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Protein Kinase C Theta (PKCθ): A Key Player in T Cell Life and Death

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Abstract

Protein kinase C-theta (PKCθ) is a member of the novel, Ca^{2+}-independent PKC subfamily, which plays an important and non-redundant role in several aspects of T cell biology. Much progress has been accomplished in understanding the function of PKCθ in the immune system and its unique translocation to the immunological synapse in Ag-stimulated T lymphocytes. Biochemical and genetic approaches revealed that PKCθ is required for the activation of mature T cells as well as for their survival. Mutation of the *PKCθ* gene leads to impaired receptor-induced stimulation of the transcription factors AP-1, NF-κB and NFAT, which results in defective T cell activation, and to aberrant expression of apoptosis-related proteins, resulting in poor T cell survival. Furthermore, PKCθ-deficient mice display defects in the differentiation of T helper subsets, particularly in Th2 and Th17-mediated inflammatory responses. Therefore, PKCθ is a critical enzyme that regulates T cell function at multiple stages, and it represents an attractive drug target for allergic and autoimmune diseases.

Keywords

PKCθ; T cell; signal transduction; TCR; CD28

Introduction

PKCθ was isolated as a member of the Ca^{2+} independent, novel PKC (nPKC) subfamily (which also includes PKCδ, ε and η) expressed mainly in T cells, muscle cells and platelets[1-3]. Other PKC subfamilies include the conventional PKCs (cPKCs; α, β and γ), which are activated by Ca^{2+} and the second messenger diacylglycerol (DAG), and the atypical PKCs (aPKCs; λ and ι), which are not activated by Ca^{2+} or DAG. Since its discovery, PKCθ has attracted considerable interest among T cell biologists because of its unique regulation and ability to mediate non-redundant functions in T cell receptor (TCR) signaling[4-6]. Biochemical analyses suggested that PKCθ plays an important role in T cell activation. Consistent with such a role, mature T cells from PKCθ-deficient ($PKC\theta^{-/-}$) mice display defects in TCR-induced proliferation, cytokine production and differentiation. The activation of several transcription factors essential for IL-2 production and T cell proliferation is impaired in $PKC\theta^{-/-}$ T cells. In addition, recent work revealed that PKCθ is also required for the survival of activated peripheral T lymphocytes. These findings have some important implications for the control of

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certain immunological diseases and possibly cancer. In this review, we discuss recent insight on the biological roles of PKCθ and its potential as a therapeutic target.

Structure of PKCθ

PKCθ possesses several functional domains that are conserved in the Ca^{2+}-independent nPKC subfamily. It contains, beginning in its NH_2 terminus, C2-like, pseudosubstrate (PS), DAG-binding (C1), unique V3, and kinase domains. The C2-like domain is similar in its overall structure to the Ca^{2+}-binding C2 domain found in cPKCs, but it does not bind Ca^{2+}. Instead, it is thought to be involved in regulatory phospholipid binding and/or protein-protein interactions. The C2-like domain contains a tyrosine residue (Tyr-90), which is phosphorylated by the T cell tyrosine kinase Lck[7]. The PS sequence, which resembles an ideal PKC substrate with the exception that it contains an alanine residue instead of a substrate serine residue is bound to the kinase domain in the resting state. As a result, PKCθ is maintained in a closed, inactive state, which is inaccessible to cellular substrates. Upon receptor stimulation, phospholipase-γ1 (PLCγ1) is activated and hydrolyzes membrane inositol phospholipids to generate the membrane lipid second messenger DAG, which binds the C1 domain of PKCθ, thereby enhancing its plasma membrane association of PKCθ. This association and, most likely, other regulatory interactions, lead to a change in PKCθ conformation into an open, active state whereby it can now access its substrates and phosphorylate them. The precise nature of these changes, which lead to kinase activation, is not entirely clear, but phosphorylation of Tyr-90 may contribute to this process.

The unique cellular localization of PKCθ

In addition to PKCθ, T cells express several other members of the PKC family, including cPKCs (α and β), nPKCs (ε, η) and atypical PKCs (ζ)[8]. However, PKCθ displays several unique properties, which distinguish it from other T cell-expressed PKCs, prominent among which, is its unique cellular localization in defined subregions of the T cell. Thus, PKCθ is unique in its translocation to the T cell plasma membrane at the site of contact between antigen (Ag)-specific T cells and Ag-presenting cells (APCs)[9], the so-called immunological synapse (IS)[10]. The IS serves as a platform for delivering sustained activation signals required for productive T cell activation. Formation of a functional IS involves the assembly of signaling complexes consisting of TCRs, costimulatory accessory receptors (such as CD28, CD4/CD8 or LFA-1), and intracellular signaling effector proteins[11], reorganization of the actin cytoskeleton[12-14], and clustering of specialized membrane microdomains termed lipid rafts[15-17].

A more detailed analysis of the T cell-APC contact region revealed compartmentalization of molecules in at least three distinct identifiable areas of the synapse, the so called central SMAC (cSMAC), peripheral SMAC (pSMAC) and distal SMAC (dSMAC)[18, 19]. PKCθ colocalizes with the TCR in the cSMAC[18]. This localization occurs at a high stoichiometry and persists for several hours. The pSMAC is enriched with the LFA-1 integrin[18], while the dSMAC is characterized by the concentration of the CD45 phosphotyrosine phosphatase[19]. The spatial organization and stability (or duration) of the IS determine the functional outcome of TCR engagement and underlie the fundamental phenomenon of differential T cell signaling, *i.e.*, ability of the triggering of a single receptor (the TCR) to stimulate highly variable functional responses, which can range from proliferation and cytokine production to cell death. The cSMAC clustering of PKCθ correlated with its catalytic activation, and it only occurred upon productive activation of T cells, *i.e.*, upon exposure to APCs that were fed with optimal Ag concentrations leading to efficient proliferation[18]. In contrast, altered peptide ligands or low peptide concentrations that induced weak or no detectable proliferation, did not promote

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PKCθ recruitment to the cSMAC. Therefore, localization of PKCθ in the IS is highly related to T cell activation.

Our work revealed that upon Ag stimulation, PKCθ translocates to membrane lipid rafts and, furthermore, that these lipid rafts preferentially localize to the IS[20], a finding recently confirmed by another group[21]. The structural basis for the association of PKCθ with lipid rafts is unclear since PKCθ does have any obvious raft-targeting motifs such as palmitoylation sites. It is, therefore, possible that this lipid raft localization reflects the association of PKCθ with another raft-targeted signaling protein, *e.g*., the Lck tyrosine kinase[20], which is known to reside in lipid rafts by virtue of its dual acylation (myristoylation and palmitoylation), and which we found to be associated with PKCθ in stimulated T cells[7]. We also found that translocation of PKCθ to the IS and lipid rafts is enhanced by CD28 costimulation, and that it requires functional Lck but not ZAP-70[20]. Another recent study revealed that CD28 costimulation is uniquely important for the selective concentration of PKCθ in the cSMAC, while other modes of costimulation, *i.e*., by LFA-1 engagement, induced a more diffuse IS localization of PKCθ, which was less restricted to the cSMAC[22].

Unlike PKCθ, lipid rafts are not restricted to the cSMAC. Thus, the lipid raft translocation of PKCθ *per se* is unlikely to entirely account for the very specific concentration of the enzyme in the cSMAC during Ag stimulation. Nevertheless, receptor-induced clustering of lipid raft may serve as an important driving force that promotes the initial translocation of PKCθ to the IS, where additional mechanism(s) may function to selectively recruit it to the cSMAC. This notion is supported by our finding that disruption of cholesterol-rich lipid rafts by pretreatment with relatively low concentrations of β-methyl cyclodextrin, which still allow some conjugate formation between Ag-specific T cells and APCs, prevented the translocation of PKCθ to the IS[20]. Thus, raft clustering at the IS may be especially important in promoting the proper cSMAC localization of PKCθ and, perhaps, the organization of a mature IS under physiological conditions of low TCR occupancy.

Function of PKCθin T cell activation

Many studies have indicated that PKCθ plays a critical role in mature T cell activation. Activation of T lymphocytes requires sustained physical interaction of the TCR with a major histocompatibility complex (MHC)-presented peptide Ag. TCR engagement by a peptide/MHC complex transduces the signal for T cell activation. However, productive T cell activation depends on an additional signal that can be provided by a number of costimulatory receptors. The major costimulatory signal for T cell activation is provided by interaction of the T cell surface molecule CD28 with its CD80/CD86 (B7-1/B7-2) ligands on APCs[23]. The combination of signals from TCR and CD28 leads to activation of downstream transcription factor, followed by the activation of *IL-2* gene promoter. In the absence of a CD28 signal, T cells enter a stable state of unresponsiveness termed anergy[24].

The original analysis of PKCθ$^{-/-}$ T cells[25] revealed that two transcription factors, *i.e*., nuclear factor κB (NF-κB) and activator protein-1 (AP-1) are targets of PKCθ in TCR/CD28-costimulated T cells, but failed to reveal any substantial defect in the activation of another critical transcription factor, *i.e*., nuclear factor of T cells (NFAT). These transcription factors are all essential for activation of the *IL-2* gene, reflecting their obligatory binding to distinct regions in the corresponding gene promoter, including the CD28 response element (RE), which binds NF-κB and AP-1 in a combinatorial manner[26]. The PKCθ-mediated activation of these two transcription factors requires the integration of signals generated by the TCR and CD28. Later analysis of independently generated PKCθ$^{-/-}$ mice as well as further functional dissection of the original PKCθ$^{-/-}$ mice revealed, however, that Ca^{2+} signals and, consequently, NFAT activation, are also impaired in PKCθ$^{-/-}$ T cells[27, 28]. Consistent with the important role of

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PKCθ in *IL-2* promoter activation, the mature T cells of PKCθ$^{-/-}$ mice display a severe defect in TCR/CD28-induced proliferation and IL-2 secretion, defects that can largely be rescued by the addition of exogenous IL-2[25, 28].

Among the transcription factors that are positively regulated by PKCθ, the pathway leading to NF-κB activation has been analyzed in most detail. Ag receptor-mediated activation of the canonical NF-κB (NF-κB1) pathway involves the action of several adaptor proteins, including caspase recruitment domain (CARD), membrane-associated guanylate kinase (MAGUK) protein-1 (CARMA1, also termed CARD11), B-cell lymphoma-10 (Bcl10) and mucosa-associated lymphoid tissue-1 (MALT1), as well as the IKK complex[29, 30]. The latter consists of two enzymatic components, IKKα and IKKβ, and a regulatory subunit, IKKγ (also known as NEMO). Deletion of the *CARMA1* gene in mice results in impaired receptor- and PKC-mediated T cell proliferation and cytokine production resulting from a selective defect in NF-κB and JNK activation[31, 32]. CARMA1 is constitutively associated with lipid rafts, and becomes further enriched in these rafts after TCR stimulation. Two groups demonstrated that PKCθ directly phosphorylates CARMA1 *in vitro* on several serine residues in its linker region and, moreover, that mutation of two of these residues abolished the ability of CARMA1 to activate NF-κB[33, 34]. These findings indicate that phosphorylation of CARMA1 by PKCθ is crucial for NF-κB activation. PKCθ-phosphorylated CARMA1 recruits the Bcl10-MALT1 complex. The activated Bcl10-MALT1 complex then activates IKK by inducing ubiquitination of IKKγ, a process that may itself be regulated by two additional enzymes, *i.e.*, the TRAF6 ubiquitin ligase and the TAK1 Ser/Thr kinase[35]. This degradation allows 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[36].

PKCθ also plays an indirect role in activation of the non-canonical NF-κB (NF-κB2) pathway, in which the NF-κB-inducing kinase (NIK) and IKKα (but not IKKβ or γ) play an important role. In this pathway, NIK-dependent proteolytic processing of a p100 precursor generates translocation-competent p52-containing NF-κB complexes, which induce a gene program distinct from that activated by NF-κB1. Thus, we found that TCR/CD28 costimulation induced higher p52 protein levels in T cells, but this effect was secondary to enhanced *de novo* synthesis of p100, not to enhanced processing of extant p100; PKCθ deficiency impaired the signal-dependent accumulation of p52 because of defects in p100 production, which is itself activated by NF-κB1 binding to its gene promoter and, hence, is indirectly PKCθ-dependent[37].

The pathway leading from PKCθ to AP-1 activation is less clearly understood. We reported originally that AP-1 was a target of PKCθ and, moreover, that the PKCθ-mediated AP-1 activation required Ras[38]. AP-1 activation depends on several mitogen-activated protein (MAP) kinases, particularly JNK but also ERK and, perhaps, p38[39]. However, the TCR/CD28-induced activation of these three MAP kinases in PKCθ$^{-/-}$ T cells was reported to be intact[25, 28]. Nevertheless, we found more recently that PKCθ$^{-/-}$ CD8^{+} T cells displayed impaired ERK and JNK activation in response to specific Ag stimulation[40], raising the possibility that the artificially strong signal provided by costimulation with saturating concentrations of anti-CD3/CD28 antibodies masks the requirement for PKCθ in AP-1 activation.

In a search for PKCθ-interacting proteins, we recently isolated SPAK, a Ste20-related MAP3K, as a direct substrate of PKCθ in the pathway leading to AP-1, but not NF-κB, activation[41] The C-terminal domain of SPAK directly bound PKCθ, and this association was substantially enhanced by CD3/CD28 costimulation. Moreover, PKCθ, but not PKCα, directly phosphorylated SPAK *in vitro*, and Ser-311 was found to be the major phosphorylation site. In addition, the TCR/CD28-induced activation of SPAK was largely impaired in PKCθ$^{-/-}$ T cells. Lastly, transfected SPAK synergized with a constitutively active PKCθ mutant to activate

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AP-1 but not NF-κB and, conversely, siRNA-mediated silencing of SPAK expression suppressed PKCθ-mediated AP-1 (but not NF-κB) activation[41]. Thus, SPAK is most likely a mediator of PKCθ signals leading to AP-1 activation, but the intermediates mediating AP-1 activation in this pathway downstream of SPAK remain to be identified and functionally characterized.

Least understood of all is the mechanism that links PKCθ to the activation of NFAT. We found that the TCR/CD28-induced activation of Tec, a T cell-expressed member of the Tec tyrosine kinase family, which positively regulates the activation of PLCγ1 and, hence, Ca^{2+} signaling, is reduced in PKCθ$^{-/-}$ T cells[42]. Consistent with this result, the inducible tyrosine phosphorylation of PLCγ1, which reflects its catalytic activation, was also inhibited in PKCθ$^{-/-}$ T cells. Furthermore, we documented (by coimmunoprecipitation) an association between PKCθ and Tec[42]. On the basis of these results, we proposed that there exists a positive regulatory feedback loop, in which PKCθ, whose membrane recruitment requires binding of its cysteine-rich C1 domain to membrane-localized DAG generated by activated PLCγ1, feeds back to associate with and sustain the activation of Tec (and/or another Tec family member), thereby promoting PLCγ1 activity that, in turn, generates IP_3, the second messenger initiating the Ca^{2+} response.

Regulation of PKCθ localization and activation

The mechanism responsible for the selective localization and function of PKCθ in T cells is not entirely clear. Similar to cPKCs and other nPKCs, PKCθ contains a cysteine-rich C1 domain that binds the membrane-localized second messenger DAG. The recently reported presence of a high local DAG concentration in the IS[43] may facilitate recruitment of PKCθ to the IS upon Ag stimulation. However, it does not easily explain why other DAG-binding PKCs, which are expressed in T cells, are not recruited to the IS under similar conditions. Therefore, PKCθ is likely to be regulated by some unique pathway, which does not operate on other PKCs. Such a pathway may be dictated by the presence of specific protein- or lipid-interacting sequences in PKCθ. This notion is consistent with observations that the C2-like domain of nPKCs can function as a protein-protein interaction domain. Thus, interaction of the C2 domain of PKCθ with a specific receptor for activated C kinase (RACK) protein[44] could play an important role in recruiting PKCθ to the IS, thereby allowing it to mediate its specific functions in T cells. Furthermore, the C2-like domain of PKCδ, which shares substantial homology with PKCθ, was recently found to constitute a novel phosphotyrosine-binding domain, and the amino acids critical for pTyr binding in PKCδ are conserved in PKCθ[45]. Lck-mediated phosphorylation of PKCθ on Tyr-90[7] may also create a binding site for a partner having an SH2 or a pTyr-binding (PTB) domain.

In searching for a mechanism that regulates the localization and activity of PKCθ, we found that PKCθ is regulated by Vav1, a guanine nucleotide exchange factor for Rac and Cdc42 that plays an important role in T cell development and activation[46]. Thus, a dominant negative PKCθ mutant blocked a number of growth signals, which are normally induced by overexpression of Vav1, namely, activation of JNK, the *IL-2* gene promoter and NFAT or AP-1 reporter genes. Conversely, a dominant negative Vav1 mutant did not significantly inhibit the same signaling events induced by a constitutively active PKCθ mutant[46], tentatively placing PKCθ downstream of Vav1 in these growth signaling pathways.

Since activation of phosphatidylinositol 3-kinase (PI3-K) has been linked to Vav1 in T cells, we also addressed the role of PI3-K in the activation of PKCθ. A selective pharmacological inhibitor of PI3-K, LY294002, effectively inhibited the anti-CD3/CD28-induced membrane translocation of PKCθ in peripheral blood T cells. In contrast, several strategies, which inhibit PLCγ1, were less effective in this regard[47]. One potential PI3-K target that could play a role

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in the membrane recruitment of PKCθ is PI3-K-dependent kinase-1 (PDK1), which associates with, and is responsible for activation loop phosphorylation of, different PKC enzymes[48]. Therefore, a potential scenario can be envisaged, in which PKCθ associates with PDK1, and is then recruited to the membrane. Such a scenario is supported by a report that, upon its stimulus-induced translocation to the T cell IS, PDK1 functions as a scaffold to recruit PKCθ and CARMA1 on one hand, and the Bcl10-MALT1 complex on the other, thereby bringing the components essential for NF-κB activation to the same microenvironment in the T cell membrane to enable NF-κB1 activation[49]. However, these findings need to be interpreted with caution given the report that transphosphorylation of PKCθ on Thr-538, which was considered in that study to be a marker of PKCθ activation, actually appears to be a constitutive event, which can also be found in naïve, resting T cells[50]. Moreover, we found that coexpression of PDK1 with PKCθ did not enhance the PKCθ-induced activation of NF-κB and AP-1 reporter genes[47]. Therefore, the role of PDK1 in regulating PKCθ localization and activation remains questionable, although it may be involved in the early maturation of PKCθ by phosphorylating it at Thr-538.

PKCθ substrates

Understanding of the biological functions of PKCθ in T cells and other tissues where it is expressed would benefit highly from the identification and characterization of its physiological substrates. However, as with other members of the PKC family, progress in this regard has been relatively slow. The ability of PKCθ to phosphorylate a given protein *in vitro* does not necessarily indicate biological relevance since kinases tend, in general, to be more promiscuous *in vitro* than in intact cells, reflecting spatiotemporal restrictions placed on the accessibility of substrates to their phosphorylating enzymes in the cellular milieu. On the other hand, the ability of an ectopically expressed kinase to phosphorylate a protein in intact cells or, conversely, impaired phosphorylation of a given cellular protein in cells lacking a certain kinase does not necessarily indicate that the kinase in question directly phosphorylates the protein, since that kinase could activate another, downstream kinase, which then phosphorylates the substrate proteins. Therefore, identification of physiological kinase substrates requires a combination of independent *in vitro* and cellular approaches, prominent among which are the use of animals or cells genetically deficient for a defined kinase, advanced phosphoproteomic approaches, and bioinformatic tools that can predict consensus sites recognized by given kinases. In that regard, consensus phosphorylation sites by most members of the PKC family have been determined and, in general, they depend on the presence of positively charged amino acid residues immediately upstream of the phosphorylated Ser/Thr residues[51].

Recent studies have indicated several potential candidate substrates of PKCθ. The PKCθ-mediated phosphorylation of SPAK[41] and CARMA1[52], which is required for proper activation of the AP-1 and NF-κB signaling pathways, respectively, in T cells, has been discussed earlier. The first PKCθ substrate to be identified was moesin[53], a member of the ezrin-radixin-moesin (ERM) family of cytoskeletal proteins, which serve to link intracellular signaling proteins to the actin cytoskeleton. Interaction of ERM proteins with actin is regulated by phosphorylation of a conserved Thr residue near their C-terminus, and that conserved residue (Thr-558) was found to be phosphorylated by PKCθ[53]. Of interest, other tested PKC enzymes did not phosphorylate moesin[53]. However, it is not clear whether PKCθ phosphorylates moesin (or other ERM proteins) in intact T cells and, if so, whether other kinases can phosphorylate ERM proteins. The biological role of moesin phosphorylation in the context of T cell activation is not clear, but it may be involved in remodeling of the actin cytoskeleton, which occurs following the interaction of an Ag-specific T cells with an APC and the organization of the IS, given the findings that upon assembly of the IS, ERM proteins translocate to the T cell membrane at a site opposite the IS[54-56].

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PKCθ may potentially regulate actin cytoskeleton remodeling in Ag-stimulated T cells by phosphorylating another substrate, *i.e.*, WASP-interacting protein (WIP)[57]. Actin polymerization following TCR engagement is dependent on Wiskott-Aldrich syndrome protein (WASP), which is one of the effectors of the small GTPase Cdc42, and is critical for T cell activation[58]. WIP associates with WASP and thereby inhibits its Cdc42-mediated activation. TCR engagement was found to lead to PKCθ-dependent phosphorylation of WIP, most likely on Ser-488, resulting in dissociation of WIP from WASP and, consequently, WASP activation[57]. The importance of PKCθ for WIP phosphorylation was evident from the finding that WIP phosphorylation was largely impaired in PKCθ$^{-/-}$ T cells[57].

Insulin receptor substrate 1 (IRS1), an adapter protein, which links the insulin receptor to its downstream effectors, is another potential substrate of PKCθ[59]. Obesity and stress inhibit insulin action by activating protein kinases that phosphorylate IRS1 on serine and have thus been linked to insulin resistance and the development of type 2 diabetes. A recent study demonstrated that PKCθ phosphorylates IRS1 on Ser-1101, thereby blocking its tyrosine phosphorylation and downstream activation of the Akt pathway. Mutation of Ser-1101 to alanine rendered IRS1 insensitive to the effect of PKC and restored insulin signaling in cultured cells[59]. These findings are consistent with another report demonstrating that PKCθ$^{-/-}$ mice are protected from fat-induced insulin resistance in their muscle tissue[60], and suggest that PKCθ is a potential therapeutic target for the treatment of type 2 diabetes.

Lastly, PKCθ most likely phosphorylates itself on several residues in a manner that positively regulates its activity and/or localization. The potential autophosphorylation sites include Ser-676 (in a turn motif) and Ser-695 (in a hydrophobic motif) near the C-terminus[61], and Thr-219, which is located between the two tandem lipid-binding C1 domains of PKCθ[62].

Functions of PKCθ in vivo and its role in T cell differentiation

Based on the grossly impaired activation of PKCθ$^{-/-}$ T cells stimulated *in vitro* with anti-CD3/CD28 antibodies[25, 28] it could perhaps be anticipated that PKCθ$^{-/-}$ mice would display a severe depression in their ability to mount immune responses *in vivo*. This question remained unresolved for quite some time after PKCθ$^{-/-}$ mice were first generated and characterized. However, beginning in 2004, various studies have analyzed the effects of PKCθ deletion on *in vivo* immune functions. Surprisingly, these studies demonstrated a differential requirement for PKCθ in different immune responses. In particular, analysis of antiviral responses (which depend on Th1/Tc1 cells) revealed that PKCθ$^{-/-}$ mice generated intact lymphocytic choriomeningitis virus (LCMV)-specific primary and recall cytotoxic T lymphocyte (CTL) responses as well as an antiviral (vesicular stomatitis virus)) antibody response following virus infection[63]. However, when the mice were immunized with gp33, a peptide representing an immunodominant CTL epitope of the LCMV glycoprotein, late peptide-specific proliferative and CTL responses were reduced but could be rescued by IL-2 addition[63]. Similar results were obtained in another study, which, in addition to LCMV, also analyzed *in vivo* responses to influenza and vaccinia virus infection[64]. Similarly, the ability of PKCθ$^{-/-}$ mice to clear MHV-68 infection, maintain long-term control of latency and generate CTL responses was also largely normal, although antiviral antibody and T helper (Th) cell cytokine production were significantly lower in PKCθ$^{-/-}$ mice[65]. These findings suggest that alternative signals, *e.g.*, innate immunity, compensate for the lack of PKCθ *in vivo* and allow an adequate protective response. Indeed, a more recent study demonstrated that increased activation signals delivered *in vivo* by highly activated dendritic cells, as present during viral infections, overcame the requirement for PKCθ during CD8^{+} T cell antiviral responses[64].

Two groups reported that PKCθ is required for developing Th2-dependent immunity *in vivo*, including asthma, but is less critical for Th1 responses[66, 67]. The first study documented

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severely impaired airway hyperresponsiveness (AHR), eosinophilia, IgE and Th2 cytokine responses to inhaled Ag or to *Nippostongylus brasiliensis* infection, but intact Th1-mediated protection against *Leishmania major* infection[66]. Reduction in IL-4, but not IFNγ, expression was also observed in Ag-primed and restimulated TCR-transgenic PKCθ$^{-/-}$ T cells, and exogenous IL-2 partially restored IL-4 production. Similarly, we found that PKCθ$^{-/-}$ mice were strongly compromised in generating Th2 cells, and exhibited reduced airway eosinophilia, AHR, mucus production and Th2 cytokines in the lungs[67]. However, leukocyte infiltration and Th1 cytokine production were largely intact in a Th1-mediated lung inflammation. The requirement for PKCθ in development of Th2 responses may reflect its important role in upregulating the expression of GATA-3, the master transcription factor that promotes Th2 differentiation and development[68]. Indeed, it was recently found that the expression of GATA-3 was significantly reduced in PKCθ$^{-/-}$ T cells and, furthermore, that overexpression of GATA-3 by retroviral infection rescued the Th2 defect manifested by these cells[69].

Although several studies demonstrated that PKCθ plays a less important role in Th1 responses[66, 67], and we reported that Ag-stimulated CD8^{+} T cells produce relatively intact levels of IFNγ (but greatly reduced IL-2 and TNFα levels)[40], several recent studies demonstrated that PKCθ$^{-/-}$ mice immunized with myelin oligodendrocyte glycoprotein (MOG) were resistant to the development of clinical experimental autoimmune encephalitis (EAE)[70-72] and to Ag-induced arthritis[73], diseases traditionally considered to be Th1-mediated. CD45RB^{high} T cells from PKCθ$^{-/-}$ mice were also found to be deficient in their ability to induce another type of an experimental autoimmune disease, considered to be a model of inflammatory bowel disease, in recipient SCID mice[72]. A potential resolution of this apparent discrepancy comes from the recent discovery of a novel subset of Th cells, *i.e.*, IL-17-producing Th17 cells, which represent a distinct lineage from Th1 and Th2 cells, and have now been shown to be essential for the development of inflammatory autoimmune diseases[74, 75]. Development of Th17 cells depends on cytokine signals provided by TGFβ and IL-6[76] and, moreover, the cytokine products of Th1 and Th2 cells antagonize the development of Th17 cells[74, 75]. Indeed, the resistance to EAE development in PKCθ$^{-/-}$ mice was associated with impaired IL-17 expression *in vitro* and *in vivo*[71]. Therefore, analysis of the mechanism through which PKCθ controls the development, expansion and/or function of Th17 cells is likely to reveal important clues about the molecular basis of Th17-mediated inflammatory diseases and potentially provide novel drug targets for these diseases. Nevertheless, the possibility that under certain immunization conditions, Th1 responses may also be moderately reduced as a result of PKCθ deletion cannot be discounted, consistent with findings of reduced expression of IFNγ and T-bet[73], the Th1-inducing master transcription factor[77].

In addition to impaired responses in experimental models of autoimmune diseases, PKCθ$^{-/-}$ mice also display reduced expression of several cytokines (IL-2, IL-4, IFNγ, and TNFα) at early times (1-3 hours) after *in vivo* intraperitoneal challenge with an anti-CD3 antibody[72]. This defect was evident both by reduced plasma cytokine levels and spleen mRNA levels. This rapid cytokine response is most likely mediated by rapidly activated NKT cells. However, despite the finding that PKCθ is required for thymic NKT cell development, the number of peripheral NKT cells was not affected by PKCθ deletion[78].

In summary, the ability of innate immunity signals provided by viruses to overcome the requirement for PKCθ in antiviral immunity on one hand, and the apparent critical role of PKCθ in Th2- and Th17-mediated responses suggests that pharmacological approaches to inhibit PKCθ activity may be beneficial in treatment of allergic and autoimmune diseases, without compromising the ability of patients to mount antiviral responses.

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PKCθ and T cell survival

Members of the PKC family can mediate anti-apoptotic signals in different cell types. Given the fact that CD28 costimulation can provide a survival signal that protects T cells from apoptosis[79], PKCθ may also provide a T cell survival signal. This question was initially analyzed in transformed T cells through the use of PKCθ mutants and a pharmacological PKCθ inhibitor (rottlerin), and it was found that PKCθ promotes T cell survival and antagonizes apoptotic signals[80, 81], predominantly by phosphorylation and inactivation of BAD[81]. Indeed, a very recent study confirmed the phosphorylation of BAD by PKCθ and documented an interaction between these two proteins[77].

Later on, we showed that PKCθ is essential for the survival of activated primary T cells[40]. Somewhat unexpectedly, the Ag-induced proliferation of TCR-transgenic PKCθ$^{-/-}$ T cells assessed by CFSE dilution was intact, but their differentiation into effector CTLs was nevertheless grossly defective, reflecting enhanced and accelerated apoptosis. This poor survival correlated with reduced expression of the survival-promoting proteins Bcl-2 and Bcl-xL and, conversely, with abnormally high basal expression of the related, pro-apoptotic protein Bim[40]. Forced retroviral expression of Bcl-xL or Bcl-2 partially rescued the survival of PKCθ$^{-/-}$ T cells. Some, but not all, survival defects were secondary to the impaired production of IL-2 (and perhaps other survival-promoting cytokines) by PKCθ$^{-/-}$ T cells[40]. A more recent study similarly found that PKCθ-mediated signals enhance CD4^{+} T cell survival by upregulating Bcl-xL expression[82]. The important role of PKCθ as a T cell survival factor suggests that pharmacological inhibition of PKCθ may promote apoptosis of activated, self-reactive T cells, which mediate autoimmunity. This notion is consistent with findings that a general PKC inhibitor (bisindolylmaleimide VIII) promoted apoptosis of activated, but not resting, T cells in a Fas-dependent manner and, in parallel, prevented the development of two experimental autoimmune diseases in rats, *i.e.*, EAE and adjuvant arthritis[79]. In addition, given the often abundant expression of PKCθ in transformed T cells (leukemias and lymphomas), PKCθ inhibitors may also facilitate the elimination of malignant T cells by promoting their apoptosis[76, 80].

Conclusions

Past studies have established the important place of PKCθ in controlling several fundamental processes in T cell biology, including integration of TCR and CD28 signals leading to activation of transcription factors (NF-κB and AP-1), which are essential for productive T cell activation and proliferation, differentiation and effector function of Th subsets, particularly Th2 and Th17 cells, and T cell survival. One of the most significant observations resulting from the analysis of *in vivo* immune functions of PKCθ$^{-/-}$ T mice is the finding, reported independently by several groups, that despite its important role in TCR/CD28-induced T cell activation, PKCθ is not required for mounting effective antiviral responses[63-65], most likely reflecting the ability of innate immunity-activating signals generated by infectious viruses to overcome the requirement for PKCθ[64]. This finding implies that pharmacological inhibition of PKCθ may represent a therapeutically valuable approach to block undesired, pathological immune responses such as Th2-mediated allergic inflammation and Th17-mediated autoimmune diseases without interfering with protective antiviral responses. Selective inhibitors of the catalytic activity of PKCθ may represent one approach to accomplish such inhibition, but approaches that interfere with the specific recruitment of the enzyme to the IS and/or its interaction with regulatory proteins also offer substantial promise. However, in order to fully realize the potential of PKCθ as a drug target, it would be important to elucidate the mechanisms that control the activation and unique localization of PKCθ, including the regulatory function of its C2-like domain, identify its physiological substrates, and expand the

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analysis of the effects of *PKC*θ deletion to additional models of immunologically relevant diseases.

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