Review

Selective Function of PKC-θ in T cells

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T cell activation is a critical process in initiating adaptive immune response since only through this process the naïve antigen specific T cells differentiate into armed effector T cells that mediate the actual immune response. During T cell activation, naïve T cells undergo clonal expansion and acquire the capability to kill target cells infected with pathogens or produce cytokines essential for regulating immune response. Inappropriate activation or inactivation of T cells leads to autoimmunity or severe immunodeficiencies. PKC-θ is selectively expressed in T cells and required for mediating T cell activation process. Mice deficient in PKC-θ exhibit defects in T cell activation, survival and activation-induced cell death. PKC-θ selectively translocates to immunological synapse and mediates the signals required for activation of NF-κB, AP1 and NFAT that are essential for T cell activation. Furthermore, *PKC-θ*$^{-/-}$ mice displayed multiple defects in the development of T cell-mediated immune responses *in vivo*. PKC-θ is thus a critical molecule that regulates T cell function at multiple stages in T cell-mediated immune responses *in vivo*. *Cellular & Molecular Immunology*. 2006;3(4):263-270.

Key Words: T cell activation, PKC-θ, TCR signaling, homeostasis, AICD

Introduction

T cells play a critical role in adaptive immune response. Defects or deregulation of T cell activation or function leads to immunodeficiencies or autoimmunity. Coordinated activation of T cells in response to antigen leads to activation, clonal expansion, and differentiation of antigen specific T cells. T cell activation is controlled by numerous signaling pathways initiated by T cell receptor (TCR) and co-stimulatory molecules (1). Biochemical signals initiated by TCR determine the specificity of T cell activation and the signaling pathways mediated by co-stimulatory molecules modulate the threshold for T cell activation (2). Protein kinase C isoforms are central components in signaling pathways that regulate numerous cellular processes in both adaptive and innate immunity (3). Protein kinase C (PKC) is a family of serine/threonine kinases that specifically phosphorylates its substrates at serine/threonine residues. There are 12 different isoforms in PKC family, and each one has unique roles in the regulation of cellular functions (4). PKC-θ is a member of novel PKCs that are Ca^{2+}-independent PKC subfamily. PKC-θ is expressed primarily in T lymphocytes and muscle (5, 6). The critical role of PKC-θ in mediating T cell activation *in vivo* has been demonstrated by two-independently generated strains of knockout mice (7, 8). In both the cases, *PKC-θ*$^{-/-}$ naïve T cells displayed defects in T cell activation due to lack of NFAT, NF-κB and AP1 activation. Interestingly, thymocyte development is normal in *PKC-θ*$^{-/-}$ mice, suggesting the specific function of PKC-θ only in the peripheral T cells, and other isoforms of PKC might compensate for the loss of PKC-θ in thymocytes. Cell type specific function of PKC-θ is presumably mediated through the interactions with T cell specific factors that act to scaffold and selectively recruit PKC-θ during T cell antigenic stimulation (9). Studies using pharmacological reagents and knockout mice showed that PKC-θ specifically translocates to the immunological synapse (IS) upon CD3 crosslink (10). This review will detail the roles of PKC-θ in TCR-mediated signaling pathways. The important roles of PKC-θ in T cell mediated *in vivo* immune responses will also be discussed.

TCR-mediated signaling and PKC-θ activation

Engagement of TCR by major histocompatibility complex (MHC) molecules with antigen peptide on the antigen presenting cells (APCs) initiates TCR signals, resulting in the activation of receptor associated Src family protein tyrosine kinase (PTK) LCK (1). Activated LCK then phosphorylates the immunoreceptor tyrosine-based motif (ITAM) of the

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TCR-CD3 complex, which acts as docking sites for ZAP70, a Syk family PTK. Recruitment of ZAP70 leads to its activation and phophorylation of adaptor molecules such as LAT, SLP76 and VAV. Phosphorylated form of LAT acts as docking site of SLP76, VAV and Grb2, and thereby recruits PI3K and PLCγ1 (11, 12). Both PI3K and PLCγ1 are key enzymes critical for the activation of PKC-θ. In addition, PLCγ1 can also bind to LAT indirectly through the adaptor proteins, SLP76 and GADS, *via* a proline rich domain of SPL76 and a SH3 domain of GADS (11). Dual phosphorylation by ZAP70 and Itk (a PTK of Tec family) triggers the activation of PLCγ1 (12). Activation of PLCγ1 leads to the production of second messengers namely inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 induces Ca^{2+} influx whereas DAG activates PKCs (1). Ionomycin (Ca^{2+} mobilizer) in combination with phorbol esters (PKC activators) mimics the signals required for T cell activation (13), indicating that IP_3-induced Ca^{2+} influx and DAG-mediated PKC activation cooperate with each other to mediate T cell activation. TCR signals together with additional signaling cascades initiated through the co-stimulatory molecules eventually lead to the activation of multiple transcription factors, including NFAT, NF-κB and AP-1, which in turn regulate T cell activation, proliferation, survival and differentiation.

Intracellular location of PKC-θ is critical for its function in mediating TCR signals. In resting T cells, PKC-θ is mostly localized in cytoplasm. Whereas upon TCR stimulation, PKC-θ translocates to the membrane detergent insoluble regions called lipid rafts (14). Stimulation of T cells with antigen-pulsed APC results in the activation of PKC-θ and translocation of PKC-θ to the inner regions of the immunological synapse (9, 10). Like other PKC isoforms, PKC-θ contains several functional domains that are implicated in regulating its memberance translocation, activation and function (15). Translocation and activation of PKC-θ are regulated by both tyrosine and serine/threonine phosphorylation of regulatory and kinase domain (16, 17). Several biochemical studies have shown that the activation and regulation of kinase activity of PKC-θ upon T cell activation are dependent on several critical components. First, PKC-θ has to be translocated to the membrane, a critical process that requires lipid second messenger, DAG, which is generated upon TCR-mediated activation of PLCγ1 (18). In addition, a non-conventional PI3K- and VAV-dependent pathway also mediates the selective translocation of PKC-θ in T cells (19, 20). However, involvement of this pathway in PKC-θ activation is not fully understood. In addition, translocation of PKC-θ to membrane and immunological synapse is dependent on LCK, but not Fyn, although both tyrosine kinases interacts with PKC-θ (16, 21). Upon membrane translocation, PKC-θ is phosphorylated at Tyr90 by LCK (16). Site specific mutation of this site in PKC-θ abolished its abilities to translocate to the membrane, to interact with DAG resulting in defective activation NF-κB, AP1 and NFAT in Jurkat T cells. Observation from these studies suggested that LCK plays a critical role in modulating PKC-θ effector function by tyrosine phosphorylation. In addition, both PI3K/VAV and ZAP-70/SLP76 pathways are also implicated in TCR-mediated membrane translocation of PKC-θ, but detailed molecular mechanisms remain unknown. These studies have clearly shown that T cell-activation-induced translocation of PKC-θ to lipid rafts and immunological synaspe is crucial for its function in T cell activation. Recently it has been shown that PDK1 interacts with and phosphorylates PKC-θ at threonine 538 located in activation loop (22). Phosphorylation of this site is critical for PKC-θ kinase activity, and its ability to activate NF-κB. Recently, Thuille et al. have shown that PKC-θ undergoes auto-phosphorylation at threonine 219 in the regulatory domain upon T cell activation. Mutation of this site in PKC-θ prevented the proper recruitment of PKC-θ in the activated T cells, but does not affect its catalytic activity or DAG binding ability (23). Activation of PKC-θ is thus carefully regulated by multiple mechanisms during T cell activation.

Role of PKC-θ in the activation of NF-κB

Transcription factor NF-κB is activated upon TCR crosslinking, and is critical for T cell survival and activation (24, 25). In unstimulated T cells, NF-κB is sequestered in the cytoplasm by IκB. T cell activation results in phosphorylation and degradation of IκB, leading to translocation of NF-κB to the nucleus (26). Phosphorylation of IκB is mediated by IκB kinase (IKK) complex, which contains two catalytic subunits, IKKα and IKKβ, and one regulatory subunit, IKK-γ. Previous studies in several T cell lines had shown that PKC-θ is essential for activation of NF-κB upon TCR-mediated stimulation (26-28). In agreement with these studies, primary *PKC-θ*$^{-/-}$ T cells displayed defects in NF-κB activation upon TCR stimulation (7, 8). *PKC-θ*$^{-/-}$ T cells failed to activate IKK complex or degrade IκB (8). In contrast, TNF-α and IL-1 mediated activation of NF-κB was normal in *PKC-θ*$^{-/-}$ T cells, suggesting the specific role of PKC-θ in TCR-mediated signaling. Similar to PKC-θ- deficient T cells, PKC-β deficient B cells exhibit defects in the activation of NF-κB upon BCR-stimulation (29). These studies clearly suggest that B and T cells thus utilize different isoforms of PKC in the regulation of NF-κB pathway. Several independent studies have shown CARMA1 and Bcl10 as the possible link between PKC-θ and IKK activation (30, 31). CARMA1 (also known as CARD11 or Bimp3) is selectively expressed in lymphocytes and associates constitutively with lipid rafts (32). Similar to *PKC-θ*$^{-/-}$ T cells, CARMA1 and Bcl10-deficient T cells are impaired in TCR-mediated, but not TNF-α and IL-1-mediated, activation of NF-κB (30, 33-35). Whereas, *Bcl10*$^{-/-}$ T cells showed normal AP1 activation (35), suggesting that Bcl10 acts specifically downstream of PKC-θ in the activation of NF-κB, but not AP1. It turns out that both TCR and BCR-induced activations of NF-κB are dependent on CARMA1 and Bcl10. Upon TCR

activation, Bcl10 translocates to lipid rafts where it associates with CARMA1 *via* their respective CARD domains. T cells deficient in CARMA1 or the CARD domain or with a mutation in the coiled-coil domain of CARMA1 are defective in the TCR and BCR-mediated activation of NF-κB, and recruitment of Bcl10 to the lipid rafts. Therefore, CARMA1 is necessary to recruit Bcl10 to the lipid rafts where it is phosphorylated by an unknown mechanism, a step critical for the activation of IKK complex. It is believed that upon translocation to lipid rafts, PKC-θ phosphorylates and activates Bcl10, resulting in subsequent activation of NF-κB pathway. In support of this, T cells obtained from CARMA1-deficient mice are defective in recruitment of Bcl10 to TCR complexes and lipid rafts (34). Recent study has shown that PKC-θ interacts with and phosphorylates CC domain of CARMA1 (33, 36). CARMA1 is required for translocation of PKC-θ to lipid rafts, as T cells deficient in CARMA1 displayed defective translocation of PKC-θ to lipid rafts (34). Although CARMA1/Bcl10 is known to act downstream of PKC-θ, and upstream of IKK in the signaling cascades, how Bcl10/MALT1 complex transmits signal to IKK complex are not fully understood. It is also not clear whether the translocation of CARMA1/Bcl10 and IKK is dependent on PKC-θ. In PKC-β-deficient B cells, IKK translocation to the lipid rafts is impaired (29), suggesting that PKC-β facilitates the translocation of IKK. It is thus possible that PKC-θ promotes IKK activation by promoting its translocation to lipid rafts. It is also possible that PKC-θ can phosphorylate IKKβ subunit, although there is no direct evidence to support this possibility. Alternatively, Bcl10 may promote the activation of IKK complex *via* its ability to bind and oligomerize MALT1.

Several biochemical studies have shown that PDK1 interacts with PKC-θ and phosphorylates threonine 538 (T538) in the activation loop of PKC-θ (17, 22). Phosphorylation of T538 is critical for PKC-θ activation (17). The same study has also shown that PDK1 is required for PKC-θ-mediated activation of IKK in Jurkat T cells. In addition, silencing of PDK1 expression in Jurkat cells completely abolished phosphorylation of PKC-θ at T538, and activation of IKK and NF-κB. PDK1 also binds to CARMA1, and is required for the recruitment of CARMA1 and Bcl10 to the lipid rafts. Observation from this study demonstrated the dual functions of PDK1 in the activation of NF-κB. First, through the activation of PKC-θ, PDK1 facilitates the recruitment of IKK complex to the lipid membrane. Second, through its interaction with CARMA1, PDK1 regulates the recruitment of CARMA1 and Bcl10 to the lipid rafts. Lee et al. also showed the recruitment of IKK complex to PKC-θ is not affected by the CARMA1 deficiency (22). In contrast, deficiency of CARMA1 affects the recruitment of IKK complex to the lipid rafts.

Another critical player in TCR-mediated activation of NF-κB is caspase-8, a protease involved in apoptosis. Caspase-8-deficient Jurkat cells and patients carrying mutant form of caspase-8 are defective in TCR-mediated activation of NF-κB (37). Furthermore, caspase-8-deficient mice exhibit defects in NF-κB activation, T-cell proliferation and immune response to viruses (38). Recently Su et al. showed that caspase-8 is an essential component in the activation of IKK complex by linking CARMA1/Bcl10/MALT1 to IKK (37). It seems that caspase-8 activity, but not the auto-processing ability, is critical for NF-κB activation. Absence of caspase-8 does not affect the formation of CARMA1/Bcl10/MALT1 complex, but affects the ability of this complex to recruit IKK (37). However, it is not clear exactly where caspase-8 fits into the IKK pathway.

Role of PKC-θ in the activation of AP1

Another essential transcription factor that is activated in response to TCR stimulation is AP1. AP1 is required for T cell activation and cytokine secretion. During T cell activation, c-Jun and c-fos, two critical components of AP1 complexes, are up-regulated (39). T cell activation also triggers MAPK cascades, resulting in the phosphorylation of c-Jun and c-fos. c-Jun and c-fos then form an active heterodimer (AP1) complex. Previous *in vitro* studies using Jurkat cells have implicated JNK in linking PKC-θ to AP1 (40). However, JNK activation is normal in *PKC-θ*$^{-/-}$ T cells (8), suggesting that an alternative pathway may be involved in PKC-θ-regulated AP1 activation. This finding was consistent with the report that JNK1/2 are not required for the primary T cell activation and IL-2 production (41). Since AP1 transcription factors are regulated both at the transcriptional and translational levels, it is also possible that PKC-θ regulates AP1 at transcription level. A recent study has showed that PKC-θ regulates AP1 activation through SPAK pathway (42). PKC-θ was found to interact directly with SPAK, and phosphorylates SPAK at serine 311 *in vitro*. Overexpression of mutant form of SPAK or knockdown of SPAK resulted in reduced PKC-θ-mediated activation of AP1, but not NF-κB. However, whether phosphorylation of serine 311 is dependent on T cell activation remains unknown. Observation from this study strongly suggests that SPAK lies downstream of PKC-θ in the regulation of AP1 activation.

Role of PKC-θ in the activation of NFAT

NFAT is a family of transcription factors that include at least five members. NFAT is essential for T cell activation, proliferation and differentiation. In addition, NFAT signaling controls AICD and T cell homeostasis. NFAT is regulated by Ca^{2+}/calcineurin-dependent signaling pathway (43, 44). Under resting condition, phosphorylated form of NFAT is sequestered in the cytoplasm. TCR stimulation leads to an increase in intracellular calcium, which activates the serine/threonine phosphatase calcineurin. Activated calcineurin then dephosphorylates NFAT to expose its nuclear localization sequences, leading to rapid translocation of NFAT into nucleus. Recently, several studies including ours have shown that PKC-θ enhances the activation of NFAT *via* stimulating

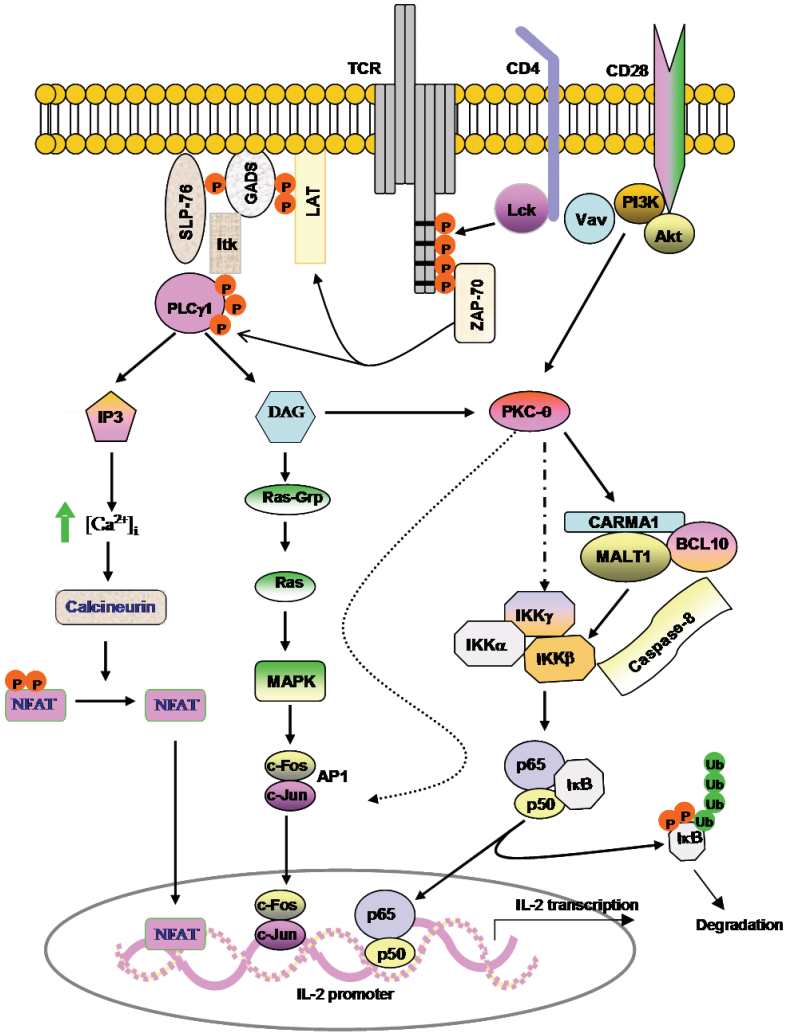

Figure 1. Schematic illustration of PKC-θ-regulated signaling pathways.

Ca^{2+} influx (7, 18, 45). *PKC-θ*$^{-/-}$ T cells displayed defective activation of PLCγ1, production of IP_3, influx of Ca^{2+} and translocation of NFAT to nucleus. The mechanisms underlying PKC-θ-mediated activation of PLCγ1, however, are not clear. Tec family kinase, Itk, is a potential link between PKC-θ and PLCγ1. PLCγ1 is reported to be a target for Itk, as overexpression of Itk strongly activates PLCγ1 as well as NFAT, even without TCR crosslinking (46). Similar to *PKC-θ*$^{-/-}$ T cells, T cells from mice deficient in the Tec family kinases Itk and Rlk display defective IP_3 production and Ca^{2+} influx due to reduced PLCγ1 activity (47-49). Altman et al. demonstrated that Tec mediates the activation of PLCγ1 in restimulated *PKC-θ*$^{-/-}$ T cells (45). In contrast to Itk, Tec is expressed at very low levels in naïve T cells, but is up-regulated in restimulated T cells (46). PKC-θ and Itk were found in the same complex. Moreover, Itk activation is reduced in *PKC-θ*$^{-/-}$ T cells upon TCR stimulation (unpublished data). It is thus likely that PKC-θ regulates Ca^{2+} influx *via* Itk in naïve T cells, whereas effector T cells depend on Tec to regulate PKC-θ-mediated Ca^{2+} influx and NFAT activation. PKC-θ has thus been demonstrated to regulate multiple signaling pathways as illustrated in Figure 1. How PKC-θ coordinates these pathways is one of the future directions in understanding the molecular mechanisms underlying PKC-θ-regulated T cell function.

Potential PKC-θ substrates

Several potential PKC-θ substrates have been identified by biochemical studies. PKC-θ has been shown to interact with and phosphorylate adaptor protein Cbl (50), pro-apoptotic protein Bad (51), and the cytoskeleton linker meiosin (52). A recent study showed that PKC-θ binds to and phosphorylates SPAK are critical for the activation of AP1 (42). In addition, PKC-θ also interacts with hematopoietic protein tyrosine phosphatase (HePTP), and phosphorylates HePTP at Ser225 upon antigen stimulation of T cells (53). This phosphorylation is believed to regulate the translocation of HePTP to

the lipid rafts. Mutation of S225A in HePTP enhanced PKC-θ-mediated activation of NFAT/AP1. Observation from this study demonstrated that PKC-θ-mediated phosphorylation of HePTP is critical for regulating TCR-mediated signals required for activation of NFAT and AP1 pathways. However, the function of these identified substrates in T cell activation *in vivo* has not been illustrated.

Survival function of PKC-θ

T cell activation leads to up-regulation of anti-apoptotic proteins such as Bcl-xL, A1/Bfl-1, c-IAP1 and FLIP (54, 55). NF-κB plays a critical role in the survival of activated T cells by up-regulating these anti-apoptotic proteins. Recently, we and others have shown PKC-θ is necessary for the survival of activated T cells (24, 56). *PKC-θ*$^{-/-}$ T cells undergo apoptosis in response to TCR stimulation, correlating with the reduced expression of NF-κB-dependent Bcl-xL. Forced expression of Bcl-xL and Bcl-2 restored the survival of *PKC-θ*$^{-/-}$ T cells. In addition, exogenous IL-2 can also partially overcome the defective survival and proliferation of *PKC-θ*$^{-/-}$T cells. Similar to the primary T cells, PKC-θ is necessary for the survival of T cell lines such as Jurkat, Hut 78, CEM and MOLT-4 (unpublished observation). It has been shown that PKC-θ is able to promote survival by phosphorylation and inactivation of Bad in Jurkat cells (51). However, primary T cells deficient in PKC-θ show comparable levels of Bad phosphorylation to that of wild type T cells (56), suggesting that the observed apoptosis in *PKC-θ*$^{-/-}$ mice is not likely due to lack of Bad phosphorylation. Expression of Bcl-2 or Bcl-xL cannot completely rescue the survival of *PKC-θ*$^{-/-}$ T cells (56), additional death pathways may also contribute to the observed apoptosis. Indeed, pro-apoptotic molecule Bim, but not Bax, is up-regulated in *PKC-θ*$^{-/-}$ T cells (56). Wan et al. reported that inhibition of NF-κB lead to an increase in the expression of pro-apoptotic protein p73 (54). It remains to be determined whether p73 pathway contributes to the apoptosis of *PKC-θ*$^{-/-}$ T cells.

Role of PKC-θ in AICD and T cell homeostasis

Activation-induced cell death (AICD) is one of the mechanisms for eliminating effector T cells that are no longer in use (57, 58). Fas (CD95) and Fas ligand (FasL, CD95L)-mediated apoptosis plays an important role in deleting effector T cells, as mice lacking Fas or FasL display defective deletion of peripheral T cells (59, 60), and eventually develop autoimmune disorders (61-63). In addition to its well-established role in AICD, Fas also plays a very important role in lymphocyte proliferation. Furthermore, FasL-induced apoptosis has been shown to be responsible for protecting immune privileged sites from cellular immune-mediated damage (64, 65). Resting T cells express low levels of Fas and FasL. T-cell activation results in up-regulation of Fas, and repeated stimulation of activated T-cells up-regulates FasL. The cytokine IL-2 sensitizes activated T cells to AICD by promoting the expression of FasL and concurrent down-regulation of c-FLIP (66, 67). However, the mechanisms by which IL-2 regulates FasL expression may be different from TCR-mediated regulation. By overexpression of PKC-θ or PKC inhibitor treatment, PKC-θ was found to promote TCR-induced activation of FasL promoter in Jurkat cells (68). Consistent with this finding, we observed that FasL failed to be fully up-regulated in *PKC-θ*$^{-/-}$ mice treated with SEB, indicating the role of PKC-θ in stimulating FasL expression *in vivo* (69). FasL expression is regulated by multiple transcription factors such as NFAT, NF-κB and AP1 (58). Furthermore, we observed that stimulation of FasL expression depends on PKC-θ-mediated activation of NFAT pathway in addition to activation of NF-κB and AP1. Similar to PKC-θ-deficient T cells, Itk-deficient T cells are also defective in FasL expression and AICD. Interestingly, *PKC-θ*$^{-/-}$ T cells displayed resistance to Fas-mediated apoptosis as well as activation-induced cell death. Interaction of Fas with FasL results in the activation of initiator caspase, caspase-8. In the absence of PKC-θ, Fas-induced activation of apoptotic molecules such as caspase-8, caspase-3 and Bid was not efficient. PKC-θ is thus required for promoting both FasL expression and Fas-mediated apoptosis.

Role of PKC-θ in Th1 and Th2 response

Activated naïve CD4$^+$ T cells differentiate into two distinct subsets, Th1 and Th2 cells, which are responsible for cell and humoral immune responses. These subsets are defined based on their cytokine expression profile. Th1 cells express interferon-γ, IL-17 and IL-2, whereas Th2 cells produce IL-4, IL-5, IL-9, IL-10 and IL-13. The balance between these two subsets is critical for mediating proper immune response to pathogens. Imbalanced Th1/Th2 leads to autoimmunity (excess Th1 response) or hypersensitivity (excess of Th2 response). TCR-mediated signals guide Th-cell polarization. Given the well-established role of PKC-θ in the regulation of T cell activation and proliferation, it is not surprising that PKC-θ plays a role in the regulation of Th1- and Th2-mediated immune responses (70-73). *PKC-θ*$^{-/-}$ mice displayed impaired Th2 response *in vivo* after infection with *Nippostrongylus brasiliensis*, whereas *PKC-θ*$^{-/-}$ mice developed normal Th1 response against *Leishmania major* (70). Similar to PKC-θ-deficient mice, CD28- and Itk-deficient mice are capable of mounting effective CD4$^+$ and CD8$^+$ T cell responses upon infection with LCMV or *Leishmania major*. However, CD28-deficient mice are not able to mount a CTL response against abortively replicating vesicular stomatitis virus, when antigen levels are limiting. One explanation is that infections by the former pathogens provide sustained high peptide densities on antigen-presenting cells and effectively stimulate the innate immune system because of extensive replication. Such strong activation may well overcome the requirement for the specific signaling component. *In vivo* confirmation of such findings is difficult considering the complex interactions between multiple cell

types and cytokines in *in vivo* microenvironments. Indeed, TCR signaling is not the only pathway for T cell activation triggered during anti-viral immune response, other pathways of T cell activation are able to overcome the defects in PKC-θ-mediated signals. For example, proinflammatory cytokines IL-1 and TNF-α can activate NF-κB *via* a PKC-θ-independent pathway (8, 26). It is possible that cytokines produced during immune responses compensate for the PKC-θ function under certain circumstances.

PKC-θ is required for induction of allergic asthma. T cells play a critical role in the initiation and maintenance of airway inflammation during allergic asthma. In addition, cytokines secreted by T cells regulate the action of mast cells, eosinophils and macrophages, which are the main effector cells in the inflammation of airway. Using murine model for allergic asthma, two recent studies have shown that PKC-θ plays a critical role in the development of immunological symptoms in allergic asthmatic model (70, 72). Following induction of allergic asthma, PKC-θ-deficient mice displayed drastically reduced lung inflammation, esinophil infiltration, and mucus production. In addition, infiltration of T cells into the lungs is reduced in the *PKC-θ*$^{-/-}$ mice. *PKC-θ*$^{-/-}$ T cells also exhibited reduced proliferation and cytokine production such as IL-5 and IL-13. These studies suggest that PKC-θ regulates the secretion of Th2 cytokines IL-5 and IL-13. PKC-θ thus may be an attractive target for anti-asthmatic drug.

PKC-θ is required for full development of experimental allergic encephalomyelitis (EAE). $CD4^+$ Th1 subset plays a crucial role in the pathogenesis of many autoimmune diseases such as multiple sclerosis, rheumatoid arthritis (RA), type I diabetes, systemic lupus erythromatosus (SLE), myocarditis, thyroditis and uveitis. EAE is also a Th1-mediated autoimmune disease characterized by the inflammatory demylination of central nervous system (CNS). EAE can be induced in susceptible strains of mouse, rodents and primates by injecting antigens such a myelin basic protein (MBP), myelin oligodendrocyte protein (MOP), and proteolipid protein (PLP). Recently, two studies have showed that PKC-θ is critical for the development of the disease (71, 73). *PKC-θ*$^{-/-}$ mice failed to develop EAE upon injection with myelin oligodendrocyte glycoprotein. *PKC-θ*$^{-/-}$ T cells are defective in the production of Th1 proinflammatory cytokines such as IFN-γ, TNF-α and IL-17, and failed to infiltrate the CNS. Observations from these studies clearly show a critical role of PKC-θ in the generation and effector function of autoimmune Th1 cells. Interestingly, PKC-θ was shown to be critical for Th2, but not Th1, responses in several other disease models as we previously mentioned (70, 72). The question is why *PKC-θ*$^{-/-}$ mice are able to develop efficient Th1 immunity in other models, whereas fail to induce Th1-dependent tissue damage in EAE. Fas/FasL-mediated apoptosis contributes to the tissue damage of central nervous system associated with EAE, as lpr and gld mice are relatively resistant to the development of clinical EAE (74, 75). It is possible that impaired FasL/Fas pathway observed in *PKC-θ*$^{-/-}$ mice (69) may also contribute to the resistance to EAE in *PKC-θ*$^{-/-}$ mice in addition to impaired Th1 responses. Besides T cell activation and survival, PKC-θ-regulated FasL/Fas pathway is thus another layer of control in PKC-θ-regulated immune responses.

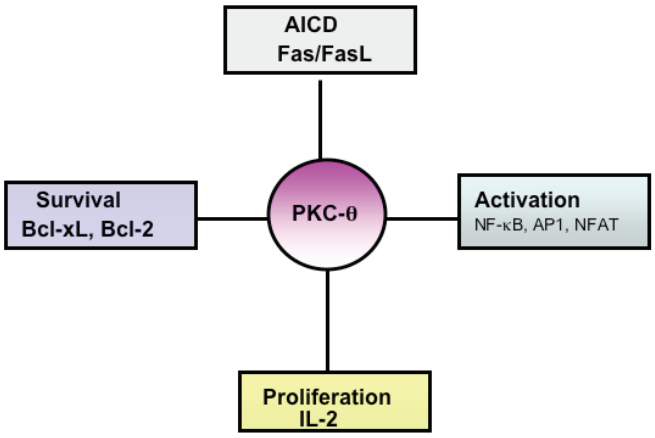

Figure 2. Multiple roles of PKC-θ in the regulation of T cell function. PKC-θ plays important roles in T cell activation, survival, apoptosis and IL-2 production.

In conclusion, PKC-θ mediates critical signals required for T cell activation and survival both *in vitro* and *in vivo*. PKC-θ is thus a critical molecule that regulates T cell function at multiple stages in T cell-mediated immune responses as summarized in Figure 2.

Thus, understanding of the mechanisms by which PKC-θ functions may lead to therapeutic strategies which manipulate signaling pathways and alter T-cell function. Further, PKC-θ is potentially a drug target for controlling T cell activation in the treatment of immunological diseases and transplant rejection.

Acknowledgements

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