REVIEW ARTICLE

Emerging and diverse roles of protein kinase C in immune cell signalling

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Members of the protein kinase C (PKC) family are expressed in many different cell types, where they are known to regulate a wide variety of cellular processes that impact on cell growth and differentiation, cytoskeletal remodelling and gene expression in the response to diverse stimuli. The broad tissue distribution and redundancy of *in vitro* function have often hampered the identification of definitive roles for each PKC family member. However, recent *in vivo* studies of PKC isoenzyme-selective knockout and transgenic mice have highlighted distinct functions of individual PKCs in the immune system. These genetic analyses, along with biochemical studies utilizing PKC isoenzyme-specific cDNA (wild-type, constitutively active and dominant-negative), antisense oligonucleotides (ASO), RNA interference (RNAi), and pharmacological inhibitors, indicate that PKC-regulated signalling pathways play a significant role in many aspects of immune responses, from development, differentiation, activation and survival of lymphocytes to macrophage activation. The importance of PKCs in cellular immune responses suggests that improved understanding of the molecular events that govern their actions could point to new avenues for development of treatments for immune disorders.

Key words: cytokine, immune cell signalling, inflammation, macrophage, protein kinase C (PKC), T-cell receptor (TCR).

INTRODUCTION

Immune cells respond to various stimuli, including microbial antigens, mitogens and cytokines, by using signal transduction pathways to mediate gene expression and immune function. Fine-tuning of the signalling threshold is vitally important for the host; overreactions to self-antigens can lead to devastating autoimmune disease, whereas inadequate reaction to foreign antigens can result in increased susceptibility to infection or tumour formation. The protein kinase Cs (PKCs) are particularly important mediators of immune intracellular signalling (Scheme 1), with recent studies of gene-knockout mice generated through homologous recombination increasingly emphasizing non-redundant roles of individual PKC isoenzymes in different immune cell types (Table 1).

Originally identified in 1977 by Nishizuka and colleagues [1] as a cyclic nucleotide-independent protein kinase that phosphorylated histone and protamine in bovine cerebellum, the PKC family comprises at least ten mammalian isoenzymes of serine/threonine protein kinases with a broad range of tissue distribution and differential cellular localization [2]. Bearing in mind that there are PKC isoforms resulting from alternatively processed PKC transcripts [2,3], PKC can be grouped into three categories according to the presence or absence of motifs that dictate cofactor requirements for optimal catalytic activity (Figure 1). Whereas conventional [cPKC: α, βI–II (spliced variants) and γ] and novel (nPKC: δI–III, ε, η and θI–II) PKCs bind diacylglycerol (DAG), which stimulates kinase catalytic activity, atypical [aPKC: ζ, PKMζ (catalytic fragment of PKCζ), and ι/λ] PKCs do not interact with DAG. Ca^{2+} is an additional requirement for cPKCs, but not for nPKCs.

Although most PKCs are fully phosphorylated shortly after translation, they usually remain catalytically inactive due to the pseudosubstrate domain. In resting cells or in the absence of lipid hydrolysis, PKCs are localized primarily to the cytosol. Activation leads to hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), generating DAG and *myo*-inositol 1,4,5-trisphosphate (IP_3). DAG binds to the C1 domain (Figure 1) and increases the affinity of PKC for membrane phospholipids. As a consequence, not only is the residence time of PKC on the membrane increased, but also the conformational changes associated with phospholipid binding displaces the pseudosubstrate moiety from the catalytic domain, thus enabling PKC to phosphorylate protein substrates.

PKCs demonstrate relatively broad *in vitro* substrate specificity, yet clearly have distinct *in vivo* functions. The diverse and distinct roles of individual PKCs are, at least in part, attributed to differences in their structural features and the mechanisms that modulate their activation. For the sake of brevity, the reader is referred to reviews of the PKC family that discuss the lipid, protein partner and phosphorylation controls that operate in conjunction with different PKC family members to determine latent activity, location and agonist responsiveness [4–6]. These various regulatory mechanisms working together to define PKC isoenzyme-, cell- and tissue-selective functions are evident in the context of immune cell signalling through PKC as discussed below.

FUNCTIONS OF PKCs IN T-CELLS

PKCθ and T-cell receptor (TCR) antigen signalling

Proper activation of T-lymphocytes is central to the establishment of adaptive immunity. This process is initiated by the engagement

Abbreviations used: AP-1, activator protein-1; APC, antigen-presenting cell; BCR, B-cell receptor; Btk, Bruton's tyrosine kinase; CARD, caspase recruitment domain; CARMA1, CARD/membrane-associated guanylate kinase; CAT, catalytic domain of PKC; DAG, diacylglycerol; ERK, extracellular-signal-regulated kinase; ICAM-1, intercellular cell-adhesion molecule 1; IFNγ, interferon-γ; IκB, inhibitory κB; IKK, IκB kinase; IL, interleukin; IS, immunological synapse; JNK, c-Jun N-terminal kinase; LFA-1, lymphocyte-function-associated antigen-1; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NFAT, nuclear factor of activated T-cells; PKC, protein kinase C; aPKC, atypical PKC; cPKC, conventional PKC; nPKC, novel PKC; RNAi, RNA interference; SFK, Src family protein tyrosine kinase; TCR, T-cell receptor; TNFα, tumour necrosis factor α; WASP, Wiskott–Aldrich syndrome protein; WIP, WASP-interacting protein.

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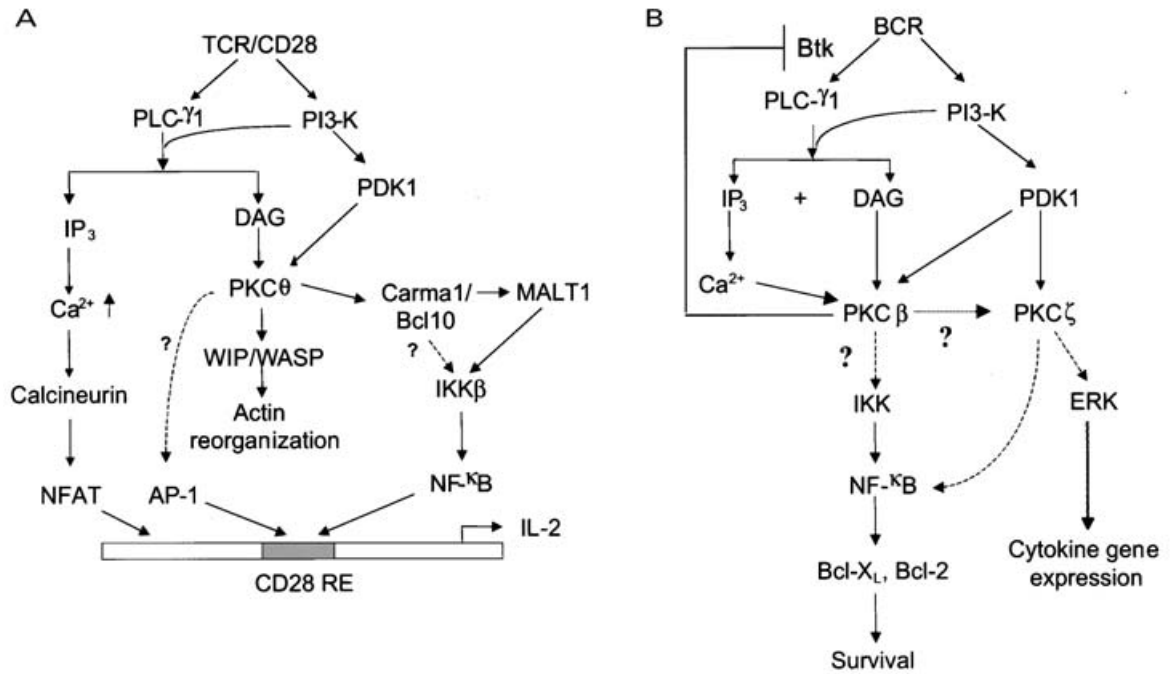

Scheme 1 Signal transduction pathways involving PKC in T- and B-lymphocytes

(**A**) PKC-mediated TCR signalling. (**B**) PKC-mediated B-cell survival pathway. See text for details of each pathway. MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; PDK1, phosphoinositide-dependent kinase 1; PLC-γ1, phospholipase C-γ1; RE, response element.

Table 1 Sampling of PKC isoenzyme-binding proteins and substrates, and summary of immunological phenotypes of PKC isoenzyme-knockout mice

ASIP, aPKC-specific interacting protein; β'-COP, coat protein; CREB, cAMP-response-element-binding protein; GAP43, growth-associated protein 43; hnRNP, heterogeneous nuclear ribonucleoprotein; ND, not determined; NELL, Nel-like protein; PICK1, protein interacting with protein kinase C; PKK, PKC-associated kinase; RACK1, receptor for activated C kinase; RBCK1, RING-B box-coiled-coil (RBCC) protein interacting with PKC 1; SRBC, sheep red blood cell; VHL, von Hippel–Lindau factor; ZIP, PKCζ-interacting protein.

Genetic strategy	Predominant tissue expression*	Interacting proteins†	PKC substrates†	PKC-knockout phenotype	References
cPKC subfamily					
PKCα	Ubiquitous, high in T-cells	Caveolin, Sdr, syndecan-4, PICK1, tropomodulin, vinculin	Sdr, syndecan-4, tropomodulin, vinculin	ND; transgenic PKCα display hyperproliferative T-cells	[42]
PKCβ	Ubiquitous, high in B-cells	RACK1, RBCK1, Btk, PKK, NELL	Btk, PKK, NELL	BCR signalling and survival defects; mast cell defects	[50,73]
nPKC subfamily					
PKCδ	Ubiquitous	SRBC, GAP43, syndecan, Fyn	SRBC, GAP43, hnRNP K	Hyperproliferative B- and mast cells; B-cell anergy defect	[66,67,75]
PKCε	Ubiquitous	RACK1 and 2 (β'-COP), Btk, caveolin	Btk	Macrophage activation defect	[71]
PKCθ	T-lymphocytes, skeletal muscle	Fyn, Lck, 14-3-3τ, Vav, Akt, IKKβ, Btk, HIV Nef	WIP, Moesin, CREB	TCR signalling defect	[14,15]
aPKC subfamily					
PKCζ	Ubiquitous	RBCK1, ASIP, caveolin, VHL, p62/ZIP, tubulin, 14-3-3β and θ	14-3-3β and θ	BCR signalling defect	[59]

* Reviewed in [3].

† Determined from the literature and reviewed in [5]. Generic PKC substrates include MARCKS (myristoylated alanine-rich C-kinase substrate) and adducin.

of major histocompatibility complex (MHC)–peptide complexes and B7 molecules on antigen-presenting cells (APCs) to the TCRs and CD28 receptors on T-cells respectively [7]. This leads to the formation of a supramolecular complex containing reoriented TCR, adhesion molecules and signalling components at the interface of the T-cell and the APC, known as the immunological synapse (an analogous structure has been described for B-cells). Although the importance of PKC in regulating T-cell activation has been well characterized, the role of individual PKCs in activation and developmental events in T-cells remains largely undefined. The nPKC member PKCθ was originally suspected to play an important role in TCR-induced activation because its expression is largely restricted to T-lymphocytes (and skeletal muscle), where it co-localizes with the TCR in the T-cell immunological synapses [3,8,9]. Furthermore, overexpression and inhibition studies of PKCθ, but not other PKC isoenzymes, show that PKCθ mediates activation of the transcription factors activator protein-1 (AP-1) and nuclear factor κB (NF-κB) in response to TCR/CD28 co-stimulation in a cell type (T-cell lines)-dependent manner [10–13].

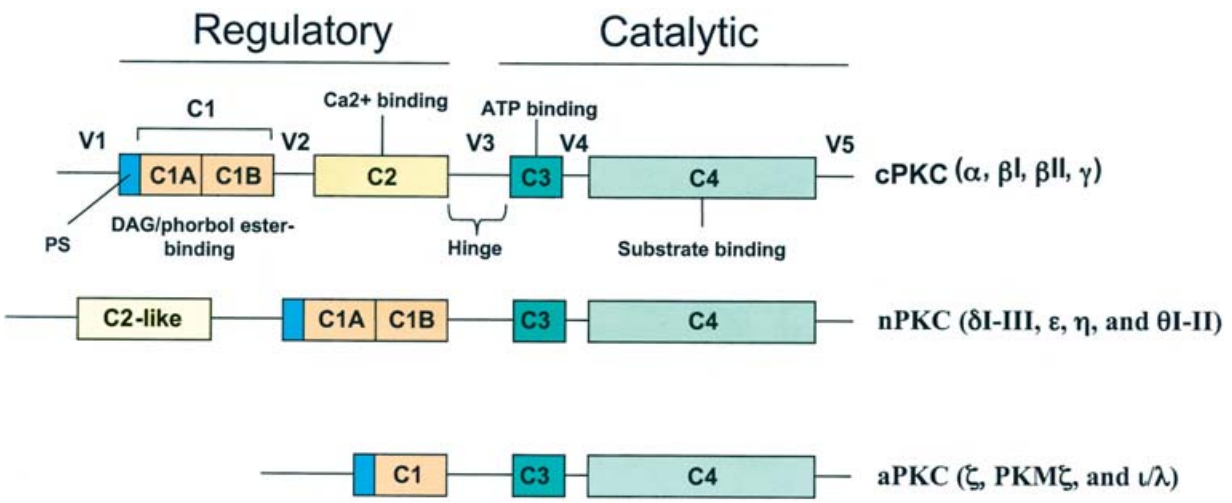

Figure 1 Structural characteristics of the PKC family

Schematic representation of the structure of the PKC family, which all share various conserved domains. Each PKC contains a highly homologous C-terminal catalytic domain and an N-terminal regulatory domain that is composed of key motifs, which mediate cofactor binding and substrate accessibility. The DAG/phorbol-ester-binding C1 domain, which is defined by the presence of two repeated cysteine-rich zinc-finger motifs (C1A and C1B), functional in cPKCs and nPKCs, but not aPKCs, form a hydrophobic surface that mediates binding to the neutral lipid DAG and phorbol esters. The C2 domain mediates Ca^{2+} binding in cPKCs, but differences in key residues abolish this function in nPKCs. aPKCs have single modified C1 domains. C3 and C4 form the ATP- and substrate-binding lobes of the kinase core. The autoinhibitory pseudosubstrate sequence (PS) present in the regulatory domain of all PKC isoenzymes interacts directly with the substrate-binding cavity in the catalytic domain, thereby sterically blocking access of substrates to the active site. The intramolecular binding between the PS domain and catalytic domain is dependent on the overall PKC conformation and is highly regulated by membrane interactions and protein phosphorylation.

The essential role of PKCθ for TCR activation and signalling *in vivo* was recently demonstrated using two independent PKCθ-knockout mouse lines [14,15]. In both cases, peripheral T-cells deficient in PKCθ exhibited reduced proliferation and interleukin (IL)-2 production. In addition, TCR/CD28-induced AP-1 and NF-κB activation were significantly impaired. These defects are specific to TCR/CD28-driven signalling as tumour necrosis factor α (TNFα)- and IL-1-induced NF-κB DNA-binding activity was intact in PKC$\theta^{-/-}$ T-cells [14]. Although the two different PKCθ-knockout mouse cell lines support the role of PKCθ in TCR signalling, there is a notable difference in TCR/CD28-induced activation of NFAT (nuclear factor of activated T-cells): it was abrogated in one strain [15], but not in the other [14]. Although the reason for the discrepancy between the two studies remains to be determined, the NFAT defect is in keeping with the observed impairment in the TCR-induced Ca^{2+} mobilization in PKCθ-deficient T-cells [15] and previous results implicating PKCθ in synergistic activation of NFAT with calcineurin [16–18]. Interestingly, neither thymocyte differentiation nor NF-κB activation was impaired in thymocytes of PKC$\theta^{-/-}$ mice, suggesting that specific functions of PKCθ might be dictated by development stages, and that other PKC members, such as PKCα (see below), might compensate the loss of PKCθ function in thymocytes.

The cell-type-selective function of PKCθ is presumably mediated by T-cell-specific factors that act to scaffold and selectively recruit PKCθ and/or that serve as a downstream substrate/effector for the protein kinase during T-cell antigen stimulation. With respect to the former scenario, a study using pharmacological inhibitors and knockout mice showed that translocation of PKCθ to lipid rafts (cholesterol- and sphingolipid-rich plasma membrane microdomains) within the immunological synapse (IS) upon CD3 cross-linking [19,20] is dependent on Lck, but not Fyn, although both tyrosine kinase members have been shown to associate with PKCθ [21]. However, it is possible that the overall structure of the IS might be dependent on Lck as well and the lack of PKCθ recruitment is therefore epi-phenomenal to the defect in the IS architecture. Interestingly, an active PKCθ catalytic domain (CAT) failed to partition into lipid rafts and to activate NF-κB, whereas addition of an Lck-derived membrane/lipid raft localization sequence to the CAT of PKCθ restored its translocation and NF-κB activation [22]. Furthermore, the lipid-raft-resident fraction of PKCθ was transiently tyrosine-phosphorylated by Lck, predominantly on Tyr^{90} in the regulatory domain (REG) [23]. Although these studies suggest that the lipid raft integrity is important for the inducible tyrosine phosphorylation of PKCθ, the role of Tyr^{90} phosphorylation in regulation of PKCθ raft localization and effector function has not been addressed. In addition to Lck, both the phosphoinositide 3-kinase (PI3-K)/Vav (a Rac-1 guanine nucleotide exchange factor) [24] and ZAP-70 [ζ-chain (TCR)-associated protein kinase of 70 kDa]/SLP-76 (Src homology 2-domain-containing leucocyte protein of 76 kDa) pathways [25] were also implicated in regulation of PKCθ membrane translocation, although the detailed mechanisms have not been defined. In any event, these results indicate that T-cell-activation-induced translocation of PKCθ to lipid rafts, where PKCθ is localized to the T-cell IS, is crucial for the proper function of PKCθ.

Although additional studies will be required to address the mechanism that selectively mediates the translocation of PKCθ to lipid rafts, recent genetic studies from several independent labs have identified members of the family of caspase recruitment domain (CARD)-containing proteins, CARMA1 (CARD/membrane-associated guanylate kinase) and Bcl-10, in coupling PKCθ to NF-κB activation in T-cells [26–28]. Mechanisms of NF-κB activation have been reviewed in depth elsewhere [29]. Briefly, NF-κB activation requires the phosphorylation and degradation of inhibitory κB (IκB) protein. The phosphorylation of IκB is mediated by the IκB kinase (IKK) complex, which contains two catalytic subunits, IKKα and IKKβ, and one regulatory subunit, IKKγ. CARMA1 (also known as CARD11 or Bimp3), which is selectively expressed in lymphocytes and associates constitutively with lipid rafts [26], is required for TCR-, but not TNFα- or IL-1-, induced NF-κB activation in Jurkat cells [27,28]. Upon TCR

activation, the ubiquitously expressed Bcl-10 protein apparently translocates to lipid rafts where it associates with CARMA1 via their respective CARD domains [26]. This is thought to lead to the observed PKCθ-dependent phosphorylation/activation of Bcl-10 and subsequent NF-κB activation [26]. In line with this view, T-cells from mutant mice lacking CARMA1 expression are defective in recruitment of Bcl-10 to clustered TCR complexes and lipid rafts [30]. These mice have defects in antigen-mediated cell proliferation and cytokine production in T-cells (as well as in B-cells), possibly owing to impaired NF-κB [30,31] and c-Jun N-terminal kinase (JNK) activation [31]. Furthermore, Bcl-10-null mice also display impaired activation of NF-κB in the context of TCR and B-cell receptor (BCR) stimulation [32].

It is not clear, however, whether or not PKCθ directly phosphorylates Bcl-10 and how Bcl-10 transmits TCR/CD28 signals to NF-κB activation. Furthermore, there is no evidence that Bcl-10 phosphorylation is of importance. It is possible that the CARMA1–Bcl-10 complex may link PKCθ to the IKK signalosome, which has been shown to localize to membrane lipid rafts and form a complex with PKCθ in activated T-cells [33]. PKCθ recruitment to lipid rafts is thought to promote IKK activation through phosphorylation of the IKKβ subunit [11,12], although evidence for direct phosphorylation of IKKβ by PKCθ is not available. Alternatively, Bcl-10 may promote IKK activation via its ability to bind and mediate oligomerization of the caspase-like protein MALT1 (mucosa-associated lymphoid tissue lymphoma translocation gene 1) [34]. In addition, the exact role of CARMA1 as a regulator of JNK following antigen receptor stimulation remains unclear: although inhibition of CARMA1 via the use of a dominant-negative mutant of CARMA1 in Jurkat cells blocks TCR/CD28-induced JNK activation [26], a CARMA1-deficient Jurkat cell line has normal JNK activation in response to TCR/CD28 stimulation [27]. The former finding is consistent with the phenotype of CARMA1-null mice [31], whereas the latter observation is more in line with the phenotype of PKCθ-knockout mice [14].

Relatively little is known about the signalling pathway that connects PKCθ to AP-1 activation. Although previous studies have implicated JNK in linking PKCθ to AP-1 activation, TCR-induced JNK activation appeared intact in PKCθ-deficient T-cells, suggesting that an alternative pathway may be responsible for coupling PKCθ to AP-1 [14]. This would be consistent with the reports that JNK1/2 are not required for primary T-cell activation and IL-2 production [35,36]. PKCθ has also been shown to physically interact with and functionally co-operate with Akt1/protein kinase Bα (PKBα) in NF-κB signalling in TCR/CD28-stimulated T-cells [37], although the functional significance has yet to be ascertained by *in vivo* studies.

Finally, PKCθ can also impact T-cell activation through regulation of the complex interaction of the actin cytoskeleton with signalling molecules downstream of TCR [38]. A recent study showed that the Wiskott–Aldrich syndrome protein (WASP)-interacting protein (WIP) was phosphorylated by PKCθ in response to TCR engagement [39]. WIP forms a complex with WASP, which plays a critical role in promoting TCR-induced actin nucleation and polymerization in both T- and B-lymphocytes [40]. Upon TCR stimulation, the WIP–WASP complex translocates to lipid rafts, where PKCθ phosphorylates WIP, thereby leading to its dissociation from WASP [39]. Consistent with these observations, the phenotype of WIP$^{-/-}$ mice is similar to that of PKC$\theta^{-/-}$ mice in that the former also exhibit defects in T-cell (but not B-cell) activation, proliferation and IL-2 production in response to TCR ligation [41]. It remains to be determined how the PKCθ-dependent actin nucleation and polymerization affects downstream signalling pathways.

Potential role of PKCα in thymocyte development

The cPKC member, PKCα, is one of the major PKC isoenzymes expressed in thymocytes. It has been reported previously that thymocytes from PKCα-overexpressing transgenic mice exhibit extensive cell proliferation and IL-2 production in response to TCR stimulation [42]. The overexpressed PKCα proteins appeared to localize predominantly to the membrane fractions of transgenic thymocytes, suggesting that PKCα translocation to the cell membrane of thymocytes acts as an active second messenger of TCR-induced proliferation and IL-2 production. In a more recent study, Trushin et al. [43] demonstrated, using kinase mutants, RNA interference (RNAi) and pharmacological inhibitors, that PKCα, but not PKCβ, is required for NF-κB activation following TCR/CD28-induced T-cell activation in Jurkat cells. Interestingly, PKCα-dependent activation of NF-κB was abrogated by rottlerin, a pharmacological inhibitor of PKCθ (and PKCδ), or by expression of a dominant-negative form of PKCθ. These results suggest that PKCα acts upstream of PKCθ to activate NF-κB during CD3/CD28 activation, although this remains to be demonstrated *in vivo*. Finally, a transfection study of foetal thymuses using either a constitutively active or a dominant-negative form of PKCα showed that PKCα can influence both allelic exclusion and differentiation during thymocyte development [44]. In view of the importance of pre-TCR-mediated activation of NF-κB activation in the selection of thymocytes [45–47], the role of PKCα in this context warrants further investigation and is likely to be PKCθ-independent, since neither thymocyte differentiation nor NF-κB activation was impaired in thymocytes of PKCθ-null mice.

Does PKCβ have a role in T-cell migration?

During inflammation, T-cells enter the peripheral inflamed tissues and roll along microvascular endothelial cells to sample inflammatory signals. T-cell recruitment is mediated by the integrin receptor known as lymphocyte-function-associated antigen-1 (LFA-1), which binds with high affinity to the counter-receptor intercellular cell-adhesion molecule 1 (ICAM-1) expressed by endothelium at inflammatory sites. T-cells arrested in the target tissues undergo cell spreading and polarization and subsequent migration across the vascular wall to seek out their extravascular targets in the inflamed tissue. LFA-1-induced signalling is thought to involve the PKCs as ICAM-1-mediated cross-linking of LFA-1 was demonstrated to recruit PKCβ(I) and PKCδ to microtubules [48]. Moreover, expression studies showed that PKCβ(I) promoted cell polarization and enhanced motility of an otherwise defective PKCβ-deficient T-cell clone [49]. These results implicate a role for PKCβ(I) in the migration of T-cells, although this remains to be demonstrated *in vivo*.

FUNCTIONS OF PKCs IN B-CELLS

PKCβ and B-cell survival

The first *in vivo* evidence for a role of the cPKC member, PKCβ, in B-cell functions came from the work of Leitges et al. [50], who showed that PKCβ-knockout mice have reduced splenic B-cells, a significantly lower number of B-1 lymphocytes and low levels of serum IgM and IgG3. The mutant mice also exhibit defective IgM-induced B-cell proliferation, despite normal T-cell activation in response to TCR stimulation (PKCβ is expressed in T-cells). The impaired B-cell activation and ineffective T-independent immune response phenotypes of PKCβ deficient mice are analogous with those observed in mice deficient for Bruton's

tyrosine kinase (Btk) or X-linked immunodeficient mice (Xid), which carry a missense mutation in Btk [51]. Btk has been implicated previously in BCR-induced NF-κB induction [52,53] and has been shown to interact with PKCβ, whereby the interaction might be important for activation of PKCβ [54]. In a negative feedback mechanism, activated PKCβ down-modulates the Btk activity via direct phosphorylation of Btk and regulation of its membrane localization [55].

Recently, two independent groups demonstrated that BCR-dependent cell proliferation and survival in PKCβ-knockout mice are significantly impaired owing to defective induction of the cell survival genes Bcl-2 and Bcl-x_L, which are known to be regulated by NF-κB activation [56,57]. How does PKCβ regulate the survival of peripheral B-cells through BCR-induced NF-κB activation? PKCβ has been suggested to control the formation of IKK raft complexes, since BCR stimulation of PKC$\beta^{-/-}$ B-cells fails to induce the translocation of the IKK complex to lipid rafts [57]. Furthermore, IκB degradation is impaired in the PKCβ-deficient B-cells activated by IgM cross-linking, but not when stimulated through CD40 ligation [56,57]. Using anti-phospho-specific antibodies to activated forms of IKKα/IKKβ, Saijo et al. [57] found that the PKC$\beta^{-/-}$ B-cells lack phosphorylated IKKα proteins both under basal conditions and when stimulated by BCR cross-linking, whereas phosphorylation of IKKβ is relatively unaffected, but not sustained, in these cells. Thus, in contrast with IKKβ-dependent PKCθ-induced NF-κB activation upon TCR stimulation, PKCβ appears to promote BCR-induced NF-κB activation by modulating IKKα activity. However, this hypothesis is inconsistent with the respective roles of IKKα and IKKβ in B-cell survival: IKKβ, but not IKKα, is essential for B-cell survival [58]. Furthermore, it is unclear how PKCβ may be involved in Btk-mediated NF-κB activation upon BCR cross-linking, as the substrate specificity of Btk for tyrosine residues would preclude a direct connection between Btk and the IKK complex.

PKCζ: a partner in crime?

Targeted disruption of the PKCζ gene in mice indicates that the role of this aPKC within the immune system is also specific to B-cell function [59]. B-cells from PKCζ-deficient mice showed increased spontaneous apoptosis, and impaired proliferation and survival in response to IgM cross-linking, whereas both peripheral T-cells and thymocytes seemed to develop and proliferate normally. The defective survival of B-cells in these mice correlated with defects in the activation of extracellular-signal-regulated kinase (ERK) (but not p38 MAPK or JNK) and the transcription of NF-κB-dependent genes, including Bcl-x_L, IκB and IL-6. Furthermore, transcription of these NF-κB-dependent genes, but not NF-κB nuclear translocation, was inhibited in B-cells stimulated with IgM. PKCζ-null mice were unable to mount an optimal T-cell-dependent immune response, in spite of the fact that, as adults, they exhibited no major defects in the subpopulations of B-cells, indicating that this is a post-B-cell maturation phenomenon. Although the possibility of a PKC cascade involving both PKCβ and PKCζ has not been excluded, recent findings showed that PKCζ can regulate NF-κB via an IKK-independent pathway, by directly phosphorylating Ser^{311} of the p65 subunit (RelA) [60,61].

PKCλ and pre-B-cell development

Although neither PKCβ nor PKCζ deficiency alters B-cell development in the bone marrow, a recent study suggests PKCλ may have a role in NF-κB activation during early B-cell development [62]. Unlike most PKCs expressed in B-lineage cells, whose activity is partly regulated by serine/threonine phosphorylation, the activity of PKCλ is additionally controlled by tyrosine kinase phosphorylation within its CAT [63]. This phosphorylation event is induced by anti-Igβ-induced stimulation of pro-B-cells, but not pro-B-cells from mice deficient in Src family protein tyrosine kinases (SFKs), namely Blk, Fyn and Lyn, suggesting that PKCλ tyrosine phosphorylation may be mediated by SFKs [62]. Moreover, triple-deficient pro-B-cells displayed markedly reduced IKK phosphorylation and NF-κB activation, and this defect was restored by paclitaxel, which can activate PKCλ. These results suggest that PKCλ is a downstream target of SFKs in pre-BCR-mediated NF-κB activation. In support of PKCλ-mediated IKK activation as a potential mechanism, previous studies have demonstrated that PKCλ is capable of phosphorylating both IKKα and IKKβ [64]; however, direct measurement of IKK activity in the absence of PKCλ remains to be performed.

PKCδ and B-cell tolerance

Self-reactive B-cells normally undergo either clonal deletion or tolerance to self-antigens (B-cell anergy), which is essential for the prevention of autoimmune disease [65]. The physiological role of PKCδ, the closest related PKC member to PKCθ, in the control of B-cell tolerance has recently been uncovered by characterization of PKCδ-knockout mice generated independently by two laboratories [66,67]. Loss of PKCδ in mice leads to significant splenomegaly and lymphadenopathy because of increased numbers of peripheral B-cells, although no noteworthy abnormalities are observed in T-cells [66]. The mice die prematurely due to severe autoimmune disease, which is characterized by the detection of autoreactive antibodies, indicating that PKCδ is essential for the prevention of autoimmune disease. Furthermore, PKCδ deficiency prevents B-cell anergy, allowing maturation and differentiation of self-reactive B-cells, attributed to a defect in NF-κB activation, at least as judged by inefficient IκB degradation in the cytoplasm [67].

Although the above studies suggest that PKCδ is involved in negative regulation of proliferation, there is no consensus on the mechanism. Mecklenbrauker et al. [67] reported that the B-cells from their PKCδ-deficient mice have normal responses to antigenic stimulation and thereby concluded that PKC$\delta^{-/-}$ B-cells have a specific defect in the induction of anergy. In contrast, Miyamoto et al. [66] showed that the proliferation of the B-cells from their PKC$\delta^{-/-}$ mice was increased in response to several mitogenic stimuli, suggesting a generalized enhancement of signalling events. Whereas NF-κB activation remained unaffected, increased production of the growth-promoting cytokine IL-6, as well as the DNA-binding activity of the nuclear factor IL-6 (NF-IL-6) transcription factor, was detected in the PKC$\delta^{-/-}$ B-cells, suggesting PKCδ might negatively regulate B-cell growth through transcriptional regulation of the IL-6 gene. Although the contradictory results remain to be reconciled, the results suggest that BCR engagement results in the activation of not only the pro-mitogenic PKCβ, but also of the anti-mitogenic PKCδ in regulation of B-cell immunity in a specific manner, possibly allowing for fine-tuning of the immune reaction.

ROLE OF PKC IN INNATE IMMUNITY

PKCε in macrophage activation

Previous studies have implicated a role of PKCε as an important mediator of macrophage function. Macrophage-specific inhibition of PKCε has been linked to an inhibition of IL-4-induced NO production [68], whereas expression of PKCε was sufficient to

induce NO synthesis in RAW 264.7 macrophage cell line [69]. In addition, there is evidence for a role for PKCε in macrophage lipopolysaccharide (LPS) signal transduction, possibly in part through induction of mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) [70]. The recent generation and characterization of mice with a targeted disruption of the PKCε gene indeed supports an important role for PKCε in macrophage biology [71]. In contrast with the previous PKC-isoenzyme-knockout mice studies, there was no obvious defect observed in thymocytes, T-cell proliferation or B-cell function in the PKC$\varepsilon^{-/-}$ mice. Whereas the loss of PKCε did not appear to affect differentiation of monocytes and macrophages from bone marrow precursors, elicited peritoneal macrophages generated dramatically reduced levels of NO, prostaglandin E_2 (PGE_2), TNFα and IL-1β in response to LPS and interferon-γ (IFNγ) co-stimulation. Macrophages from PKC$\varepsilon^{-/-}$ mice extravasated normally in response to thioglycollate and the knockout mice were generally healthy, but females had occasional infertility due to Gram-negative bacterial infections in the uterus. In fact, PKCε-deficient mice were unable to clear bacterial infections and demonstrated a significantly decreased period of survival. Further analyses revealed LPS-treated PKCε-deficient macrophages had reduced NO synthase (NOS)-2 expression and attenuated IKK and NF-κB activation, whereas ERK and p38 MAPK were partially inhibited.

Interestingly, PKCε also appears to play a similar role in mediated LPS signalling in dendritic cells [70]. Experiments using PKC-isoenzyme-specific pseudosubstrate-based peptide inhibitors demonstrated that PKCε, but not PKCα or PKCβ, was critical for LPS-induced IKK and NF-κB activation as well as production of TNFα and IL-12 in monocyte-derived dendritic cells [72]. Finally, a recent study showed that PKCε-deficient mouse embryonic fibroblasts display a reduced response to IFNγ as a function of integrin engagement, and this correlated with impaired IFNγ-induced tyrosine phosphorylation of the STAT1 (signal transducer and activator of transcription 1) transcription factor [73]. Taken together, these results suggest that PKCε plays a critical role in integrating different signalling cascades that impact on the establishment of an effective innate immune response.

Yin and yang role of PKC in mast cell degranulation

In addition to their role in B-cell functions described above, additional phenotypes in PKCβ- and PKCδ-null mice have been identified by other laboratories, including a defect in mast cell degranulation [74–76]. Antigen-mediated activation of mast cells by means of IgE antibodies bound to the cell surface triggers complex formations involving the FcεRI (high-affinity IgE receptor) cytoplasmic domains and various intracellular proteins, which subsequently initiates diverse signal transduction pathways resulting in the immediate release of pro-inflammatory agents. Degranulation is a key feature of FcεRI-induced mast cell activation, which requires both PKC and Ca^{2+} for maximal activity. Mast cells derived from PKCβ-deficient mice not only displayed a substantial decrease in degranulation, but also produced less IL-6 in response to IgE–antigen or Ca^{2+} ionophore stimulation, which is in agreement with a previous study reporting an increased level of IL-6 (and IL-2) mRNA in PKCβ-overexpressing mast cells [77]. In contrast, PKCδ-deficient mast cells exhibited a more sustained Ca^{2+} mobilization and a significantly higher level of degranulation, suggesting that PKCδ is a negative regulator of antigen-induced mast cell degranulation [75]. The molecular basis for the opposing function of the two PKC isoenzymes in mast cell degranulation is not clear, but may involve PKCβ-dependent Ras and ERK activation, and TNFα secretion [76] and PKCδ-dependent phosphorylation of the FcεR1 γ-chain that triggers efficient FcεR1 endocytosis after receptor engagement [78]. Clearly, more in-depth studies of the downstream signal transduction pathways leading to transcriptional activation of IL-6 and degranulation by IgE–antigen will be required to advance this area.

CONCLUDING REMARKS AND PERSPECTIVES

Chronic inflammation is increasingly considered to be an important underlying factor in pathological processes in the development of various diseases, including cancer, insulin resistance, artherosclerosis and rheumatoid arthritis. Given the emerging and cell-specific functions of the PKCs in the regulation of the cellular immune responses, selective inhibition of individual PKC isoenzymes might be effective in treating a wide range of human diseases associated with aberrant immune functions. It would be important to utilize these animals to examine the effect of targeted disruption of relevant PKC isoenzymes in disease models to provide *in vivo* validation of distinct PKC isoenzymes as potential targets for pharmaceutical intervention. The phenotype of various PKC-knockout mice suggests that the toxicity of PKC inhibition is anticipated to be rather limited. In this context, it is particularly encouraging to note the recent development of PKC-specific inhibitors, some of which are showing promising results in clinical trials [79,80].

A general concern with the PKC-knockout mice studies, however, is the uncertainty of whether the observed phenotypes are due to developmental effects of null mutation or due to loss of the enzyme activity in adult tissues. Furthermore, a defect in the recruitment or formation of signal-transducing complex molecules, which could result in the decreased activation of downstream targets observed in these mutant cells, has not been excluded. In addition, the phenotype of the PKC-isoenzyme-deficient mice does not rule out compensatory or complementary functions for PKC members. The generation of tissue-specific PKC-isoenzyme-selective conditional knockout and knock-in mice, as well as the use of antisense oligonucleotides (ASO), RNAi and selective pharmacological inhibitors to specifically knock down the levels or kinase activity of individual PKC in relevant cell lines should help address these potential caveats.

The exact molecular basis for the non-redundant function of individual PKCs in different immune cell types remains to be determined, although probable explanations include distinct subcellular localization or stimulus-induced relocalization, different capacities to interact with adaptor proteins or substrates, and different kinetics of activation. Do PKC isoenzymes have a role in other cell types of the immune system and are they dictated by development stages? What are the specific PKC-binding proteins and substrates of PKC, and the structural determinants of their interaction with PKC isoenzymes that dictate their regulation and function in a given cell type or stimulus response? The availability of PKC-isoenzyme-selective knockout mice should now allow one to validate the various reported PKC protein substrates (Table 1). Another major challenge in the PKC signalling field will be to clarify the intersecting and diverging branches of antigen/ligand-receptor signalling pathways, to provide a better understanding of how distinct immune outcomes are regulated by PKCs. Do activation of most PKCs ultimately lead to NF-κB activation and, if so, what are the immediate PKC substrates directly involved in this cascade? It is conceivable that PKCs merely play an indirect, yet critical, role in controlling the localization of signalling proteins or their assembly into functional complexes. The use of systems biology approaches, including cDNA microarray,

proteomics and targeted mutagenesis of phosphorylation sites, should help advance our understanding of the complex mechanisms of PKC signalling in immune responses and may yield novel insights into development of therapeutic agents to selectively modulate PKC activity to treat immune disorders.

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