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Receptor signaling in immune cell development and function

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Abstract

Immune cell development and function must be tightly regulated through cell surface receptors to ensure proper responses to pathogen and tolerance to self. In T cells, the signal from the T-cell receptor is essential for T-cell maturation, homeostasis, and activation. In mast cells, the high-affinity receptor for IgE transduces signal that promotes mast cell survival and induces mast cell activation. In dendritic cells and macrophages, the toll-like receptors recognize microbial pathogens and play critical roles for both innate and adaptive immunity against pathogens. Our research explores how signaling from these receptors is transduced and regulated to better understand these immune cells. Our recent studies have revealed diacylglycerol kinases and TSC1/2-mTOR as critical signaling molecules/regulators in T cells, mast cells, dendritic cells, and macrophages.

Keywords

T-cell receptor; T-cell development; Anergy; Regulatory T cells; Dendritic cell (DC); Macrophages; Mast cells; Toll-like receptor (TLR); FcεRI; Diacylglycerol kinase (DGK); Mammalian target of rapamycin (mTOR); Tuberous sclerosis 1

Regulating T-cell receptor signaling for T-cell development and function

During T-cell maturation in the thymus, a functional T-cell receptor (TCR) is generated through proper V(D)J recombination [1]. Proper TCR signaling is critical for T-cell maturation and survival. Absence of the pre-TCR or the TCR signal results in developmental blockage at the CD4⁻CD8⁻ double negative (DN) stage and CD4⁺CD8⁺ double positive (DP) stage, respectively [2–4]. In addition, the strength of TCR signal influences the outcome of thymic selections. DP thymocytes expressing TCRs with high affinities to self-peptide MHC complexes are negatively selected due to programmed cell death. Negative selection deletes highly self-reactive T cells to establish central tolerance. Thymocytes express TCRs with low and moderate affinities to self-peptide-MHC complexes are positively selected to mature [5]. Proper thymic selection is critical for generating a repertoire T cells for effective immune responses against foreign antigens and, at the same time, self-tolerant. Furthermore, specialized TCRs such as the γδTCR and the invariant Vα14 Jα18 TCR in mouse may transduce signals different from the conventional αβTCR to direct the differentiation of precursors to distinct T-cell lineages [6]. In the periphery, tonic T-cell receptor signal maintains normal T-cell homeostasis [7], but does not cause full T-cell activation. In response to pathogen infection, TCR signal triggers naïve T-cell activation after recognizing foreign peptides presented by dendritic cells and other antigen-presenting cells (APCs). During early T-cell activation and further differentiation to effector T cells, T cells undergo drastic but characteristic changes, including enlargement of cell sizes, entry into cell cycle, synthesis of effector molecules including cytokines and granzymes, altered cell surface molecules, and trafficking between lymph nodes and tissues to mount effective immune responses. Abnormal TCR signaling can cause severe consequences. Defects in TCR signaling can result in impairment of immune function due to failure to generate T cells or insufficient activation of T cells [8]. It can also result in autoimmunity due to impairment of negative selection and shift of T-cell repertoire. Abnormally elevated TCR signaling can lead to autoimmunity due to uncontrolled T-cell activation [9]. While it is becoming clear that proper TCR signaling is essential for normal T-cell development and function, the mechanisms that regulate TCR signaling remain poorly understood.

T-cell receptor signaling

An outline of signal transduction from the TCR has been illustrated through the studies by many laboratories in the last 20 years (Fig. 1). Following TCR engagement, the Src family tyrosine kinase Lck is activated following dephosphorylation by Csk [10]. Lck phosphorylates the ITAMs on CD3, enzymes, and adaptors, leading to the activation and recruitment of Zap70 to the TCR ζ chain and subsequent formation of multi-molecule signaling complexes that are nucleated by LAT and SLP76 [11–14]. These proximal signaling events lead to the activation of PLCγ1 [15]. Activated PLCγ1 hydrolyzes phosphatidylinositol 4,5-bisphosphate [PIP2] to generate two important second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphates (IP3), that activate multiple downstream signaling cascades [16]. IP3 binds to its receptor in the endoplasmic reticulum (ER) to trigger the release of Ca⁺⁺ from the ER to the cytosol. Depletion of Ca⁺⁺ in the ER induces STIM1 conformation change and oligomerization, which further recruits Orai protein to open CRAC channels and Ca⁺⁺ influx. Ca⁺⁺ binds to calmodulin and triggers the activation of calcineurin, which dephosphorylates NFAT. Dephosphorylated NFAT

translocates the nuclei to induce transcription [17]. NFAT participates in transcription of genes involved in T-cell activation and T-cell tolerance. DAG associates with and activates multiple effector molecules, including the classic type protein kinase Cs (PKCs), the novel type PKCs, protein kinases D (PKDs), RasGRPs, Munc13 s, and chimerins [18–20]. In T cells, DAG binds to PKC θ to induce its translocation to immune synapse and activation [21]. PKC θ phosphorylates Carma1 to allow recruitment of Bcl10 and Malt1 to lipid raft. Both the Carma1/Bcl10/Malt1 complex and PKC θ associate with the IKK complex to induce its activation [22]. Activated IKK complex phosphorylates I κ B to trigger its ubiquitination and degradation, allowing nuclear translocation of NF κ B to activate transcription [23]. In addition, DAG associates with RasGRP1 to activate Ras directly or by priming Sos for guanyl nucleotide-releasing activity [24]. GTP-bound Ras further activates the Raf-Mek1/2-Erk1/2-AP1 pathway. PKC θ also promotes TCR-induced Ras-Erk1/2 pathway by phosphorylating RasGRP1 at threonine 184 to increase its activity [25]. Numerous studies have demonstrated that both PKC θ - and RasGRP1-mediated signaling pathways play critical roles in T-cell development and peripheral T-cell function [26]. Given the importance of DAG-mediated signaling for T cells, it is important to determine how DAG is regulated and the importance for tight control of DAG concentration in T cells. Dysregulated DAG signaling can be detrimental, which is suggested by the action of phorbol esters, functional analogues of DAG, which can cause maximal T-cell activation and are carcinogens.

Diaclyglycerol kinases as critical regulators for T cells

Diaclyglycerol kinases (DGKs) are evolutionarily conserved enzymes that catalyze the phosphorylation of DAG to generate phosphatidic acid (PA). Similar to DAG, PA is also a second messenger implicated as a mediator of the mitogenic action of various growth factors and hormones in several types of mammalian cells [27]. PA associates with and regulates many effector molecules, such as SHP-1 [28], mTOR [29], PDE4 (cAMP-specific phosphodiesterase) [30], PP-1 (protein phosphatase-1) [31], PI5 K (type I phosphatidylinositol 4-phosphate 5-kinase) [32], Sos [33], and p47^{phox} [34]. DGKs have been hypothesized to play important roles in receptor signaling and cellular function by regulating DAG and PA concentration. However, the physiological functions of DGKs have been poorly understood. Each of the ten mammalian DGKs contains a kinase domain and at least two cysteine-rich C1 domains that are homologous to the DAG/phorbol ester-binding domain of protein kinase Cs (PKCs). In addition, DGK isoforms also contain distinct structural motifs that are used to define mammalian DGKs into five types [19, 35, 36] (Fig. 2). Most DGK isoforms do not have obvious preference for specific acyl chains of DAG [37]. DGKs may associate with proteins or lipids through these distinct structural motifs to control their subcellular localizations and to perform specific functions. Most DGK isoforms are expressed in multiple tissues, particularly in the hematopoietic system and the neuronal system. Within one tissue, multiple DGK isoforms may be present. Transcripts of at least five DGK isoforms can be detected in T cells, macrophages, and mast cells (our unpublished data).

Synergistic and crucial role of DGK α and ζ for T-cell development—The facts that defects in generating DAG or in DAG effectors cause abnormal T-cell development suggest the importance of DAG-mediated signaling. PLC γ 1 deficiency in T cells results in the impairment of both positive and negative selection [38]. The RasGRP1-Ras-Erk1/2 pathway is crucial for positive selection of conventional $\alpha\beta$ T cells but appears to be dispensable for innate CD8 T cell or Treg development [26, 39]. While the PKC θ -Carma1/Bcl10-IKK-NF κ B pathway is not essential for positive selection of conventional $\alpha\beta$ T cells [22], it plays important roles for *i*NKT cell and Treg generation [40]. These findings have led us to hypothesize that DAG-mediated signaling must be tightly controlled for proper T-cell maturation.

To test our hypothesis, we have generated and analyzed mice deficient of DGK α , ζ , or both. In the absence of either DGK α or ζ , T-cell numbers in the thymus and peripheral lymphoid organs are not obviously altered [41, 42]. However, absence of both DGK α and ζ (DGK $\alpha\zeta$ DKO) results in a severe decrease in CD4⁺CD8⁻ and CD4⁻CD8⁺ SP thymocytes. Positive selection but not negative selection is impaired in DGK $\alpha\zeta$ DKO mice [43]. These data provide the first evidence that two DGK isoforms synergistically control a biological process. In CD4⁺CD8⁺ DP thymocytes of DGK $\alpha\zeta$ DKO mice, TCR stimulation induces elevated DAG concentration and increased Ras and Erk1/2 activation as well as activation of the PKC θ -IKK-NF κ B pathway. During in vitro thymic organ culture of DGK $\alpha\zeta$ DKO thymi, addition of PA increases CD4 and CD8 SP thymocytes, revealing that DGK-derived PA is important for DP thymocyte maturation to the SP stage [43]. Thus, DGKs may function as a signal switch during T-cell development by turning off DAG-mediated signaling and at the same time turning on PA-mediated signaling. Further studies should determine how DGK-derived PA exerts its function in developing thymocytes.

In addition to promoting positive selection of conventional α/β T cells, DGK α and ζ also synergistically promote the development of the invariant V α 14-J α 18 NKT (*i*NKT) cell development. In the absence of either DGK α or ζ , *i*NKT cell numbers are not obviously abnormal compared to WT mice. *i*NKT cell numbers are drastically decreased in the thymus, spleen, and liver of DGK $\alpha\zeta$ DKO mice. In contrast to conventional α/β T cells and *i*NKT cells, regulatory T-cell generation, γ/δ T-cell development as well as β -selection appears not inhibited by DGK α and ζ deficiency, suggesting that DGK α and ζ is differentially required for the development of these distinct T-cell lineages or developmental checkpoints (reference 43 and unpublished data). It would be important to determine further whether DGK activity does not participate for these developmental events or DGK isoforms other than DGK α and ζ may be involved.

Regulation of T-cell activation and tolerance by DGKs—T-cell-mediated immune response is critical for host defense against microbial infection and for tumor surveillance. However, T-cell function also needs to be tightly regulated to ensure immune responses are properly controlled. Dysregulated T-cell responses can be detrimental to the host [44]. Similar to developing T cells in the thymus, DAG-mediated signaling is critical for T-cell activation. Inhibition of DAG-mediated signaling pathways results in the impairment of T-cell activation and effector T-cell function [45]. In contrast, treatment of T cells with phorbol esters induces maximal T-cell activation. To determine the role of DGKs in T-cell activation, we first used a gain-of-function approach by overexpression of DGK α or ζ in T cell lines. Enhanced DGK function can potentially inhibit TCR-induced Ras-Erk1/2-API activation [46]. Gajewski's and Merida's groups have revealed that increased DGK activities inhibit the recruitment of RasGRP1 to the immune synapse or cytoplasmic membrane [47, 48]. Complementary to these gain-of-function data, studies in mice deficient of either DGK α or ζ result in hyperresponsive T cells in response to TCR stimulation, correlating with decreased conversion of DAG to PA and enhanced activation of the Ras-Erk1/2-API pathway [41, 42]. Although DGK α or ζ -deficient T cells are hyper-proliferative in response to TCR stimulation, they remain in a naïve state in vivo. DGK α - or ζ -deficient mice also do not manifest obvious autoimmune diseases. However, DGK $\alpha\zeta$ DKO T cells display spontaneous activation phenotypes in vivo and in vitro. They express high levels of T-cell activation markers, display effector/memory T-cell phenotype, and readily produce cytokines. DGK $\alpha\zeta$ DKO T cells proliferate more vigorously than WT T cells in response to TCR stimulation and even proliferate in vitro without TCR stimulation (our unpublished data). Together, these observations establish that DGK α and ζ are physiological inhibitors of TCR signaling and T-cell activation and synergistically control normal T-cell homeostasis.

While negative selection depletes majority of self-reactive T cells, this process is not complete. Some self-reactive T cells escape negative selection in the thymus and populate the periphery. Peripheral tolerance mechanisms ensure these self-reactive T cells from causing unnecessary tissue damage. One of the peripheral T-cell tolerance mechanisms is the induction of anergy. For naïve T cells to become fully activated, both TCR signaling and a costimulatory signal, such as CD28, are required [49, 50]. In the absence of costimulatory signaling, TCR signal itself can induce T cells into an anergic state [51–54]. In addition, anergic T cells can be induced by stimulation with partial agonist peptides in the presence of co-stimulation [55], or by treatment with the Ca^{++} ionophore ionomycin [51, 56–58]. Anergic T cells do not respond to antigen restimulation in the presence of appropriate co-stimulation. They do not make IL-2 or divide following re-stimulation, even though they express all the necessary receptors [44, 59, 60].

While the mechanisms involved in anergy induction may vary among different models, anergic T cells usually manifest impaired activation of the RasGRP1-Ras-Erk1/2 pathway but normal Ca^{++} influx and NFAT activation following TCR stimulation [61–63]. Treatment of T cells with ionomycin induces Ca^{++} influx, leading to activation of NFAT and anergy induction by promoting transcription of anergy-promoting molecules, such as FasL, Erg2/3 (early growth response 2/3), and several E3 ubiquitin ligases (Cbl-b, Itch, and Grail) [56, 58, 64–66]. While ionomycin alone induces T-cell anergy, ionomycin plus a DAG analogue, such as PMA, induces full T-cell activation. Since DAG and IP_3 are produced by PLC γ 1 at an equal molar ratio following TCR engagement, it has been hypothesized that DGK activity may contribute to T-cell anergy selectively terminating DAG-mediated signaling. In supporting this hypothesis, DGK α and ζ are expressed in high levels in naïve and anergic T cells but down-regulated in activated T cells. In the absence of either DGK α or ζ , T cells display resistance to anergy induction in both in vitro and in vivo, supporting an important role of DGK activity for T-cell anergy [42].

TSC1/2-mTOR signaling in T cells

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that integrates numerous environmental stimuli including growth factors, nutrients, and stress-activated signals to regulate cell metabolism, survival, growth, and proliferation [67]. mTOR associates with different proteins to form two signaling complexes, mTOR complexes 1 and 2 (mTORC1 and mTORC2) with different sensitivity to rapamycin and signaling properties [68]. mTORC1 is sensitive to rapamycin inhibition, while mTORC2 is resistant to acute rapamycin treatment. mTORC1 phosphorylates and activates the 70 kDa ribosomal S6 kinase (S6K1) and the translational repressor 4 elongation factor binding protein 1 (4E-BP1) [69, 70] to promote cell growth and proliferation. Activated S6K1 phosphorylates S6 and other translational regulators such as eIF2 kinase and eIF-4B to regulate the translation initiation [71, 72] and ribosomal biogenesis. Phosphorylation of 4E-BP1 releases eukaryotic initiation factor 4E (eIF4E) to promote the recruitment of ribosome machinery in protein translation [73]. The mTORC2 phosphorylates Akt at serine 473 to increase Akt activity that further promotes nutrient uptake and cell survival [74, 75].

Both genetic and pharmacological studies have demonstrated that mTOR plays crucial roles during T-cell activation, anergy, lineage commitment, and other immune responses [76]. A decrease in mTOR signaling due to chemical inhibition or genetic manipulation has been associated with T-cell anergy [77], induction of regulatory T cells [78–82], impairment of effector T-cell generation [82, 83], and, surprisingly, enhanced memory T-cell responses to microbial pathogens [84]. In addition, mTOR has been shown to play an important role in T-cell trafficking in vivo by regulating the expression of CCR7 [85].

While it is becoming clear that mTOR plays critical roles in T cells and that TCR stimulation induces the mTOR activity [86], how TCR signaling leads to mTOR activation and the importance of tight control of mTOR signaling in T cells have been not fully understood. To investigate signaling pathways that are important for mTOR activation, we utilized genetically manipulated mice in the RasGRP1-Ras-Erk1/2 pathway. In the absence of RasGRP1, T-cell development is severely blocked at the CD4⁺CD8⁺ DP stage. We have found that in RasGRP1^{-/-} thymocytes, TCR-induced activation of mTORC1 and mTORC2 is impaired. In contrast, increased Ras activity results in elevated activation of both mTORC1 and mTORC2 in thymocytes. Further manipulation of Mek1/2 activity in thymocytes with chemical inhibitors and in T cell lines with constitutively active and dominant negative Mek1/2 reveals that Mek1/2 activity is critical for TCR-induced mTORC1 and mTORC2 activity (Gorentla and Zhong, unpublished data). Together, these observations demonstrate that the RasGRP1-Ras-Mek1/2-Erk1/2 pathway is critical for TCR-induced mTOR activation. Consistent with these observations, we have found that DGK α and ζ deficiency causes enhanced mTOR activation that can be inhibited by Mek1/2 inhibitors (Gorentla and Zhong, unpublished data). It has been reported that PA can associate with mTOR to promote mTOR signaling. Both PLD- and DGK ζ -derived PA have been shown to participate in mTOR activation in cell line models [29, 87]. At present, it is unclear how absence of DGK-derived PA may affect TCR-induced mTOR signaling. However, our data point to a dominant inhibitory role of DGK α and ζ in TCR-induced mTOR activation by negative control of DAG-mediated activation of the RasGRP1-Ras-Erk1/2 pathway. As the RasGRP1-Ras-Erk1/2 pathway also controls Rsk1 activation and AP1 activity, it remains to determine how this pathway may affect T-cell biology through the mTOR signaling.

Since the Ras-Mek1/2-Erk1/2 pathway controls multiple effector molecules besides mTOR, neither the DGK α ζ DKO nor the caKRas model would provide clear information with regard to how dysregulated mTOR signaling may affect T cells. To selectively manipulate mTOR signaling, we have decided to utilize a conditional TSC1-deficient mouse model. mTORC1 is activated by GTP-bound RheB (the Ras homologue enriched in brain). RheB is negatively regulated by an upstream Tuberous sclerosis complex 1/2 (TSC1/2), a bipartite protein complex of Hamartin (TSC1) and Tuberin (TSC2) [88, 89]. TSC1 stabilizes TSC2 to prevent its ubiquitination and degradation. TSC2 inhibits RheB through its GAP activity and thus functions as negative regulator of mTORC1 signaling. In cell line models, the PI3 K-Akt pathway has been shown to activate mTORC1 signaling through phosphorylation of TSC2 [90–92]. Phosphorylation of TSC2 triggers the dissociation of TSC2 from TSC complex, leading to the activation of mTORC1 signaling via Rheb [93]. To investigate the role of TSC1 in T cells, we have conditionally deleted TSC1 out in T cells. In TSC1-deficient T cells, TSC2 protein is hardly detectable, indicating critical role of TSC1 for TSC2 stability in T cells and a virtual TSC1/TSC2 double deficiency in TSC1 knockout T cells. In the absence of TSC1, TCR-induced mTORC1 activity is elevated and T-cell sizes are increased. However, mTORC2 and Akt activities are decreased, leading to increased T-cell death and decreased T-cell numbers in peripheral lymphoid organs (O'Brien and Zhong, unpublished data). Thus, TSC1 differentially controls mTORC1 and mTORC2 signaling in T cells and plays important roles in maintaining normal homeostasis of T cells.

Regulation of TLR signaling and innate immunity by DGKs and TSC1/2 mTOR signaling

Adaptive immune responses are tightly regulated by innate immune cells. TLRs are a family of evolutionarily conserved receptors that recognize specific microbial PAMPs and constitute a major mechanism to respond to microbial infection by the hosts [23, 24, 94, 95]. Although diverse in specificities of ligand recognition, TLRs appear to share several

common intracellular signaling pathways. A MyD88-dependent pathway is utilized by most TLRs [96]. This pathway is initiated after association of MyD88 with the TLRs during microbial recognition. MyD88 in turn recruits IRAK1 and 4 (IL-1R-associated kinase 1 and 4), TRAF6 (TNF receptor-associated factor 6), and other signaling molecules to the cytoplasm membrane, leading to the activation of IKK α / β / γ (I κ B kinase) complex [97–99]. IKK α / β / γ phosphorylates I κ B, causing degradation and nuclear translocation of NF κ B to induce expression of its target genes such as IL-12 and TNF α [100]. In addition, MyD88-mediated proximal signaling events also lead to the activation of JNK, p38, and Erk1/2 MAPKs through Tak1 [101]. JNK and p38 activities are important for proinflammatory cytokine production [102–104]. The TRIF-dependent pathway is mediated by two TIR-domain containing adaptor molecules TRIF and TRAM [105]. This pathway is utilized by TLR3 and TLR4 [106, 107], leading to phosphorylation and activation of IRF3 (IFN-regulatory factor 3) [108]. IRF3 activates transcription of type I IFNs [109]. The class I $_A$ PI3 Ks can negatively control TLR-induced responses. DCs deficient of the p85 α regulatory subunit of the class I $_A$ PI3 Ks express a high level of IL-12 following stimulation of TLR2, 4, 5, and 9. Mice deficient in p85 α in BALB/c background become resistant to *Leishmania major* infection due to enhanced Th1 responses [110].

While DGK ζ -deficient T cells are hyperresponsive to TCR stimulation, we were surprised that DGK ζ -deficient mice are more susceptible to *Toxoplasma gondii* infection than WT control mice [111]. It has been well established that host resistance to *T. gondii* is dependent on Th1 immune response and IFN γ . Dendritic cells play critical role in generating effective adaptive immune response against *T. gondii* through TLR-mediated recognition of the pathogen and subsequent production of IL12. As there is no intrinsic defect of DGK ζ -deficient T cells to differentiate to Th1 lineage, we investigated whether DGK ζ is involved in TLR-mediated innate immunity and found that TLR-induced production of proinflammatory cytokines such as IL12 by DGK ζ -deficient DCs and BMM ϕ is diminished. In DGK ζ ^{-/-} bone marrow-derived macrophages (BMM ϕ), PI3 K and Akt activation is enhanced following LPS stimulation. Importantly, treatment of DGK ζ ^{-/-} BMM ϕ with a PI3 K inhibitor or phosphatidic acid can restore TLR-induced IL12 production, indicating that DGK ζ inhibits PI3 K/Akt signaling to promote TLR-induced proinflammatory cytokine production. It would be of interest to further determine whether PA exerts its effect by regulating PI3 K activity or through other mechanisms. These observations not only establish a critical role of DGK ζ in TLR-mediated innate immune responses but also emphasize the importance of innate immune responses in the control of adaptive immunity as enhanced activation of DGK ζ ^{-/-} T cells is not sufficient to confer resistance to *T. gondii*.

Growing evidence has also implicated mTOR in innate immune responses. Rapamycin treatment blocks TLR9-MyD88 interaction and IRF7 phosphorylation in plasmacytoid dendritic cells (pDCs) following CpG stimulation, leading to the inhibition of type I IFNs production and impaired antiviral immune responses in vivo. Concordantly, 4E-BP1 and 4E-BP2 double-deficient MEF cells express high levels of IRF7 and type I IFNs, rendering resistance to viral infection [112]. However, rapamycin treatment has also been found to have either inhibitory or stimulating effects on proinflammatory cytokine and IL-10 production in human dendritic cells and monocytes [113, 114]. Using TSC1 conditional knockout mice, we have found that TSC1 plays critical roles in mTOR signaling in dendritic cells and macrophages and controls TLR-induced proinflammatory cytokine production (Pan and Zhong, unpublished data). The study establishes a critical role of TSC1 in TLR-mediated innate immune responses.

Diacylglycerol kinases and TSC1/2-mTOR signaling in mast cells

Mast cells are the central effectors in immune pathogenesis of asthma and other allergic disorders and play important roles in host defense against helminth infection [115–119]. The high-affinity receptor for IgE FcεRI plays critical role for mast cell activation by inducing mast cell degranulation, synthesis and release of lipid mediators, and transcriptional activation and secretion of cytokines [120]. The paradigm of FcεRI signaling mimics TCR signaling in many aspects, such as activation of the Src family members Lyn and Fyn, the tyrosine kinase Syk, the Tec family kinase Btk, and P I3Ks [121–130], and the formation of multi-molecular signaling complexes by adaptor molecules such as LAT and SLP76. These proximal events lead to the activation of PLCγ [131–134], PKCs [135], MAPKs [136, 137], and mTOR [138, 139]. Similar to T cells, DAG is generated following FcεRI engagement and controls mast cell function by activating multiple signaling cascades. DAG activates PKCs and RasGRP1 that are important for mast cell degranulation and cytokine production [140–148]. Both in vitro and in vivo evidence has suggested a critical role of DAG in regulating mast cell function as defects in DAG generation or in DAG-mediated signaling pathways greatly impact mast cell function [142, 149–151].

Mast cells express at least five DGK isoforms at the mRNA levels (our unpublished data). FcεRI stimulation induces DAG phosphorylation and PA production in mast cells. In the absence of DGKζ, FcεRI-induced PA production is decreased, indicating that DGKζ is involved in DAG conversion to PA. While DGKζ deficiency appears to be dispensable for mast cell generation in vivo or differentiation from bone marrow progenitor cells in vitro, DGKζ differentially regulates mast cell degranulation and cytokine production. DGKζ-deficient BMMCs produce elevated amount of IL-6 following FcεRI stimulation. However, their degranulation is impaired when compared to WT BMMCs in vitro. DGKζ-deficient mice display diminished anaphylactic responses in vivo. DGKζ^{-/-} BMMCs show enhanced Erk1/2 and PKCβ activation but decreased PLCγ phosphorylation and Ca influx, which may contribute to the differential effects DGKζ deficiency on cytokine production and degranulation in mast cells [111].

The role of TSC1/2-mTOR signaling in mast cells has been much less clear. A few studies have suggested potential roles of mTOR in mast cells. FcεRI stimulation induces phosphorylation of mTOR, S6K1, and 4E-BP1 that is dependent on PI3 K activity. Rapamycin inhibits FcεRI-induced S6K1 and 4E-BP1 phosphorylation and cytokine production without affecting degranulation [138, 139]. We have found that FcεRI stimulation induces both mTORC1 and mTORC2 activation that are differentially regulated by TSC1 (Shin and Zhong, unpublished data).

Summary

In summary, recent studies have revealed that DGKs and tight regulation of mTOR signaling are important for proper immune cell development and function. Mice with altered DGKs and the TSC1/2-mTOR pathway should provide important model systems to investigate the regulation of innate and adaptive cell functions and to develop new strategies to modulate immune cell function for the treatment of autoimmune and allergic diseases and for cancer immunotherapy.

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Fig. 1.
Schematic illustration of T-cell receptor signaling (see text for details)

Fig. 2.

Structural features of mammalian DGKs. Mammalian DGKs are divided into five types based on their structural features. *CA* catalytic domain, *CI* cysteine-rich diacylglycerol/phorbol ester-binding domain, *RVH* recoverin homology domain, *EF* EFhand, *PH* pleckstrin homology domain, *SAM* sterile alpha motif domain, *PBM* PDZ domain-binding motif, *M* myristoylated alanine-rich C-kinase substrate (MARCKS) motif, *AAAA* ankyrin-repeats, *G/P* glycine/proline-rich region