PKC-theta in regulatory and effector T-cell functions

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One of the major goals in immunology research is to understand the regulatory mechanisms that underpin the rapid switch on/off of robust and efficient effector (Teffs) or regulatory (Tregs) T-cell responses. Understanding the molecular mechanisms underlying the regulation of such responses is critical for the development of effective therapies. T-cell activation involves the engagement of T-cell receptor and co-stimulatory signals, but the subsequent recruitment of serine/threonine-specific protein Kinase C-theta (PKC-θ) to the immunological synapse (IS) is instrumental for the formation of signaling complexes, which ultimately lead to a transcriptional network in T cells. Recent studies demonstrated that major differences between Teffs and Tregs occurred at the IS where its formation induces altered signaling pathways in Tregs. These pathways are characterized by reduced recruitment of PKC-θ, suggesting that PKC-θ inhibits Tregs suppressive function in a negative feedback loop. As the balance of Teffs and Tregs has been shown to be central in several diseases, it was not surprising that some studies revealed that PKC-θ plays a major role in the regulation of this balance. This review will examine recent knowledge on the role of PKC-θ in T-cell transcriptional responses and how this protein can impact on the function of both Tregs and Teffs.

Keywords: PKC-θ, immune synapse, regulatory T cells, effector T cells, immune interventions

INTRODUCTION

Current global health challenges demand not only more effective and safer therapies to dampen undesired immune responses as in autoimmune diseases, inflammation, and transplant rejection, but also aim at boosting desired responses such as in cancer and infections. Hence, a major goal of immunology research has been to understand the regulatory mechanisms that underpin the rapid switch on/off of robust and efficient effector (Teffs) or regulatory (Tregs) T-cell responses. Thus, understanding the molecular mechanisms underlying the regulation of such responses is critical for the development of effective therapies.

Strong T-cell activation involves the engagement of T-cell receptor (TCR) and co-stimulatory signals. Subsequent recruitment of the serine/threonine-specific protein Kinase C-theta (PKC-θ) to the immunological synapse (IS) is instrumental for the formation of CARMA/BCL10/MALT (CBM) signaling complex in the cytoplasm (1–4). PKC-θ is the first PKC family member described to be recruited to the IS (5) and it plays an integral role in activating a range of signaling cascades that ultimately results in a transcriptional network in T cells. More recently, PKC-η was also described in immune synapse (6, 7) as well as PKC-ε (8).

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recruitment of PKC-θ (9), suggesting that PKC-θ inhibits Tregs suppressive function in a negative feedback loop.

Expression level and stability of the transcription factor Foxp3 (forkhead box P3) are known to be critical for the development and function of bona fide Tregs. However, recent comprehensive analyses such as genome-wide and proteomics analysis revealed possible involvement of other molecular mechanisms in the development of Tregs. Fu and colleagues reported that combinations of Foxp3 with other transcription factors are able to induce a common Treg-type gene expression pattern, which cannot be achieved solely by Foxp3 (10). Molecules such as Smad3, NFAT, and AP-1 have been identified to initiate and/or enhance Foxp3 transcription. While some gene expression in Tregs is directly modulated by the binding of Foxp3 to their promoters or enhancers, other gene expression requires interaction of Foxp3 with other transcription factors. It remains to determine whether PKC-θ can directly modulate Foxp3 transcription to then inhibit Tregs suppressive activity or requires implication of other transcription factors.

Signaling kinases have emerged as a new class of chromatin-associated enzymes that act as an intermediary between cytoplasmic and chromatin modifications. This is exemplified by Hog1 in yeast or the human homolog, p38α, which activates target gene expression during mitotic stress by interacting with ATP-dependent chromatin remodelers and other kinases, e.g., MSK1/2 to phosphorylate H3 Ser10 and 28 (11). Due to their nuclear-localizing signal (NLS) (12), PKC family members represent a novel class of chromatin-associated kinases that alternate between the cytoplasm and nucleus (13–16). Their role in T-cell transcriptional responses needs to be unequivocally proven. Therefore, further investigations are needed.

**PKC-θ and T-Cell Responses: Importance of Immune Synapse Formation**

Despite the fact that different PKC isoforms, including PKC-α, δ, ε, η, and θ, are expressed at various levels in T cells (26, 27), PKC-θ is the most studied protein kinase. Its presence in the IS following antigen stimulation of T cells is well determined (28). However, besides PKC-θ, PKC-η, and PKC-ε are both present in IS (6–8). The role of PKC-θ is getting more attention in the recent years as reviewed recently elsewhere (29).

A digital three-dimensional imaging analysis during T cell and antigen-presenting cells (APCs) interactions revealed a bull’s eye structure of the IS. Three distinct subregions were identified: central supramolecular activation cluster (cSMAC), peripheral SMAC (pSMAC), and distal SMAC (dSMAC) (30, 31). An intermediate ring of pSMAC surrounds the central core cSMAC, which is enriched with cognate integrin lymphocyte function-associated antigen 1 (LFA-1, also known as α4β7 integrin) and intercellular adhesion molecule 1 (ICAM-1) (32).

The initial signaling from TCR/CD28 stimulation was found to induce the DAG accumulation on plasma membrane, which recruits PKC-θ to the IS by binding to its C1 domains (24). However, DAG–C1 domain interaction itself does not seem sufficient for the selective translocation of PKC-θ to the cSMAC. Other coeffect engagement is required for the selective PKC-θ recruitment to cSMAC (Figure 2). Particularly, Lck tyrosine kinase plays an essential dual role in regulating CD28–PKC-θ complex formation and PKC-θ conformational change in this IS translocation. First, Lck serves as a linker/adaptor in CD28–PKC-θ complex. Also, Src-homology (SH)2 or SH3 domains in Lck is associated with a phosphotyrosine (pTyr)-containing C-terminal proline (Pro)-rich motif (Pro–pTyr188–Ala–Pro) in mature CD28 (1, 33). More recently, Kong et al. have also shown that Pro-rich motif within the V3 domain binds to the SH3 domain of Lck (18). Therefore, the most likely binding model was suggested as a PKC-θ/Lck/CD28 trimolecular signaling complex, in which the SH2 domain of Lck...
interacts with phosphorylated Tyr-188 of the CD28 cytoplasmic tail and the SH3 domain of Lck binds to Pro-rich motif in the V3 domain of PKC-θ (32). Moreover, when PKC-θ V3 pro-rich motif is mutated, it disrupts the CD28–PKC-θ complex formation and impairs PKC-θ-mediated downstream T-cell activation and differentiation in Th2 and Th17 cells (18). In addition, Lck has also shown the ability of directly phosphorylating PKC-θ at Tyr-90 in its C2-like domain both in vitro and in vivo (1). However, there is no direct evidence to show that Tyr-90 phosphorylation participates in PKC-θ conformational alteration and kinase activation. Along with DAG binding and Tyr-90 phosphorylation initiated active conformation of PKC-θ, Thr-538 is directly phosphorylated by GLK in the activation loop that is responsible for the stability of PKC-θ active conformation. Although Thr-538 may not directly regulate the IS recruitment of PKC-θ, it enables the accessible kinase domains to undergo autophosphorylation at other phosphorylation site, such as Thr-219, which is required for proper translocation of PKC-θ to the cSMAC (24, 34).

In contrast to the long-lived symmetric IS formation that is required for productive T-cell activation and differentiation, transient asymmetric synapse is able to induce T-cell anergy in response to weak signal input, such as in the absence of CD28 co-stimulatory signal (35). The conversion between IS and kinapses was found to be regulated by PKC-θ and Wiscott Aldrich Syndrome protein (WASp). PKC-θ negatively controls the stability of IS, while WASp restores the IS in the absence of PKC-θ activity (36).

It is well documented that PKC-θ plays a critical role in T-cell activation, proliferation, and differentiation. Ex vivo studies have shown that PKC-θ is involved in the activation of NF-κB, activation protein-1 (AP1), and nuclear factor of T cells (NFAT) (37–41). In resting T cells, NF-κB is sequestered in the cytoplasm by IκB that binds to its NLS. Upon the TCR/CD28 activation, PKC-θ phosphorylates membrane-associated guanylate kinase (MAGUK) domain-containing protein 1 (CARMA1) on its serine residues, resulting in the recruitment of B-cell lymphoma/leukemia 10 (BCL10) and mucosa-associated lymphoid tissue 1 (MALT1) to form an active CARMA1–BCL10–MALT1 signaling complex. Then it promotes the activation of IKK complex to phosphorylate the inhibitory IκB for its degradation, leading to NF-κB nuclear translocation for transcriptional programs required for T-cell activation (38). Recently, PKC-θ has been identified as an essential component in OX40 signalosome, containing molecules such as OX40, TRAF2, RIP2, IKKα/β/γ, as well as the CBM complex. This process has been shown to be independent of TCR engagement (42).

Although the importance of PKC-θ catalytic activity is extensively addressed in T cells, the chromatin-associated role of this signal transduction kinase is still poorly understood. Upon T-cell activation, PKC-θ is translocated to the nucleus via NLS (12), forming an active chromatin-anchored complex that includes RNA polymerase II, the histone kinase MSK-1, lysine specific demethylase 1 (LSD1), and the adaptor molecule, 14-3-3ζ. This complex then localizes to the proximal promoter and coding regions of inducible immune responsive genes in

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**FIGURE 1 | PKC-θ/PKC family structure important to its catalytic modulation and cellular translocation.**

- **Y90 phosphorylation site**: Required for PKC-θ recruitment to the IS by Lck phosphorylation.
- **T219 autophosphorylation site**: Required for membrane translocation of PKC-θ necessary for downstream signalling.
- **T538 phosphorylation site in the activation loop**: Interacts with αC helix to maintain the active formation of kinase domain of PKC-θ, it is essential for PKC-θ activation by GLK phosphorylation.
- **S676 autophosphorylation site in turn motif**: May regulate PKC-θ catalytic activity.
- **S695 autophosphorylation site in hydrophobic motif**: Required for PKC-θ catalytic activity, and enhances T-cell activation.

**Diagram:**
- Proteins and motifs involved in PKC-θ signaling.
- Key phosphorylation sites and their roles.
- Diagrammatic representation of PKC-θ structure and functional domains.
human T cells (16). Moreover, the formation of this nuclear PKC-θ-containing transcriptional complex at regulatory regions of gene targets is persistent, which contrasts to its rapid association with signaling molecules at the IS. In addition, a chromatin immunoprecipitation (ChIP)-on-ChIP assay showed that PKC-θ also negatively regulates a distinct cluster of microRNA transcription by tethering at their promoter regions (16). More recent studies indicated that chromatin-associated NF-κB is required for the assembly of PKC-θ-containing active transcription complex. Moreover, NF-κB negatively regulates miR-200 transcription by forming a repressive complex on target gene impeding the formation of PKC-θ active transcription complex (43). However, further studies are required to determine the functional differences between cytoplasmic and nuclear-targeted PKC-θ regulation and their contribution to transcriptional regulations in T cells.

The role of PKC-θ is evidently more diverse since in vivo studies on Prkcq−/− mice in different disease models showed differential requirements by distinct T-cell subsets. The studies have shown that PKC-θ is dispensable for the differentiation and effector function of Th1 cells (44, 45). PKC-θ-deficient mice showed intact CTL responses against intracellular bacterium Leishmania major (44), LCMV (46), and murine gamma-herpesvirus 68 infection (47). However, Th2 cell proliferation and differentiation is significantly defective in Prkcq−/− mice, which may reflect its important role in upregulating the GATA-3 expression (48). PKC-θ was also found to be essential for the induction of effective Th2 response against allergens or helminth infection (44). More recent studies showed that PKC-θ was required in the induction of graft-versus-host disease (GvHD) and alloreactive T-cell mediated immune response. However, it was dispensable for inducing graft-versus-leukemia (GvL) response in bone marrow transplantation (BMT) mice model (49). In sharp contrast to the positive role of PKC-θ in the promotion of Th2 effective immune response, recent studies indicated that PKC-θ negatively regulates the Tregs cells suppressive function.

**PKC-θ AND Tregs**

Regulatory T cells play a pivotal role in immune homeostasis. This CD4+ T-cell subset is important in autoimmunity prevention but also for avoiding any exaggerated immune responses that would be harmful to the host (50). Tregs could also be deleterious as is the case in cancer where the suppression of effector T cells (Teffs) responses might lead to tumor growth (51). In case of HIV infection, their role is ambiguous. In this context, the suppression of immune activation is considered beneficial while the suppression of HIV-specific responses, deleterious (52). Tregs develop in the thymus but some are induced in the periphery following differentiation of naïve CD4+ T cells after acquiring appropriate signals. The expression of transcription factor FoxP3 as well as several other molecules such as CTLA-4, CD39, and CD25 (IL-2Rα) are associated with Tregs function (53). Interleukin 2 (IL-2) is crucial for their expansion, survival as well as function (54). As is the case for Teffs, Tregs also need TCR stimulation to exert their function...
through contact-dependent mechanisms (55). However, the signaling pathways in Tregs are slightly different from Teffs. A recent study which analyzed kinase-based signaling networks in Tregs and Teffs reported that only 11 out of 185 kinases are differentially expressed between these two subsets (56). Although it is thought that PKC-θ plays an important role in Tregs, its function has not been extensively studied. By far, the most comprehensive analysis of its role comes from Zanin-Zhorov et al. (9). To study signaling, Gupta et al. (57) who used a knock-out mice model to study the role of PKC-θ in Tregs function. This study revealed that Tregs from PKC-θ-deficient mice were equally suppressive to Tregs from wt mice (57). However, they showed that PKC-θ is important in Tregs development as they were significantly reduced in PKC-θ-deficient mice. This deficiency is probably due to the enhancing effect PKC-θ has on Foxp3 expression, through calcineurin/NFAT pathway (57). The other possibility is the reduced IL-2 production by Teffs in PKC-θ-deficient mice that impacts Tregs survival, proliferation, and function (54). These discrepancies between the two studies are most probably due to major differences in the model/system used. Also, targeted inhibition, either by PKC inhibitor or siRNA, which was used by Zanin-Zhorov and colleagues, might have not been specific only for PKC-θ but influenced other pathways that could have impacted Tregs' function (58).

As mentioned before, Tregs can be of thymic origin but can also be induced in the periphery iTregs. Ma et al. (59) studied the role of PKC-θ in the development of iTregs and found that PKC-θ-mediated signals through Akt-Foxo1/3A pathway inhibit the differentiation of iTregs from naïve CD4+ T cells in the presence of TGF-β. Blocking PKC-θ either by an inhibitor or by gene knockdown reversed the inhibition of iTregs differentiation. Altogether these studies suggest an important role for PKC-θ in fine-tuning the balance between Teffs and Tregs responses (Figure 3). This makes this protein kinase an attractive drug target, a feature that will be further discussed in another chapter below. In addition, the association between kinase PKC-η with CTLA-4 and its recruitment to the Tregs’ IS has recently been described (60). Defective activation of this complex in PKC-η-deficient Tregs cells was associated with reduced depletion of CD86 from APCs by Tregs. These results reveal a CTLA-4-PKC-η signaling axis required for contact-dependent suppression and implicate this pathway in the regulation of the balance between regulatory and effector mechanisms in different diseases.

**ROLE OF PKC-θ IN HUMAN DISEASES**

As discussed above, PKC-θ impacts on the function of both Tregs and Teffs. As the balance of these subsets has been shown to be central in several diseases, it was not surprising to find that PKC-θ plays a major role in these processes.

**Autoimmunity**

Autoimmunity often results from an aberrant immune response following the activation of self-reactive T cells. Very valuable information about the implication of PKC-θ in autoimmune diseases came from PKC-θ-deficient mice. It was shown in several studies that PKC-θ-deficient mice were resistant to experimental autoimmune encephalomyelitis after injection of myelin oligodendrocyte glycoprotein (MOG) (61–63). These mice presented less T-cell infiltration as well as diminished production of proinflammatory cytokines IFN-γ, TNF, and IL-17 following the immunization. Similar role was observed in other autoimmune syndromes such as collagen-induced arthritis (64), colitis (9, 62), and myosin-induced autoimmune myocarditis (65). Despite the discrepancies reported from the studies on the role of PKC-θ in Tregs suppressive function (9, 57), it has been reported that in the colitis model, the blockage of Tregs’ PKC-θ is highly protective (9). Interestingly, when Teffs were treated with the same inhibitor before transfer, mice were not protected from colitis indicating the preferential role for Tregs-mediated suppression of the disease through PKC-θ pathway. These findings generated large interest to study PKC-θ in human autoimmune diseases. Genome-wide association studies (GWAS) identified specific single nucleotide polymorphisms (SNP) within Prkcq locus associated with type 1 diabetes (T1D), rheumatoid arthritis (RA), and celiac disease (66–69). Recent findings showed an important impairment of Tregs in RA patients (70). Zhanin-Zhorov et al. used samples from patients with various diseases’ severity and isolated Tregs. They showed that inhibition of PKC-θ increased the suppressive
activity of these cells (9). Moreover, they found that PKC-θ inhibition renders Tregs resistant to inhibition by TNF-α that is known to inhibit Tregs activity by down-regulating FoxP3 (70).

**Cancer**

On the other side of the spectrum from the autoimmune diseases, at least from immune standpoint, is cancer. Whereas in autoimmune settings Tregs play a major beneficial role, in cancer settings, they are considered to be deleterious as they are shown to suppress anti-tumor responses (71). Can fine-tuning of PKC-θ expression and its positioning in immune synapse be a potential target in cancer drug trials is still under question.

Several studies describe the role of PKC-θ in cancer settings. In gastrointestinal stromal tumors (GISTs) and Ewing’s sarcoma PKC-θ could be used as a specific marker of the disease (72–75). These studies suggest that PKC-θ overexpression facilitates the diagnostics of tumors even in the case where traditional markers are absent. However, the role in the pathogenesis is still unclear.

The implication of many other PKCs in cancer is also identified and several clinical trials are already ongoing (76). Whether there is a role for PKC-θ pathway in antitumoral T-cell response and what are the implications for drug development is still to be answered. The fact that PKC-θ seems to be expressed also by the tumor cells invites caution.

**HIV**

T-cell activation is a crucial step in HIV infection and replication. Synthesis of dNTPs, increased ATP levels, and activation of transcription factors such as NF-kB, NF-AT, and AP-1 are all shown to be necessary for HIV replication (77). Therefore, being essential for several T-cell activation pathways, PKC-θ could play a major role in HIV infection.

Very early studies showed that Nef interacts preferentially with PKC-θ. This interaction is independent of calcium and enhanced by phospholipid activators of PKC. More importantly, the authors found a net loss of PKC-θ in Nef-expressing cells following stimulation. The conclusion was that this phenomenon may contribute to the various impairments of T-cell function associated with HIV infection (78).

More recent studies have shown that HIV infection employs PKC-θ to enhance HIV-1 replication. In turn, it also upregulates PKC-θ phosphorylation in its activation loop as a positive feedback in CD4+ T cell. Moreover, HIV-1 replication was reduced in CD4+ T cells in the absence of PKC-θ activity, either by using pharmacological inhibitor, rottlerin, or employing RNA interference (RNAi) strategy (79).

However, how PKC-θ affects T-cell responses against the virus itself was not studied. Some suggest that PKC-θ is an interesting target for decreasing immune activation in HIV-infected patients and that this goal could be achieved without major immunosuppression (80).

**PKC-θ AND TARGETING FOR IMMUNE INTERVENTIONS**

Impairing the PKC-θ activity is believed to be a promising therapeutic strategy against the undesired immune response, such as Th2-mediated allergies, Th17-associated autoimmune diseases (63), and GvHD (49), meanwhile preserving the beneficial Th1 and CTL anti-pathogen immunity (47, 81) as well as GvL response in BMT (49). Therefore, pharmaceutical companies have dedicated considerable efforts to develop PKC-θ inhibitors.

The main PKC-θ inhibitors were designed as ATP competitors to block PKC-θ kinase activity. So far, the most successful is sotras-taurin (AEB071) (see Table 1), which has reached substantial progress in phase I clinical trials in psoriasis and phase II clinical trial in renal transplantation (82–84). AEB071 is a “multikinase” inhibitor with strong specificity for PKC-θ, PKC-α, and PKC-β at low picomolar concentration and less preference for other nPKCs of PKC-δ, PKC-ε, and PKC-η at nanomolar concentration (84). Consistent with previous findings of selective regulation of T-cell development in PKC-θ−/− animal model, AEB071 inhibited TCR/CD28-mediated T-cell proliferation, GvHD and allograft rejection (85–87), but retained T-cell antiviral response (83). Although both PKC-θ and PKC-α are inhibited following AEB071 treatment, NFAT activation seems not impaired (88).

Several other attractive PKC-θ inhibitors can be valuable in the treatment of autoimmune diseases. PKC-θ-specific inhibitory compound C20 has been reported to increase the suppressive function of Tregs cells from RA patients (9). In another study, PKC inhibitor R524 was designed to inhibit both PKC-θ and PKC-α catalytic activity at the nanomolar concentration. Haarberg and colleagues (89) showed that R524 impaired CD4+ T-cell proliferation and cytokine production, and significantly attenuated GvHD symptoms in myeloablative preclinical mouse models of allogeneic hematopoietic cell transplantation (HCT). Another potential PKC inhibitor is enzastaurin (Ly317615), which is orally bioavailable ATP inhibitor originally identified as PKC-β inhibitor (90). This study has shown that Ly317615 has long-term activity on anti-proliferation and pro-apoptosis in both solid and hematologic cancer. Currently, Ly317615 has been evaluated in different clinical trials, including phase II trials in multiple myeloma (91) and diffuse large B-cell lymphoma (92). However, in vitro Upstate kinase profiler data showed that Ly317615 inhibits PKC-θ fivefold more potently than PKC-β at 1 μmol/L concentration (90). Therefore, Ly317615 may act as PKC-θ inhibitor to prevent GvHD while retaining GvL response (93). It is suggested that highly specific inhibition of PKC-θ will promote a better efficacy and safety in the treatment of autoimmune diseases without causing overt immunosuppression (94). However, since PKC-θ and PKC-δ share highly conserved ATP-active region with only a single residue difference (Tyr108 in PKC-θ and Phe108 in PKCδ), it has been a challenging task to develop a small molecule compound with a high degree of specificity toward PKC-θ. In 2013, Jimenez et al. designed a novel compound 27 (C27), with an excellent selectivity toward PKC-θ comparing to other PKC isoforms and non-kinase targets. Moreover, it did not show cross-reactivity against other proximal TCR kinases. C27 inhibitor showed encouraging success at the preclinical level to effectively inhibit IL-2 production in a mouse model of staphylococcal enterotoxin B-induced IL-2 release (SEB IL-2 model) (95), which makes it a potent and specific PKC-θ inhibitor candidate for therapy in autoimmune diseases.

In addition to the PKC-θ inhibitors that act as ATP competitors, other inhibitors that negatively regulate PKC-θ
phosphorylation have been developed and studied in several disease models. 4-hydroxy-3-methoxycinnamaldehyde (4H3MC) was identified as a potential PKC isotypes inhibitor, preferentially inhibiting PKC-α, PKC-θ, and PKCζ. 4H3MC ablates PKC-θ phosphorylation and impairs its translocation to the IS, subsequently inhibiting IL-2 production in Jurkat T cells and human leukocytes. In addition, it also blocks the phosphorylation of ERK and p38, which results in impairing the activation of AP-1, NFAT, and NF-κB (96). This suggests that it may be an attractive PKC inhibitor candidate against T-cell-mediated autoimmune diseases. Two PKC-θ-specific inhibitors – CGX1079 and CGX0471 – were investigated as potential therapeutic adjuvants for the antiretroviral therapy (ART) in HIV infection (80). These two inhibitors impair PKC-θ kinase activity by blocking PKC-θ phosphorylation at T538 and prevent its translocation to the IS, which subsequently impairs the activation of NF-κB, AP-1, and NFAT and decreases viral transcription. Moreover, CGX1079 and CGX0471 retardation of T-cell proliferation, these compounds did not completely compromised T-cell function, particularly CD8 anti-viral activity, avoiding general immunosuppression. Altogether, CGX1079 and CGX0471 are promising PKC-θ inhibitors that can reduce reservoir size and preserve CTL function against HIV-1 infection (80).

However, there are still some challenges that need to be taken into consideration for developing the full therapeutic potential of PKC-θ specific inhibitors in clinical applications. First, the double-edged sword feature of PKC-θ regulation in Teffs and Tregs. Tregs cell function enhancement may be favored in autoimmune diseases, while impaired Teffs cell function is not desired in tumor-specific T-cell responses. Second, as the kinase domain is well conserved among all PKC isoforms, even crossover other protein kinase members, using ATP competitors to specifically target PKC-θ would be difficult. Therefore, allosteric kinase inhibitors emerge as more specific and less toxic therapeutic candidates in the context of human diseases. As allosteric kinase inhibitors target more divergent regulatory regions and regulate the conformational changes required for kinase activation, rather than highly conserved catalytic regions of PKC-θ, their use would be more suitable.

Moreover, the pro-rich motif in the V3 hinge domain of PKC-θ that was recently identified by Kong et al. may serve as an attractive target for allosteric inhibition. This study indicated that the V3 hinge region is sufficient to trigger PKC-θ translocation into the IS or cSMAC. Importantly, this pro-rich motif in the V3 hinge domain is unique to PKC-θ and promises a high specificity in kinase activity inhibition (18). Taken together, those less conserved and much more flexible hinge regions may become potential targets for optimal PKC inhibitor design.

**Perspectives**

The chromatin-tethered role of PKC-θ has been recently identified to be essential in the regulation of inducible gene transcription in human T cells by forming an active chromatin-anchored complex that associates with RNA polymerase II, the histone kinase MSK-1, LSD1, and the adaptor molecule, 14-3-3ζ (16). In addition, blocking PKC-θ nuclear translocation impairs T-cell activation and inducible gene expression by targeting its C-terminal NLS motif, which suggests the importance of its nuclear role in T-cell activation (Li et al., unpublished data). Therefore, it may provide an alternative approach to inhibit PKC-dependent T-cell function by inhibiting its nuclear role besides the catalytic activity. However, further studies are required because very little is known about the molecular mechanism of nuclear-tethered PKC-θ in regulation of T-cell immune responses.

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