Published in final edited form as:

Trends Immunol. 2011 August ; 32(8): 358–363. doi:10.1016/j.it.2011.04.007.

PKC- θ and the immunological synapse: mechanisms and implications

Alexandra Zanin-Zhorov¹, Michael L Dustin^{1,*}, and Bruce R. Blazar^{2,*}

¹Molecular Pathogenesis Program, Helen and Martin Kimmel Center for Biology and Medicine, Skirball Institute of Biomolecular Medicine, Department of Pathology, New York University School of Medicine, New York, NY 10016, USA

²University of Minnesota Cancer Center and Department of Pediatrics, Division of Bone, Blood and Marrow Transplantation, Minneapolis, MN 55455, USA

Abstract

Protein kinase C- θ has emerged as a central regulator of conventional effector T cell (Teff) and CD4⁺FoxP3⁺ regulatory T cell (Treg) function. In the years since this initial description PKC- θ regulation has taken on even greater complexity, as it has been implicated in negative regulation of immunological synapse stability and in mediating development and negative feedback regulation in natural Tregs. This review will examine current knowledge about PKC- θ in the immunological synapse, recent evidence for its distinct localization in Tregs and the therapeutic implications of targeting PKC- θ in Teffs and Tregs.

PKC- θ location in the immunological synapse

The protein kinase C (PKC) family of serine/threonine kinases has a critical role in immune regulation through signal transduction networks that coordinate practically every move and decision. There are three families of PKCs, the conventional, which are activated by Ca²⁺ and diacylglycerol, the novel, which are activated by diacylglycerol and the atypical, which are insensitive to both Ca²⁺ and diacylglycerol ¹. In T cells, all sub-families are represented. PKC- θ is a novel PKC that is most abundant in hematopoietic cells ². PKC- θ rose to prominence as the only PKC isoform that is selectively recruited to the conventional effector T cell (Teff) immunological synapse (IS) ³ (Figure 1). The IS is a stable cell-cell junction formed between a Teff and an antigen presenting cell (APC) and composed of a peripheral supramolecular activation cluster (pSMAC) and central supramolecular activation cluster (cSMAC) where LFA-1 and TCR microclusters converge, respectively ⁴⁻⁷ (Figure 1). Several groups have demonstrated that PKC- θ is recruited to the cSMAC in a CD28 co-stimulation dependent manner ^{3,8-10}. In addition, we have demonstrated that PKC- θ can be recruited to the IS in a multifocal pattern without CD28 engagement and negatively regulates IS stability by favoring symmetry breaking of the pSMAC in a manner counteracted by expression of Wiscott Aldrich Syndrome protein (WASp) ¹¹. Furthermore, stabilization of the IS by PKC- θ inhibition can enhance killing by CD4⁺ cytotoxic T cells ¹². The detailed mechanism by which PKC- θ stimulates symmetry breaking in Teffs is not known, but in other systems symmetry breaking involves cooperative myosin II activation ¹³.

Correspondence to: Bruce R. Blazar, M.D, blaza001@umn.edu.

*equal contributors

CD4⁺ FoxP3⁺ regulatory T cells (Tregs) play a central role in suppressing inflammatory responses in TCR dependent manner¹⁴. There is evidence for stable Treg-DC interactions in vivo that play an important role in Treg function^{15–17}, but the organization of the Treg IS has been less studied. In contrast to Teffs, PKC-θ is sequestered to the distal pole complex away from the Treg IS¹⁸. Moreover, we found that Tregs form more stable IS than Teffs on supported planar bilayers presenting anti-CD3 antibodies and ICAM-1. This is consistent with the localization of PKC-θ at the distal pole of the IS, which may favor higher IS stability in Tregs. Thus, the location and regulation of PKC-θ is different in Teffs and Tregs.

PKC-θ signaling pathways

PKC-θ plays a central role in controlling T cell function by regulation of signaling pathways leading to activation of transcription factors, *i.e.*, nuclear factor κB (NF-κB), activation protein-1 (AP-1) and nuclear factor of T cells (NFAT) (Figure 2). PKC-θ activates NF-κB through phosphorylation and activation of the membrane associated guanylate kinase (MAGUK) Carma1 (also called Card11)¹⁹. The MAGUK module consists of a PDZ domain, and SH3 domain and a non-enzymatically active guanylate kinase domain. The N-terminus of Carma1 contains a caspase recruitment domain (CARD) and a coiled-coil domain²⁰. Phosphorylation of Carma1 by PKC-θ initiates assembly of an oligomeric complex with CARD domain containing adapter Bcl10 and the death domain (DD) containing paracaspase Malt1 that mediates activation of NF-κB transcription factors¹⁹. PKC-θ also phosphorylates the ste20-family kinase SPAK, which is important for AP-1 transcription factor activation²¹. While one group found that Ca²⁺ and NFAT activation was relatively normal in PKC-θ deficient T cells in vitro²², another group found that the Ca²⁺/NFAT pathway was also defective²³. The role of PKC-θ in NF-κB signaling is selective for the TCR/CD28 costimulation signaling pathways^{24,25} as inflammatory cytokine mediated activation of NF-κB is PKC-θ independent²². PKC-θ is essential for IL-2 production and downstream of TCR signals²³ and also appears to mediate the enhanced IL-2 production in response to CD28 co-stimulation^{8,10}. This pathway could be considered the canonical pathway for function of PKC-θ in T cell activation.

PKC-θ and T cell function

Conventional T cells

Reverse genetic studies in mice have revealed that the critical roles of PKC-θ in TCR signaling have variable penetrance *in vivo*. The development of conventional naïve CD8 and CD4 T cells appears grossly normal in PKC-θ-deficient mice^{22,23}. PKC-θ deficient mice have profound defects in generating Th2 responses to *Nippostrongylus brasiliensis* and are also resistant to induction of pulmonary allergic hypersensitivity responses to model antigens^{26,27}. In addition, PKC-θ deficient T cells had a reduced capacity to induce autoimmune colitis²⁸. In contrast, PKC-θ deficient mice can clear *Listeria monocytogenes*, *Leishmania major*, lymphocytic choriomeningitis virus (LCMV) and murine γ-herpesvirus 68 infections similarly to wild type mice^{26,29–31}. The surprising competence of PKC-θ deficient mice in combating bacteria and viral pathogens is explained at least in part by complementation of the TCR-dependent NF-κB activation defect by toll-like receptors responding to innate cues from microbial pathogens³². Autoimmune and Th2 responses to multicellular parasites may lack of strong, NF-κB-activating innate signals leading to greater reliance on TCR signaling and a stronger phenotype in PKC-θ deficient mice.

Regulatory T cells

Tregs are a subset of CD4⁺ T cells that are central in maintaining a balance between immune tolerance to self-antigens and anti-tumor responses¹⁴. There are two developmentally distinct populations of CD4⁺ Treg, both of which depend upon the transcription factor

FoxP3³³⁻³⁵. Natural Treg (nTreg) are produced in the thymus and express that additional transcription factor Helios³⁶. Induced Treg are generated from naïve Teff in the periphery and do not express Helios³⁶. Foxp3 deficiency leads to defects in Treg function, which manifest as a multi-organ fatal inflammatory disease in mice^{37,38}. The unique feature of Tregs is their ability to suppress a large number of different target cell types that includes CD4⁺ and CD8⁺ subsets of T cells, B cells, NK cells and dendritic cells^{14,39}. The proposed mechanisms of Treg-mediated suppression include suppressor cytokine secretion, IL-2 consumption, target cell cytolysis and cell surface molecules^{14,39}.

In mice lacking PKC- θ (PRKCQ gene knockout), Treg development in the thymus is impaired, but the per cell function of peripheral Treg was intact⁴⁰. The requirement of PKC- θ for Treg development may relate to the stronger nature of the TCR ligands that drive differentiation of Tregs as compared to conventional T cells and the role of PKC- θ in augmenting signal transduction in TCR ligated cells. For example, the PKC- θ downstream target cRel is critical for Treg development and expansion⁴¹. Furthermore, PKC- θ deficiency impairs IL-2 production by Teffs as noted above, and Tregs need high levels of IL-2 to function, survive and proliferate^{14,38,42}. Therefore, reduced IL-2 in PKC- θ deficient mice may contribute to Treg defects^{40,41}.

Pre-treatment of mouse Tregs with PKC- θ inhibitor increases Treg engraftment measured one week after injection into TCR $\alpha^{-/-}\beta^{-/-}$ mice in presence of CD4⁺CD25⁺CD45RB^{high} subset of Teff¹⁸. Since Treg divided multiple times after introduction into lymphopenic mice⁴³, we can conclude that Tregs with inhibited PKC- θ show no major defect in proliferation in vivo, and may even proliferate more than Treg with fully active PKC- θ . In contrast, inhibition of PKC- θ abolished the ability of human Treg to proliferate in vitro upon anti-CD3/CD28 stimulation in presence of high levels of IL-2¹⁸. This discrepancy between the role of PKC- θ in Treg expansion in vivo and in vitro might be explained by additional factors contributing to IL-2 production and potentially other growth factors present in vivo. Thus, in Tregs, PKC- θ is required for thymic development, but dispensable for in vivo homeostatic expansion and suppressive function.

TCR signals are required for Treg suppressive function and these signals can be received through a stable IS. Human Tregs form more stable IS compared to Teffs¹⁸. This suggested a potential defect in PKC- θ signaling at the IS¹¹. Consistent with this, the Treg IS displays reduced markers of signaling down-stream of TCR including phosphorylated Src family kinases, PKC- θ and CARMA-1 (Figure 2). Furthermore, inactivation of PKC- θ in Treg with an irreversible small molecule inhibitor resulted in ~4-fold increase in Treg function¹⁸. Knock-down of PKC- θ by siRNA generated a similar increase in Treg function. The mechanism by which PKC- θ signaling mediates negative feedback in Treg is not known, but may involve NF- κ B mediated transcription in the presence of FoxP3 or other Treg associated transcription factors. Many questions remain about how the role of PKC- θ /NF- κ B in Treg development in the thymus relates to the role of those molecules in maintenance of the Treg phenotype and function.

Targeting PKC- θ in disease

Tregs dysfunction or insufficiency has been linked to autoimmune disease in human and animal models⁴⁴⁻⁴⁶. We were able to fully reproduce the findings from the Lipsky and Shevach lab that Treg isolated from peripheral blood of patients with rheumatoid arthritis (RA) have reduced function due to negative regulation by tumor necrosis factor- α (TNF- α)^{18,46}. Interestingly, we found that while conventional pathways for TNF- α signaling are not PKC θ dependent²², TNF α treatment results in re-localization of PKC- θ in Treg to a more Teff like pattern¹⁸. Moreover, PKC- θ inhibition protects Treg from TNF- α -mediated

suppression and partially restores the diminished suppressive activity of Tregs purified from RA patients^{18,46}. Finally, PKC- θ inhibition in Treg significantly increases their potential to protect mice against inflammatory colitis *in vivo*^{18,47}. It was quite remarkable that pretreating Treg with a small molecule inhibitor, washing away the free drug in infusing the cells into a lymphopenic host would have a sufficiently durable effect to change the course of a disease that develops over a period of weeks. Since Treg expand rapidly in lymphopenic hosts and PKC- θ inhibition does not block this expansion, the drug is almost certainly diluted out. Thus, these effects may take place early and then set the stage for the development of disease, perhaps by determining the final ratio of Treg to Teff in the animal after reconstitution. Thus, PKC- θ suppressed, more potent Treg could change this ratio both by inhibition proliferation of the Teff and by proliferating faster.

The recent success of small molecule inhibitors of PKC- θ in phase I clinical trials for psoriasis raises the possibility of reducing Teff and increasing Treg function a single small molecule⁴⁸. It is useful to consider both the role of PKC- θ in Teff and Treg. PKC- θ deficient Teffs are more prone to apoptosis, a finding associated with reduced levels of BclxL expression. However, forced expression of BclxL in Treg did not improve their survival⁴⁰. The absence of PKC- θ results in a reduced survival of CD4+ and to a greater extent CD8+ T cells^{49–51}. These data predicted that PKC theta inhibition may be a useful approach to inhibit Teff responses such as those that would occur during allogeneic transplantation of solid or hematopoietic organs or autoimmunity (reviewed in⁵²). Thus, inhibiting PKC- θ would function to either reduce the number of activated Teffs by failing to protect Teffs from apoptosis and activation induced cell death or ablate CD28 signals, predisposing T cells to become anergic. Conversely, blocking CD28/B7 ligand interaction using an anti-CD28 monoclonal antibody results in PKC- θ and Jnk suppression, along with long-term cardiac allograft survival⁵³.

Regulation of IL-2 production and alloreactivity *in vivo* is influenced by both PKC- α and - θ , which affect NFAT translocation⁵⁴. Whereas PKC- α or - θ deficient recipients have only minimal prolongation of cardiac allograft survival, doubly deficient recipients have additive effects on allograft survival, indicating that PKC- α and - θ are non-redundant in controlling alloreactivity *in vivo*. PKC theta deficiency is partially compensated in PKC- θ deficient recipients as cardiac allograft rejection is delayed but not eliminated. The strength of the partial compensation is only modest since cardiac allografts in PKC- θ deficient recipients are hypersusceptible to blockade with anti-CD154 antibody or CTLA4-Ig [Manicassamy, 2008 #41]. In T cell adoptive transfer experiments, PKC- θ deficient T cells fail to reject cardiac allografts unless Bclxl is expressed by transgenesis⁵⁰.

Consistent with the finding that PKC- θ regulates CD4+ and CD8+ T cell survival, alloreactivity and IL-2 production, graft-versus-host disease (GVHD) lethality in irradiated recipients of allogeneic CD8+ or CD4+ and CD8+ T cells was severely ameliorated³¹. Despite the high degree of systemic inflammation and tissue destruction that is characteristic of GVHD, PKC- θ deficient T cells were hypoproliferative and had a higher frequency of annexin V staining after adoptive transfer in irradiated allogeneic recipients, giving rise to significantly fewer splenic CD4+ and CD8+ T cells early post-BMT transplant. Interestingly, neither TLR4 nor TLR9 ligation could overcome the profound defect of PKC- θ deficient T cells in GVHD induction. This contrasts with the situation in control of bacterial and viral infection in which TLR stimulation could compensate for PKC- θ deficiency. This is important because it suggests that PKC- θ inhibition may effectively block GVHD without eliminating anti-microbial defenses. Moreover, the presence of strong proinflammatory signals such as those derived from a mitogenic anti-CD40 antibody or lipopolysaccharide enhanced, but did not fully restore, the *in vivo* expansion and cytotoxicity of TCR transgenic CD8+ T cells administered to recipients expressing the

relevant high affinity ligand. Skewing of cytokine responses away from a Th2²⁶, which have been associated with decreased GVHD in some models, was not evident. Intriguingly despite these profound defects in CD8+ T cell proliferation and cytotoxicity, clearance of a viral pathogen and leukemia cell line that are controlled by CD8+ T cells was intact. Relevant to bone marrow transplantation, Ca²⁺ signaling via calcineurin augments PKC-θ expression (Ref). Thus, calcineurin inhibitors may operate to reduce GVHD in part via downregulation of PKC-θ expression in donor T cells. Reduced PKC-θ expression may lead to lower FasL expression levels⁵⁵ that may also contribute to the lower GVHD lethality seen in recipients given PKC-θ deficient vs sufficient T cells. Taken together, these results suggest that the sterile inflammatory signaling driving GVHD may be insufficient activators of NF-κB expression in donor T cells early post-transplant. In contrast, the innate sensing mechanisms in graft-versus-leukemia may be better activators of NF-κB, resulting in complementation of the PKC-θ defect in TCR signaling. Alternatively, the reduced T cell expansion and cytotoxicity of PKC-θ deficient T cells may be insufficient to cause GVHD lethality due to absolute cell number, tissue location, or persistence of cells but sufficient to reduce viral load and tumor burden. Regardless of the mechanism, pharmacological targeting of PKC-θ may be an ideal approach to decrease GVHD lethality while preserving anti-infection and anti-tumor T cell responses post-transplant.

With respect to autoimmune diseases, PKC-θ deficient recipients are resistant to the development of experimental autoimmune encephalomyelitis^{56–58} and myosin-induced autoimmune myocarditis, associated with reduced IL-17 secretion⁵⁹. Under these conditions, TLR9 signaling can act directly on PKC-θ ko T cells to activate NF-κB, resulting in near normal T cell expansion under Th1 polarizing conditions, restoring bclxL expression and promoting the generation of autoimmune myocarditis⁵⁹. However, PKC-θ inhibition may prove to be useful in preventing autoimmune colitis as was discussed above.^{18,28}. Thus, PKC-θ inhibition could be highly useful in preventing and treating autoimmune diseases via targeting Teffs and Tregs to reduce and conversely increase their in vivo survival and function.

Concluding remarks

Our attention was initially called to PKC-θ by its unique localization in the Teff cell immunological synapse^{3,4}. A dozen years later application of a second-generation technology to study the immunological synapse revealed that PKC-θ is excluded from the Treg synapse¹⁸ (Figure 3). These studies led to our current understanding that PKC-θ has dual roles in Teff and Treg cells that can be targeted to reduce inflammation in the context of alloreactivity and autoimmunity. In Teffs PKC-θ promotes inflammation by increasing activation of the NF-κB transcription factors at the immunological synapse to promote growth and survival^{22,52}. In Tregs, PKC-θ is sequestered to distal complex away from the immunological synapse and mediates a negative feedback on Treg suppressive function¹⁸. The dual role of PKC-θ in controlling T cell function represents a therapeutic potential of PKC-θ inhibition as a powerful tool to block undesired inflammatory responses.

References

1. Steinberg SF. Structural basis of protein kinase C isoform function. *Physiol Rev.* 2008; 88 (4):1341–1378. [PubMed: 18923184]
2. Baier G, et al. Molecular cloning and characterization of PKC theta, a novel member of the protein kinase C (PKC) gene family expressed predominantly in hematopoietic cells. *J Biol Chem.* 1993; 268 (7):4997–5004. [PubMed: 8444877]
3. Monks CR, et al. Selective modulation of protein kinase C-theta during T-cell activation. *Nature.* 1997; 385 (6611):83–86. [PubMed: 8985252]

4. Dustin ML, et al. A novel adapter protein orchestrates receptor patterning and cytoskeletal polarity in T cell contacts. *Cell*. 1998; 94:667–677. [PubMed: 9741631]
5. Kaizuka Y, et al. Mechanisms for segregating T cell receptor and adhesion molecules during immunological synapse formation in Jurkat T cells. *Proc Natl Acad Sci U S A*. 2007; 104 (51): 20296–20301. [PubMed: 18077330]
6. Monks CR, et al. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature*. 1998; 395 (6697):82–86. [PubMed: 9738502]
7. Varma R, et al. T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity*. 2006; 25 (1):117–127. [PubMed: 16860761]
8. Huang J, et al. CD28 plays a critical role in the segregation of PKC theta within the immunologic synapse. *Proc Natl Acad Sci U S A*. 2002; 99 (14):9369–9373. [PubMed: 12077322]
9. Sanchez-Lockhart M, Miller J. Engagement of CD28 outside of the immunological synapse results in up-regulation of IL-2 mRNA stability but not IL-2 transcription. *J Immunol*. 2006; 176 (8):4778–4784. [PubMed: 16585571]
10. Yokosuka T, et al. Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters and protein kinase C theta translocation. *Immunity*. 2008; 29 (4):589–601. [PubMed: 18848472]
11. Sims TN, et al. Opposing Effects of PKCtheta and WASp on Symmetry Breaking and Relocation of the Immunological Synapse. *Cell*. 2007; 129 (4):773–785. [PubMed: 17512410]
12. Beal AM, et al. Protein kinase Ctheta regulates stability of the peripheral adhesion ring junction and contributes to the sensitivity of target cell lysis by CTL. *J Immunol*. 2008; 181 (7):4815–4824. [PubMed: 18802085]
13. Yam PT, et al. Actin-myosin network reorganization breaks symmetry at the cell rear to spontaneously initiate polarized cell motility. *J Cell Biol*. 2007; 178 (7):1207–1221. [PubMed: 17893245]
14. Sakaguchi S, et al. Regulatory T cells and immune tolerance. *Cell*. 2008; 133 (5):775–787. [PubMed: 18510923]
15. Tadokoro CE, et al. Regulatory T cells inhibit stable contacts between CD4+ T cells and dendritic cells in vivo. *J Exp Med*. 2006; 203 (3):505–511. [PubMed: 16533880]
16. Tang Q, et al. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat Immunol*. 2006; 7 (1):83–92. [PubMed: 16311599]
17. Wing K, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008; 322 (5899): 271–275. [PubMed: 18845758]
18. Zanin-Zhorov A, et al. Protein kinase C-theta mediates negative feedback on regulatory T cell function. *Science*. 2010; 328 (5976):372–376. [PubMed: 20339032]
19. Blonska M, Lin X. CARMA1-mediated NF-kappa B and JNK activation in lymphocytes. *Immunological Reviews*. 2009; 228:199–211. [PubMed: 19290929]
20. Lin X, Wang D. The roles of CARMA1, Bcl10, and MALT1 in antigen receptor signaling. *Semin Immunol*. 2004; 16 (6):429–435. [PubMed: 15541657]
21. Li Y, et al. SPAK kinase is a substrate and target of PKCtheta in T-cell receptor-induced AP-1 activation pathway. *EMBO J*. 2004; 23 (5):1112–1122. [PubMed: 14988727]
22. Sun Z, et al. PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. *Nature*. 2000; 404 (6776):402–407. [PubMed: 10746729]
23. Pfeifhofer C, et al. Protein kinase C theta affects Ca2+ mobilization and NFAT cell activation in primary mouse T cells. *J Exp Med*. 2003; 197 (11):1525–1535. [PubMed: 12782715]
24. Coudronniere N, et al. NF-kappa B activation induced by T cell receptor/CD28 costimulation is mediated by protein kinase C-theta. *Proc Natl Acad Sci U S A*. 2000; 97 (7):3394–3399. [PubMed: 10716728]
25. Lin X, et al. Protein kinase C-theta participates in NF-kappaB activation induced by CD3-CD28 costimulation through selective activation of IkappaB kinase beta. *MolCell Biol*. 2000; 20 (8): 2933–2940.

26. Marsland BJ, et al. Protein kinase C theta is critical for the development of in vivo T helper (Th)2 cell but not Th1 cell responses. *Journal of Experimental Medicine*. 2004; 200 (2):181–189. [PubMed: 15263025]
27. Salek-Ardakani S, et al. Differential regulation of Th2 and Th1 lung inflammatory responses by protein kinase C theta. *J Immunol*. 2004; 173 (10):6440–6447. [PubMed: 15528385]
28. Anderson K, et al. Mice deficient in PKC theta demonstrate impaired in vivo T cell activation and protection from T cell-mediated inflammatory diseases. *Autoimmunity*. 2006; 39 (6):469–478. [PubMed: 17060026]
29. Berg-Brown NN, et al. PKC{theta} Signals Activation versus Tolerance In Vivo. *J Exp Med*. 2004; 199 (6):743–752. [PubMed: 15024044]
30. Giannoni F, et al. Protein kinase C theta is not essential for T-cell-mediated clearance of murine gammaherpesvirus 68. *J Virol*. 2005; 79 (11):6808–6813. [PubMed: 15890920]
31. Valenzuela JO, et al. PKCtheta is required for alloreactivity and GVHD but not for immune responses toward leukemia and infection in mice. *J Clin Invest*. 2009; 119 (12):3774–3786. [PubMed: 19907075]
32. Marsland BJ, et al. Innate signals compensate for the absence of PKC-{theta} during in vivo CD8(+) T cell effector and memory responses. *Proc Natl Acad Sci U S A*. 2005; 102 (40):14374–14379. [PubMed: 16186501]
33. Fontenot JD, et al. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol*. 2003; 4 (4):330–336. [PubMed: 12612578]
34. Hori S, et al. Control of regulatory T cell development by the transcription factor Foxp3. *Science*. 2003; 299 (5609):1057–1061. [PubMed: 12522256]
35. Zheng Y, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. *Nat Immunol*. 2007; 8 (5):457–462. [PubMed: 17440451]
36. Thornton AM, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *J Immunol*. 2010; 184 (7):3433–3441. [PubMed: 20181882]
37. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol*. 2005; 6 (4):345–352. [PubMed: 15785760]
38. Shevach EM. Regulatory/suppressor T cells in health and disease. *Arthritis Rheum*. 2004; 50 (9):2721–2724. [PubMed: 15457438]
39. Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity*. 2009; 30 (5):636–645. [PubMed: 19464986]
40. Gupta S, et al. Differential requirement of PKC-theta in the development and function of natural regulatory T cells. *Mol Immunol*. 2008; 46 (2):213–224. [PubMed: 18842300]
41. Long M, et al. Nuclear factor-kappaB modulates regulatory T cell development by directly regulating expression of Foxp3 transcription factor. *Immunity*. 2009; 31 (6):921–931. [PubMed: 20064449]
42. Furtado GC, et al. Interleukin 2 signaling is required for CD4(+) regulatory T cell function. *J Exp Med*. 2002; 196 (6):851–857. [PubMed: 12235217]
43. Gavin MA, et al. Homeostasis and anergy of CD4(+)CD25(+) suppressor T cells in vivo. *Nat Immunol*. 2002; 3 (1):33–41. [PubMed: 11740498]
44. Lafaille JJ, et al. High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell*. 1994; 78 (3):399–408. [PubMed: 7520367]
45. Powrie F, et al. Regulatory interactions between CD45RB^{high} and CD45RB^{low} CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J Exp Med*. 1994; 179 (2):589–600. [PubMed: 7905019]
46. Valencia X, et al. TNF downmodulates the function of human CD4+CD25^{hi} T-regulatory cells. *Blood*. 2006; 108 (1):253–261. [PubMed: 16537805]
47. Ding Y, et al. Beta-catenin stabilization extends regulatory T cell survival and induces anergy in nonregulatory T cells. *Nat Med*. 2008; 14 (2):162–169. [PubMed: 18246080]

48. Skvara H, et al. The PKC inhibitor AEB071 may be a therapeutic option for psoriasis. *J Clin Invest.* 2008; 118 (9):3151–3159. [PubMed: 18688284]
49. Barouch-Bentov R, et al. Protein kinase C-theta is an early survival factor required for differentiation of effector CD8+ T cells. *J Immunol.* 2005; 175 (8):5126–5134. [PubMed: 16210616]
50. Manicassamy S, et al. A critical role for protein kinase C-theta-mediated T cell survival in cardiac allograft rejection. *J Immunol.* 2008; 181 (1):513–520. [PubMed: 18566417]
51. Saibil SD, et al. CD4+ and CD8+ T cell survival is regulated differentially by protein kinase C-theta, c-Rel, and protein kinase B. *J Immunol.* 2007; 178 (5):2932–2939. [PubMed: 17312138]
52. Isakov N, Altman A. Protein kinase C(theta) in T cell activation. *Annu Rev Immunol.* 2002; 20:761–794. [PubMed: 11861617]
53. Jang MS, et al. A blocking anti-CD28-specific antibody induces long-term heart allograft survival by suppression of the PKC theta-JNK signal pathway. *Transplantation.* 2008; 85 (7):1051–1055. [PubMed: 18408588]
54. Gruber T, et al. PKC theta cooperates with PKC alpha in alloimmune responses of T cells in vivo. *Mol Immunol.* 2009; 46 (10):2071–2079. [PubMed: 19356803]
55. Villalba M, et al. Protein kinase ctheta cooperates with calcineurin to induce Fas ligand expression during activation-induced T cell death. *J Immunol.* 1999; 163 (11):5813–5819. [PubMed: 10570264]
56. Healy AM, et al. PKC-theta-deficient mice are protected from Th1-dependent antigen-induced arthritis. *J Immunol.* 2006; 177 (3):1886–1893. [PubMed: 16849501]
57. Salek-Ardakani S, et al. Protein kinase C-theta controls Th1 cells in experimental autoimmune encephalomyelitis. *J Immunol.* 2005; 175 (11):7635–7641. [PubMed: 16301673]
58. Tan SL, et al. Resistance to experimental autoimmune encephalomyelitis and impaired IL-17 production in protein kinase C theta-deficient mice. *J Immunol.* 2006; 176 (5):2872–2879. [PubMed: 16493044]
59. Marsland BJ, et al. TLR ligands act directly upon T cells to restore proliferation in the absence of protein kinase C-theta signaling and promote autoimmune myocarditis. *J Immunol.* 2007; 178 (6):3466–3473. [PubMed: 17339441]

Figure 1.
Signaling molecules linking the TCR to NF- κ B activation in T cells.

Figure 2.

Immunological synapse (IS) stability and localization of NF- κ B activating complex in Teff and Treg cells. The NF- κ B activating complex consisting of PKC- θ , Carma-1 and other components is localized to the center of the IS in Teff and the distal pole complex in Treg. The IS is destabilized by PKC- θ in Teff leading to more broken IS patterns, whereas exclusion of PKC- θ from the IS of Treg stabilizes there IS. Red = ICAM-1, green = TCR, white = actin.

Figure 3.