

The role of class I phosphoinositide 3-kinase in T-cell function and autoimmunity

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Abstract

PI3K (phosphoinositide 3-kinase) regulates diverse cellular responses in the immune system, and members of this enzyme family are considered attractive drug targets for modulating allergy, inflammation and leukaemia. Clearly it is important to understand the function of PI3K in T-lymphocytes, cells that regulate nearly every aspect of immunity. However, the precise role of PI3K in T-cell development and function has been difficult to determine. In this review, I summarize current knowledge of PI3K function in T-cells, focusing on the class I subgroup of PI3K catalytic and regulatory isoforms. I discuss gene disruption studies in mice that reveal redundant or limited roles for individual isoforms, along with evidence for potential autoimmunity when class IA PI3K signalling is reduced.

PI3K (phosphoinositide 3-kinase) isoforms

Due to space constraints, I refer the reader to other reviews with a detailed description of PI3K isoform structure, regulation and activation mechanisms in T-cells [1,2]. To briefly summarize, there are eight PI3K catalytic isoforms in the mammalian genome, which are divided into class IA, IB, II and III. Class IA and IB isoforms are the only forms that produce the critical second messenger $\text{PtdIns}(3,4,5)\text{P}_3$, and are the best studied in immune cells. Class IA isoforms are heterodimers of a catalytic subunit (either $\text{p}110\alpha$, $\text{p}110\beta$ or $\text{p}110\delta$) and a regulatory subunit ($\text{p}85\alpha$, $\text{p}55\alpha$, $\text{p}50\alpha$, $\text{p}85\beta$ or $\text{p}55\gamma$). There is a single class IB catalytic isoform ($\text{p}110\gamma$) that forms a dimer with either $\text{p}101$ or $\text{p}84$ regulatory subunits. Class IA enzymes are activated primarily by signals emanating from tyrosine kinases, whereas class IB PI3K is activated downstream of GPCRs (G-protein-coupled receptors). Hence, in T-lymphocytes it is expected that TCR (T-cell receptor)-initiated signals activate primarily class IA PI3K, whereas chemokines activate mainly class IB PI3K.

PI3K inhibitors in T-cells

The compounds wortmannin and LY294002 have long been used to investigate the role of PI3K catalytic activity in cellular responses. Overall, studies of these compounds in primary T-cells have suggested a relatively limited role for PI3K [2]. For example, antigen-dependent synapse formation and Ca^{2+} mobilization are unaffected by these compounds. The effects of these drugs on chemokine-driven T-cell motility varies depending on the chemokine but are generally lesser than the effects on corresponding responses in neutrophils [3].

Key words: autoimmunity, knockout mouse, phosphoinositide 3-kinase (PI3K), regulatory T-cell (Treg), T-cell, thymocyte.

Abbreviations used: ERK, extracellular-signal-regulated kinase; GPCR, G-protein-coupled receptor; IL-2, interleukin-2; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; TCR, T-cell receptor; Treg, regulatory T-cell.

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LY294002 is a potent inhibitor of T-cell proliferation, more so than is wortmannin, a difference that is likely to be the result of off-target effects of LY294002 [4].

PI3K gain-of-function mutants impair T-cell homeostasis

Until recently, the best evidence for an important role of PI3K and its downstream effectors in T-cell function was derived from mouse models of gain of function [2]. Mice heterozygous for PTEN (phosphatase and tensin homologue deleted on chromosome 10), the phosphatase mainly responsible for opposing PI3K function, develop T-cell lymphoproliferation, autoimmunity and increased incidence of leukaemia. $\text{PTEN}^{-/-}$ T-cells show aberrant development in the thymus and loss of peripheral self-tolerance. Broadly similar phenotypes are observed in mice expressing transgenes that cause constitutive activation of class IA PI3K or its downstream target, Akt (also known as protein kinase B). Indeed, studies of T-cells expressing active Akt have suggested a variety of functions of Akt in promoting T-cell survival, proliferation and differentiation. However, studies of Akt loss-of-function mice have so far failed to confirm a role for individual Akt isoforms in T-cell function.

Class I knockout phenotypes in T-cells

Table 1 provides a summary of published studies of mice lacking individual class I PI3K isoforms, and can be used for reference throughout this section. For simplicity the phenotypes in non-T-cells are not discussed here except where directly relevant. The first reported PI3K-knockout mouse strains were targeted for the regulatory subunit gene *Pik3r1* and lack either $\text{p}85\alpha$ alone or $\text{p}85\alpha/\text{p}55\alpha/\text{p}50\alpha$ [5,6]. To date there is no published evidence for T-cell-intrinsic phenotypes in mice lacking *Pik3r1* gene products. This is somewhat surprising given that all three proteins are abundantly

Table 1 | Phenotypes of mice lacking individual class I PI3K isoforms

pAkt, phosphorylated Akt.

Targeted gene	Class	Type	Thymocyte development	T-cell signalling and function
P110 δ [8,9,18] (kinase dead)	IA	Catalytic	Normal	\downarrow pAkt and Ca ²⁺ \downarrow Ag/specific proliferation/expansion \downarrow T-helper differentiation \downarrow Treg numbers and function Inflammatory bowel disease
P85 α [5,6]	IA	Regulatory	Normal	Normal
P85 β [7]	IA	Regulatory	Normal	Normal signalling \uparrow Survival
P110 γ [11–13]	IB	Catalytic	\downarrow Cellularity \downarrow DP Δ CD4/CD8	Normal signalling \downarrow Cytokine secretion \downarrow T-cell function and memory <i>in vivo</i> Resistant to lupus

expressed in T-cells and/or thymocytes, and these mice have severe defects in development of B-cells and mast cells. Mice lacking *Pik3r2* (p85 β) exhibit T-cell development and function that is largely normal as well [7]. However, these T-cells show resistance to apoptosis under some conditions, an unexpected finding given that PI3K signalling normally opposes cell death.

Overall, studies of mice lacking *Pik3r1* or *Pik3r2* have suggested that class IA regulatory subunits have either limited or redundant functions in T-cells. However, studies of mice with kinase-dead p110 δ (p110 δ^{D910A}) have provided clear evidence that class IA PI3K in general, and this catalytic isoform in particular, regulates important aspects of T-cell function. The initial report from Okkenhaug and co-workers [8] showed that p110 δ -deficient T-cells develop normally but show significant defects in TCR-mediated signal transduction. Along with a more recent study from this group, the data show a large reduction in PI3K signalling output as judged by Akt phosphorylation and other signalling endpoints [9]. These defects correlate with a major decrease in T-cell proliferation and clonal expansion in response to a specific antigen, although apoptosis does not appear to increase. p110 δ^{D910A} T-cells also show decreased ability to differentiate into either Th1 or Th2 effectors. p110 α or p110 β are also expressed in T-cells but studies of T-cells lacking either isoform have not been reported. However, it seems likely that these isoforms mediate residual PI3K signalling in p110 δ^{D910A} T-cells [9] and might be up-regulated to compensate in T-cells from mice with p110 δ -null alleles.

The class IB catalytic isoform p110 γ is well established as a regulator of chemotaxis in neutrophils [2]. In T-cells, p110 γ appears to be a more minor player in chemokine-driven homing and motility, for which another signalling pathway regulated by DOCK2 (dedicator of cytokinesis 2) seems to play a larger role [10]. Nevertheless, p110 γ does mediate important aspects of T-cell development and function. Mice lacking p110 γ show impaired thymocyte selection leading to fewer double-positive cells and an altered ratio of CD4

and CD8 single-positives [11,12]. The GPCR(s) responsible for p110 γ signalling in thymocytes has not been identified but might be an adenosine receptor. Peripheral T-cells lacking p110 γ exhibit impaired proliferation and cytokine production in response to TCR engagement [11]. Consistent with this finding, selective inhibitors of p110 γ inhibit T-cell proliferation driven by TCR/CD28 cross-linking, to a similar extent as wortmannin [4]. Recent studies of p110 γ -knockout mice also suggest a role for this isoform in the development of T-cell memory [13]. It will be important to identify the receptors or intracellular cross-talk responsible for engaging the p110 γ isoform in antigen-driven T-cell responses. The polarized accumulation of chemokine receptors at the 'immunological synapse' provides one possible mechanism for augmenting PI3K lipid production via the class IB enzyme during antigen recognition [14].

Double knockout phenotypes in T-cells

To address possible redundancy among class IA isoforms in T-cells, several groups have now begun to study mice with compound deletion or inactivation of PI3K genes (Table 2). Mice lacking both p110 γ and p110 δ show profound defects in thymocyte development and survival [15,16]. This indicates that class IA and IB PI3Ks serve partially redundant functions in thymocytes, with either subgroup sufficient to produce a threshold of PI3K signalling that can largely maintain survival.

My laboratory has generated mice with a null allele for *Pik3r2* (p85 β) and conditional deletion of *Pik3r1* (p85 α /p55 α /p50 α) in thymocytes and T-cells (termed r1 Δ T/r2n mice) [4]. In T-cells from r1 Δ T/r2n mice, catalytic isoform expression is greatly reduced and Akt phosphorylation is essentially undetectable following stimulation through the TCR or TCR/CD28. ERK (extracellular-signal-regulated kinase) phosphorylation appears more severely reduced than reported for p110 δ^{D910A} T-cells. Nevertheless, thymocyte development is largely normal and peripheral T-cell numbers

Table 2 | Phenotypes of mice lacking multiple class I PI3K isoforms

n.d., not determined; pAkt, phosphorylated Akt; pERK, phosphorylated ERK.

Targeted genes	Class	Type	Thymocyte development	T-cell signalling and function
p110 γ /p110 δ [15,16]	IA/IB	Catalytic	↓↓↓Cellularity	n.d.
p85 α /p85 β [4,17]	IA	Regulatory	Normal	↓pAkt, pERK and Ca ²⁺ ↓Proliferation/cytokine Altered Th2 differentiation ↓Treg numbers Sjögren's syndrome-like disease

and subsets are unimpaired in young animals. This suggests that class IA PI3K is mostly dispensable for T-cell development and homeostasis, although this needs to be studied more carefully using TCR transgenic models and adoptive transfer experiments. *In vitro*, r1 Δ T/r2n T-cell proliferation is nearly abrogated in response to TCR cross-linking. However, co-cross-linking of CD28 or addition of IL-2 (interleukin-2) partially restores cell division. This suggests that IL-2 receptors and CD28 can deliver substantial costimulatory signals in the absence of PI3K. r1 Δ T/r2n T-cells display an aberrant cytokine secretion pattern following differentiation under Th2 conditions, but unlike p110 δ ^{D910A} T-cells, r1 Δ T/r2n T-cells show robust Th1 differentiation [17]. *In vivo*, r1 Δ T/r2n T-cells generate reduced help to B-cells for antibody responses to protein antigen, but mediate normal primary responses to viral infection [4]. Overall, our analysis of r1 Δ T/r2n mice supports analysis of p110 δ ^{D910A} mice in demonstrating a role for class IA PI3K in selective T-cell functions. However, considerable T-cell-mediated immunity is retained. While it may be true that gain of function in the PI3K/Akt pathway can promote T-cell proliferation and break tolerance, it is worth emphasizing that loss of class IA PI3K function in T-cells does not cause profound T-cell deficiency and may contribute to autoimmunity, as described in the next section.

Class IA PI3K and autoimmunity

Deletion of class IA PI3K isoforms causes profound B-cell immunodeficiency as well as greatly impaired differentiation and function of mast cells. Hence, inhibitors of this subclass could be beneficial for the treatment of allergic diseases as well as antibody-driven autoimmune disorders. However, studies of T-cells with reduced class IA PI3K function suggest that inhibiting this subclass might have the paradoxical effect of enhancing susceptibility to autoimmunity. This was first suggested by the initial study of p110 δ ^{D910A} mice, which develop inflammatory bowel disease [8]. More recent work from this group indicates that Treg (regulatory T-cell) function is impaired in these mice [18]. Thus residual function of effector T-cells in these mice might be sufficient to promote colon inflammation when the influence of Tregs is diminished.

r1 Δ T/r2n mice also develop an autoimmune syndrome [17]. With nearly complete penetrance, adult mice aged

2–12 months develop an exocrinopathy characterized by lymphocyte infiltration into the lacrimal and salivary glands. Inflammation of other organs is less frequent (including the colon), and there is no evidence of immune complex disease in the kidney. Serum from these mice exhibits a high frequency of antinuclear antibodies, in most cases with specific reactivity towards SS-A and SS-B antigens. Considered together, the pathology and serology are highly similar to the human disease Sjögren's syndrome. At present, the cellular defects responsible for disease pathogenesis are not clear. r1 Δ T/r2n T-cells also show aberrant cytokine production that is similar to the phenotype reported for T-cells from humans with Sjögren's syndrome [17]. Similar to p110 δ ^{D910A} mice, r1 Δ T/r2n mice show reduced frequencies of Tregs in the periphery but we have not yet evaluated Treg function extensively. It is also possible that negative selection of autoreactive thymocytes is defective in these mice, as in another mouse model of Sjögren's syndrome [19]. It is important to consider that p85 β is absent in all tissues of these mice, and the disease might therefore arise from a combination of factors both T-cell-intrinsic and -extrinsic.

Implications for drug development

The autoimmunity that develops in mice with class IA PI3K-deficient T-cells would seem to suggest a note of caution in plans to develop therapies targeting this subclass or p110 δ specifically. Clearly, the possibility of autoimmunity should be carefully monitored should any such agents enter the clinic. However, it might turn out that acute treatment with such agents would not have the same effects as the genetic loss-of-function models might suggest. For example, Treg development and maintenance might be determined early in life and unaffected by later modulation of class IA PI3K signalling, perhaps even with chronic treatment. On the other hand, ongoing activation of effector lymphocytes and mast cells might still be sensitive to inhibition of this pathway, providing an adequate therapeutic window for treatment.

The class IB isoform p110 γ is emerging as an attractive target whose inhibition might provide relief from autoimmunity and inflammation [20]. Initially, interest arose in p110 γ as a therapeutic target primarily due to its central role in migration and activation of neutrophils and macrophages. However, subsequent work showed an important function for p110 γ in mast cell degranulation *in vitro*. Studies of

p110 γ inhibitors in mice have further indicated a role for this isoform in the generation of CD4 T-cell memory [21]. Considering the central role of memory CD4 T-cells in most autoimmune diseases, including systemic syndromes such as lupus, p110 γ inhibitors show much promise for treatment of these conditions.

A challenge for the development of inhibitors targeting either class IA or IB would seem to be finding a balance between dampening the pathology and avoiding broad immunosuppression. For example, compounds selective for p110 δ might be expected to deplete mature B-cells and block humoral immunity to pathogens. Inhibitors of p110 γ might increase susceptibility to a variety of pathogens for which a robust inflammatory response is essential. Both classes of inhibitors might impair T-cell function to some degree. Further investigation of mouse models will continue to provide important clues about the likely benefits and challenges of PI3K-targeted therapy.

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References

- 1 Vanhaesebroeck, B., Leevers, S.J., Ahmadi, K., Timms, J., Katso, R., Driscoll, P.C., Woscholski, R., Parker, P.J. and Waterfield, M.D. (2001) *Annu. Rev. Biochem.* **70**, 535–602
- 2 Deane, J.A. and Fruman, D.A. (2004) *Annu. Rev. Immunol.* **22**, 563–598
- 3 Ward, S.G. (2006) *Trends Immunol.* **27**, 80–87
- 4 Deane, J.A., Kharas, M.G., Oak, J.S., Stiles, L.N., Luo, J., Moore, T.I., Ji, H., Rommel, C., Cantley, L.C., Lane, T.E. and Fruman, D.A. (2006) *Blood*, doi:10.1182/blood-2006-07-038620
- 5 Fruman, D.A., Snapper, S.B., Yballe, C.M., Yu, J.Y., Davidson, L., Alt, F.W. and Cantley, L.C. (1999) *Science* **283**, 393–397
- 6 Suzuki, H., Terauchi, Y., Fujiwara, M., Aizawa, S., Yazaki, Y., Kadowaki, T. and Koyasu, S. (1999) *Science* **283**, 390–392
- 7 Deane, J.A., Trifilo, M.J., Yballe, C.M., Choi, S., Lane, T.E. and Fruman, D.A. (2004) *J. Immunol.* **172**, 6615–6625
- 8 Okkenhaug, K., Bilancio, A., Farjot, G., Priddle, H., Sancho, S., Peskett, E., Pearce, W., Meek, S.E., Salpekar, A., Waterfield, M.D. et al. (2002) *Science* **297**, 1031–1034
- 9 Okkenhaug, K., Patton, D.T., Bilancio, A., Garcon, F., Rowan, W.C. and Vanhaesebroeck, B. (2006) *J. Immunol.* **177**, 5122–5128
- 10 Nombela-Arrieta, C., Lacalle, R.A., Montoya, M.C., Kunisaki, Y., Megias, D., Marques, M., Carrera, A.C., Manes, S., Fukui, Y., Martinez-A., C. and Stein, J.V. (2004) *Immunity* **21**, 429–441
- 11 Sasaki, T., Irie-Sasaki, J., Jones, R.G., Oliveira-dos-Santos, A.J., Stanford, W.L., Bolon, B., Wakeham, A., Itie, A., Bouchard, D., Koziarzki, I. et al. (2000) *Science* **287**, 1040–1046
- 12 Rodriguez-Borlado, L., Barber, D.F., Hernandez, C., Rodriguez-Marcos, M.A., Sanchez, A., Hirsch, E., Wymann, M., Martinez-A., C. and Carrera, A.C. (2003) *J. Immunol.* **170**, 4475–4482
- 13 Barber, D.F., Bartolome, A., Hernandez, C., Flores, J.M., Fernandez-Arias, C., Rodriguez-Borlado, L., Hirsch, E., Wymann, M., Balomenos, D. and Carrera, A.C. (2006) *J. Immunol.* **176**, 589–593
- 14 Molon, B., Gri, G., Bettella, M., Gomez-Mouton, C., Lanzavecchia, A., Martinez-A., C., Manes, S. and Viola, A. (2005) *Nat. Immunol.* **6**, 465–471
- 15 Swat, W., Montgrain, V., Doggett, T.A., Douangpanya, J., Puri, K., Vermi, W. and Diacovo, T.G. (2006) *Blood* **107**, 2415–2422
- 16 Webb, L.M., Vigorito, E., Wymann, M.P., Hirsch, E. and Turner, M. (2005) *J. Immunol.* **175**, 2783–2787
- 17 Oak, J.S., Deane, J.A., Kharas, M.G., Luo, J., Lane, T.E., Cantley, L.C. and Fruman, D.A. (2006) *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16882–16887
- 18 Patton, D.T., Garden, O.A., Pearce, W.P., Clough, L.E., Monk, C.R., Leung, E., Rowan, W.C., Sancho, S., Walker, L.S., Vanhaesebroeck, B. and Okkenhaug, K. (2006) *J. Immunol.* **177**, 6598–6602
- 19 Li, H., Dai, M. and Zhuang, Y. (2004) *Immunity* **21**, 51–60
- 20 Wymann, M.P., Zvelebil, M. and Laffargue, M. (2003) *Trends Pharm. Sci.* **24**, 366–376
- 21 Barber, D.F., Bartolome, A., Hernandez, C., Flores, J.M., Redondo, C., Fernandez-Arias, C., Camps, M., Ruckle, T., Schwarz, M.K., Rodriguez, S. et al. (2005) *Nat. Med.* **11**, 933–935

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