The role of protein kinase C\(\eta\) in T cell biology

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INTRODUCTION

Protein kinase C (PKC) is a large family of serine/threonine kinases that can be divided into three subfamilies based on their structural homology and requirement of cofactors for activation (Baier, 2003). The conventional PKC subfamily contains \(\alpha, \beta, \beta I, \gamma, \delta, \varepsilon, \theta, \lambda, \zeta\), and \(\eta\) isoforms, requiring calcium and diacylglycerol (DAG) for activation. The novel PKC subfamily contains \(\delta, \epsilon, \theta, \eta, \zeta\), and \(\eta\) isoforms, and requires DAG but not calcium for activation. In contrast, the atypical PKC subfamily (i.e., \(\xi\) and \(\lambda\)) requires neither DAG nor calcium for their activation (Pfeifhofer et al., 2003). Studies using PKC isoform-specific knockdown mice have shown differential roles of each isoform in T cell development and function (Sun et al., 2000; Thuille et al., 2004; Gruber et al., 2005a; Pfeifhofer et al., 2006). For example, PKC\(\alpha\)-deficient mice have a normal T cell development phenotype. In peripheral T cells, PKC\(\alpha\) is dispensable for normal T cell activation and IL2 production, but it is required for proliferation and IFN-\(\gamma\) production (Pfeifhofer et al., 2006). PKC\(\beta I\) is dispensable for normal T cell development and function (Thuille et al., 2004), although it was found to be important for LFA-1-mediated T cell locomotion in a PKC\(\beta I\)-deficient cell line (Volkov et al., 2001). PKC\(\beta I\) is a negative regulator of T cell activation, as PKC\(\beta I\)-deficient T cells are hyperproliferative and produce more IL2 cytokine upon stimulation (Gruber et al., 2005a). This negative regulatory role is also reflected in PKC\(\delta\)-deficient B cells (Mecklenbrauker et al., 2002; Miyamoto et al., 2002). In striking contrast, PKC\(\theta\)-deficient T cells completely lose the ability to proliferate or to produce IL2 after stimulation through the T cell receptor (TCR) in in vitro assays (Sun et al., 2000; Pfeifhofer et al., 2003), even though both \(\delta\) and \(\theta\) have the closest identity (60%) within the novel PKC subfamily (Kong et al., 2011; Quann et al., 2011). PKC\(\eta\) was dispensable for T cell development and activation (Gruber et al., 2005b). In PKC\(\eta\)-deficient mice, there is no overt defect in T cell development (Leitges et al., 2001), but these mice showed impaired Th2 cell differentiation (Martin et al., 2005). Interestingly, although discovered more than two decades ago (Osada et al., 1990), and like PKC\(\delta\), highly expressed in T cells (Baier, 2003; Figure 1: data from www.biogps.org (Su et al., 2004; Wu et al., 2009) the role of PKC\(\eta\) had never been thoroughly examined in T cells until the recent study from our group (Fu et al., 2011). This despite the fact that PKC\(\eta\)-deficient mice have existed for almost 10 years (Chida et al., 2003). Meanwhile, although discovered only a little later than PKC\(\alpha\), PKC\(\eta\) is considered paramount in T cell function. Our recent work on PKC\(\eta\) has significantly filled this gap by showing both isoform-specific and redundant (with PKC\(\beta I\)) roles of PKC\(\eta\).
in T cell development and function (Fu et al., 2011; Fu and Gascoigne, 2012). In this article, we will first briefly review some earlier studies on PKCγ, then mainly focus on four subjects currently under study: (1) the recruitment of PKCγ to the immunological synapse; (2) its role in T cell development; (3) its role in T cell function; (4) its role in TCR signaling. Finally, we would like to share...
some of our thoughts with the readers about future investigations regarding PKC\(\eta\).

**COMPARISON OF PKC\(\eta\) AND PKC\(\theta\) MOLECULES**

In the novel PKC subfamily, PKC\(\delta\) and PKC\(\theta\) are closely related (60% identity), as are PKC\(\epsilon\) and PKC\(\eta\) (also 60% identity; Baier, 2003; Quann et al., 2011). A cross comparison between PKC\(\eta\) and PKC\(\theta\) reveals that these two isoforms bear 42% identity (Figure 2A). The overall domain structure of PKC\(\eta\) and PKC\(\theta\) proteins shows a high degree of similarity. This domain architecture is shown in Figure 2B. In both isoforms, there is a “C2-like” domain near the amino-terminal of the protein, which cannot bind calcium, unlike the C2 domains in conventional PKC isoforms (Baier, 2003). Following the C2-like domain, there are tandem repeats of two DAG binding C1 domains and the V3 hinge region. This is the most different region between PKC\(\eta\) and PKC\(\theta\) (Figure 2C). In PKC\(\theta\), V3 is important in association of the kinase with CD28 and as a result is required to mediate PKC\(\theta\)’s localization in the central synapse (Kong et al., 2011). The motif within PKC\(\theta\) V3 domain that is required for CD28 interaction, including the conserved PXXP sequence (Kong et al., 2011), is missing in PKC\(\eta\) (Figure 2C). The C2-like, C1, and V3 domains together form a regulatory region, which likely performs the isoform-specific functions, as the carboxyl-terminal serine/threonine kinase domain is rather conserved across all PKC isoforms. The difference between the V3 domains of PKC\(\theta\) and PKC\(\eta\) suggests that this may be responsible for their different localization in the immunological synapse.

**A BRIEF HISTORY OF PKC\(\eta\) STUDIES**

PKC\(\eta\) was originally identified from a mouse epidermis cDNA library and found to be highly expressed in mouse tissues such as skin, lung, and heart (Osada et al., 1990). Because of this tissue-specific expression pattern, most studies regarding PKC\(\eta\) were historically focused on keratinocyte proliferation and differentiation (Ohba et al., 1998; Cabodi et al., 2000). However, development of skin was normal in PKC\(\eta\)-deficient mice in steady state (Chida et al., 2003). In contrast, under challenging conditions, these PKC\(\eta\)-deficient mice were susceptible to skin tumor induction and showed impaired wound healing (Chida et al., 2003). In immune cells, PKC\(\eta\) is highly expressed in mouse macrophages and T cells, but not B cells (Figure 1). However, interestingly, potential roles of PKC\(\eta\) in B cells were suggested in a number of studies (Morrow et al., 1999; Oda et al., 2008). For example, PKC\(\eta\) was shown to be specifically transcribed in pro-B but not pre-B cells, and a pro-apoptotic role of PKC\(\eta\) in B cells was suggested (Morrow et al., 1999). In another study, PKC\(\eta\) was shown to direct IRF4 expression and Igk gene rearrangement in pre-BCR signaling (Oda et al., 2008). Surprisingly, nothing was known about the specific role of PKC\(\eta\) in T cells until quite recent work from our group and others (Singleton et al., 2009; Fu et al., 2011; Quann et al., 2011; Sewald et al., 2011), which is the topic we address below.

**RECRUITMENT OF PKC\(\eta\) TO THE IMMUNOLOGICAL SYNAPSE**

The immunological synapse or supramolecular activation cluster (SMAC) forms at the interface between a T cell and an...
antigen-presenting cell (APC; or a surrogate), and is the site at which early signaling events occur (Grakoui et al., 1999). The widely accepted importance of PKCθ in T cells is largely due to its identification as the only PKC isofrom recruited to the immunological synapse (Monks et al., 1997), and particularly to the central synapse region (cSMAC), along with TCR and other molecules (Monks et al., 1998). Since then, PKCθ has served as a landmark for defining the immunological synapse. However, studies from our group and others challenged the view that only PKCθ is recruited to the synapse (Singleton et al., 2009; Fu et al., 2011; Quann et al., 2011). PKCη is recruited to the immunological synapse upon T cell recognition of its cognate antigenic peptide-MHC (pMHC), but not non-stimulatory pMHC, presented by APCs (Figure 3; Fu et al., 2011). More interestingly, PKCη and PKCθ showed different recruitment patterns, as PKCη forms a diffuse pattern at the immunological synapse, whereas PKCθ concentrates into the central region (Figure 3; Singleton et al., 2009; Fu et al., 2011), suggesting different functions in time and space of these two PKC isofroms. In addition to PKCη and PKCθ, PKCs is also recruited to the immunological synapse (Quann et al., 2011). In this study, polarization of the T cell microtubule-organizing center (MTOC) is directed by diacylglycerol (DAG) at the immunological synapse via three PKC isofroms, in two sequential steps. Initially, PKCs and PKCη accumulate in a broad region of the interface between T cell and APC, followed by PKCθ concentrating in a smaller, central, zone (Quann et al., 2011). It seems that in different cell types, recruitment of PKC isofroms could also be different. For example, it has been shown that, in contrast to the immunological synapse-localization in effector T cells, PKCθ is sequestered away from the immunological synapse in regulatory T cells (Treg), and thus mediates negative feedback on Treg cell function (Zanin-Zhorov et al., 2010). This intriguing observation may be also worth examination for PKCs and PKCη.

PKCη IN T CELL DEVELOPMENT

Our initial speculation that PKCη may play a role in T cell development was based on the finding that PKCη mRNA expression was upregulated during thymocyte positive selection (Mick et al., 2004; Niederberger et al., 2005). These observations were surprising given the established important role of PKCθ in T cell biology, but intriguing because PKCθ-deficient mice have only a very minor defect in thymocyte development. Initial phenotyping of PKCθ-deficient mice did not identify any defects in thymocyte development (Sun et al., 2000; Pfeifhofer et al., 2003), although later studies did find a mild thymocyte development defect in such mice (Morley et al., 2008; Fu et al., 2011). However, pheno-typing of PKCθ-deficient mice showed rather normal thymocyte development. This was not completely unexpected given the multiple novel PKC isofroms co-expressed in T cells, and redundancy could play a role to compensate for the absence of any particular isofrom. We also noted that induction of PKCη mRNA is much higher and earlier in PKCθ-deficient mice than in wild-type mice (i.e., induction during positive selection in wild-type mice, but induction before positive selection in PKCθ-deficient mice), suggesting a compensatory effect due to redundancy of function between PKCη and PKCθ (Fu et al., 2011). In accord with this notion, PKCη is recruited to the immunological synapse in immature CD4+CD8+ (DP) thymocytes in the PKCθ−/− mice, as is PKCθ in the PKCθ-sufficient DP cells. In PKCθ-sufficient cells, PKCη is only recruited to the synapse in mature CD4+ or CD8+ (SP) thymocytes. These results are only suggestive of redundant function, but clear redundancy between PKCη and PKCθ in thymocyte development was confirmed when we phenotyped PKCη−/−θ−/− mice. Positive selection of thymocytes in these double-knockout mice was more severely impaired than either single PKC-knockout mice. However, the blockade of thymocyte development in PKCη−/−θ−/− mice was not complete, as SP cell numbers were only reduced by about 50% (Figure 4A; Fu et al., 2011). Therefore, it is possible that other PKC isofroms than PKCη and θ can still compensate for their deficiency, perhaps most likely those members within the same subfamily (e.g., PKCs).

It is natural to speculate that in a multimember protein family, there are some overlapping functions between individual members (i.e., redundancy), as well as isofrom-specific functions. In our study, we found that PKCη and PKCθ had opposite effects on the CD4 to CD8 T cell ratios in the secondary lymphoid organs (Figure 4B). PKCθ-deficient mice had a higher CD4/CD8 ratio than wild-type mice, whereas PKCθ-deficient mice had a lower ratio, indicating an isofrom-specific role of these PKCs in balancing CD4 and CD8 T cell homeostasis. Interestingly, these effects are “neutralized” by each other in that PKCη−/−θ−/− mice exhibited normal CD4/CD8 ratios. Multiple factors can affect the CD4/CD8 T cell ratio during thymocyte development (Corbella et al., 1994; Suzuki et al., 1995; Sim et al., 1998a,b). The
**PKCη IN PERIPHERAL T CELL HOMEOSTASIS AND RESPONSE TO ANTIGEN**

For the sake of simplicity, we focused on CD8 T cells for most functional studies on PKCη−/− mice (Fu et al., 2011). PKCη-deficient CD8 T cells showed a mild proliferation defect compared to wild-type T cells upon anti-CD3 antibody stimulation. In contrast, under the same conditions, PKCθ-deficient CD8 T cells were completely non-proliferative, as previously reported (Sun et al., 2000; Pfeifhofer et al., 2003). However, this striking difference between PKCη−/− and PKCθ−/− CD8 T cells was blurred under more physiological conditions. For example, when we used APCs pulsed with antigenic peptide to stimulate these PKC-deficient CD8 T cells, both PKCη−/− and PKCθ−/− CD8 T cells still proliferated less well than wild-type cells, but the relative difference between PKCη−/− and PKCθ−/− CD8 T cells is much more subtle than with anti-CD3 crosslinking (Fu et al., 2011). In general, we observed that antigen-specific proliferation of PKCη-deficient T cells was more severely reduced compared to wild-type cells than was anti-CD3 antibody induced proliferation. It may be that this is because the anti-CD3 stimulation does not involve the formation of the immunological synapse, whereas the synapse is important in the antigen-specific responses. The proliferation defect of PKCη−/− CD8 T cells was also confirmed in *in vivo* experiments, where wild-type and PKCη−/− CD8 T cells were co-transferred into recipient mice and stimulated by antigenic peptide (Figure 5A; Fu et al., 2011).

Therefore the proliferation defect of PKCη−/− CD8 T cells is consistent both *in vitro* and *in vivo*. However, in the case of PKCθ−/− CD8 T cells, *in vivo* reductions in responses were much less severe than those observed *in vitro*. For instance, the absence of PKCθ does not impair antigen-specific proliferation (Barouch-Bentov et al., 2005) or antiviral immune responses, in which PKCθ−/− CD8 T cells were found to proliferate normally (Berg-Brown et al., 2004; Marsland et al., 2005). The role of PKCθ in the *Listeria* infection model is controversial, with one group showing PKCθ is not important (Valenzuela et al., 2009) and another group claiming the opposite (Sakovicz-Burkiewicz et al., 2008). These conflicting results may be due to the different bacterial infection doses used between these two groups. One common explanation of PKCθ’s dispensable role in these infection models is that *in vivo* innate signals can compensate for the absence of PKCθ (Marsland et al., 2005; Valenzuela et al., 2009), however it is also possible that PKCη functions in place of PKCθ in these cases.

In stark contrast, in an experiment to measure T cell homeostatic proliferation, we found that PKCη, but not PKCθ, is required (Figure 5B; Fu et al., 2011). In these experiments, no matter whether we used polyclonal T cells or monoclonal TCR transgenic T cells as donor cells, only PKCη−/− CD8 T cells showed impaired proliferation in lymphopenic animals, whereas PKCθ−/− CD8 T cells showed normal homeostatic proliferation. The non-essential role of PKCθ in T cell homeostatic proliferation was also independently reported by others (Valenzuela et al., 2009). This was indeed an unexpected result: one would have assumed that defective homeostatic proliferation might occur in PKCθ−/− T cells, at least as a reflection of strong deficiency in *in vitro* proliferation. Both TCR mediated signaling and the cytokines IL7 and IL15 are required to support normal homeostatic proliferation (Jameson, 2002; Surh and Sprent, 2005). However, we think...
altered responsiveness to these cytokines is unlikely to contribute to the defective homeostatic proliferation in PKCη-deficient T cells, because the amounts of IL7Rα (CD127) and IL15R (CD122) on the PKCη-deficient T cells were the same as those of wild-type T cells (Fu et al., 2011). We were also unable to find any difference in the numbers of apoptotic cells between PKCη-deficient and -sufficient mice, suggesting that the requirement for PKCη for homeostatic proliferation is not due to differential cell survival. PKCθ has been found to be a survival factor for CD8 T cells. In contrast to antigen-specific T cell proliferation, which is the clonal expansion of particular T cells recognizing their cognate antigen, homeostatic proliferation is the response of T cells to self-MHCp complexes for survival. Therefore the strength of TCR signaling is different in these two scenarios. It is possible that PKCη and PKCθ play dominant roles in homeostatic and antigen-specific proliferation respectively. PKCθ may be more important in antigen-specific activation because of its reported role in breaking the “symmetry” of the synapse (Sims et al., 2007). This is required for T cell movement, such as during scanning over the surface of an APC.

**PKCθ IN T CELL RECEPTOR SIGNALING**

Compared to the very well characterized mechanisms regarding PKCθ in the molecular signaling machinery in T cells (Egawa et al., 2003; Wang et al., 2004; Roose et al., 2005; Manicassamy et al., 2006), similar studies of PKCη are at a very early stage. In our study, we showed that Ca2+ flux and NFκB nuclear translocation were impaired in PKCη−/− T cells, but that TCR-proximal signaling pathways were intact. These signaling defects are similar to those defects reported in PKCθ−/− T cells (Sun et al., 2000; Pfeifhofer et al., 2003). Thus two questions remain: First, if the signaling defects are the same in PKCη and PKCθ-deficient T cells, why are the defects in PKCθ-deficient T cells not as strong as PKCθ-deficient T cells, at least in vitro? One possibility is that more signaling pathways are interrupted by PKCθ-deficiency compared to PKCη-deficiency, in addition to NFκB (Sun et al., 2000) and NFAT (i.e., Ca2+ signaling-related) defects (Pfeifhofer et al., 2003). For example, it was recently shown that PKCθ can bind to CD28 and thus mediates a co-stimulation-driven signaling pathway from the immunological synapse (Yokosuka et al., 2008; Kong et al., 2011). More importantly, are there non-overlapping or distinct pathways between PKCη and PKCθ? The answer is likely yes. First of all, as shown in our study, PKCη and PKCθ have distinct roles in homeostatic proliferation, with η being required but θ being dispensable (Fu et al., 2011). Second, the different spatio-temporal localization of PKCη and PKCθ in T cells needs to be considered, because the localization of TCR, CD28, and other molecules plays important roles in T cell activation and proliferation.

**FIGURE 5 | Requirement of PKCθ in T cell proliferation.** (A) PKCθ is required for efficient antigen-specific T cell proliferation in vivo. (B) PKCθ but not PKCη is required for T cell homeostatic proliferation in vivo. Adapted from Fu et al. (2011).

**Table 1 | Comparison of PKCθ and PKCη in T cell biology.**

| T cell development in KO mice | PKCθ | PKCη |
|-------------------------------|------|------|
| MATURE T CELLS IN KO MICE    |      |      |
| CD4/CD8 ratio                 | Lower than WT² | Higher than WT² |
| Proliferation                 |      |      |
| to aCD3 in vitro              | Severely impaired²⁻⁴ | Mildly impaired² |
| to PMA/Inomycin               | Normal⁴ or Impaired³ | Normal³ |
| to antigen in vivo            | Normal⁵⁻⁸ or Impaired⁶ | Impaired⁶² |
| to antigen in vitro           | Impaired¹⁻⁴⁻⁵ | Impaired²⁵ |
| Homeostatic proliferation     |      |      |
| Non-tg CD8 T cells            | Normal²⁻⁷ | Impaired² |
| OT-1 tg CD8 T cells           | Normal² | Impaired² |

**SIGNALLING EVENTS IN KO CELLS**

| Calcium flux                  | Impaired³ | Impaired² |
| NFKB                          | Impaired³⁻⁴ | Impaired² |
| NFAT                          | Normal³ or Impaired⁴ | Not available |
| AP-1                          | Impaired³⁻⁴ | Not available |

**IMMUNOLOGICAL SYNAPSE (IS)**

| In effector T cells           | Recruited to IS¹⁰⁻¹²⁻¹⁵ | Recruited to IS²⁻¹² |
| Spatial pattern               | Central region¹¹⁻¹² | Diffuse pattern²⁻¹² |
| Temporal kinetic             | Late, after η¹³ | Early, before θ¹³ |
| Domaínal required            | V3 domain¹⁴ | Not available |
| In regulatory T cells         | Not recruited to IS¹⁵ | Not available |

¹Morley et al. (2008), ²Fu et al. (2011), ³Sun et al. (2000), ⁴Pfeifhofer et al. (2003), ⁵Berg-Brown et al. (2004), ⁶Barouch-Bentov et al. (2009), ⁷Valenzuela et al. (2009), ⁸Marland et al. (2005), ⁹Marland et al. (2004), ¹⁰Monks et al. (1997), ¹¹Monks et al. (1998), ¹²Singleton et al. (2009), ¹³Quann et al. (2011), ¹⁴Kong et al. (2011), ¹⁵Zannin-Zhorov et al. (2010).
PKCθ in the immunological synapse, with η showing an earlier and more diffuse pattern and θ showing a later and more concentrated pattern in the central region of the synapse (Singleton et al., 2009; Fu et al., 2011; Quann et al., 2011). Finally, there is a study showing PKCη and PKCθ having differential downstream functions in EL4 thymoma cells (Resnick et al., 1998). Collectively, these results strongly indicate the existence of an at least partially independent signaling pathway involving PKCθ.

**FUTURE DIRECTIONS**

As mentioned earlier, the study of PKCη in T cell biology and the immune system in general, is far behind the state of knowledge we have on its cousin PKCθ (Fu and Gascoigne, 2012). Several recent studies have finally brought PKCη under the spotlight (Singleton et al., 2009; Suzuki et al., 2009; Fu et al., 2011; Quann et al., 2011; Sewald et al., 2011). In Table 1, we summarize the available results regarding PKCη in comparison with PKCθ. However, much more work needs to be done before we have a comprehensive understanding of the role of PKCη. First, what molecular machinery is involved in PKCη signaling? Does PKCη share the same signaling complex with PKCθ, such as the CAMA1/MALT1/Beclin complex? Second, what drives PKCθ to the immunological synapse and what is the importance of differential localization of PKCθ compared to PKCη in the synapse? A recent study shows that the V3 domain is required for PKCη recruitment to the immunological synapse (Kong et al., 2011). Is this also true for PKCθ, considering their generally similar structures, or is the diffuse synapse-localization of PKCη due to the lack of the relevant motif in the V3 domain? Since PKCη interacts with CD28 through a V3 motif, does PKCη also interact with CD28, or if not, is it due to the different V3 sequences? Does PKCη interact with other co-stimulatory molecules? Third, what roles does PKCη have in other T cell subsets or in other immune cells? In mice, it has been shown that PKCθ-deficiency impairs regulatory T cell development (Schmidt-Supprian et al., 2004), and in humans it has been shown that PKCθ plays a negative feedback role in regulatory T cell function, which is in contrast to its positive feedback role in naïve conventional T cells (Zanin-Zhorov et al., 2010). Thus it may be informative to check the role of PKCη in Treg cell development and function. PKCθ-deficiency has been shown to specifically impair Th2 cell responses but not Th1 responses, and thus has various effects in anti-pathogen immune responses (Berg-Brown et al., 2004; Marsland et al., 2004, 2005; Sakowicz-Burkiewicz et al., 2008). Could PKCη play an opposing role in these cases or a redundant role? What effects may PKCη have on CD4 T-helper cell subset differentiation? The role of PKCη in infection models and autoimmune diseases is another area that clearly needs attention. A simple but very informative study would be to directly compare the immune responses in η−/−, θ−/−, or ηθ-double deficient mice to the same viral and bacterial pathogens to get a full picture of the role of these two PKC isoforms in immunity. All these questions deserve more systematic studies in the future.

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