaling from the TCR and in localization and function of proteins residing proximal to the TCR, and they localize at the IS (Harder, Rentero, Zech, & Gaus, 2007; Kabouridis & Jury, 2008). Despite many studies on the role of lipid rafts in T cell activation, their importance in this process is still somewhat controversial (Kenworthy, 2008), and TCR microcluster formation is, in fact, independent of lipid raft clustering (Saito & Yokosuka, 2006). Imaging analysis demonstrated that lipid rafts preferentially accumulate in the cSMAC. However, quantitative analyses indicated that the level of lipid rafts recruitment to the cSMAC is relatively small, suggesting that rearrangement of lipid rafts from the pSMAC into the cSMAC can account for this accumulation (Burack, Lee, Holdorf, Dustin, & Shaw, 2002).

NIH-PA Author Manuscript

We found that T cell stimulation by anti-receptor antibodies or by peptide-MHC complexes induces translocation of PKCθ to membrane lipid rafts, which localized to the IS. This translocation was mediated by the regulatory domain of PKCθ, was dependent on Lck (but not ZAP-70) kinase, and a PKCθ-Lck complex was present in the lipid rafts (Bi, et al., 2001). The catalytic domain of PKCθ did not partition into rafts and was incapable of activating NF-κB, but addition of an Lck-derived acylation signal, which targeted the catalytic domain into lipid rafts, restored these functions. Thus, physiological T cell activation translocates PKCθ to rafts, and this translocation is important for its function (Bi, et al., 2001).

VI. PKCθ, CD28 Costimulation, and T Cell Anergy

NIH-PA Author Manuscript

T cell anergy is an important mechanism of peripheral immune tolerance, whereby T cells primed by antigen fail to respond to restimulation with the same antigen (Fathman & Lineberry, 2007; Schwartz, 2003). TCR signaling events are aberrant in anergic T cells, and the underlying mechanisms are complex (Saibil, Deenick, & Ohashi, 2007). However, one dominant theme that has emerged from recent studies is the importance of the Ca^{2+} signaling pathway involving NFAT activation in anergy induction (Heissmeyer, et al., 2004; Heissmeyer & Rao, 2004; Macian, et al., 2002; Macian, Im, Garcia-Cozar, & Rao, 2004). Thus, T cell anergy ensues when NFAT is activated by partial TCR signals in the absence of AP-1 and/or NF-κB activation, reflecting the induction of a unique anergy-associated gene

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

program (Heissmeyer, et al., 2004; Macian, et al., 2002), which involves the upregulation of E3 ubiquitin ligases and the resulting degradation of early signal transducing proteins (Fathman & Lineberry, 2007; Heissmeyer & Rao, 2004; Macian, et al., 2004).

Based on the "two-signal hypothesis", which states that productive T cell activation requires a TCR signal (signal 1) and an additional costimulatory signal (signal 2) (Bretscher & Cohn, 1968, it was found that provision of a TCR signal in the absence of costimulation induces T cell anergy {Jenkins, 1990 #297)(Harding, McArthur, Gross, Raulet, & Allison, 1992; Jenkins, Chen, Jung, Mueller, & Schwartz, 1990). Subsequently, CD28 was identified as the major costimulatory receptor in naïve T cells {Harding, 1992 #300). In this context, it is interesting to note that many of the TCR signaling events that are impaired in anergic T cells, such as the activation of Ras, MAP kinases, AP-1 and NF-κB (Fathman & Lineberry, 2007; Saibil, Deenick, et al., 2007; Schwartz, 2003), represent downstream targets of PKCθ, raising the intriguing possibility that PKCθ plays a key role in determining the balance between productive T cell activation and anergy. Several lines of evidence support this notion. First, it is now clear that PKCθ integrates signals from both the TCR and CD28, a requirement for its functional activity (Coudronniere, et al., 2000) and proper localization in the T cell IS (Huang, et al., 2002; Kong, et al., 2011; Sedwick, et al., 1999). Second, *Prkcq*$^{-/-}$ T cells display an anergic phenotype upon antigen challenge, similar to that of *Cd28*$^{-/-}$ mice (Berg-Brown, et al., 2004). And, third, a blocking anti-CD28-specific antibody was reported to induce long-term heart allograft survival by suppressing the PKCθ-JNK signaling pathway (Jang, et al., 2008). Given the less severe inhibitory effect of *Prkcq* deletion on the Ca^{2+}-NFAT signaling pathway relative to the AP-1 and NF-κB pathways, it is therefore conceivable that in the absence of PKCθ, there would be sufficient residual NFAT activation but nearly absent AP-1 and NF-κB activation, conditions that would favor the induction of T cell anergy (Heissmeyer, et al., 2004). Hence, selective inhibition of PKCθ function could potentially achieve the beneficial effect of inducing anergy (tolerance) to organ and bone marrow transplants.

VII. Unique Function of PKCθ in Treg Development and Function: The Yang

Tregs play an indispensable role in maintaining immune homeostasis and immunological unresponsiveness to self-antigens, as well as in suppressing excessive immune responses deleterious to the host, such as autoimmune and autoinflammatory disorders, allergy, acute and chronic infections, cancer, and metabolic inflammation. Tregs are generated in the thymus as a functionally mature subpopulation of T cells termed natural Tregs (nTregs) and can also be induced from naive T cells in the periphery by an appropriate cytokine milieu to differentiate into induced Tregs (iTregs). Foxp3 serves as an essential master transcription factor that determines Treg lineage specification (Josefowicz, et al., 2012; Josefowicz & Rudensky, 2009; Rudensky, 2011; Sakaguchi, et al., 2008).

PKCθ was found to be required for the thymic development of nTregs (Gupta, et al., 2008; Schmidt-Supprian, et al., 2004). This requirement is not absolute, however, since *Prkcq*$^{-/-}$ mice still have ~20% of the nTregs found in wild-type mice. Moreover, *ex vivo* Tregs isolated from *Prkcq*$^{-/-}$ mice display intact suppressive activity (Gupta, et al., 2008) (K. -F. Kong, unpublished data). The requirement of PKCθ reflected its important role in activating the NF-κB signaling pathway because, similar to PKCθ deletion, deletion of IKKβ and Bcl10, two critical components in the canonical NF-κB pathway, reduced nTreg development (Schmidt-Supprian, et al., 2004). The importance of NF-κB in Treg development is also evident from the finding that the transcription factor c-Rel initiates *Foxp3* transcription in thymic Treg precursors (Hori, 2010). The nTreg development defect in *Prkcq*$^{-/-}$ mice was not related to a missing survival signal since transgenic expression of the anti-apoptotic survival protein Bcl_{xL} could not restore the Treg cell population in these

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

mice (Gupta, et al., 2008). In addition, CN-A_{β}-deficient mice also had a decreased Treg cell population similar to that observed in *Prkcq*$^{-/-}$ mice, suggesting that NFAT also plays an important role in nTreg development (Gupta, et al., 2008).

One prominent Treg-mediated suppressive mechanism is dependent upon its contact with APCs. This physical contact promotes the formation of a specialized signaling platform, the IS, at the Treg-APC interface (Sakaguchi, et al., 2008; Sarris, Andersen, Randow, Mayr, & Betz, 2008; Zanin-Zhorov, et al., 2010). A recent study explored the characteristics of the Treg IS and, surprisingly, reported the intriguing finding that in contrast to Teff cells, PKCθ is excluded form the Treg IS and, instead, it localizes to the distal pole in human Tregs (Zanin-Zhorov, et al., 2010). Furthermore, a selective small molecule PKCθ inhibitor (C20) enhanced the suppressive activity of Tregs, implying a negative regulatory role for PKCθ on Treg function. Similarly, pharmacological inhibition of NF-κB also increased human Treg suppressive activity, suggesting that PKCθ targets NF-κB in a pathway leading to inhibition of Treg function. This contrasts with the positive regulatory function of the NF-κB pathway in thymic Treg development (Hori, 2010; Schmidt-Supprian, et al., 2004). Pharmacological inhibition of PKCθ protected Tregs from inactivation by TNFα, rescued the defective activity of Tregs from rheumatoid arthritis (RA) patients, and enhanced protection of mice from inflammatory colitis (Zanin-Zhorov, et al., 2010). However, the PKCθ inhibitor abolished the ability of human Tregs to proliferate *in vitro* in response to anti-CD3 plus -CD28 antibodies in the presence of high IL-2 concentrations (Zanin-Zhorov, et al., 2010; Zanin-Zhorov, et al., 2011). Our preliminary finding that a dominant negative PKCθ V3 domain, which inhibits the differentiation of Th2 and Th17 cells (Kong, et al., 2011), enhances the *in vitro* differentiation of naïve T cells into FoxP3^{+} Tregs (K. F. Kong & E. Y. Zhang, unpublished data), supports the inhibitory role of PKCθ in Treg function. Another, indirect support for this notion comes from a very recent study, which demonstrated that embryonic stem cells-derived factors, which have been known to modulate immune activation, inhibited the phosphorylation of PKCθ and the activation of its target, NF-κB, in Teff cells, while at the same time upregulating Treg markers such as FoxP3, TGFβ and IL-10 in CD4^{+}CD25^{+} cells (Mohib, AlKhamees, Zein, Allan, & Wang, 2012).

Another very recent study also addressed the role of PKCθ in Treg differentiation (Ma, Ding, Fang, Wang, & Sun, 2012). These authors reported that PKCθ-mediated signals inhibit iTreg differentiation *in vitro* via an Akt-Foxo1/3A pathway. This conclusion was based on findings that TGFβ-induced iTreg differentiation was enhanced in *Prkcq*$^{-/-}$ T cells or in wild-type T cells treated with a selective PKCθ inhibitor, and that *Prkcq*$^{-/-}$ T cells displayed reduced Akt kinase activity. Furthermore, knockdown or overexpression of the Akt targets Foxo1 and Foxo3a inhibited or promoted the iTreg differentiation of *Prkcq*$^{-/-}$ T cells, respectively. By contrast, we found that naïve T cells from *Prkcq*$^{-/-}$ mice displayed a severely impaired differentiation into Foxp3^{+} Treg cells when cultured under similar conditions to those used by Ma et al. (Ma, et al., 2012), *i.e*., anti-CD3/CD28 antibody stimulation in the presence of TGFβ and IL-2 (K. -F. Kong, unpublished data) suggesting that PKCθ is, in fact, indispensable for the *in vitro* differentiation of CD4^{+}Foxp3^{+} T cells. The reason for these apparently contradictory findings is unclear, but it is important to note that important caveats need to be taken into consideration when assessing the effect of pharmacological PKCθ inhibitors or *Prkcq* gene deletion on the differentiation of Tregs (or T cells in general): In the first case, small molecule kinase inhibitors do not have absolute selectivity and, therefore, functional effects of PKCθ inhibition could conceivably be due to the inhibition of other kinases. In the second case, embryonic deletion could affect T cell developmental processes, and these effects could be carried over to the peripheral T cells. Hence, it would be useful to generate conditional *Prkcq* gene knockout mice where PKCθ expression is abolished post T cell development, *i.e*., only in the mature peripheral T cells.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Another issue that merits consideration when studying the function of PKCθ in Treg development, differentiation, and function has to do with the role of CD28 in Tregs, given the importance of this costimulatory receptor in the IS localization and function of PKCθ in Teff cells. In contrast to thymic Treg development that requires high-affinity and avidity TCR interaction together with a CD28 costimulatory signal, peripheral Treg induction depends on suboptimal TCR stimulation together with TGFβ, but in the absence of CD28 costimulation (Curotto de Lafaille & Lafaille, 2009; Josefowicz & Rudensky, 2009). In fact, CD28 signals can inhibit iTreg differentiation (Ma, et al., 2012; Zanin-Zhorov, et al., 2010), most likely reflecting the importance of CD28 in promoting activation of NF-κB (Coudronniere, et al., 2000), consistent with the negative effect of NF-κB on iTreg differentiation (Zanin-Zhorov, et al., 2010).

VIII. PKCθ in Human Disease

The establishment of *Prkcq*$^{-/-}$ mice (Pfeifhofer, et al., 2003; Sun, et al., 2000) made it possible to systematically dissect the critical functions of PKCθ at the molecular, cellular and *in vivo* levels under physiological and pathological conditions. However, the potential role of PKCθ in the pathogenesis of human diseases represents a much more challenging question. Nonetheless, PKCθ has consistently been reported to be associated with several human diseases, autoimmune diseases and cancer.

Recent genome-wide association studies (GWAS), which compare single nucleotide polymorphisms (SNP) between thousands of diseased and healthy individuals followed by powerful statistical analyses, have identified specific SNPs within the *Prkcq* locus that are significantly associated with type 1 diabetes (T1D), RA, and celiac disease (Cooper, et al., 2008; Raychaudhuri, et al., 2008; Stahl, et al., 2010; Zhernakova, et al., 2011). For example, the SNP rs947474 has been reproducibly reported as a risk factor for the development of T1D (Cooper, et al., 2008; Reddy, et al., 2011). On the other hand, the C or G single nucleotide variation at rs4750316 is consistently associated with RA (Raychaudhuri, et al., 2008; Stahl, et al., 2010). These novel findings provide a framework for formulating tangible hypotheses and testable models in order to understand the PKCθ-dependent molecular pathways pertinent to human diseases. For example, the T1D susceptibility associated with ChIP SNP rs947474, which is located 78 kb downstream of the *Prkcq* gene, is positioned within the gene regulatory region. Incidentally, a comprehensive genome-wide mapping study using ChIP followed by deep sequencing (ChIP-seq) revealed that the same SNP lies within a vitamin D receptor (VDR)-binding site (Ramagopalan, et al., 2010). The VDR is a transcription factor, which, upon binding to its ligand, exerts pleiotropic biological effects on immune cells, including T cells (Baeke, Takiishi, Korf, Gysemans, & Mathieu, 2010). Thus, this disease-associating SNP could possibly affect the occupancy and/or function of the VDR. With this knowledge, it would be feasible and interesting to examine whether the single nucleotide variation at rs947474 can modulate the expression and/or function of human PKCθ and, as a result, increase the risk of developing T1D.

Another intriguing development concerning the association of PKCθ with human disease has recently emerged in cancer studies. Gastrointestinal stromal tumors (GISTs) represent a specific group of tumors involving the mesenchymal tissues of the gastrointestinal tract. Gain-of-function mutations in the *c-Kit* protooncogene that result in its constitutive activity account for about 85% of cases and, hence, c-Kit expression is the standard marker for GIST diagnosis (Hirota, et al., 1998). Treatment of GIST patients with the tyrosine kinase inhibitor Imatinib is effective, although its long-term use can lead to drug resistance (Gschwind, Fischer, & Ullrich, 2004). In a small subset of GIST patients, the expression of c-Kit is less prominent and, therefore, in an effort to identify new markers for c-Kit-negative GIST, several groups found that PKCθ was expressed in all forms of GISTs, but not in other

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

mesenchymal or epithelial tumors, including non-GIST c-Kit-positive tumors. Thus, PKCθ can serve as a sensitive and specific marker for GISTs (Blay, et al., 2004; Debiec-Rychter, et al., 2004; Duensing, et al., 2004). Subsequent studies in different cancer centers have corroborated this finding (H. E. Lee, Kim, Lee, Lee, & Kim, 2008; Motegi, et al., 2005). Does the aberrant expression of PKCθ in GISTs play a role in the development of these tumors? In fact, *in vitro* experiments demonstrated that PKCθ acts upstream to regulate the expression of c-Kit since knockdown of PKCθ in GIST cell lines using RNA interference caused a reduction of c-Kit expression, inhibition of the PI3K/Akt signaling pathway, upregulation of the cyclin-dependent kinase inhibitors p21 and p27, cell arrest at the G1 phase of the cell cycle, and apoptosis (Ou, Zhu, Demetri, Fletcher, & Fletcher, 2008). Hence, these findings suggest that PKCθ could promote GIST development and, therefore, its inhibition could potentially be therapeutically beneficial.

The link between the aberrant expression of PKCθ and GIST might represent merely the tip of the iceberg. Earlier preliminary studies and a recent more focused report indicate that the PKCθ could also be detected in Ewing's sarcoma, which is a rare group of bone neoplasms affecting mainly children and adolescents (Blay, et al., 2004; Kang, Kim, Park, & Kang, 2009). In a small subset of Ewing's sarcomas, PKCθ appeared to have a characteristic dot-like localization, suggesting its utility as a prognostic marker (Kang, et al., 2009). However, this interesting observation warrants further careful study. PKCθ was also implicated as a critical regulator of c-Rel-driven mammary tumorigenesis, as PKCθ activation inhibited the FOXO3a/ERα/p27Kip1 axis that normally maintains an epithelial cell phenotype and induces c-Rel target genes, thereby promoting proliferation, survival, and more invasive breast cancer (Belguise & Sonenshein, 2007).

Other examples for the potential involvement of PKCθ in human disease include its reported role in mediating insulin resistance (Griffin, et al., 1999; Itani, et al., 2000; Kim, et al., 2004; Serra, et al., 2003), the high level expression of GLK, a direct PKCθ-activating kinase, in T cells of systemic lupus erythematosus patients (Chuang, et al., 2011) and, as mentioned earlier, the restoration of the impaired Treg activity in RA patients by a PKCθ inhibitor (Zanin-Zhorov, et al., 2010). Altogether, these emerging reports on the association between PKCθ and human diseases highlight the need to better understand the function of this enzyme in the human immune system and to seek approaches that could inhibit its function in humans (see below).

IX. Is PKCθ a Promising Drug Target?

Since its discovery, PKCθ has garnered considerable amount of attention as a potential therapeutic target (Baier, 2003; Baier & Wagner, 2009). Previous sections of this review have provided several strong arguments that support, at least from a theoretical standpoint, a strong case for considering PKCθ as an attractive drug target for selective T cell immunosuppression. Perhaps the strongest argument is provided by the now well-established documentation of the selective requirement of PKCθ in T cell-dependent immune responses (Table I). Particularly intriguing and exciting are the findings that PKCθ is critical for harmful immune responses, namely, Th2-meditaed allergies, Th17-mediated autoimmune diseases, and GvHD, but is dispensable for beneficial immune responses such as protection against pathogen infection mediated by Th1 cells and CTLs and GvL responses in BMT recipients. Of particular interest are the findings that pathogenic T cells depend on PKCθ, whereas Tregs are, in fact, negatively regulated by it. Thus, strategies to inhibit PKCθ would be expected to achieve a synergistic outcome of simultaneously inhibiting inflammatory T cells and promoting Treg function, a highly desirable scenario in autoimmune diseases and transplantation. However, promotion of Treg function and

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

inhibition of effector T cell function represent a double-edged sword because they would be desirable in, *e.g*., autoimmune diseases, but not in tumor-specific T cell responses.

Second, based on studies reviewed earlier, it is likely that inhibition of PKCθ would also promote anergy induction by preventing (or diminishing) the activation of AP-1 and NF-κB, two transcription factors that control the balance between anergy (induced by NFAT activation alone) and productive T cell activation in favor of the latter. This scenario can be contrasted with CN inhibitors, which are widely used for immunosuppression, because, unlike PKCθ, which almost certainly antagonizes anergy induction, NFAT (the target of CN inhibitors) is required for maintaining anergy to organ transplants. Hence, PKCθ inhibition could be expected to interfere with transplant rejection by donor Teff cells and, at the same time, enable anergy against the transplant to become stably established. Third, PKCθ provides a survival signal, particularly to activated and potentially pathogenic T cells as well as to leukemic T cells. Therefore, it is conceivable that PKCθ inhibition would promote the apoptosis of pathogenic T cells and perhaps even T cell leukemias. Last but not least, PKCθ has a relatively narrow range of tissue distribution with predominant expression in T cells and, therefore, minimal toxic side effects of PKCθ inhibitory drugs can be expected in tissues other than T cells. This expectation is supported by the generally intact health status and fertility of *Prkcq*$^{-/-}$ mice, and is, again, in sharp contrast to the considerable toxicity of CN inhibitors, which reflects the ubiquitous tissue distribution and functions of NFAT.

On the other hand, it is also important to consider whether highly selective inhibition of PKCθ alone would be sufficient to achieve desirable and effective therapeutic effects. This question arises because of the possibility of functional redundancy among T cell-expressed PKCs. Despite the fact that *Prkcq*$^{-/-}$ T cells display severe activation defects, which are somewhat milder in the mice generated by Baier *et al*. (Pfeifhofer, et al., 2003), and the nearly absolute requirement of PKCθ in certain murine immune responses (*e.g*., Th2, Th17), there is substantial evidence for cooperation and functional redundancy between PKCθ and other T cell-expressed PKCs (Baier & Wagner, 2009)). The following are several examples for this redundancy: First, PKCε enhances NF-κB, NFAT and AP1 signaling pathways leading to IL-2 expression (Genot, Parker, & Cantrell, 1995; Szamel, Appel, Schwinzer, & Resch, 1998), promotes proliferation of human CD4^{+} T cell by attenuating the inhibitory effects of TGFβ1 (Mirandola, et al., 2011), and also has an anti-apoptotic effect in T cells (Bertolotto, et al., 2000; Villalba, et al., 2001). Second, PKCα cooperates with PKCθ to downregulate TCR expression (von Essen, et al., 2006) and to promote certain aspects of T cell activation, *e.g*., alloimmune responses and IFNγ production (Gruber, et al., 2009); PKCα is also required for Th1-dependent IgG2a/2b antibody responses (Pfeifhofer, et al., 2006). Third, double knockout mice deficient in both PKCη and PKCθ have a significant defect in T cell development, which is not observed in the corresponding single knockout mice and, furthermore, PKCη promotes the activation of mature CD8^{+} T cells and homeostatic proliferation (Fu, et al., 2011). Fourth, PKCβ positively regulates T cell migration (Volkov, Long, & Kelleher, 1998; Volkov, Long, McGrath, Ni Eidhin, & Kelleher, 2001), expression of the activation markers CD69 and CD25 and secretion of IL-8 and TNFβ (Cervino, Lopez-Lago, Vinuela, & Barja, 2010), and IL-2 exocytosis in T cells (Long, Kelleher, Lynch, & Volkov, 2001). Finally, PKCζ has been shown to control Th2 cell function and allergic airway inflammation (Martin, et al., 2005). Thus, the potential contribution of other PKC family members to the activation, differentiation and function of T cells (and other immune cells) has to be taken into account when considering the development of PKCθ-based therapeutics for clinical use, especially given the fact that little is known about the functions of this enzyme in human T cells.

In view of the above considerations, it is not surprising that pharmaceutical drug companies have dedicated substantial efforts to identify and characterize small molecule inhibitors of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

PKCθ catalytic activity. Recent studies reported the development and characterization of compounds that display various degree of selectivity toward PKCθ (Cole, et al., 2008; Cywin, et al., 2007; Mosyak, et al., 2007). These small molecules function as ATP competitive inhibitors, *i.e*., they bind to the ATP-binding pocket of the kinase. A majority of kinase inhibitors developed to date target this same site. However, because this site is conserved among kinases, it is difficult to obtain highly selective inhibitors. For example, imatinib, a Bcr-Abl kinase inhibitor that is used to treat chronic myelogenous leukemia patients, was found to inhibit several unrelated tyrosine kinases, and the concept of "multi-kinase" inhibition as a beneficial rather than an undesired effect is gaining some prominence (Kontzias, Laurence, Gadina, & O'Shea, 2012). Furthermore, since catalytic kinase inhibitors in current clinical use 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.

Among recent PKCθ inhibitors, the compound AEB071 (sotrastaurin) seems to have reached the most advanced development stage, and it has entered clinical trials in psoriasis and organ transplantation (Budde, et al., 2010; Friman, et al., 2011; Skvara, et al., 2008). AEB071 inhibits not only PKCθ, but also other novel (Ca^{2+}-independent δ, ε, and η; nPKC) and conventional (Ca^{2+}-dependent α and β; cPKC) members at sub-nanomolar to low nanomolar concentrations, with a 1,000–10,000-fold lower selectivity for other kinases (Evenou, et al., 2009; Skvara, et al., 2008). It also effectively inhibited anti-TCR/CD28-stimulated human and mouse T cell proliferation and cytokine production (Evenou, et al., 2009; Matz, et al., 2010), as well as local GvHD and allograft rejection in rats and non-human primates (Bigaud, et al., 2012; Kamo, Shen, Ke, Busuttil, & Kupiec-Weglinski, 2011; Weckbecker, et al., 2010), and was well tolerated. Consistent with the findings that PKCθ is dispensable for antiviral immunity (Berg-Brown, et al., 2004; Giannoni, et al., 2005; Marsland, et al., 2005; Valenzuela, et al., 2009), PKC inhibition with AEB071 did not lead to increased infections in renal transplant patients enrolled in a phase II clinical trial (Friman, et al., 2011). However, it remains to be seen whether AEB071 will be sufficiently effective as a monotherapy. Since it reportedly does not inhibit Ca^{2+} signaling (Evenou, et al., 2009), combination therapy with other immunosuppressive agents such as cyclosporine A at low, suboptimal concentrations (Bigaud, et al., 2012; Budde, et al., 2010; Evenou, et al., 2009; Weckbecker, et al., 2010) may be useful, provided it does not cause global, potentially harmful immunosuppression

It has been argued that the ability of AEB071 to broadly inhibit PKCs underlies its inhibition of T cell activation, since this broad activity prevents potential compensation by other PKC isoforms (Baier & Wagner, 2009; Friman, et al., 2011). Indeed, as mentioned earlier, functional cooperation and partial redundancy between PKCθ and other PKCs, including PKCα (Gruber, et al., 2009) has been demonstrated (Baier & Wagner, 2009; Pfeifhofer-Obermair, et al., 2012). However, the finding that the combined deletion of PKCθ and PKCα primarily affects NFAT activation (Gruber, et al., 2009) is inconsistent with findings that AEB071 does not inhibit NFAT activation (Evenou, et al., 2009). Thus, some other PKC besides PKCθ or PKCα, or even a non-PKC kinase that is not inhibited by AEB071, may be important. Moreover, it is hard to predict whether potent inhibition of other PKC family members besides PKCθ may be beneficial by overcoming kinase redundancy or, conversely, may have the undesired effect of inducing global immunosuppression or some toxicity. In fact, adverse effects, particularly those affecting the gastrointestinal tract, were reported with higher incidence in renal transplant recipients in a phase II AEB071 clinical trial (Friman, et al., 2011). Hence, it remains unclear whether lower selectivity toward PKC family members would represent a therapeutic advantage or disadvantage, and further clinical trials are required to determine if the therapeutic benefits of AEB071 outweigh its side effects.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Given the potential toxicity and side effects of small molecule kinase inhibitors, there has recently been an increased interest in allosteric kinase inhibitors, *i.e*., compounds that do not bind to the catalytic pocket of the kinase but, rather, to another, regulatory region and, by doing so prevents kinase activation, most likely by interfering with a conformational change required for opening of the catalytic pocket and, hence, full activity (Lamba & Ghosh, 2012). Because allosteric inhibitors bind to much less conserved sites in kinases, they are likely to be much more selective and less toxic. Consideration of PKCθ as a potential target for allosteric inhibition requires that at least two criteria are met: First, the enzyme should contain a defined allosteric site that is necessary for its activation and downstream functions in order to ensure efficacy. Second, this allosteric site should be unique, *i.e*., have low sequence homology to other PKCs (or kinases in general) to ensure a high degree of specificity (with the proviso that exquisite specificity is indeed an advantage, as opposed to the potential benefits of "multi-kinase" inhibition). We propose that the proline-rich motif in the V3 domain of PKCθ, which we found recently to be essential for targeting it to the IS and the cSMAC, and enabling it to activate its downstream targets (Kong, et al., 2011), meets these criteria. The V3 domain is the most divergent region among members of the PKC family, and the critical proline-rich motif is found only in PKCθ. V3 domains of PKCs were initially considered to represent a flexible hinge region for the "opening" of PKC and its change from a resting state to the active conformation for substrate binding and kinase activity (Steinberg, 2008). However, it is becoming clear that the hinge regions of PKCs have additional functions, including protein-protein interactions. Indeed, in addition to PKCθ, it has been reported that the G(D/E)E motif located in the V3 region of PKCα and PKCε is essential for the selective targeting of these isoforms (Quittau-Prevostel, Delaunay, Collazos, Vallentin, & Joubert, 2004).

X. Conclusions and Future Perspectives

Studies on PKCθ since its discovery about 20 years ago have revealed an extensive amount of information about its expression, regulation and function, especially in T cells where it is expressed most abundantly. It is now clear that PKCθ plays important roles in T cell activation and survival by activating several downstream signaling pathways, with the NF-κB and AP-1 signaling pathways representing major targets. The early characterization of *Prkcq*$^{-/-}$ mice, which was conducted *in vitro*, implied a global role in T cell activation, reflected by severe defects in TCR-induced activation, proliferation, and cytokine production. This notion raised some doubts regarding the utility and advantage of PKCθ as a drug target over other, widely used immunosuppressive drugs such as CN inhibitors, reflecting the concern that like, *e.g*., tacrolimus, it would globally inhibit immune responses, including protective responses against pathogens. However, later analyses by many groups of the ability of *Prkcq*$^{-/-}$ mice to mount various *in vivo* immune responses, including the use of experimental disease models, have led to the surprising, but clinically promising, conclusion that the requirement of PKCθ in the immune system is quite selective. These findings, combined with the predominant expression of PKCθ in T cells, make a strong case, at least from a theoretical standpoint, for its potential utility as a target for drugs that would display high selectivity and low toxicity. Indeed, progress in developing selective PKCθ inhibitors and early clinical trials have been reported, and there is clearly a sense that interest in this enzyme as a drug target for selective and beneficial immunosuppression is not waning but, rather, is on the upswing. Nevertheless, it is clear that there are still substantial gaps in our knowledge, which need to be explored and resolved before the full therapeutic potential of PKCθ-inhibiting strategies can be realized in the clinical arena.

1) Of prime importance among the unresolved questions is the importance of PKCθ in the human immune system. The overwhelming majority of PKCθ-related studies have been conducted in mice, and very little is known about the role and importance of this enzyme in

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

human T cells. Interestingly, despite major progress over the past ~20 years in elucidating the molecular basis of many forms of human immunodeficiency, an immunodeficiency associated with impaired PKCθ expression or function has not yet been reported (to the best of our knowledge). Nevertheless, the limited amount of reports addressing the function of PKCθ in human T cells provides a basis for cautious optimism. First, early stage small molecule catalytic inhibitors of PKCθ inhibit the activation and proliferation of human T cells *in vitro*, subject to the caveat that these inhibitors likely inhibit other kinases in addition to PKCθ. Second, early clinical trials with one such inhibitor (AEB071), despite being inconclusive are encouraging. Third, the *Prkcq* gene has been tentatively identified as a potential risk factor in a few human autoimmune and inflammatory diseases. Fourth, and perhaps most relevant in this regard, is the report that a selective small molecule PKCθ inhibitor reversed the impaired suppressive activity of Tregs from RA patients (Zanin-Zhorov, et al., 2010). These reports should serve as a strong impetus for exploring more extensively the importance of PKCθ in the human immune system. The *in vitro* use of various PKCθ inhibitors that are becoming available or RNAi-based knockdown strategies, as well as the expansion of clinical trials with PKCθ inhibitors, either alone or as components in combination with low, less toxic doses of conventional immunosuppressive drugs such as tacrolimus could be very informative in this regard.

2) The seminal finding that PKCθ is excluded from the IS of Tregs and, more importantly, that PKCθ negatively regulates Treg-mediated suppression need to be extended in order to determine the mechanistic basis for its IS exclusion and negative regulation of Treg suppressive function. A possible explanation for the exclusion of PKCθ from the Treg IS was provided by Yokosuka *et al*. (Yokosuka, et al., 2010), who showed that CTLA-4 competes with CD28 in recruitment to the cSMAC, thereby displacing the PKCθ-CD28 complex (Kong, et al., 2011) from the IS. However, it is equally possible, at least theoretically, that PKCθ, perhaps in complex with some other partner(s), plays an active signaling role to inhibit the function of Tregs when it is localized in the distal T cell pole.

3) Although mouse *Prkcq*$^{-/-}$ T cells display severe activation defects, it is possible that effective immunosuppressive strategies based on PKCθ may have to take into consideration the need to inhibit other PKC family members that may play a compensatory role. This notion is supported by the reported cooperativity between PKCθ and other PKCs as described earlier, and the suggestion that the effectiveness of AEB071 in inhibiting T cell activation results from its ability to inhibit several other PKCs in addition to PKCθ (Evenou, et al., 2009; Skvara, et al., 2008). In this context, it is important to note that the use of pharmacological kinase inhibitors can result in functional outcomes quiet distinct from those observed in mice lacking that same kinase, emphasizing again the importance of conducting inhibitor studies in human T cells.

4) Despite the promise of early small molecule catalytic PKCθ inhibitors, the use of similar kinase inhibitors in general is less than optimal because of their lack of absolute specificity, which often leads to toxic side effects. Therefore, development and exploration of allosteric inhibitors for PKCθ (and other PKCs that may participate in T cell activation) is a worthy goal. Our recent study (Kong, et al., 2011) demonstrates a new potential approach for attenuating PKCθ-dependent functions utilizing allosteric compounds based on the critical proline-rich motif in the V3 domain of PKCθ that will block its Lck-mediated association with CD28 and recruitment to the IS, an association obligatory for its downstream signaling functions. The pursuit of this new approach is worthwhile.

5) Lastly, it would be important to elucidate the mechanisms that render some types of immune responses, particularly T cell-mediated antiviral immunity, PKCθ-independent. In this regard, it has been demonstrated that inclusion of a TLR9 agonist in a T cell vaccine can

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

rescue impaired T cell responses in *Prkcq*$^{-/-}$ mice, suggesting that certain TLR signaling pathway(s) can compensate for the lack of PKCθ (Marsland, et al., 2007). One likely candidate is the NF-κB signaling pathway, which is a major PKCθ target in T cells, but is also activated by engaged TLRs. Therefore, it would be interesting to determine whether this compensatory activity of TLR ligands is shared by other TLRs.

These unresolved questions pave the way and provide directions for future high priority studies that will improve our understanding of the role of PKCθ in the human immune system, and guide the development of what is likely to be a new generation of drugs that target PKCθ to induce desirable selective forms of immunosuppression. Such drugs may be able to eliminate or dampen deleterious immune responses such as autoimmunity and GvHD without impacting the ability of treated patients to eliminate harmful infections. In the coming years, we should see important and exciting advances along these lines and, hopefully, will realize the potential of PKCθ as a novel and highly useful drug target.

Acknowledgments

This is publication number 1555 from the La Jolla Institute for Allergy and Immunology. Work from the authors' laboratory described in this article was supported by grant CA035299 from the National Institutes of Health. We thank our many past laboratory members who have contributed to the work on PKCθ.

List of non-standard abbreviations

BMT	bone marrow transplantation
ChIP	chromatin immunoprecipitation
CN	calcineurin
CTL	cytotoxic T lymphocyte
GIST	gastrointestinal stromal tumor
GvHD	graft-*versus*-host disease
GvL	graft-*versus*-leukemia (response)
IS	immunological synapse
PI3K	phosphatidylinositol 3-kinase
PKC	protein kinase C
SMAC	supramolecular activation cluster
SNP	single nucleotide polymorphism
TCR	T cell receptor
TLR	toll-like receptor
Teff	effector T cell
Treg	regulatory T cell
VSV	vesicular stomatitis virus
WASp	Wiskott-Aldrich Syndrome protein

References

Abb J, Bayliss GJ, Deinhardt F. Lymphocyte activation by the tumor-promoting agent 12-O-tetradecanoylphorbol-13-acetate (TPA). J. Immunol. 1979; 122:1639–1642. [PubMed: 221578]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Aguilo JI, Garaude J, Pardo J, Villalba M, Anel A. Protein kinase C-θ is required for NK cell activation and *in vivo* control of tumor progression. J Immunol. 2009; 182:1972–1981. [PubMed: 19201850]

Altman A, Kaminski S, Busuttil V, Droin N, Hu J, Tadevosyan Y, et al. Positive feedback regulation of PLCγ1/Ca^{2+} signaling by PKCθ in restimulated T cells via a Tec kinase-dependent pathway. Eur J Immunol. 2004; 34:2001–2011. [PubMed: 15214048]

Anderson K, Fitzgerald M, Dupont M, Wang T, Paz N, Dorsch M, et al. Mice deficient in PKC θ demonstrate impaired *in vivo* T cell activation and protection from T cell-mediated inflammatory diseases. Autoimmunity. 2006; 39:469–478. [PubMed: 17060026]

Anel A, Aguilo JI, Catalan E, Garaude J, Rathore MG, Pardo J, et al. Protein kinase C-θ (PKC-θ) in natural killer cell function and anti-tumor immunity. Front Immunol. 2012; 3:187. [PubMed: 22783260]

Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Current Opin Pharmacol. 2010; 10:482–496.

Baier G. The PKC gene module: molecular biosystematics to resolve its T cell functions. Immunol Rev. 2003; 192:64–79. [PubMed: 12670396]

Baier G, Telford D, Giampa L, Coggeshall KM, Baier-Bitterlich G, Isakov N, et al. Molecular cloning and characterization of PKC θ, a novel member of the protein kinase C (PKC) gene family expressed predominantly in hematopoietic cells. J Biol Chem. 1993; 268:4997–5004. [PubMed: 8444877]

Baier G, Wagner J. PKC inhibitors: potential in T cell-dependent immune diseases. Curr Opin Cell Biol. 2009; 21:262–267. [PubMed: 19195860]

Baier-Bitterlich G, Uberall F, Bauer B, Fresser F, Wachter H, Grunicke H, et al. Protein kinase C-θ isoenzyme selective stimulation of the transcription factor complex AP-1 in T lymphocytes. Mol Cell Biol. 1996; 16:1842–1850. [PubMed: 8657160]

Balogh G, de Boland AR, Boland R, Barja P. Effect of 1,25OH(2)-vitamin D3 on the activation of natural killer cells: role of protein kinase C and extracellular calcium. Exp Mol Pathol. 1999; 67:63–74. [PubMed: 10527758]

Barouch-Bentov R, Lemmens EE, Hu J, Janssen EM, Droin NM, Song J, et al. Protein kinase C-θ is an early survival factor required for differentiation of effector CD8+ T cells. J Immunol. 2005; 175:5126–5134. [PubMed: 16210616]

Bauer B, Krumbock N, Ghaffari-Tabrizi N, Kampfer S, Villunger A, Wilda M, et al. T cell expressed PKCθ demonstrates cell-type selective function. Eur J Immunol. 2000; 30:3645–3654. [PubMed: 11169407]

Beal AM, Anikeeva N, Varma R, Cameron TO, Norris PJ, Dustin ML, et al. Protein kinase C θ regulates stability of the peripheral adhesion ring junction and contributes to the sensitivity of target cell lysis by CTL. J Immunol. 2008; 181:4815–4824. [PubMed: 18802085]

Belguise K, Sonenshein GE. PKCθ promotes c-Rel-driven mammary tumorigenesis in mice and humans by repressing estrogen receptor α synthesis. J Clin Invest. 2007; 117:4009–4021. [PubMed: 18037997]

Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001; 27:20–21. [PubMed: 11137993]

Berg-Brown NN, Gronski MA, Jones RG, Elford AR, Deenick EK, Odermatt B, et al. PKCθ signals activation versus tolerance *in vivo* . J Exp Med. 2004; 1999:743–752. [PubMed: 15024044]

Bertolotto C, Maulon L, Filippa N, Baier G, Auberger P. Protein kinase C θ and ε promote T-cell survival by a rsk-dependent phosphorylation and inactivation of BAD. J Biol Chem. 2000; 275:37246–37250. [PubMed: 10976111]

Bi K, Tanaka Y, Coudronniere N, Hong S, Sugie K, van Stipdonk MJB, et al. Antigen-induced translocation of PKC-θ to membrane rafts is required for T cell activation. Nat. Immunol. 2001; 2:556–563. [PubMed: 11376344]

Bigaud M, Wieczorek G, Beerli C, Audet M, Blancher A, Heusser C, et al. Sotrastaurin (AEB071) alone and in combination with cyclosporine A prolongs survival times of non-human primate

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

recipients of life-supporting kidney allografts. Transplantation. 2012; 93:156–164. [PubMed: 22179400]

Blay P, Astudillo A, Buesa JM, Campo E, Abad M, Garcia-Garcia J, et al. Protein kinase C θ is highly expressed in gastrointestinal stromal tumors but not in other mesenchymal neoplasias. Clin Cancer Res. 2004; 10:4089–4095. [PubMed: 15217944]

Bretscher PA, Cohn M. Minimal model for the mechanism of antibody induction and paralysis by antigen. Nature. 1968; 220:444–448. [PubMed: 5686155]

Bromley SK, Burack WR, Johnson KG, Somersalo K, Sims TN, Sumen C, et al. The immunological synapse. Annu Rev Immunol. 2001; 19:375–396. [PubMed: 11244041]

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001; 27:68–73. [PubMed: 11138001]

Budde K, Sommerer C, Becker T, Asderakis A, Pietruck F, Grinyo JM, et al. Sotrastaurin, a novel small molecule inhibiting protein kinase C: first clinical results in renal-transplant recipients. Am J Transplant. 2010; 10:571–581. [PubMed: 20121745]

Burack WR, Lee KH, Holdorf AD, Dustin ML, Shaw AS. Cutting edge: quantitative imaging of raft accumulation in the immunological synapse. J Immunol. 2002; 169:2837–2841. [PubMed: 12218094]

Campi G, Varma R, Dustin ML. Actin and agonist MHC-peptide complex-dependent T cell receptor microclusters as scaffolds for signaling. J Exp Med. 2005; 202:1031–1036. [PubMed: 16216891]

Carrasco S, Merida I. Diacylglycerol-dependent binding recruits PKCθ and RasGRP1 C1 domains to specific subcellular localizations in living T lymphocytes. Mol Biol Cell. 2004; 15:2932–2942. [PubMed: 15064353]

Cartwright NG, Kashyap AK, Schaefer BC. An active kinase domain is required for retention of PKCθ at the T cell immunological synapse. Mol Biol Cell. 2011; 22:3491–3497. [PubMed: 21795397]

Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J Biol Chem. 1982; 257:7847–7851. [PubMed: 7085651]

Cervino MC, Lopez-Lago MA, Vinuela JE, Barja P. Specific inhibition of protein kinase Cβ expression by antisense RNA affects the activation of Jurkat T lymphoma cells. J Biol Regul Homeost Agents. 2010; 24:273–285. [PubMed: 20846475]

Chan AC, Shaw AS. Regulation of antigen receptor signal transduction by protein tyrosine kinases. Curr Opin Immunol. 1996; 8:394–401. [PubMed: 8794001]

Chang JD, Xu Y, Raychowdhury MK, Ware JA. 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. 1993; 268:14208–14214. [PubMed: 7686153]

Chuang HC, Lan JL, Chen DY, Yang CY, Chen YM, Li JP, et al. The kinase GLK controls autoimmunity and NF-κB signaling by activating the kinase PKC-θ in T cells. Nat Immunol. 2011; 12:1113–1118. [PubMed: 21983831]

Cole DC, Asselin M, Brennan A, Czerwinski R, Ellingboe JW, Fitz L, et al. Identification, characterization and initial hit-to-lead optimization of a series of 4-arylamino-3-pyridinecarbonitrile as protein kinase C θ (PKCθ) inhibitors. J Med Chem. 2008; 51:5958–5963. [PubMed: 18783200]

Cooper JD, Smyth DJ, Smiles AM, Plagnol V, Walker NM, Allen JE, et al. Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. Nat Genet. 2008; 40:1399–1401. [PubMed: 18978792]

Coudronniere N, Villalba M, Englund N, Altman A. NF-κB activation induced by T cell receptor/CD28 costimulation is mediated by protein kinase C-θ. Proc Natl Acad Sci USA. 2000; 97:3394–3399. [PubMed: 10716728]

Coussens L, Parker PJ, Rhee L, Yang-Feng TL, Chen E, Waterfield MD, et al. Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. Science. 1986; 233:859–866. [PubMed: 3755548]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity. 2009; 30:626–635. [PubMed: 19464985]

Cywin CL, Dahmann G, Prokopowicz AS 3rd, Young ER, Magolda RL, Cardozo MG, et al. Discovery of potent and selective PKC-θ inhibitors. Bioorg Med Chem Lett. 2007; 17:225–230. [PubMed: 17055721]

Davis DM, Chiu I, Fassett M, Cohen GB, Mandelboim O, Strominger JL. The human natural killer cell immune synapse. Proc Natl Acad Sci USA. 1999; 96:15062–15067. [PubMed: 10611338]

Debiec-Rychter M, Wasag B, Stul M, De Wever I, Van Oosterom A, Hagemeijer A, et al. Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. J Pathol. 2004; 202:430–438. [PubMed: 15095270]

Dienz O, Hehner SP, Droge W, Schmitz ML. Synergistic activation of NF-κB by functional cooperation between Vav and PKCθ in T lymphocytes. J. Biol. Chem. 2000; 275:24547–24551. [PubMed: 10862755]

Dienz O, Moller A, Strecker A, Stephan N, Krammer PH, Droge W, et al. Src homology 2 domain-containing leukocyte phosphoprotein of 76 kDa and phospholipase C γ1 are required for NF-κB activation and lipid raft recruitment of protein kinase C θ induced by T cell costimulation. J Immunol. 2003; 170:365–372. [PubMed: 12496421]

Duensing A, Joseph NE, Medeiros F, Smith F, Hornick JL, Heinrich MC, et al. Protein Kinase C θ (PKCθ) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). Cancer Res. 2004; 64:5127–5131. [PubMed: 15289315]

Dustin ML. Adhesive bond dynamics in contacts between T lymphocytes and glass-supported planar bilayers reconstituted with the immunoglobulin-related adhesion molecule CD58. J Biol Chem. 1997; 272:15782–15788. [PubMed: 9188475]

Dustin ML. T-cell activation through immunological synapses and kinapses. Immunol Rev. 2008; 221:77–89. [PubMed: 18275476]

Dustin ML, Allen PM, Shaw AS. Environmental control of immunological synapse formation and duration. Trends Immunol. 2001; 22:192–194. [PubMed: 11274923]

Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. Nat Immunol. 2000; 1:23–29. [PubMed: 10881170]

Erdel M, Baier-Bitterlich G, Duba C, Isakov N, Altman A, Utermann G, et al. Mapping of the human protein kinase C-θ (PRKCQ) gene locus to the short arm of chromosome 10 (10p15) by FISH. Genomics. 1995; 25:595–597. [PubMed: 7790001]

Evenou JP, Wagner J, Zenke G, Brinkmann V, Wagner K, Kovarik J, et al. 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. 2009; 330:792–801. [PubMed: 19491325]

Fang X, Wang R, Ma J, Ding Y, Shang W, Sun Z. Ameliorated ConA-induced hepatitis in the absence of PKC-θ. PLoS One. 2012; 7:e31174. [PubMed: 22347449]

Fathman CG, Lineberry NB. Molecular mechanisms of CD4+ T-cell anergy. Nat Rev Immunol. 2007; 7:599–609. [PubMed: 17612584]

Freeley M, Volkov Y, Kelleher D, Long A. Stimulus-induced phosphorylation of PKC θ at the C-terminal hydrophobic-motif in human T lymphocytes. Biochem Biophys Res Commun. 2005; 334:619–630. [PubMed: 16009340]

Friman S, Arns W, Nashan B, Vincenti F, Banas B, Budde K, et al. Sotrastaurin, a novel small molecule inhibiting protein-kinase C: randomized phase II study in renal transplant recipients. Am J Transplant. 2011; 11:1444–1455. [PubMed: 21564523]

Fu G, Hu J, Niederberger-Magnenat N, Rybakin V, Casas J, Yachi PP, et al. Protein kinase C η is required for T cell activation and homeostatic proliferation. Sci Signal. 2011; 4:ra84. [PubMed: 22155788]

Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Curr Opin Rheumatol. 2003; 15:430–435. [PubMed: 12819471]

Garaude J, Kaminski S, Charni S, Aguilo JI, Jacquet C, Plays M, et al. Impaired anti-leukemic immune response in PKCθ-deficient mice. Mol Immunol. 2008; 45:3463–3469. [PubMed: 18462800]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Genot EM, Parker PJ, Cantrell DA. Analysis of the role of protein kinase C-α, -ε, and -ζ in T cell activation. J Biol Chem. 1995; 270:9833–9839. [PubMed: 7730364]

Ghosh S, Karin M. Missing pieces in the NF-κB puzzle. Cell. 2002; 109(Suppl):S81–S96. [PubMed: 11983155]

Giannoni F, Lyon AB, Wareing MD, Dias PB, Sarawar SR. Protein kinase C θ is not essential for T-cell-mediated clearance of murine gammaherpesvirus 68. J Virol. 2005; 79:6808–6813. [PubMed: 15890920]

Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, et al. The immunological synapse: a molecular machine controlling T cell activation. Science. 1999; 285:221–227. [PubMed: 10398592]

Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. Diabetes. 1999; 48:1270–1274. [PubMed: 10342815]

Griffith JW, O'Connor C, Bernard K, Town T, Goldstein DR, Bucala R. Toll-like receptor modulation of murine cerebral malaria is dependent on the genetic background of the host. J Infect Dis. 2007; 196:1553–1564. [PubMed: 18008236]

Gruber T, Hermann-Kleiter N, Pfeifhofer-Obermair C, Lutz-Nicoladoni C, Thuille N, Letschka T, et al. PKC θ cooperates with PKC α in alloimmune responses of T cells in vivo. Mol Immunol. 2009; 46:2071–2079. [PubMed: 19356803]

Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Cancer. 2004; 4:361–370. [PubMed: 15122207]

Gupta S, Manicassamy S, Vasu C, Kumar A, Shang W, Sun Z. Differential requirement of PKC-θ in the development and function of natural regulatory T cells. Mol Immunol. 2008; 46:213–224. [PubMed: 18842300]

Harder T, Rentero C, Zech T, Gaus K. Plasma membrane segregation during T cell activation: probing the order of domains. Curr Opin Immunol. 2007; 19:470–475. [PubMed: 17628460]

Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature. 1992; 356:607–609. [PubMed: 1313950]

Hayashi K, Altman A. Protein kinase C θ (PKCθ): a key player in T cell life and death. Pharmacol Res. 2007; 55:537–544. [PubMed: 17544292]

Healy AM, Izmailova E, Fitzgerald M, Walker R, Hattersley M, Silva M, et al. PKC-θ-deficient mice are protected from Th1-dependent antigen-induced arthritis. J Immunol. 2006; 177:1886–1893. [PubMed: 16849501]

Heissmeyer V, Macian F, Im SH, Varma R, Feske S, Venuprasad K, et al. Calcineurin imposes T cell unresponsiveness through targeted proteolysis of signaling proteins. Nat Immunol. 2004; 5:255–265. [PubMed: 14973438]

Heissmeyer V, Rao A. E3 ligases in T cell anergy--turning immune responses into tolerance. Sci STKE. 2004; 2004:pe29. [PubMed: 15252218]

Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998; 279:577–580. [PubMed: 9438854]

Hori S. c-Rel: a pioneer in directing regulatory T-cell lineage commitment? Eur J Immunol. 2010; 40:664–667. [PubMed: 20162555]

Huang J, Lo PF, Zal T, Gascoigne NR, Smith BA, Levin SD, et al. CD28 plays a critical role in the segregation of PKCθ within the immunologic synapse. Proc. Natl. Acad. Sci. USA. 2002; 99:9369–9373. [PubMed: 12077322]

Hug H, Sarre TF. Protein kinase C isoenzymes: divergence in signal transduction? Biochem J. 1993; 291(Pt 2):329–343. [PubMed: 8484714]

Inoue M, Kishimoto A, Takai Y, Nishizuka Y. 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. 1977; 252:7610–7616. [PubMed: 199594]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Isakov N, Altman A. Tumor promoters in conjunction with calcium ionophores mimic antigenic stimulation by reactivation of alloantigen-primed murine T lymphocytes. J. Immunol. 1985; 135:3674–3680. [PubMed: 2999231]

Isakov N, Altman A. PKC-theta-mediated signal delivery from the TCR/CD28 surface receptors. Front Immunol. 2012; 3:273. [PubMed: 22936936]

Itani SI, Zhou Q, Pories WJ, MacDonald KG, Dohm GL. Involvement of protein kinase C in human skeletal muscle insulin resistance and obesity. Diabetes. 2000; 49:1353–1358. [PubMed: 10923637]

Jang MS, Pan F, Erickson LM, Fisniku O, Crews G, Wynn C, et al. A blocking anti-CD28-specific antibody induces long-term heart allograft survival by suppression of the PKC theta-JNK signal pathway. Transplantation. 2008; 85:1051–1055. [PubMed: 18408588]

Jenkins MK, Chen CA, Jung G, Mueller DL, Schwartz RH. Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. J. Immunol. 1990; 144:16–22. [PubMed: 2153162]

Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012; 30:531–564. [PubMed: 22224781]

Josefowicz SZ, Rudensky A. Control of regulatory T cell lineage commitment and maintenance. Immunity. 2009; 30:616–625. [PubMed: 19464984]

Kabouridis PS, Jury EC. Lipid rafts and T-lymphocyte function: implications for autoimmunity. FEBS Lett. 2008; 582:3711–3718. [PubMed: 18930053]

Kaibuchi K, Takai Y, Nishizuka Y. Protein kinase C and calcium ion in mitogenic response of macrophage-depleted human peripheral lymphocytes. J. Biol. Chem. 1985; 260:1366–1369. [PubMed: 3155734]

Kamo N, Shen XD, Ke B, Busuttil RW, Kupiec-Weglinski JW. Sotrastaurin, a protein kinase C inhibitor, ameliorates ischemia and reperfusion injury in rat orthotopic liver transplantation. Am J Transplant. 2011; 11:2499–2507. [PubMed: 21883905]

Kane LP, Lin J, Weiss A. Signal transduction by the TCR for antigen. Curr. Opin. Immunol. 2000; 12:242–249. [PubMed: 10781399]

Kang GH, Kim KM, Park CK, Kang DY. PKC-θ expression in Ewing sarcoma/primitive neuroectodermal tumour and malignant peripheral nerve sheath tumour. Histopathology. 2009; 55:368–369. [PubMed: 19723158]

Kenworthy AK. Have we become overly reliant on lipid rafts? Talking Point on the involvement of lipid rafts in T-cell activation. EMBO Rep. 2008; 9:531–535. [PubMed: 18516088]

Kikkawa U, Takai Y, Tanaka Y, Miyake R, Nishizuka Y. Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters. J Biol Chem. 1983; 258:11442–11445. [PubMed: 6311812]

Kim JK, Fillmore JJ, Sunshine MJ, Albrecht B, Higashimori T, Kim DW, et al. PKC-θ knockout mice are protected from fat-induced insulin resistance. J Clin Invest. 2004; 114:823–827. [PubMed: 15372106]

Kong KF, Yokosuka T, Canonigo-Balancio AJ, Isakov N, Saito T, Altman A. 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. 2011; 12:1105–1112. [PubMed: 21964608]

Kontzias A, Laurence A, Gadina M, O'Shea JJ. Kinase inhibitors in the treatment of immune-mediated disease. F1000 Med Rep. 2012; 4:5. [PubMed: 22403586]

Krzewski K, Chen X, Orange JS, Strominger JL. Formation of a WIP-, WASp-, actin-, and myosin IIA-containing multiprotein complex in activated NK cells and its alteration by KIR inhibitory signaling. J Cell Biol. 2006; 173:121–132. [PubMed: 16606694]

Kwon MJ, Ma J, Ding Y, Wang R, Sun Z. Protein kinase C-θ promotes Th17 differentiation via upregulation of Stat3. J Immunol. 2012; 188:5887–5897. [PubMed: 22586032]

Lamba V, Ghosh I. New directions in targeting protein kinases: focusing upon true allosteric and bivalent inhibitors. Curr Pharm Des. 2012; 18:2936–2945. [PubMed: 22571662]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Lee HE, Kim MA, Lee HS, Lee BL, Kim WH. Characteristics of KIT-negative gastrointestinal stromal tumours and diagnostic utility of protein kinase C θ immunostaining. J Clin Pathol. 2008; 61:722–729. [PubMed: 18381383]

Lee KH, Dinner AR, Tu C, Campi G, Raychaudhuri S, Varma R, et al. The immunological synapse balances T cell receptor signaling and degradation. Science. 2003; 302:1218–1222. [PubMed: 14512504]

Letschka T, Kollmann V, Pfeifhofer-Obermair C, Lutz-Nicoladoni C, Obermair GJ, Fresser F, et al. PKC-θ selectively controls the adhesion-stimulating molecule Rap1. Blood. 2008; 112:4617–4627. [PubMed: 18796635]

Li Y, Hu J, Vita R, Sun B, Tabata H, Altman A. SPAK kinase is a substrate and target of PKCθ in T-cell receptor-induced AP-1 activation pathway. EMBO J. 2004; 23:1112–1122. [PubMed: 14988727]

Lin X, O'Mahony A, Mu Y, Geleziunas R, Greene WC. Protein kinase C-θ participates in NF-κB activation induced by CD3-CD28 costimulation through selective activation of IκB kinase β. Mol Cell Biol. 2000; 20:2933–2940. [PubMed: 10733597]

Lin X, Wang D. The roles of CARMA1, Bcl10, and MALT1 in antigen receptor signaling. Semin Immunol. 2004; 16:429–435. [PubMed: 15541657]

Liu Y, Graham C, Li A, Fisher RJ, Shaw S. Phosphorylation of the protein kinase C-θ activation loop and hydrophobic motif regulates its kinase activity, but only activation loop phosphorylation is critical to in vivo nuclear-factor-κB induction. Biochem J. 2002; 361(Pt 2):255–265. [PubMed: 11772397]

Liu Y, Graham C, Parravicini V, Brown MJ, Rivera J, Shaw S. Protein kinase C θ is expressed in mast cells and is functionally involved in Fcepsilon receptor I signaling. J Leukoc Biol. 2001; 69:831–840. [PubMed: 11358993]

Liu Y, Witte S, Liu YC, Doyle M, Elly C, Altman A. Regulation of protein kinase Cθ function during T cell activation by Lck-mediated tyrosine phosphorylation. J Biol Chem. 2000; 275:3603–3609. [PubMed: 10652356]

Long A, Kelleher D, Lynch S, Volkov Y. Cutting edge: protein kinase C β expression is critical for export of IL-2 from T cells. J Immunol. 2001; 167:636–640. [PubMed: 11441065]

Ma J, Ding Y, Fang X, Wang R, Sun Z. Protein kinase C-θ inhibits inducible regulatory T cell differentiation via an AKT-Foxo1/3a-dependent pathway. J Immunol. 2012; 188:5337–5347. [PubMed: 22539794]

Macian F, Garcia-Cozar F, Im SH, Horton HF, Byrne MC, Rao A. Transcriptional mechanisms underlying lymphocyte tolerance. Cell. 2002; 109:719–731. [PubMed: 12086671]

Macian F, Im SH, Garcia-Cozar FJ, Rao A. T-cell anergy. Curr Opin Immunol. 2004; 16:209–216. [PubMed: 15023415]

Manicassamy S, Gupta S, Huang Z, Sun Z. Protein kinase C-θ-mediated signals enhance CD4+ T cell survival by up-regulating Bcl-xL. J Immunol. 2006; 176:6709–6716. [PubMed: 16709830]

Manicassamy S, Sadim M, Ye RD, Sun Z. Differential roles of PKC-θ in the regulation of intracellular calcium concentration in primary T cells. J Mol Biol. 2006; 355:347–359. [PubMed: 16309697]

Manicassamy S, Yin D, Zhang Z, Molinero LL, Alegre ML, Sun Z. A critical role for protein kinase C-θ-mediated T cell survival in cardiac allograft rejection. J Immunol. 2008; 181:513–520. [PubMed: 18566417]

Marsland BJ, Nembrini C, Grun K, Reissmann R, Kurrer M, Leipner C, et al. TLR ligands act directly upon T cells to restore proliferation in the absence of protein kinase C-θ signaling and promote autoimmune myocarditis. J Immunol. 2007; 178:3466–3473. [PubMed: 17339441]

Marsland BJ, Nembrini C, Schmitz N, Abel B, Krautwald S, Bachmann MF, et al. Innate signals compensate for the absence of PKC-θ during in vivo CD8+ T cell effector and memory responses. Proc Natl Acad Sci USA. 2005; 102:14374–14379. [PubMed: 16186501]

Marsland BJ, Soos TJ, Spath G, Littman DR, Kopf M. Protein kinase C θ is critical for the development of *in vivo* T helper (Th)2 cell but not Th1 cell responses. J Exp Med. 2004; 2004:181–189. [PubMed: 15263025]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Martin P, Villares R, Rodriguez-Mascarenhas S, Zaballos A, Leitges M, Kovac J, et al. Control of T helper 2 cell function and allergic airway inflammation by PKCζ. Proc Natl Acad Sci USA. 2005; 102:9866–9871. [PubMed: 15987782]

Matz M, Weber U, Mashreghi MF, Lorkowski C, Ladhoff J, Kramer S, et al. Effects of the new immunosuppressive agent AEB071 on human immune cells. Nephrol Dial Transplant. 2010; 25:2159–2167. [PubMed: 20100729]

Meller N, Altman A, Isakov N. New perspectives on PKCθ, a member of the novel subfamily of protein kinase C. Stem Cells. 1998; 16:178–192. [PubMed: 9617893]

Meller N, Elitzur Y, Isakov N. Protein kinase C-θ (PKCθ) distribution analysis in hematopoietic cells: proliferating T cells exhibit high proportions of PKCtheta in the particulate fraction. Cell Immunol. 1999; 193:185–193. [PubMed: 10222061]

Mellor H, Parker PJ. The extended protein kinase C superfamily. Biochem J. 1998; 332(Pt 2):281–292. [PubMed: 9601053]

Mirandola P, Gobbi G, Masselli E, Micheloni C, Di Marcantonio D, Queirolo V, et al. Protein kinase Cε regulates proliferation and cell sensitivity to TGF-1β of CD4+ T lymphocytes: implications for Hashimoto thyroiditis. J Immunol. 2011; 187:4721–4732. [PubMed: 21964026]

Mohib K, AlKhamees B, Zein HS, Allan D, Wang L. Embryonic stem cell-derived factors inhibit T effector activation and induce T regulatory cells by suppressing PKC-θ activation. PLoS One. 2012; 7:e32420. [PubMed: 22412871]

Monaco G, Pignata C, Rossi E, Mascellaro O, Cocozza S, Ciccimarra F. DiGeorge anomaly associated with 10p deletion. Am J Med Genet. 1991; 39:215–216. [PubMed: 2063928]

Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. Nature. 1998; 395:82–86. [PubMed: 9738502]

Monks CR, Kupfer H, Tamir I, Barlow A, Kupfer A. Selective modulation of protein kinase C-θ during T-cell activation. Nature. 1997; 385:83–86. [PubMed: 8985252]

Morley SC, Weber KS, Kao H, Allen PM. Protein kinase C-θ is required for efficient positive selection. J Immunol. 2008; 181:4696–4708. [PubMed: 18802072]

Mosyak L, Xu Z, Joseph-McCarthy D, Brooijmans N, Somers W, Chaudhary D. Structure-based optimization of PKCθ inhibitors. Biochem Soc Trans. 2007; 35(Pt 5):1027–1031. [PubMed: 17956269]

Motegi A, Sakurai S, Nakayama H, Sano T, Oyama T, Nakajima T. PKC θ, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. Pathol intl. 2005; 55:106–112.

Newton AC. Regulation of protein kinase C. Curr Opin Cell Biol. 1997; 9:161–167. [PubMed: 9069266]

Niedel JE, Kuhn LJ, Vandenbark GR. Phorbol diester receptor copurifies with protein kinase C. Proc Natl Acad Sci USA. 1983; 80:36–40. [PubMed: 6296873]

Nishanth G, Sakowicz-Burkiewicz M, Handel U, Kliche S, Wang X, Naumann M, et al. Protective Toxoplasma gondii-specific T-cell responses require T-cell-specific expression of protein kinase C-θ. Infect Immun. 2010; 78:3454–3464. [PubMed: 20498263]

Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J. 1995; 9:484–496. [PubMed: 7737456]

Ohayon A, Golenser J, Sinay R, Tamir A, Altman A, Pollack Y, et al. Protein kinase C θ deficiency increases resistance of C57BL/6J mice to *Plasmodium berghei* infection-induced cerebral malaria. Infect Immun. 2010; 78:4195–4205. [PubMed: 20660606]

Osada S, Mizuno K, Saido TC, Suzuki K, Kuroki T, Ohno S. A new member of the protein kinase C family, nPKC θ, predominantly expressed in skeletal muscle. Mol Cell Biol. 1992; 12:3930–3938. [PubMed: 1508194]

Ou WB, Zhu MJ, Demetri GD, Fletcher CD, Fletcher JA. Protein kinase C-θ regulates KIT expression and proliferation in gastrointestinal stromal tumors. Oncogene. 2008; 27:5624–5634. [PubMed: 18521081]

Page KM, Chaudhary D, Goldman SJ, Kasaian MT. Natural killer cells from protein kinase C $\theta^{-/-}$ mice stimulated with interleukin-12 are deficient in production of interferon-γ. J Leuk Biol. 2008; 83:1267–1276.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Pardo J, Buferne M, Martinez-Lorenzo MJ, Naval J, Schmitt-Verhulst AM, Boyer C, et al. Differential implication of protein kinase C isoforms in cytotoxic T lymphocyte degranulation and TCR-induced Fas ligand expression. Int Immunol. 2003; 15:1441–1450. [PubMed: 14645153]

Parker PJ, Coussens L, Totty N, Rhee L, Young S, Chen E, et al. The complete primary structure of protein kinase C--the major phorbol ester receptor. Science. 1986; 233:853–859. [PubMed: 3755547]

Passalacqua M, Patrone M, Sparatore B, Melloni E, Pontremoli S. Protein kinase C-θ is specifically localized on centrosomes and kinetochores in mitotic cells. Biochem J. 1999; 337(Pt 1):113–118. [PubMed: 9854032]

Pfeifhofer C, Gruber T, Letschka T, Thuille N, Lutz-Nicoladoni C, Hermann-Kleiter N, et al. Defective IgG2a/2b class switching in PKC $\alpha^{-/-}$ mice. J Immunol. 2006; 176:6004–6011. [PubMed: 16670309]

Pfeifhofer C, Kofler K, Gruber T, Tabrizi NG, Lutz C, Maly K, et al. Protein kinase C θ affects Ca^{2+} mobilization and NFAT cell activation in primary mouse T cells. J Exp Med. 2003; 197:1525–1535. [PubMed: 12782715]

Pfeifhofer-Obermair C, Thuille N, Baier G. Involvement of distinct PKC gene products in T cell functions. Front Immunol. 2012; 3:220. [PubMed: 22888329]

Praveen K, Zheng Y, Rivas F, Gajewski TF. Protein kinase Cθ focusing at the cSMAC is a consequence rather than cause of TCR signaling and is dependent on the MEK/ERK pathway. J Immunol. 2009; 182:6022–6030. [PubMed: 19414753]

Quann EJ, Liu X, Altan-Bonnet G, Huse M. A cascade of protein kinase C isozymes promotes cytoskeletal polarization in T cells. Nat Immunol. 2011; 12:647–654. [PubMed: 21602810]

Quittau-Prevostel C, Delaunay N, Collazos A, Vallentin A, Joubert D. Targeting of PKCα and θ in the pituitary: a highly regulated mechanism involving a GD(E)E motif of the V3 region. J Cell Sci. 2004; 117(Pt 1):63–72. [PubMed: 14627629]

Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res. 2010; 20:1352–1360. [PubMed: 20736230]

Raychaudhuri S, Remmers EF, Lee AT, Hackett R, Guiducci C, Burtt NP, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet. 2008; 40:1216–1223. [PubMed: 18794853]

Reddy MV, Wang H, Liu S, Bode B, Reed JC, Steed RD, et al. Association between type 1 diabetes and GWAS SNPs in the southeast US Caucasian population. Genes immun. 2011; 12:208–212. [PubMed: 21270831]

Rudensky AY. Regulatory T cells and Foxp3. Immunol Rev. 2011; 241:260–268. [PubMed: 21488902]

Saibil SD, Deenick EK, Ohashi PS. The sound of silence: modulating anergy in T lymphocytes. Curr Opin Immunol. 2007; 19:658–664. [PubMed: 17949964]

Saibil SD, Jones RG, Deenick EK, Liadis N, Elford AR, Vainberg MG, et al. CD4+ and CD8+ T cell survival is regulated differentially by protein kinase Cθ, c-Rel, and protein kinase B. J Immunol. 2007; 178:2932–2939. [PubMed: 17312138]

Saito T, Yokosuka T. Immunological synapse and microclusters: the site for recognition and activation of T cells. Curr Opin Immunol. 2006; 18:305–313. [PubMed: 16616469]

Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008; 133:775–787. [PubMed: 18510923]

Salek-Ardakani S, So T, Halteman BS, Altman A, Croft M. Differential regulation of Th2 and Th1 lung inflammatory responses by protein kinase C θ. J Immunol. 2004; 173:6440–6447. [PubMed: 15528385]

Salek-Ardakani S, So T, Halteman BS, Altman A, Croft M. Protein kinase Cθ controls Th1 cells in experimental autoimmune encephalomyelitis. J Immunol. 2005; 175:7635–7641. [PubMed: 16301673]

Samelson LE. Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. Annu. Rev. Immunol. 2002; 20:371–394. [PubMed: 11861607]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Sarris M, Andersen KG, Randow F, Mayr L, Betz AG. Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. Immunity. 2008; 28:402–413. [PubMed: 18328743]

Schmidt-Supprian M, Tian J, Grant EP, Pasparakis M, Maehr R, Ovaa H, et al. Differential dependence of CD4+CD25+ regulatory and natural killer-like T cells on signals leading to NF-κB activation. Proc Natl Acad Sci USA. 2004; 101:4566–4571. [PubMed: 15070758]

Schwartz RH. T cell anergy. Annu Rev Immunol. 2003; 21:305–334. [PubMed: 12471050]

Seco J, Ferrer-Costa C, Campanera JM, Soliva R, Barril X. Allosteric regulation of PKCθ: understanding multistep phosphorylation and priming by ligands in AGC kinases. Proteins. 2012; 80:269–280. [PubMed: 22072623]

Sedwick CE, Morgan MM, Jusino L, Cannon JL, Miller J, Burkhardt JK. TCR, LFA-1, and CD28 play unique and complementary roles in signaling T cell cytoskeletal reorganization. J Immunol. 1999; 162:1367–1375. [PubMed: 9973391]

Serra C, Federici M, Buongiorno A, Senni MI, Morelli S, Segratella E, et al. Transgenic mice with dominant negative PKC-θ in skeletal muscle: a new model of insulin resistance and obesity. J Cell Physiol. 2003; 196:89–97. [PubMed: 12767044]

Shaulian E, Karin M. AP-1 as a regulator of cell life and death. Nat Cell Biol. 2002; 4:E131–E136. [PubMed: 11988758]

Sims TN, Soos TJ, Xenias HS, Dubin-Thaler B, Hofman JM, Waite JC, et al. Opposing effects of PKCθ and WASp on symmetry breaking and relocation of the immunological synapse. Cell. 2007; 129:773–785. [PubMed: 17512410]

Singleton KL, Gosh M, Dandekar RD, Au-Yeung BB, Ksionda O, Tybulewicz VL, et al. Itk controls the spatiotemporal organization of T cell activation. Sci Signal. 2011; 4:ra66. [PubMed: 21971040]

Skvara H, Dawid M, Kleyn E, Wolff B, Meingassner JG, Knight H, et al. The PKC inhibitor AEB071 may be a therapeutic option for psoriasis. J Clin Invest. 2008; 118:3151–3159. [PubMed: 18688284]

Spitaler M, Emslie E, Wood CD, Cantrell D. Diacylglycerol and protein kinase D localization during T lymphocyte activation. Immunity. 2006; 24:535–546. [PubMed: 16713972]

Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nature genetics. 2010; 42:508–514. [PubMed: 20453842]

Steinberg SF. Structural basis of protein kinase C isoform function. Physiol Rev. 2008; 88:1341–1378. [PubMed: 18923184]

Stevens L, Htut TM, White D, Li X, Hanidu A, Stearns C, et al. Involvement of GATA3 in Protein Kinase C θ-induced Th2 cytokine expression. Eur J Immunol. 2006; 36:3305–3314. [PubMed: 17111354]

Steward-Tharp SM, Song YJ, Siegel RM, O'Shea JJ. New insights into T cell biology and T cell-directed therapy for autoimmunity, inflammation, and immunosuppression. Ann N Y Acad Sci. 2010; 1183:123–148. [PubMed: 20146712]

Sun Z. Intervention of PKC-theta as an immunosuppressive regimen. Front Immunol. 2012; 3:225. [PubMed: 22876242]

Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, et al. PKC-θ is required for TCR-induced NF-κB activation in mature but not immature T lymphocytes. Nature. 2000; 404:402–407. [PubMed: 10746729]

Sutcliffe EL, Bunting KL, He YQ, Li J, Phetsouphanh C, Seddiki N, et al. Chromatin-associated protein kinase C-θ regulates an inducible gene expression program and microRNAs in human T lymphocytes. Mol Cell. 2011; 41:704–719. [PubMed: 21419345]

Szamel M, Appel A, Schwinzer R, Resch K. Different protein kinase C isoenzymes regulate IL-2 receptor expression or IL-2 synthesis in human lymphocytes stimulated via the TCR. J Immunol. 1998; 160:2207–2214. [PubMed: 9498759]

Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T, Nishizuka Y. Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J Biol Chem. 1979; 254:3692–3695. [PubMed: 438153]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Tan SL, Zhao J, Bi C, Chen XC, Hepburn DL, Wang J, et al. Resistance to experimental autoimmune encephalomyelitis and impaired IL-17 production in protein kinase C θ-deficient mice. J Immunol. 2006; 176:2872–2879. [PubMed: 16493044]

Tassi I, Cella M, Presti R, Colucci A, Gilfillan S, Littman DR, et al. NK cell-activating receptors require PKC-θ for sustained signaling, transcriptional activation, and IFN-γ secretion. Blood. 2008; 112:4109–4116. [PubMed: 18784374]

Thuille N, Heit I, Fresser F, Krumbock N, Bauer B, Leuthaeusser S, et al. Critical role of novel Thr-219 autophosphorylation for the cellular function of PKCθ in T lymphocytes. EMBO J. 2005; 24:3869–3880. [PubMed: 16252004]

Touraine JL, Hadden JW, Touraine F, Hadden EM, Estensen R, Good RA. Phorbol myristate acetate: a mitogen selective for a T-lymphocyte subpopulation. J. Exp. Med. 1977; 145:460–465. [PubMed: 299884]

Truneh A, Albert F, Golstein P, Schmitt-Verhulst AM. Calcium ionophore plus phorbol ester can substitute for antigen in the induction of cytolytic T lymphocytes from specifically primed precursors. J. Immunol. 1985a; 135:2262–2267. [PubMed: 2411789]

Truneh A, Albert F, Golstein P, Schmitt-Verhulst AM. Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. Nature. 1985b; 313:318–320. [PubMed: 3918270]

Tseng SY, Liu M, Dustin ML. CD80 cytoplasmic domain controls localization of CD28, CTLA-4, and protein kinase Cθ in the immunological synapse. J Immunol. 2005; 175:7829–7836. [PubMed: 16339518]

Tseng SY, Waite JC, Liu M, Vardhana S, Dustin ML. T cell-dendritic cell immunological synapses contain TCR-dependent CD28-CD80 clusters that recruit protein kinase C theta. J Immunol. 2008; 181:4852–4863. [PubMed: 18802089]

Valenzuela JO, Iclozan C, Hossain MS, Prlic M, Hopewell E, Bronk CC, et al. PKCθ is required for alloreactivity and GVHD but not for immune responses toward leukemia and infection in mice. J Clin Invest. 2009; 119:3774–3786. [PubMed: 19907075]

Vardhana S, Choudhuri K, Varma R, Dustin ML. Essential role of ubiquitin and TSG101 protein in formation and function of the central supramolecular activation cluster. Immunity. 2010; 32:531–540. [PubMed: 20399684]

Varma R, Campi G, Yokosuka T, Saito T, Dustin ML. T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. Immunity. 2006; 25:117–127. [PubMed: 16860761]

Verma RS, Macera MJ, Krishnamurthy M, Abramson J, Kapelner S, Dosik H. Chromosomal abnormalities in adult T-cell leukemia/lymphoma (ATL). A report of six cases with review of the literature. J Cancer Res Clin Oncol. 1987; 113:192–196. [PubMed: 2881933]

Villalba M, Altman A. Protein kinase C-θ (PKCθ), an example of a drug target for therapeutic intervention with human T cell leukemias. Curr. Cancer Drug Targets. 2002; 2:125–134. [PubMed: 12188914]

Villalba M, Bi K, Hu J, Altman Y, Bushway P, Reits E, et al. 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. 2002; 157:253–263. [PubMed: 11956228]

Villalba M, Bushway P, Altman A. PKCθ mediates a selective T cell survival signal via phosphorylation of BAD. J. Immunol. 2001; 166:5955–5963. [PubMed: 11342610]

Villalba M, Coudronniere N, Deckert M, Teixeiro E, Mas P, Altman A. A novel functional interaction between Vav and PKCθ is required for TCR-induced T cell activation. Immunity. 2000; 12:151–160. [PubMed: 10714681]

Villalba M, Kasibhatla S, Genestier L, Mahboubi A, Green DR, Altman A. Protein kinase Cθ cooperates with calcineurin to induce Fas ligand expression during activation-induced T cell death. J Immunol. 1999; 163:5813–5819. [PubMed: 10570264]

Villunger A, Ghaffari-Tabrizi N, Tinhofer I, Krumbock N, Bauer B, Schneider T, et al. 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. 1999; 29:3549–3561. [PubMed: 10556809]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Volkov Y, Long A, Kelleher D. Inside the crawling T cell: leukocyte function-associated antigen-1 cross-linking is associated with microtubule-directed translocation of protein kinase C isoenzymes βI and δ. J Immunol. 1998; 161:6487–6495. [PubMed: 9862672]

Volkov Y, Long A, McGrath S, Ni Eidhin D, Kelleher D. Crucial importance of PKC-βI in LFA-1-mediated locomotion of activated T cells. Nat Immunol. 2001; 2:508–514. [PubMed: 11376337]

von Essen M, Nielsen MW, Bonefeld CM, Boding L, Larsen JM, Leitges M, et al. Protein kinase C (PKC) α and PKC are the major PKC isotypes involved in TCR down-regulation. J Immunol. 2006; 176:7502–7510. [PubMed: 16751397]

Vyas YM, Mehta KM, Morgan M, Maniar H, Butros L, Jung S, et al. Spatial organization of signal transduction molecules in the NK cell immune synapses during MHC class I-regulated noncytolytic and cytolytic interactions. J Immunol. 2001; 167:4358–4367. [PubMed: 11591760]

Wang D, You Y, Case SM, McAllister-Lucas LM, Wang L, DiStefano PS, et al. A requirement for CARMA1 in TCR-induced NF-κB activation. Nat Immunol. 2002; 3:830–835. [PubMed: 12154356]

Wang X, Chuang HC, Li JP, Tan TH. Regulation of PKC-θ function by phosphorylation in T cell receptor signaling. Front Immunol. 2012; 3:197. [PubMed: 22798961]

Wange RL. LAT, the linker for activation of T cells: a bridge between T cell-specific and general signaling pathways. Sci STKE. 2000; 2000:RE1. [PubMed: 11752630]

Weckbecker G, Pally C, Beerli C, Burkhart C, Wieczorek G, Metzler B, et al. Effects of the novel protein kinase C inhibitor AEB071 (Sotrastaurin) on rat cardiac allograft survival using single agent treatment or combination therapy with cyclosporine, everolimus or FTY720. Transpl Int. 2010; 23:543–552. [PubMed: 20003043]

Weil R, Israel A. T-cell-receptor- and B-cell-receptor-mediated activation of NF-κB in lymphocytes. Curr Opin Immunol. 2004; 16:374–381. [PubMed: 15134788]

Xu ZB, Chaudhary D, Olland S, Wolfrom S, Czerwinski R, Malakian K, et al. Catalytic domain crystal structure of protein kinase C-θ (PKCθ). J Biol Chem. 2004; 279:50401–50409. [PubMed: 15364937]

Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, et al. TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science. 2005; 308:1626–1629. [PubMed: 15860593]

Yokosuka T, Kobayashi W, Sakata-Sogawa K, Takamatsu M, Hashimoto-Tane A, Dustin ML, et al. Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters and protein kinase C θ translocation. Immunity. 2008; 29:589–601. [PubMed: 18848472]

Yokosuka T, Kobayashi W, Takamatsu M, Sakata-Sogawa K, Zeng H, Hashimoto-Tane A, et al. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. Immunity. 2010; 33:326–339. [PubMed: 20870175]

Yokosuka T, Sakata-Sogawa K, Kobayashi W, Hiroshima M, Hashimoto-Tane A, Tokunaga M, et al. Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. Nat Immunol. 2005; 6:1253–1262. [PubMed: 16273097]

Zanin-Zhorov A, Ding Y, Kumari S, Attur M, Hippen KL, Brown M, et al. Protein kinase C-θ mediates negative feedback on regulatory T cell function. Science. 2010; 328:372–376. [PubMed: 20339032]

Zanin-Zhorov A, Dustin ML, Blazar BR. PKC-θ function at the immunological synapse: prospects for therapeutic targeting. Trends Immunol. 2011; 32:358–363. [PubMed: 21733754]

Zhernakova A, Stahl EA, Trynka G, Raychaudhuri S, Festen EA, Franke L, et al. PLoS Genet. 2011; 7:e1002004. [PubMed: 21383967]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table I

Selectivity of PKCθ Functions *in vivo*

***in vivo* immune responses**	**Role of PKCθ**	**Immune cells implicated in response**	**References**
Effector function/memory responses/ viral clearance upon LCMV infection	Dispensable	CTL, Th1	Berg-Brown et al., 2004
Neutralizing antibodies against VSV infection	Dispensable	B cells, Tfh help	Berg-Brown et al., 2004
Murine herpes virus-68 clearance, expansion of virus-specific $CD8^+$ T cells	Dispensable	CTL	Giannoni et al., 2005
Recall responses to vaccinia virus infection	Dispensable	CTL	Marsland et al., 2005
Murine CMV clearance, expansion of virus-specific $CD8^+$ T cells	Dispensable	CTL	Valenzuela et al., 2009
Effector CTL response to *Listeria Monocytogenes* (LM) infection[1]	Dispensable	CTL	Valenzuela et al., 2009
LM clearance, effector cell expansion[2]	Required	CTL, Th1	Sakowicz-Burkiewicz et al., 2008
Effector response against *T. gondii* infection, pathogen clearance	Required	Th1, CTL, Th2, B cells	Nishanth et al., 2010
Plasmodium berghei ANKA-induced Inflammatory cerebral malaria	Moderately required	Th1, CTL?	Ohayon et al., 2010
Leishmenia major clearance, effector response against infection	Dispensable (B6)	Th1	Marsland et al., 2004
	Required (Balb/C)	Th2	
Immunity to M-MuLV-induced leukemia	Required	CTL, Th1	Garaude et al., 2008
Rejection of engrafted MHC class I-negative tumors	Required	NK	Aguilo et al., 2009
Lung inflammation induced by ovalbumin administration	Dispensable	Th1[3]	Salek-Ardakani et al., 2004; Marsland et al. 2004
	Required	Th2[4]	
IgE, eosinophilia response to *N. Brasiliensis* infection	Required	Th2	Marsland et al., 2004
GvL response	Dispensable	Th1, CTL?	Valenzuela et al., 2009
Systemic GvHD	Required	Th1, CTL	Valenzuela et al., 2009
Local (footpad) host *vs*. graft response	Required	Th1, CTL	Anderson et al., 2006
Early (1–3 hr) cytokine response to anti-CD3 injection	Required	NKT?	Anderson et al., 2006
Cardiac allograft rejection	Mildly required; cooperates with PKCα	Th1, CTL	Gruber et al., 2009
	Required		Manicassamy et al., 2008
Coxsackie B3-induced autoimmune myocarditis	Dispensable	Th1	Marsland et al., 2007
α-myosin/CFA-induced experimental autoimmune myocarditis	Required	Th17	Marsland et al., 2007
Autoimmune colitis, EAE	Required	Th17, Th1	Anderson et al., 2006; Salek-Ardakani et al., 2005;Tan et al., 2006
Methylated BSA-induced arthritis	Required	Th1, Th2, B cells	Healy et al., 2006
Concanavalin A-induced autoimmune hepatitis	Required	NKT	Fang et al., 2012

[1] 2×10^3 CFU LM-Ova

[2] 5×10^4 CFU LM-Ova

[3]Ova/CFA immunization s.c.

[4]Ova/alum immunization i.p.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript