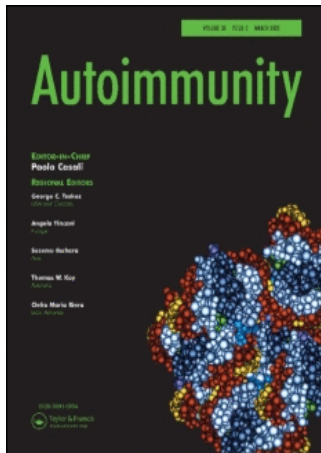


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Role of phosphoinositide 3-kinase signaling in autoimmunity

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Abstract

Activation of the phosphoinositide 3-kinase (PI3K) pathway promotes proliferation and survival in many different cell types of the immune system. PI3K acts downstream of receptors that mediate proliferation and survival in T cells, and required roles for individual class I PI3K catalytic isoforms have been established. Interestingly, mice with either augmented or diminished PI3K activity in T cells develop lymphoproliferation and signs of autoimmunity. Here, we summarize our current knowledge of mouse strains with hyperactive or reduced PI3K, different isoforms of class I PI3K in T cell-mediated immunity and autoimmunity, and the therapeutic implications for modulating this pathway for treatment of various autoimmune diseases.

Keywords: *Phosphoinositide 3-kinase (PI3K), T cells, autoimmunity, signal transduction*

Introduction

The immune system has evolved to recognize and eliminate foreign antigens while maintaining tolerance to self. This is a particular challenge for lymphocytes, since the mature T cell and B cell repertoires contain billions of clones that express unique antigen receptors generated by random DNA recombination. There are several mechanisms that prevent self-reactive lymphocytes from emerging and causing damage to self tissues. During lymphocyte development, many clones with high avidity for self antigen are eliminated or inactivated. This is referred to as central tolerance. Peripheral tolerance refers to a variety of mechanisms that ensure that autoreactive clones that do emerge do not become activated upon recognition of self antigen in peripheral tissues. Mechanisms of peripheral tolerance include clonal anergy due to lack of costimulatory signals, activation-induced cell death and suppression by regulatory T cells. Each of the mechanisms of peripheral and central tolerance requires the responding lymphocyte to interpret extracellular information with an appropriate intracellular signal transduction response. Paradoxically, many of the signaling pathways that promote

lymphocyte proliferation and effector function are also critical for mechanisms of self-tolerance. The PI3K signaling pathway is a prime example of a mechanism that must be finely tuned to ensure proper host defense without autoimmunity. An increasing body of information suggests that PI3K plays a crucial role in T cell homeostasis and tolerance. Paradoxically, either increasing or decreasing PI3K activity in T cells lead to an autoimmune phenotype in mice. In this review we will describe the various murine models used to study class I PI3K signaling in T cells, the rising interest in the unique functions of different isoforms and the possibility of targeting the PI3K pathway to treat autoimmune diseases.

Overview of PI3K signaling pathway

PI3K comprises a family of cytoplasmic lipid kinases that phosphorylate the 3'-hydroxyl group of the inositol head groups of D-*myo*-phosphatidylinositol (PtdIns) or its derivatives [1]. The members of the PI3K family are divided into three classes (I, II, III) based on sequence homology and substrate specificity. Among the different members of this family, class I

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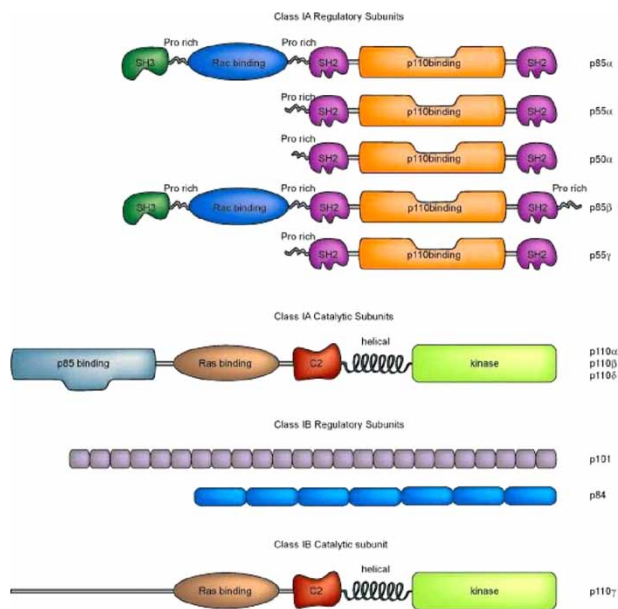


Figure 1. Schematic diagram of catalytic and regulatory subunits of class I PI3K. In addition to the kinase binding domain class IA regulatory subunits contain several other domains that regulate the localization and function of the p85/p110 heterodimer. The structures of the p101 or p84/p87 regulatory isoforms of class IB PI3K are unclear at this time.

PI3K members (Figure 1) are responsible for acute rises in the critical second messenger $\text{PtdIns}(3,4,5)\text{P}_3$ upon activation of receptors that sense various extracellular signals. $\text{PtdIns}(3,4,5)\text{P}_3$ acts as a docking site for the recruitment of proteins containing a pleckstrin-homology (PH) domain to the cell membrane (Figure 2).

Class I PI3K members are further divided into two subclasses, class IA and IB, which differ in their regulatory subunits and in the types of upstream activating receptor [1]. Class IA PI3K enzymes are activated downstream of receptor tyrosine kinases and each exists as a tightly associated heterodimer consisting of a catalytic subunit of approximately 110 kDa and a regulatory subunit. The five regulatory

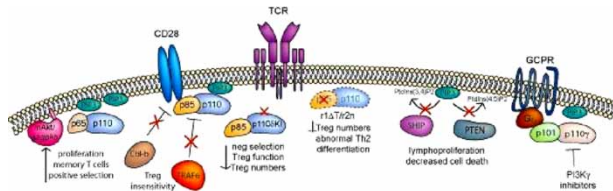


Figure 2. Class I PI3K are activated upon stimulation of receptor tyrosine kinases or G-protein coupled receptors to generate $\text{PtdIns}(3,4,5)\text{P}_3$ (sometimes termed PIP3). Various mouse genetics studies elucidate the role of class I PI3K in T cell-mediated autoimmunity; the phenotypes are shown next to the component that was studied. Both upregulation and downregulation of PI3K activity leads to autoimmune development in mice, which emphasizes the importance of balance in PI3K signaling in T cell tolerance and function.

isoforms p85 α , p55 α , p50 α , p85 β and p55 γ associate with any of the three catalytic isoforms p110 α , p110 β and p110 δ to regulate their stability, location and activity. Each regulatory subunit isoform has two Src homology-2 (SH2) domains that bind to the consensus sequence YXXM upon phosphorylation of the tyrosine residue. Class IB PI3K mediates $\text{PtdIns}(3,4,5)\text{P}_3$ generation upon activation of G-protein-coupled receptors (GPCRs) and is composed of a p110 γ catalytic subunit and either a p101 or p84/p87 regulatory subunit [2,3].

In T cells, stimulation of the T cell receptor, CD28 and other costimulatory receptors, cytokine receptors and chemokine receptors leads to the recruitment of class I PI3K to the cell membrane [4]. Treatment of T cells with pan-PI3K catalytic inhibitors such as LY294002 or wortmannin blocks T cell proliferation *in vitro* to a greater extent than targeted inactivation of individual class I PI3K genes [5–9]. This may be due to nonspecific drug target effects [10] or redundancy among different classes or isoforms of class I PI3K. On the other hand, PI3K inhibitors reduce T cell migration towards chemotactic agents to a similar extent as deletion of p110 γ [11,12], indicating that the class IB PI3K is the major isoform involved in T cell chemotaxis. p110 δ appears to play a more important role than p110 γ in B cell chemotaxis and homing [12].

Increased PI3K activity leads to autoimmunity

Given the importance of PI3K in lymphocyte proliferation and survival, one might predict that an increase in PI3K activity could lead to autoimmunity. This is indeed the case in a transgenic (Tg) model where T cells express p65^{PI3K}, a truncated isoform of the p85 α regulatory subunit that causes constitutive activation of class IA PI3K catalytic subunits [13]. In this model p65^{PI3K}, which was cloned from a thymic lymphoma, is expressed under the control of the Lck proximal promoter. Young p65^{PI3K} Tg mice show increased CD4 positive selection and emigration in the thymus that leads to an ~30% increase of CD4⁺T cells in the periphery [14]. In addition to increased production, p65^{PI3K} Tg CD4⁺ T cells have decreased apoptosis *in vivo* and *in vitro*, which leads to accumulation of CD44^{hi}CD62L^{lo}CD45RB^{lo} memory T cells in the periphery. Dysregulation of CD4⁺ memory T cell homeostasis eventually leads to the development of lymphoproliferative disease, where p65^{PI3K} Tg mice over 12 months of age develop splenomegaly, lymphadenopathy, lymphocytic infiltration of various organs, hypergammaglobulinemia and anti-dsDNA antibodies.

Aberrant Akt activity leads to autoimmunity

A number of proteins translocate to the cell membrane upon generation of $\text{PtdIns}(3,4,5)\text{P}_3$. One of the most

important downstream effectors of PI3K is the PH-domain-containing protein kinase Akt (also known as PKB), which is recruited to the membrane and phosphorylated by PDK-1 and other kinases upon PtdIns(3,4,5)P₃ formation [1,4]. In many cellular systems, Akt activity correlates with increases in cell metabolism, cell cycle progression and survival. Two studies have shown that dysregulation of Akt/PKB activity in T cells leads to multi-organ inflammation [15,16]. In the first study, Parsons et al. [15] characterized Tg mice expressing a *gag-pkb* fusion protein driven by the human CD2 promoter; this system leads to constitutive membrane localization and activation of Akt in T cells. Aged homozygous *gag-pkb* mice develop multiorgan autoimmune disease characterized by glomerulonephritis with IgA deposition, hypergammaglobulinemia, splenomegaly and lymphadenopathy. T cells skew towards the CD4 phenotype, but young *gag-pkb* mice show increases in CD4, CD8 and B cell compartments in the periphery. *gag-pkb* T cells have increased CD69 expression suggesting an activated phenotype, but unlike other strains with enhanced PI3K/Akt signaling described below, these cells proliferate normally with anti-CD3 alone and with anti-CD3/anti-CD28. B cells of these mice also show an activated phenotype and hyperproliferate upon anti-IgM stimulation. This increase in B cell activity is likely due to increased T cell help since B cells in this strain have normal Akt activity. One mechanism of lymphoproliferation is likely to be defective T cell apoptosis since these show reduced FasL-induced death *in vitro* [17].

Rathmell et al. [16] have reported similar results using myristoylated-Akt (mAkt); like the retroviral *gag* sequences in *gag-pkb*, the myristoylation sequence constitutively localizes Akt to the cell membrane and leads to its activation. Mice expressing mAkt driven by the *Lck* promoter develop autoimmune disease and die around 100–200 days of age from thymic lymphoblastic lymphoma. In accordance with the importance of Akt in cell cycle and survival, mAkt T cells show increases in cell size, metabolism and survival *in vitro*. Furthermore, these cells hyperproliferate upon TCR stimulation and do not require CD28 costimulation for optimal proliferation. CD4⁺ T cell expansion caused by activated Akt/PKB may also be partly due to increased CD4 positive selection [18]. As in the case with the *gag-pkb* mice, mAkt Tg mice also accumulate excess memory CD4 and B cells as they age and develop immunoglobulin complex deposition in the glomeruli.

Activation of Akt leads to upregulation of pro-survival molecules such as Bcl-xL and increased NF-κB activity [19]. In addition to this pathway, Akt also promotes survival by suppressing the activity of proapoptotic molecules such as members of the Foxo subfamily of forkhead transcription factors [20,21]. Foxo members regulate cell survival and

proliferation and have been implicated to play a role in lymphocyte quiescence [20,22]; phosphorylation of Foxo proteins by Akt leads to its nuclear export and inactivation. As in the case with Akt transgenic mice, Foxo3a knockout mice develop systemic autoimmunity characterized by multiorgan infiltration, hyperproliferative CD4⁺ T cells and increased Th1 and Th2 cytokine production [23]. The results of this study suggest that CD4 cell accumulation and hyperactivity seen in p65^{PI3K} Tg and the Akt Tg mice may be partly due to insufficient suppression of T helper cell activation by Foxo3a.

Decreased PTEN/SHIP activity leads to autoimmunity

Regulated dephosphorylation of PtdIns(3,4,5)P₃ is crucial for proper functioning of PI3K signaling. PTEN opposes PI3K activity by dephosphorylating the 3'-hydroxyl group of PtdIns(3,4,5)P₃ to form PtdIns(4,5)P₂ [1,24]. This phosphatase is ubiquitously expressed and the *PTEN* gene is one of the most commonly mutated tumor suppressor genes found in human cancer. Loss of PTEN in mice is embryonically lethal [25], and mice that are heterozygous for PTEN develop a wide variety of tumors as well as polyclonal lymphoproliferative disease characterized by multiorgan inflammation, hypergammaglobulinemia and anti-ssDNA autoantibodies [26]. Such symptoms are more marked in females, and PTEN^{+/-} mice die between 12 and 15 months of age from renal failure. CD4⁺, CD8⁺ and B cells expand at normal ratios in the lymph nodes of PTEN^{+/-} mice and are positive for activation markers such as CD44 and CD69 as well as Fas, which suggests a defect in activation induced cell death (AICD). Indeed, PTEN^{+/-} lymphocytes exhibit decreased Fas-mediated apoptosis that is restored by wortmannin treatment.

The strong phenotype of PTEN^{+/-} mice spurred the development of T cell-specific PTEN knockouts to further study the role of PTEN in T cell tolerance and homeostasis. *Lck-Cre* PTEN^{+/-} mice were crossed with PTEN^{-/flox} mice to generate mice that had a T cell specific deletion of PTEN [27]. PTEN^{-/flox} mice show a more severe lymphoproliferative phenotype than PTEN^{+/-} mice and die of malignant CD4⁺ T cell lymphoma by 17 weeks of age. Lymphadenopathy, splenomegaly and thymic enlargement are apparent by 6–8 weeks of age as well as spontaneous T cell activation, expansion of memory CD4 and B cells, hypergammaglobulinemia and autoantibody production. PTEN^{flox/-} T cells hyperproliferate, have reduced AICD, increased secretion of Th1 and Th2 cytokines when stimulated, and show autoreactivity *in vitro*. These findings suggest that PTEN^{flox/-} T cells have defects in central and/or peripheral tolerance. Indeed, analysis of thymic

subpopulations in the HY-TCR transgenic model, in which T cells recognize the male antigen HY, reveals a 10-fold increase of the DP population in male HY-TCR transgenic PTEN^{-flox} mice in comparison to HY-TCR PTEN^{+/+} males. PTEN deficiency also leads to defects in peripheral tolerance, as shown by the decreased deletion of V β 8⁺T cells upon injection of staphylococcal enterotoxin B. At the molecular level, PTEN^{flox/-} T cells show elevation of the anti-apoptotic protein Bcl-xL and increased phosphorylation of Akt, Erk and GSK3, which suggests that the phenotype of these mice is largely mediated by unopposed PI3K activity.

Another phosphatase that opposes PI3K activity is the 5' inositol phosphatase SHIP, which converts PtdIns(3,4,5)P₃ to PtdIns(3,4)P₂ [28]. These two lipids have partially distinct sets of downstream effectors, so SHIP modifies PI3K signaling rather than simply opposing PI3K. Loss of SHIP results in fatal myeloproliferative disease, defects in B cell development and B cell hyperactivity [29–31]. The role of SHIP activity in T cells is less apparent, however, as T cell development and function are normal in both SHIP^{+/-} and SHIP^{-/-} mice [30]. Interestingly, compound SHIP and PTEN heterozygosity exacerbates the autoimmune phenotype seen in PTEN^{+/-} mice with increases in splenomegaly, cytokine production, hypergammaglobulinemia, autoantibody production and renal pathology [32,33]. Comparative analysis of PTEN^{+/-} and SHIP^{+/-} PTEN^{+/-} mice emphasizes that T cell homeostasis and tolerance is highly sensitive to the levels of PtdIns(3,4,5)P₃.

Negative regulators of PI3K: Traf6 and Cbl-b

Traf6 is an E3 ubiquitin ligase that transduces signals from the TNF receptor and IL-1/Toll-like receptor superfamilies [34]. Activated T cells upregulate Traf6 and loss of Traf6 leads to hyperactivation of CD4⁺ T cells [35]. These results indicate that Traf6 plays a role in T cell homeostasis by limiting activation signals. One of these signals appears to be PI3K activation, as Traf6-deficient T cells display an increased basal level of phosphorylated Akt (pAkt) [36]. Mice with a T cell specific deletion of Traf6 (*Traf6-ΔT*) develop splenomegaly, lymphadenopathy, multiorgan lymphocytic infiltration and anti-dsDNA antibodies. These mice have increased B cell and activated/effector-memory CD4⁺ T cell compartments and decreased percentage of CD4⁺ T cells [36]. *Traf6-ΔT* CD4⁺ T cells hyperproliferate and do not require CD28 costimulation for optimal proliferation like T cells from other mice with genetically enhanced PI3K/Akt signaling.

A similar phenomenon is seen upon loss of another ubiquitin ligase Cbl-b; T cells from Cbl-b-deficient mice proliferate in response to TCR engagement without CD28 and display elevated basal levels of

pAkt [37,38]. Cbl-b is important for regulating PI3K activity upon CD28 costimulation by disrupting the interaction of p85 regulatory subunits with CD28 [39]. Cbl-b knockout mice develop spontaneous autoimmunity marked by lymphocytic infiltration of multiple organs, parenchymal damage, and anti-dsDNA autoantibodies [37,38]. Unlike T cells with constitutive Akt membrane localization, Cbl-b and *Traf6-ΔT* T cells show normal responses to FasL-mediated apoptosis [36–39]. CD4 effector T cells lacking either *Traf6-ΔT* and Cbl-b are resistant to regulatory T cell (Treg) suppression [36,40]. PTEN overexpression abolishes this resistance of *Traf6-ΔT* effector T cells [36], which verifies that this breach in peripheral tolerance is PI3K-dependent [41].

Class IB PI3K: A promising drug target for autoimmunity

The growing evidence that PI3K gain-of-function promotes autoimmunity has led several groups to ask whether loss-of-function mutations in specific PI3K genes, or isoform-selective inhibitors, can oppose autoimmunity. An important outcome of these studies has been the identification of class IB PI3K (p110 γ or PI3K γ) as a particularly attractive drug target for autoimmune and inflammatory conditions [42]. The class IB enzyme appears to play important roles in autoimmunity and inflammation, while being dispensable for organismal development and homeostasis. Class IB knockout mice (termed p110 γ ^{-/-} or PI3K γ ^{-/-}) are viable and fertile [8], and compounds that selectively inhibit this isoform appear to be well tolerated in mice [43]. However, PI3K γ is important for the function of many immune cell types. PI3K γ ^{-/-} mice show profound defects in neutrophil function and migration, and reduced mast cell degranulation [8,44–46]. These mice also display decreased thymic cellularity and reduced T cell function *in vitro* and *in vivo*, whereas B cell development and function are largely normal [8].

These defects in neutrophil, macrophage, and T cell function in PI3K γ ^{-/-} mice raise the possibility of using class IB PI3K inhibition to alleviate autoimmune diseases. Indeed, PI3K γ ^{-/-} mice show reduced infiltration and cartilage erosion in the collagen-induced arthritis (CIA) model of rheumatoid arthritis, and oral administration of the PI3K γ -specific inhibitor AS-605240 after onset of arthritis reduced synovial inflammation and cartilage erosion by 42 and 43%, respectively [47].

Beneficial effects of PI3K γ inhibition have also been observed in mouse models of systemic lupus erythematosus [43]. Administration of PI3K γ inhibitors to MRL/lpr mice extends the lifespan by reducing autoantibody titers that lead to glomerulonephritis and proteinuria. This effect is likely caused by reduced expansion of the CD4⁺ memory T cell compartment

that mediates renal injury. PI3K γ ablation in p65^{PI3K} Tg (p65 Tg/ γ ^{KO}) mice also increases lifespan by similar means [48]. These findings helped to define the role of class IB PI3K in memory T cell survival because deletion of PI3K γ increases spontaneous death of CD4 memory cells. Interestingly, however, while hypergammaglobulinemia and anti-dsDNA antibody titers decrease, CD4 T cell infiltration to the lung and kidney are unaltered in p65 Tg/ γ ^{KO} mice when compared to single-transgenic mice. T cell invasion that occurs in the absence of PI3K γ might be driven by the constitutively active class IA PI3K, or by PI3K-independent chemokine signals that have been described in T cells [11].

Decreased class IA PI3K activity leads to autoimmunity

The observed autoimmunity in mice with enhanced PI3K signaling implies that reduced PI3K function should restrain autoreactivity. As discussed in the previous section, this prediction seems to apply to the class IB PI3K. Likewise, immunodeficiency rather than autoimmunity has been demonstrated in mice lacking the class IA regulatory isoform p85 α [6,9]. Deletion of p85 α impairs B cell function, correlating with reduced PI3K signaling output, whereas T cell function and signaling are largely intact. Other strains have been generated that do exhibit reduced class IA PI3K signaling in T cells but unexpectedly display autoimmune features. The following paragraphs describe the immune defects and autoimmune syndromes observed in two different mouse strains with impaired class IA PI3K function.

Initial studies by Okkenhaug et al. [7] demonstrated that a D910A mutation in the catalytic subunit p110 δ , which abolishes its kinase activity, leads to defects in B cell development and function similar to those seen in p85 α -deficient mice. Although p110 δ ^{D910A/D910A} T cells develop normally, they show impaired PI3K signaling and significant defects in proliferation and cytokine secretion [7,49]. Histological analysis of p110 δ ^{D910A/D910A} mice revealed that all mice develop subclinical colitis [7]. The mechanism of autoimmunity appears to be linked to defects in both peripheral and central tolerance. There is a 20–30% reduction in the percentage of CD4⁺Foxp3⁺ Tregs in the spleen and lymph nodes, and these Tregs also show a significantly reduced ability to suppress CD4⁺CD25⁻ effector cells and secrete IL-10 *in vitro* [50]. Adoptive transfer studies confirm deficient Treg function *in vivo* where co-injection of p110 δ ^{D910A/D910A} Tregs fails to alleviate the onset of colitis in RAG1^{-/-} mice injected with CD4⁺CD45RB^{high} T cells [50].

Interestingly, there is a ~2-fold increased Treg population in the thymus of p110 δ ^{D910A/D910A} mice [50]; given that Treg development in the thymus is thought to involve interactions with self-antigens, this

finding suggests that there may be a defect in negative selection. Indeed, the proportions of V β 11⁺ and V β 12⁺ TCR clones, which react with endogenous superantigens and normally are deleted via negative selection in a BALB/c background, are increased in the thymus and spleen of p110 δ ^{D910A/D910A} mice. This decrease in deletion of autoreactive clones suggests that there is a central tolerance defect in this strain, although further studies are required to test this model in the context of peptide antigens.

Whereas the p110 δ catalytic subunit has unique functions in T cell activation and tolerance, individual class IA regulatory subunits appear to be dispensable for these processes. Deletion of either *Pik3r1* (encoding p85 α /p55 α /p50 α), or *Pik3r2* (encoding p85 β) does not impair T cell development, proliferation or homeostasis [5,6]. To test for functional redundancy of these genes, our laboratory generated T cell-specific knock-outs of both *Pik3r1* and *Pik3r2* by crossing *Pik3r1-flox* mice with *Pik3r2*-null mice and crossing the progeny (referred to as *r1f/r2n* mice) with Lck-Cre transgenic mice [10]. T cells from these mice, named *r1 Δ T/r2n*, lack the regulatory subunits p85 α , p55 α , p50 α and p85 β and show minimal levels of p110 α and p110 δ and greatly diminished p110 β expression. Like p110 δ ^{D910A/D910A} T cells, *r1 Δ T/r2n* T cells develop normally but show partially reduced proliferation and cytokine production upon stimulation *in vitro* and defective CD4⁺ T cell function *in vivo*. Unlike p110 γ ^{-/-} T cells, *r1 Δ T/r2n* T cells and p110 δ ^{D910A/D910A} T cells show no changes in survival *in vitro* [10,49].

r1 Δ T/r2n mice develop corneal opacity and eye lesions as they age with extensive leukocyte infiltration and destruction of the lacrimal glands [51]. Infiltrates include abundant CD4⁺ T cells along with some CD8⁺ T cells and B cells. Less severe infiltrates are seen in the salivary glands. This pattern of histopathology in *r1 Δ T/r2n* mice resembles that of patients with primary Sjögren's Syndrome (pSS), which usually affects both lacrimal and salivary glands but can be limited to lacrimal gland in a subset of patients [52,53]. Other similarities with pSS include the finding that sera of most *r1 Δ T/r2n* mice contain anti-nuclear antibodies (ANA) with increased titers for anti-SSA and anti-SSB autoantibodies. *r1 Δ T/r2n* CD4⁺ T cells exhibit an aberrant cytokine profile where IFN γ and IL-10 production is elevated while IL-2 and IL-4 secretion are lowered following stimulation *in vitro* under Th2-skewing conditions. This aberrant cytokine pattern is similar to that found in saliva and T cell clones of patients with Sjögren's syndrome [54,55]. Treg numbers in *r1 Δ T/r2n* mice are reduced in the spleen, indicating that, as is the case with the p110 δ ^{D910A/D910A} mice, peripheral tolerance might be defective in these mice. Further characterization of both p110 δ ^{D910A/D910A} and *r1 Δ T/r2n* mice may yield valuable information on the role of class IA PI3K in T cell tolerance.

Discussion

PI3Ks mediate multiple aspects of immune function and tolerance, and it appears that tipping the balance of PI3K signaling in either direction can lead to autoimmunity. This is most clearly seen with respect to T cell tolerance. For example, reduced class IA PI3K activity results in impaired thymocyte negative selection whereas augmented PI3K signaling increases positive selection and emigration to the periphery of cells with increased proliferation and survival capacity. Both scenarios result in autoimmunity, albeit via different mechanisms. Similarly, loss or gain of PI3K activity each lead to impaired peripheral tolerance because $p110\delta^{D910A/D910A}$ and $r1\Delta T/r2n$ mice show decreased Treg function and/or frequency whereas $Traf6\Delta T$ and Cbl-b knockout effector T cells are insensitive to Treg-mediated suppression. Together these observations indicate that the degree of PI3K activity is a critical determinant of overall TCR signaling strength. Thus, altering the strength of TCR signaling in either direction disrupts the normal regulation of thymocyte selection and peripheral activation/suppression signals.

Comparison of mice lacking either class IA or class IB PI3K illustrates the complexity of this pathway in the regulation of tolerance. Class IB knockout mice show defects in T cell development and function, and exhibit resistance to SLE correlating with reduced memory $CD4^+$ T cell survival. In contrast, $r1\Delta T/r2n$ and $p110\delta^{D910A/D910A}$ T cells show no changes in survival; in fact, $r1\Delta T/r2n$ mice develop splenomegaly and memory/activated T cell accumulation as they age. These observations suggest that class IA and IB PI3K have largely distinct functions in T cells. However, class IA and IB have partially overlapping functions in thymocytes, as mice lacking both $p110\gamma$ and $p110\delta$ have more severe defects in thymocyte development and survival than single-knockout mice [56,57]. In neutrophils and mast cells, class IA and IB PI3K can be sequentially activated to produce amplification loops that are required for the full cellular response [4]. It is important to consider possible cooperation and/or compensation between class IA and IB PI3K when designing new therapies. Nevertheless, the central importance of class IB PI3K in inflammatory cell recruitment and function, and the efficacy of class IB inhibitors in mouse models of arthritis and lupus, suggest that selective targeting of $p110\gamma$ is a promising strategy.

Hyperactive PI3K signaling enhances T cell survival and leads to memory T cell accumulation in $p65^{PI3K}$, $mAkt$ and $gag-pkb$ transgenic mice. It is interesting that these strains with increased PI3K/Akt activity in T cells have systemic autoimmunity, whereas strains with decreased class IA PI3K have tissue-specific inflammatory syndromes. It is likely that the intrinsic defects in T cell responsiveness in $p110\delta^{D910A/D910A}$

mice prevent widespread autoimmunity even though negative selection and Treg function are impaired. The decreased T cell activation and reduced Treg numbers in $r1\Delta T/r2n$ mice suggest a similar phenomenon, although further experiments are needed to evaluate Treg function and negative selection in this strain.

Studies of mice deficient in PTEN, TRAF6 or Cbl-b illustrate that the balance between negative and positive regulators of PI3K may be as important for proper T cell function as the intrinsic PI3K expression or activity levels. This is not surprising given that $PtdIns(3,4,5)P_3$ levels are very stringently controlled in other systems. For example, insulin receptor function requires class IA PI3K signaling [58] yet deletion of class IA PI3K regulatory subunits ($p85\alpha$, $p85\beta$, $p85\alpha/p55\alpha/p50\alpha$ or $p55\alpha/p50\alpha$) results in increased insulin sensitivity [59–62]. Reduced regulatory subunit expression leads to a paradoxical increase in insulin-stimulated $PtdIns(3,4,5)P_3$ production [61,63]. Studies of mice with a liver-specific $Pik3r1$ ($p85\alpha/p55\alpha/p50\alpha$) deletion revealed that the increase in $PtdIns(3,4,5)P_3$ is partially due to decreased PTEN activity [63]. This finding of $p85\alpha$ -mediated PTEN regulation emphasizes how hepatocytes have evolved compensatory mechanisms to maintain optimal levels of $PtdIns(3,4,5)P_3$. It is unknown whether T cells utilize similar mechanisms for controlling $PtdIns(3,4,5)P_3$ levels. However, there is growing evidence that class IA regulatory isoforms have adaptor functions that either promote or oppose T cell activation signals, independent of their influence on PI3K catalysis [5,64]. Further studies, in particular knock-in strategies to disrupt the function of protein domains, are necessary to decipher the molecular mechanisms by which individual PI3K isoforms regulate T cell activation.

The study of mouse models indicates that dysregulation of the PI3K pathway can lead to autoimmunity. Is this also the case in humans? Unfortunately, PI3K signaling in patients with autoimmune diseases remains largely uncharacterized. One report described a patient who presented with both Cowden's syndrome and Sjögren's syndrome, and was heterozygous for PTEN [65]. Another study showed that synovial fibroblasts from rheumatoid arthritis patients had significantly higher $pAkt$ levels than fibroblasts of osteoarthritis patients [66]. Polymorphisms in leukocyte tyrosine kinase (LTK), a member of the insulin receptor superfamily found on B cells [67], may increase PI3K recruitment to the membrane via its YXXM motif [68]. Indeed, SLE patients carry this type of gain-of-function allele at a significantly higher frequency than control subjects [69]. Class IA PI3K signaling is clearly essential for proper B cell function; either abrogation of class IA PI3K activity through inhibitor treatment, inactivation of $p110\delta$, or deletion of $Pik3r1$ or $p85\alpha$ alone leads to a severe defect in B cell

development and proliferation [6,7,9,70]. p110 δ also plays a role in B cell homing and chemotaxis [12]. Given the critical role of class IA PI3K in B cell function, it is tempting to consider using class IA PI3K inhibitors to suppress autoreactive B cells and/or autoantibody production. However, one must proceed with caution given the ubiquitous nature of class IA PI3K signaling and since a growing number of studies show that inhibition of class IA PI3K signaling in T cells could also lead to autoimmunity.

Has increased understanding of PI3K function in the immune system brought us closer to better drug therapies to treat autoimmune diseases? The answer to this question appears to be "yes", considering that a large number of pharmaceutical companies around the world have advanced programs targeting one or more class I PI3K isoform for inflammatory or autoimmune conditions [71,72]. These pharmaceutical programs were initiated largely as a result of basic research demonstrating that different regulatory and catalytic isoforms of PI3K have unique functions. At least one compound, which inhibits both p110 γ and p110 δ , is in human clinical trials for treating an inflammatory condition [73]. It seems inevitable that other companies will initiate clinical trials for other diseases soon. Meanwhile, basic researchers in the field will combine the tools of genetics and isoform-selective inhibitors to define more clearly the roles of each regulatory and catalytic isoform in animal models of immune function and autoimmunity [70,74]. There is considerable optimism in the PI3K research community that the combined efforts of basic and translational scientists will soon bring improved treatments to the clinic for autoimmune diseases.

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