Activation of PI3K (phosphoinositide 3-kinase) is a shared response to engagement of diverse types of transmembrane receptors. Depending on the cell type and stimulus, PI3K activation can promote different fates including proliferation, survival, migration and differentiation. The diverse roles of PI3K signalling are well illustrated by studies of lymphocytes, the cells that mediate adaptive immunity. Genetic and pharmacological experiments have shown that PI3K activation regulates many steps in the development, activation and differentiation of both B- and T-cells. These findings have prompted the development of PI3K inhibitors for the treatment of autoimmunity and inflammatory diseases. PI3K activation, however, has both positive and negative roles in immune system activation. Consequently, although PI3K suppression can attenuate immune responses it can also enhance inflammation, disrupt peripheral tolerance and promote autoimmunity. An exciting discovery is that a selective inhibitor of the p110δ catalytic isoform of PI3K, CAL-101, achieves impressive clinical efficacy in certain B-cell malignancies. A model is emerging in which p110δ inhibition disrupts signals from the lymphoid microenvironment, leading to release of leukaemia and lymphoma cells from their protective niche. These encouraging findings have given further momentum to PI3K drug development efforts in both cancer and immune diseases.

Key words: autoimmunity, cancer, kinase inhibitor, leukaemia, lymphocyte, lymphoma, phosphoinositide 3-kinase (PI3K).

INTRODUCTION

The adaptive immune system is crucial for protection from recurring infections by various pathogens. T- and B-lymphocytes are the key cellular mediators of this system in which the enormous diversity and exquisite specificity of the antigen receptors enable T- and B-cells to recognize virtually any foreign molecule. Antigen recognition alone, however, is insufficient to initiate effective immune responses. A key feature of the adaptive immune system is that lymphocytes depend on signals from neighbouring cells to perform specific functions in the context of various immune conditions. For example, B-cells cannot properly complete their primary task of making antibodies without signals from CD4 T-cells. Similarly, CD4 T-cells can differentiate into a variety of effector subsets depending on co-stimulatory signals and the cytokine milieu generated from other immune cells of the microenvironment. To prevent autoimmunity, lymphocyte self-tolerance must be enforced and certain conditions should apply to tumours of lymphoid origin, whose survival typically depends on both cell-intrinsic and -extrinsic signals from the microenvironment.

The PI3K (phosphoinositide 3-kinase) signalling network plays a fundamental role in signal transduction in mammalian cells [1,2]. Hence it is not surprising that PI3K is activated by diverse stimuli in lymphocytes and is required for the maintenance of proper adaptive immunity and self-tolerance [3,4]. In the present review we summarize recent advances in the understanding of PI3K signalling in B- and T-cells. These advances have been spurred largely by two technological breakthroughs: refinements in gene-targeted mouse models and discovery of PI3K isoform-selective inhibitors. We emphasize two key concepts. First, PI3K activation should not be viewed as a simple on/off switch, but rather as a ‘rheostat’ whose output must be properly balanced to promote immune responses. Integration of diverse extracellular cues to achieve the required cellular response involves a complex network of intracellular signalling events. This concept also applies to tumours of lymphoid origin, whose survival typically depends on both cell-intrinsic and -extrinsic signals from the microenvironment.
populations engage PI3K signalling pathways, as do the cells of the innate immune system. Therefore decreased PI3K signalling can lead to immunosuppression or, in some contexts, to increased inflammation and improved pathogen clearance, and even to autoimmunity. The second concept is that despite this complexity, the p110δ catalytic isoform of class I PI3K has emerged as a central driver of lymphocyte clonal selection, differentiation and trafficking. We conclude by describing progress in the development of PI3K inhibitors for therapeutic uses. Indeed, development of highly specific PI3K inhibitors for clinical use is one of the major goals in the pharmaceutical industry today. Of particular interest is the surprising success of a selective p110δ inhibitor CAL-101 in human B-cell malignancies, where CAL-101 seems to act mainly by perturbing the signals received from the tumour microenvironment.

OVERVIEW OF PI3K

PI3Ks are a family of lipid kinases that phosphorylate the 3-hydroxy group of PtdIns (phosphatidylinositol) and phosphoinositides (phosphorylated derivatives of PtdIns) [2]. Unlike yeast, whose genome encodes only one PI3K isoform [the class III PI3K Vps34 (vacuolar protein sorting 34)] whose main role is in vesicle trafficking, the mammalian PI3Ks include eight enzymes with diverse roles in both vesicle trafficking and signal transduction. These enzymes are grouped into the categories known as class I, class II and class III, on the basis of their substrate preference and structure. Only the class I PI3Ks have the ability to use PtdIns(4,5)P₂ as a substrate to generate the important second messenger PtdIns(3,4,5)P₃. Certain proteins containing a PH (pleckstrin homology) domain can specifically bind PtdIns(3,4,5)P₃ and be recruited to membranes where PI3K is active [5]. Hence class I PI3K acts as a signalling hub at the plasma membrane to change the lipid composition in a way that links transmembrane receptors to the organization of multiprotein complexes, also known as signalosomes [6]. The composition of these signalosomes and the specific PH domain-containing PI3K effector proteins recruited to these assemblies varies according to the receptor that is engaged. In most cells, the serine/threonine kinase Akt [also known as PKB (protein kinase B)] is a key PI3K effector and Akt phosphorylation is used as a common readout of PI3K activation [7]. Two amino acid residues in Akt are phosphorylated in a PI3K-dependent manner: Thr³⁸³ by PDK-1 (phosphoinositide-dependent kinase-1), and Ser²⁷³ by TORC [TOR (target of rapamycin) complex with rictor (rapamycin-insensitive companion of mammalian TOR) and other proteins] [2].

PI3K and Akt co-ordinate many aspects of the response to antigen receptor engagement. In B- and T-cells, the formation of signalosomes that drive antigen receptor-dependent Ca²⁺ mobilization is partially dependent on PI3K [6,8]. PtdIns(3,4,5)P₃ produced upon BCR (B-cell receptor) or TCR (T-cell receptor) clustering binds to PH domains in Tec family kinases to promote activation of phospholipase Cγ and subsequent hydrolysis of PtdIns(4,5)P₂. This results in release of soluble Ins(1,4,5)P₃ to initiate Ca²⁺ mobilization, and accumulation of diacylglycerol in the membrane to activate PKC (protein kinase C) isoforms and GTP exchange factors for Ras. A role for PI3K upstream of Ras in lymphocytes is supported by the observation of decreased ERK (extracellular-signal-regulated kinase) phosphorylation in PI3K-deficient T- and B-cells stimulated through antigen receptors [9]. Activated Akt has many substrates with key roles in lymphocyte activation and trafficking. The FOXO (forkhead box O) transcription factors are one key group of Akt substrates in lymphocytes [3]. Akt-mediated phosphorylation inhibits DNA binding by FOXO factors and promotes their nuclear exit and cytoplasmic sequestration and degradation. Akt also can phosphorylate TSC2 (tuberous sclerosis 2) and PRAS40 (proline-rich Akt substrate of 40 kDa) to increase the activity of TORC1, a multifunctional signalling protein that co-ordinates cell growth and metabolism [10]. However the dependence of TORC1 activation on PI3K and Akt varies in lymphocytes according to the cell subset and stimulus [11,12].

Elevation of cellular PtdIns(3,4,5)P₃ is transient and is controlled by lipid phosphatases [13,14]. PTEN (phosphatase and tensin homologue) is a phosphoinositide 3-phosphatase that converts PtdIns(3,4,5)P₃ back into PtdIns(4,5)P₂. In cells lacking PTEN, basal PtdIns(3,4,5)P₃ levels are increased and receptor stimulation causes exaggerated PI3K signalling. SHIP [SH (Src homology) 2 domain-containing inositol phosphatase] 1 and SHIP2 are 5-phosphatases that convert PtdIns(3,4,5)P₃ into PtdIns(3,4)P₂. The latter lipid species can still recruit and activate PDK-1 and Akt, but has other signalling functions that do not overlap with PtdIns(3,4,5)P₃. For example, the PH domains of Tec family kinases have a high affinity for PtdIns(3,4,5)P₃, but not PtdIns(3,4)P₂, whereas the converse is true for the adaptor proteins Bam32 (B-cell adapter molecule of 32 kDa), TAPP (tandem PH domain-containing protein) 1 and TAPP2 [15].

Class I PI3Ks are further divided into two subgroups: class IA and class IB [2]. Class IA PI3Ks contain one of three distinct 110 kDa catalytic isoforms (p110α, p110β or p110δ) that form a heterodimer with one of the five regulatory (adaptor) isoforms (p85α, p55α, p50α, p85β or p55γ). The overall structure of p110 subunits is very similar with the following domains: AB (adapter-binding domain), Ras-binding, C2, helical and lipid kinase (Figure 1). The class IA regulatory isoforms share a similar C-terminal half that contains two SH2 domains that flank a coiled-coil domain (also called ISH2) that binds tightly to the p110 ABD to form the heterodimer. The N-terminal portions of p85α and p85β contain an additional SH3 domain and a RhoGAP homology region flanked by proline-rich motifs. Class IB PI3K is composed of a single catalytic isoform (p110γ) bound to one of two regulatory isoforms (p101 or p84). In general, class IA PI3Ks are activated by TK (tyrosine kinase)-based signals, whereas class IB PI3Ks are activated by GPCRs (G-protein-coupled receptors). However, these distinctions have been challenged by numerous studies linking p110β and p110δ to GPCRs and p110γ to TKS [2,16]. Whereas p110α and p110β are expressed ubiquitously, p110δ and p110γ, compared with other cell types, are predominantly expressed in the cells of the immune system. This suggests that p110δ and p110γ may have evolved to serve specific roles in immunity, a hypothesis supported by many published studies and emphasized in the present review. The possibility that p110α and p110β isoforms might also be important in lymphocytes will also be discussed.

The mechanism of class IA PI3K activation has been clarified through structural studies and has been reviewed in detail elsewhere [17,18]. Briefly, the regulatory subunit has different functions in the absence or presence of stimulation. In the basal state, the regulatory subunit stabilizes the catalytic subunit while suppressing its activity. This suppression is mediated by several intersubunit contacts including helical(p110–N-SH2(p85) and C2(p110)–ISH2(p85) (Figure 1). The C-SH2 domain of p85β can also form an inhibitory contact with the kinase domain of p110β. In response to TK-based signals, the SH2 domains become bound to pTyr (phosphotyrosine) residues on receptors and adaptor proteins (consensus motif pTyr-X-X-Met), releasing the inhibitory contacts and bringing the catalytic subunit to the membrane where its substrates reside. Additional interactions
involving the N-terminal domains of p85 proteins might also contribute to PI3K recruitment and activation in some contexts. Naturally occurring mutations in p85α and p85β have been identified in tumors of humans and mice [19–23]; in general these are gain-of-function mutations that disrupt the inhibitory interfaces and elevate PI3K enzyme activity while maintaining the stability of the catalytic subunit. How do receptors on lymphocytes engage class I PI3K? Antigen receptors, co-stimulatory molecules, cytokine receptors and chemokine receptors can all trigger an increase in PtdIns(3,4,5)P3, and phosphorylation of Akt. Cross-linking of the BCR leads to tyrosine phosphorylation of the co-receptor CD19 and BCAP to recruit PI3K downstream of the vav exchange factors [28–30], with activation of PI3K through SH2 domain interactions [4,24,25] (Figure 2). TCR engagement also activates class IA PI3K, but the molecular details remain to be fully established. Possible mechanisms include: binding of p85 proline-rich domains to SH3 domains of Src family kinases [26,27], interaction of p85 RhoGAP domains with RacGTP downstream of the vav exchange factors [28–31], and association of SH2 domains in p85 or p50 with pTyr residues in proteins associated with TCR signalosomes [32,33]. Two co-stimulatory molecules on T-cells, CD28 and ICOS (inducible T-cell co-stimulator), recruit PI3K through pTyr-X-X-Met motifs [34]. In the case of CD28, recruitment of PI3K appears dispensable for most of the initial co-stimulatory signals, but is essential for CD28 function in effector T-cells [4]. The role of PI3K in ICOS signalling is discussed below. Other co-stimulatory molecules and cytokine receptors on B- and T-cells recruit and activate class IA PI3K through diverse mechanisms [35,36]. The class IB p110γ isoform is mainly activated by GPCR signals (i.e. chemokine receptors) in cells of the innate immune system such as neutrophils [37]. The βγ subunits of heterotrimeric G-proteins directly bind to p110γ and mediate enzyme activation through p101 or p84 adaptor subunits (Figure 1). However, the situation is more complicated in lymphocytes in that chemokines activate p110γ in T-cells, but p110δ in B-cells [38,39]. The mechanism linking GPCRs to p110δ in B-cells is not known. Furthermore, there is some evidence that TCR signalling activates p110γ, possibly via Gα proteins or the small GTPase Ras [40,41]. All of the class I catalytic subunits including p110γ contain Ras-binding domains (Figure 1), and the interaction of p110γ with Ras is required for T-cell development at the β-selection stage [41]. An interesting and largely unexplored question is whether different receptors generate distinct pools of PtdIns(3,4,5)P3 during lymphocyte activation, with selective localization and downstream effectors. This concept was validated in a study of p110γ activity in mast cells, where p101- and p84-bound p110γ produced distinct pools of PtdIns(3,4,5)P3 with separable function [42].

**TOOLS TO STUDY PI3K SIGNALLING IN LYMPHOCYTES**

Following the molecular cloning of class I PI3K isoforms in the early 1990s, PI3K signalling in lymphocytes was studied mainly using established cell lines derived from lymphoid tumours. This approach was convenient for biochemical studies because cell lines have more cytoplasm and protein content than primary T- and B-cells. Cell-line studies also avoided the problem that primary lymphocytes die rapidly in culture and cannot easily be metabolically labelled or transfected. However, tumour cells have severe drawbacks for signal transduction research as they usually have dysregulated PI3K signalling, and cell proliferation is typically uncoupled from physiological extracellular controls. Furthermore, the PI3K inhibitors used in early experiments (wortmannin and LY294002) are non-selective compounds that inhibit all PI3K isoforms as well as TOR and other lipid and

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**Figure 1** Domain structure of class IA and class IB PI3K isoforms

Black arrows indicate constitutive interactions of the heterodimers. Broken red arrows indicate inhibitory interactions between the regulatory and catalytic isoforms of class IA PI3K that maintain low basal activity of the enzyme. Phosphorylation of tyrosine (Y) residues on the conserved pY-X-X-M motifs on various receptors or adaptor proteins (CD19/BCAP for B-cells and CD28/ICOS for T-cells) recruits the regulatory isoforms via the two SH2 domains and this binding releases the inhibitory interactions. Other protein–protein interactions also contribute to class IA and IB recruitment and activation.

**Figure 2** PI3K engagement in B-lymphocytes and the rheostat concept

BCR engagement triggers tyrosine (Y) phosphorylation on CD19 and BCAP to recruit PI3K dimers mainly consisting of p85α and p110α. PI3K activation promotes signalosome assembly for Ca2+ mobilization and diacylglycerol (DAG) production, and increases the activity of Akt. BCR-dependent Ca2+ flux, Akt activation and proliferation is mainly dependent on PtdIns(3,4,5)P3 pools generated by dimers of the p110γ catalytic isoform with the p85α regulatory isoform. Cytokine (BAFF and IL-4-dependent survival signals require p110δ as well, which might generate distinct pools of PtdIns(3,4,5)P3, as shown. Two parallel membranes drawn in light brown represent a three-dimensional cell surface rather than distinct membranes. In vivo, overall PI3K activity serves as a ‘rheostat’ (grey circle) whose signal output strength determines the nature of the response. In B-cells, high PI3K activity opposes class switch recombination and promotes plasma cell differentiation. Low PI3K activity promotes class switch recombination, with p110δ inhibition selectively augmenting IgE production in mice. A similar rheostat concept applies in CD4 T-cells to generate the variety of different subsets required depending on the immune context. BAFF-R, BAFF receptor; IL-4R, IL-4 receptor; Syk, spleen tyrosine kinase.
protein kinases in cells [43–45]. A more precise understanding of PI3K signalling required better tools.

The first major technical advance was the creation of genetically modified mouse strains lacking individual class I PI3K isoforms. Germline and/or tissue-specific knockout of the genes encoding each class I catalytic subunit, and of the genes encoding p85α/p55α/p50α and p85β, have been generated and characterized (Supplementary Table S1 at http://www.BiochemJ.org/bj/442/4420465add.htm). Insights gained from these mouse strains have been summarized in many reviews (see for example [4,35,46]) and new findings are discussed in the present review. However, PI3K gene knockouts have significant limitations even when deletion is tissue-specific. One problem is that loss of one isoform of PI3K often leads to altered expression of non-targeted isoforms [47]. For example, deletion of the PIK3R1 gene encoding p85α/p55α/p50α causes reduced expression of p110 proteins and increased expression of p85β. Deletion of individual p110 isoforms results in functional compensation of other catalytic isoforms. Hence, an important step forward was the generation of knockin mice that express intact proteins with point mutations causing loss-of-function. This strategy was first used by Okkenhaug et al. [9] to generate p110δ<sup>DLR/A573K</sup> mice with a point mutation inactivating p110δ kinase function, and has been used subsequently to generate KI (kinase inactive) alleles of each of the p110 isoforms (Supplementary Table S1). This strategy prevents compensatory changes in expression or function and provides a more precise model of chemical kinase inhibition. However, the knockin strategy is still limited to some degree by the fact that the mutation can affect lymphocyte development, resulting in an altered pool of mature T- or B-cells. In addition, p110αKI mice display embryonic lethality at a stage before lymphoid precursors can be isolated. Therefore data from knockout and knockin mice are most conclusive when combined with studies of wild-type cells treated with selective PI3K inhibitors.

The discovery and validation of selective ATP-competitive PI3K inhibitors has been driven by efforts from both industry and academia. Although the kinase domains of class I PI3K isoforms are highly conserved, X-ray crystal structures have shown that the ATP-binding pockets have distinct topologies and flexibilities that can allow selective binding of distinct chemical structures [18]. These properties have allowed the development of compounds with good selectivity for single class I PI3K isoforms. There are also several compounds targeting all class I isoforms with minimal off-target effects on other kinases (termed ‘pan-class I PI3K inhibitors’). Examples of isoform-selective and pan-class I PI3K inhibitors are shown in Table 1. Many of these can be purchased from commercial vendors, and most have optimized pharmacological properties to allow dosing of animals in vivo. Despite these powerful additions to the PI3K inhibitor toolkit, many investigators continue to test PI3K function using wortmannin or LY294002. The importance of choosing the right PI3K inhibitor at the optimal concentration (or in vivo dose) cannot be overstated.

In addition to PI3K gene knockouts and knockins, other genetically engineered mouse models have been produced to study PI3K signalling in lymphocytes. These include knockout and knockin mutations in PI3K effectors such as PDK-1 and Akt, and knockouts of the genes encoding the PtdIns(3,4,5)<sub>α</sub> phosphatases PTEN and SHIP1. These models have proven very useful for dissecting PI3K signalling pathways in lymphocytes, and some recent advances are described in the present review. Mouse strains with mutations affecting TOR signalling, FOXO transcription factors and other PI3K-regulated signalling components have also yielded important advances that are discussed below. Another common approach has been to generate transgenic mice expressing membrane-targeted forms of Akt in lymphocytes. The rationale for this strategy is that it will reveal the functions of Akt, independent of other PI3K effectors. These mice exhibit dramatic phenotypes with altered lymphocyte development, lymphoproliferation and loss of immune homeostasis [48–51]. However, the phenotypes of Akt gain-of-function mutations should be interpreted with caution. In normal immune cells, Akt is recruited transiently to cell membranes and once activated it travels to different subcellular compartments to phosphorylate diverse substrates. The activation state of Akt is also controlled by a large array of phosphatases and other regulatory proteins. Therefore constitutive anchoring in the membrane does not faithfully recapitulate the functions of active Akt. Nevertheless, T-cell-specific deletion of PTEN or expression of a constitutively active PI3K also causes lymphoproliferation and autoimmunity in mice [52,53]. Together these approaches concur that artificially elevating PI3K signalling output in T-cells causes a loss of immune homeostasis.

Fluorescent bioprobes have provided valuable insights into the kinetics and subcellular localization of PI3K lipid production in lymphocytes, and will continue to be useful tools [54]. However these probes do not provide a comprehensive and quantitative view of different PI3K lipid species. Further advances in this direction are likely to emerge from the application of novel mass spectrometry technologies. The group of Hawkins and colleagues have reported a method for sensitive quantification of PtdIns(3,4,5)α<sub>3</sub> without radioactive or heavy-isotope labelling [55]. The method also allows the discrimination of PtdIns(3,4,5)α<sub>3</sub> species on the basis of distinct fatty acyl chain composition. Combining this technique with genetic or pharmacological inhibition of specific PI3K isoforms might uncover important aspects of PI3K signalling specificity in lymphocytes.

### Table 1 Selected PI3K inhibitors currently available for basic research or in clinical trials

<table>
<thead>
<tr>
<th>Targeted isoform(s)</th>
<th>Compound name</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan-class I</td>
<td>GDC-0941</td>
<td>Basic and clinical</td>
</tr>
<tr>
<td></td>
<td>ZSTK474</td>
<td>Basic and clinical</td>
</tr>
<tr>
<td>p110α high selectivity</td>
<td>XL147</td>
<td>Basic and clinical</td>
</tr>
<tr>
<td>p110αI/p50αI</td>
<td>A66</td>
<td>Basic</td>
</tr>
<tr>
<td>p106α</td>
<td>BYL-719</td>
<td>Clinical</td>
</tr>
<tr>
<td></td>
<td>PIK-75</td>
<td>Basic</td>
</tr>
<tr>
<td>p110γ</td>
<td>PIK-90</td>
<td>Basic</td>
</tr>
<tr>
<td>p110δ</td>
<td>TGX-115, TGX-221</td>
<td>Basic</td>
</tr>
<tr>
<td>p110γ</td>
<td>AZD6682</td>
<td>Basic and clinical</td>
</tr>
<tr>
<td>p110γ</td>
<td>AS202424, AS604850</td>
<td>Basic</td>
</tr>
<tr>
<td>p110δ</td>
<td>IC87114</td>
<td>Basic</td>
</tr>
<tr>
<td>p110γ</td>
<td>CAL-101/AS101</td>
<td>Clinical</td>
</tr>
<tr>
<td></td>
<td>CAL-263</td>
<td>Clinical</td>
</tr>
<tr>
<td></td>
<td>AMG-319</td>
<td>Clinical</td>
</tr>
<tr>
<td>Dual p110γ/p110δ</td>
<td>IPI-145</td>
<td>Clinical</td>
</tr>
</tbody>
</table>

### B-LYMPHOCYTES

#### B-cell development and tolerance

B-cells develop in the bone marrow from lymphoid progenitors that undergo an ordered series of gene rearrangement steps punctuated by stringent checkpoints. The Ig heavy-chain rearrangement occurs at the pro-B-cell stage and, when successful, the heavy cell pairs with surrogate light chains and
Igα–Igβ heterodimers to form the pre-BCR at the cell surface. Assembly of the pre-BCR triggers tonic (basal) signalling that is critical for repression of RAG (recombination-activating gene) expression to prevent further heavy-chain rearrangement. At this stage cells proliferate and are termed large pre-B- (or pre-B-I-) cells. Ig light-chain rearrangement proceeds at the small pre-B- (pre-B-II-) cell stage and this leads to surface expression of surface IgM containing μ heavy chains and either κ or λ light chains. Immature B-cells expressing intact surface IgM are further screened for reactivity in the bone marrow and spleen before entering peripheral B-cell pools.

The first evidence suggesting a role for PI3K in B-cell development came from the original studies of p85α and p110δ knockout, and p110δKI models [9,56–59]. The numbers of pre-B-cells are reduced in mice lacking p85α or p110δ indicating a clear role for class IA PI3K in the pro-B/pre-B transition, although residual development suggested that other isoforms might also function in early B-cell development. To address this possibility, Okkenhaug and colleagues analysed mice with lymphocyte-specific (CD2-Cre) deletion of p110α or p110β [60]. No significant B-cell deficits were observed. Crossing each conditional knockout strain with the p110δKI strain revealed that combined inactivation of p110α and p110δ results in a near complete absence of pre-B-II-cells, whereas deletion of p110β does not alter the p110δKI phenotype. Using a fluorescence-based microscopy technique, it was demonstrated that sorted pro-B-/pre-B-I-cells lacking p110α or p110δ have elevated Rag gene expression. This defect in Rag repression correlates with enhanced frequency of rearranged heavy chain alleles in sorted pro-/pre-B-cells. Thus both p110α and p110δ both seem to be important for allelic exclusion and developmental progression at the pre-BCR checkpoint, whereas p110β is not involved. In addition, p110α and p110δ were found to have overlapping, required functions in IL (interleukin)-7-driven proliferation of pro-B-cells. This is consistent with a previous report that both PI3K and Akt are required for pro-B-cell proliferation [61]. Overlapping functions of p110α and p110δ are also apparent in thymocyte development, as discussed below.

FOXO factors bind to the promoters of Rag1 and Rag2 genes and promote transcription [62]. Deletion of Foxo1 in the B lineage using Mbl-Cre reduces expression of Rag genes, causing impaired heavy- and light-chain rearrangement and a reduction in pre-B-cell numbers [63]. Together with the results of the p110α/δ deletion study this suggests a model in which tonic pre-BCR signalling activates PI3K and Akt, suppressing FOXO factors to extinguish Rag expression. In agreement, Su and colleagues reported that deletion of Sin1 (stress-activated-protein-kinase-interacting protein 1), an essential component of TORC2, prevents phosphorylation of Akt on Ser^473 and enhances Foxo1-dependent Rag expression in developing B-cells [64]. This study also identified a selective role for the Akt2 isoform in Foxo1 phosphorylation and suppression of Rag expression in cultured pro-B-cells. However a required role for Akt2 in this process in vivo is challenged by a study of fetal liver chimaeric mice reconstituted with Akt1/Akt2 double-knockout cells [65]. In this system, combined deletion of both Akt1 and Akt2 actually resulted in an increase in bone marrow pre-B- and immature B-cells. One possibility is that the Akt3 isoform can compensate for loss of Akt1 and Akt2 in early B-cell development in vivo; mRNA measurements suggest that Akt1 and Akt3 are expressed at higher levels than Akt2 at the pro-B- and pre-B-cell stages [65].

Kurosaki and colleagues reported that development of B-cells from CD19/BCAP double-knockout mice is blocked at the pre-BCR checkpoint stage [24]. Both CD19 and BCAP have multiple tyrosine residues that, when phosphorylated, represent a consensus motif for binding to p85 adaptor subunits. Reconstitution of wild-type, but not tyrosine-mutated, CD19 or BCAP into double-knockout cells restores PI3K/Akt signalling and B-cell development. These findings suggest that ligand-independent pre-BCR signalling triggers tyrosine phosphorylation of CD19 and BCAP, leading to recruitment of class IA heterodimers containing p85α and either p110α or p110δ. In order for light-chain rearrangement to commence, PI3K/Akt signalling needs to be extinguished to allow reactivation of FOXO-dependent Rag expression. Another component of the pre-BCR signalling complex, the adaptin protein SLP-65 [SH2 domain-containing leucocyte protein of 65 kDa; also known as BLNK (B-cell linker)], seems to be required for attenuation of PI3K/Akt signalling [66].

There are several tolerance mechanisms that prevent the emergence of self-reactive B-cells and the production of autoantibodies. In the bone marrow, immature B-cells expressing surface IgM recognizing multivalent self-antigen undergo receptor editing, and eventually clonal deletion if self-reactivity persists. In the absence of self-antigen recognition, tonic signalling by the mature surface IgM on immature B-cells activates PI3K to extinguish Rag expression, similar to the pre-BCR checkpoint [67]. Immature B-cells recognizing monovalent self-antigens in the bone marrow or periphery enter a state known as anergy, in which they fail to respond even to strong stimulation. Anergic B-cells have a short lifespan and are rapidly removed from the repertoire. Together, negative selection by clonal deletion and anergy induction produce a self-tolerant peripheral B-cell repertoire. Rickert and colleagues have shown that both negative selection and anergy are regulated at the level of PtdIns(3,4,5)P3 production [68]. Using a transgenic mouse model, it was observed that anergic B-cells have significantly lower production of PtdIns(3,4,5)P3 and activation of Akt upon BCR stimulation. Reduced PI3K activation correlates with diminished phosphorylation of CD19, and elevated expression of PTEN. Deletion of PTEN reverses the PI3K/Akt activation defects and prevents the induction of anergy. In a negative selection model using immature B-cells derived from non-transgenic mice, BCR cross-linking causes a block in proliferation and differentiation that can be overcome by PTEN deletion [68]. An unanswered question is whether a specific PI3K isoform is involved in generating the PtdIns(3,4,5)P3, pools responsible for preventing tolerance in PTEN-deficient B-cells. Whether PI3K inhibition can induce tolerance in autoreactive B-cells also remains to be determined, but is suggested by experiments described in the next section.

Peripheral B-cells

At an early stage in B-cell development, a lineage split occurs resulting in two subsets of peripheral B-cells: the innate-like B-1 B-cells that reside in body cavities, and B-2 B-cells in blood and secondary lymphoid organs. The B-2 B-cells are further divided into MZ ( marginal zone) B-cells, that reside in the MZ of the spleen and do not circulate, and FO (follicular) B-cells that recirculate through blood, lymph and lymphoid tissues. In mouse strains lacking p85α or p110δ, or in chimaeric mice with Akt1/Akt2-deficient B-cells, B-1 and MZ B-cells are nearly absent [9,56,58,65,69,70]. This phenotype is similar to mice lacking CD19, a component of BCR signalosomes [71]. This suggests
that a major of function of CD19 is to activate PI3K and Akt to allow commitment to the B-1 or MZ lineages at key stages of B-cell development. The CD19/PI3K/Akt signal appears to act through inactivation of Foxo1, since Foxo1 deletion in peripheral B-cells expands the MZ B-cell population and reverses the MZ deficiency in CD19 knockouts [72].

The absence of B-1 and MZ B-cells from PI3K-knockout strains made it difficult to interpret whether PI3K activity is important for the function of fully developed B-1 and MZ B-cells. Gold and colleagues addressed this question by utilizing a p110δ-selective inhibitor IC87114 (Table 1) in immune assays using normal B-1 and MZ B-cells [73]. Selective inhibition of p110δ in these cells completely inhibits Akt activation by TLR (Toll-like receptor) ligands and chemoattractants, suggesting that p110δ is the main isoform linking these extracellular signals to Akt. p110δ inhibition also suppresses chemotaxis, proliferation and antibody production by B-1 and MZ cells. Notably, treatment of mice with IC87114 disrupts the localization of B-cells with a MZ surface phenotype (IgM⁺IgD⁺), suggesting that p110δ is required for chemotactic and adhesive signals that position MZ B-cells in the MZ. B-1 and MZ cells are thought to be the major source of natural antibodies to common microbial antigens and some self-antigens. Consistent with the reduced B-1 and MZ compartments in p110δ-knockout mice, natural antibody production is reduced. Autoantibody production is also reduced in p110δ-knockout mice and in mice treated with p110δ inhibitor. These findings suggest that selective p110δ inhibitors have potential for the treatment of autoimmune diseases driven by autoantibodies. Whether p110δ inhibition enforces B-cell tolerance through anergy induction, or acts mainly by blocking proliferation and differentiation of self-reactive B-cells remains to be established. Interestingly, p110δ inactivation suppresses the expansion of B-1 cells and MZ B-cells in the absence of PTEN [74].

The main function of FO B-cells is to produce antibodies in response to T-cell-dependent (protein) antigens. It is well established that FO B-cell function is highly dependent on class IA PI3K. B-cells lacking p85α or p110δ, or expressing p110δKI show severely impaired signalling downstream of the BCR and a complete inability to proliferate following BCR cross-linking [9,56–59,70,75]. These defects are not simply due to altered development because wild-type B-cells treated with IC87114 display equivalent defects [76]. A primary role of PI3K following BCR cross-linking is to promote signalsome assembly leading to diacylglycerol production, PKCβ activation and NF-κB (nuclear factor-κB) nuclear translocation to activate gene expression (Figure 2) [3,8].

Class IA PI3K signals also contribute to antigen presentation by B-cells [77], adhesion of T-B conjugates [78] and to differentiation of Tfh (follicular helper T)-cells (see below). This predicts that PI3K inhibition would prevent the differentiation and function of T-cells capable of delivering helper signals to B-cells. It is surprising, therefore, that T-cell-dependent antibody responses, while reduced, are not abolished by genetic disruption of p110δ or p85α [9,56,59,73] nor by IC87114 treatment of mice [73,79]. It is important to note that PI3K is not required for that FO B-cell proliferation in vitro driven by CD40L (CD40 ligand) and IL-4, a model for B-cell response to T-cell help [57,80]. Proliferation of FO B-cells driven by TLR ligands is also partially preserved when PI3K is inhibited [9,56–59,80,81]. Therefore the defects in BCR signalling might be overcome by PI3K-independent signals from T-cells or TLR ligands. Furthermore, PI3K inhibitors promote Ig class switching under conditions where B-cell proliferation is maintained [e.g. CD40L and IL-4, or LPS (lipopolysaccharide)], whereas PTEN deletion suppresses class switching [79,82,83]. Ig class switching requires AID (activation-induced cytidine deaminase), whose gene transcription is controlled by Foxo1 [63]. All of these observations emphasize that PI3K is not a universal ‘on’ switch for B-cell responses or for T-cell help (Figure 2). Ultimately, the balance of positive and negative effects of PI3K inhibition probably explains the retention of a reasonably robust T-cell-dependent antibody response in vivo. Also of interest is the finding that p110δ inhibition or p85α deletion causes a selective elevation in basal and antigen-specific IgE production [79,84]. Whether this phenomenon will affect the clinical success of p110δ inhibitors, particularly in inflammatory diseases, is a topic of interest.

FO B-cell survival mainly depends on the cytokines BAFF (B-cell-activating factor) and IL-4 as well as tonic (basal) signalling through the BCR. Cytokine-dependent survival is highly dependent on p110δ activity in vitro [76,85]. However, FO B-cell numbers are only modestly reduced in p110δ-knockout mice suggesting that survival signals from cytokines and/or BCR tonic signalling in vivo are not absolutely p110δ-dependent. A study from Rajewsky and colleagues showed that in the absence of the BCR, a constitutively active form of p110δ is sufficient to maintain FO B-cell survival in the periphery [86]. The converse experiment using a conditional p110α-knockout approach showed that only in the absence of both p110α and p110δ were FO B-cells completely depleted [60] (Figure 2). These results again show the importance of dissecting PI3K signalling through both gain-of-function and loss-of-function approaches. It is likely that expression of a constitutively active p110α isoform can elevate PtdIns(3,4,5)P3 beyond the physiological level, bypassing the need for p110δ engagement by tonic signals from an intact BCR. Several interesting questions arise from this work. First, do p110α and p110δ produce distinct PtdIns(3,4,5)P3 pools with different spatial location and effector molecules? Alternatively, each isoform might contribute quantitatively to shared signalling outputs. Is the absence of FO B-cells from p110α/p110δ double knockouts due only to impaired BCR tonic signalling or also to a block in survival signals from cytokines?

An intriguing issue raised by the p110α/p110δ double-knockout study is whether acute treatment with pan-class I PI3K inhibitors would cause a significant reduction in the peripheral B-cell population, relative to selective inhibitors of individual PI3K isoforms. Given that pan-PI3K inhibitors are entering clinical trials for cancer therapy, it will be important to evaluate their impact on immune homoeostasis compared with more selective compounds. One might predict that selective p110δ inhibitors would mainly affect the B-1 and MZ compartments without eliminating FO B-cell numbers or function. Of particular interest is targeting p110α because activating mutations in the PIK3CA gene are very common in many solid tumours and are considered oncogenic drivers [87]. Development of a highly selective p110α inhibitor would perhaps have the potential to selectively act on PIK3CA mutant tumour cells while sparing B-1 and MZ B-cells and maintaining normal FO B-cell function in the periphery. Of course, the impact of different PI3K inhibitors on all immune subsets that regulate the tumour microenvironment is a subject of great significance. The issue of targeting PI3K isoforms in tumours of B-cell origin is addressed below.

**T-CELLS**

**Thymocytes**

T-cells develop in the thymus where CD4/CD8 DN (double negative) precursors arriving from the bone marrow undergo a differentiation programme characterized by sequential VDJ recombination of the TCRβ and TCRα chains. The DN precursor cells can further be divided into DN1–4 stages based on the
differential expression of CD44 and CD25. Proper rearrangement of TCRβ is required at the DN3 stage to proceed to the DN4 stage (β-selection) and subsequently to CD4+CD8+ DP (double positive) cells that now rearrange and express the TCRα chain. TCRαβ+ DP cells are subjected to positive and negative selection and CD4 or CD8 SP (single positive) cells migrate out of the thymus to populate the lymph nodes and spleen. It is now clear that PI3K activity plays a crucial role in thymocyte development especially during β-selection [88].

Genetic models have suggested roles for both class IA and IB PI3K in thymocyte development. Loss of p110α, p85α or both p85α/β does not significantly reduce overall numbers of thymocytes. However, pre-TCR signalling is attenuated in the absence of p85α or p110α [89,90]. The role of class IB PI3K is more prominent. p110β/γ knockout mice have increased apoptosis of DP thymocytes and, strikingly, p110β/γ double knockout markedly reduces the number of thymocytes due to a defect at the DN3–DN4 transition stage [91,92]. Turner and colleagues have addressed the molecular basis for this defect using genetic models and an in vitro DN3 differentiation system [89]. To a similar extent as the p110β/γ double-knockout mice, DP thymocyte numbers are reduced in mice lacking p110δ and the p101 regulatory subunit of class IB PI3K. Both p110δ and p101/p110γ are required at the DN3–DN4 stage, but act downstream of different receptors. Akt activation through the pre-TCR is entirely dependent on p110δ whereas CXCR4 [chemokine (C-X-C motif) receptor] 4-stimulated Akt activation is mainly dependent on p101/p110γ with a smaller role for p110δ. A functional role of CXCR4 in thymocyte development was confirmed using both genetic and pharmacological approaches. Using a knockin approach in which p110γ is mutated to prevent its binding to Ras, the same group has shown that CXCR4 activates p110γ in a Ras-dependent manner during thymocyte β-selection [41]. These studies strongly support the concept that antigen receptors can co-operate with chemokine receptors to activate both class IA and IB PI3K isoforms to promote cellular responses. An important question is whether this concept also applies to activation of effector T-cells, which express chemokine receptors with co-stimulatory function [93]. If so, it would suggest that dual p110δ/γ inhibitors would be more effective than isoform-selective compounds with respect to blocking effector T-cell activation. An interesting detail to address is whether p110δ and p110γ create distinct pools of PtdIns(3,4,5)P3, with different downstream effectors.

One process driven by pre-TCR-dependent PI3K activation is increased cellular metabolism driven by PDK-1 and Akt. A striking feature of thymocytes lacking PDK-1 is a severe defect in the ability to up-regulate nutrient receptors CD71 (transferrin receptor) and CD98 (amino acid transporter subunit) at the DN3 stage. Conversely, Pten-deficient thymocytes up-regulate these receptors and progress to the DN4 stage in the absence of a pre-TCR signal [94]. Increased expression of transporters might be required for the rapid proliferative expansion at this stage. Akt is likely to be the key mediator downstream of PI3K and PDK-1, since nutrient receptor up-regulation is maintained by a PDK-1 mutant (L155E) that can activate Akt, but not other PDK-1 substrates [95]. Akt1/Akt2 double-knockout DN3 cells show a defect in development and reduced glucose uptake [96].

CD4 T-cells

Resting CD4 T-cells circulate among lymphoid tissues, searching for foreign antigenic peptides presented by APCs (antigen-presenting cells). Antigen recognition with co-stimulation from dendritic cells leads to T-cell clonal expansion and differentiation into effector CD4 T-cells. These are usually termed Th (T helper)-cells as they provide help to other immune cells such as macrophages, B-cells and CD8 T-cells to orchestrate the overall immune response depending on the immune context. The functional variety of Th-cells is achieved by the differentiation of naïve uncommitted CD4 T-cells to distinct subsets including Th1, Th2, Th17 and Th cells (Figure 3). Distinct types of CD4 T-cells with suppressive potential, termed Tregs (regulatory T-cells), are also produced either during thymocyte selection [nTreg (natural Tregs)] or upon stimulation under tolerogenic conditions [iTreg (induced Tregs)]. The differentiation of CD4 T-cells to each subset is dictated by sensing of extracellular cues in the form of cytokines and cell contact-dependent signals from the microenvironment, with distinct outcomes depending on the immune context.

The proliferation of CD4 T-cells in vitro driven by antibody-mediated clustering of TCR with CD28 is largely PI3K-dependent [9,57,97–99]. It is likely that artificial TCR/CD28 clustering overrides certain physiological signalling requirements, as clonal expansion of CD4 T-cells driven by antigen (or superantigen) and APCs is markedly suppressed by PI3K blockades, with a major role for p110δ [97–99] (Figure 3). Furthermore, numerous studies indicate that CD4 T-cell differentiation is highly regulated by PI3K signalling and its downstream effectors, particularly the Akt/FOXO axis and TOR.

Th1 and Th2 cells

Th1 and Th2 cells were the first subsets of CD4 T-cells to be discovered and categorized by the distinctive cytokines they secrete. The signature cytokine produced by Th1 cells is IFNγ (interferon γ), which provides essential help to macrophages for the destruction of vesicular pathogens. IFNγ and IL-2 produced by Th1 cells also help CD8 T-cells differentiate into CTLs (cytotoxic T-lymphocytes) that are essential for cell-mediated immunity to viruses. Th2 cells secrete IL-4 and other cytokines to provide help to B-cells for optimal antibody production to combat extracellular pathogens. These general distinctions are not absolute as Th1 cytokines can drive class switching to Th2 cytokines of p110δ mice have a defect in both Th1 and Th2 differentiation and cytokine production, suggesting a main role of p110δ in this process. However, p110δ mice also are protected from Th2-mediated inflammation in a model of ovalbumin-dependent airway hypersensitivity [100]. In a more recent study Okkenhaug and colleagues used IC87114 to revisit the role of p110δ in proliferation and differentiation of both murine and human CD4 T-cells [101]. A noteworthy finding was that concentrations of IC87114 above 1 μM inhibit proliferation of p110δKO T-cells lacking p110δ kinase activity. Therefore off-target effects probably contribute to the cellular actions of IC87114 when used at >1 μM. Nevertheless, lower concentrations (0.01–1 μM) of IC87114 do partially suppress proliferation and strongly inhibit Th1 and Th2 cytokine production from naive and effector/memory T-cells from both murine and human sources (Figure 3). In vivo treatment with IC87114 also suppresses Th1 cytokine production and reduces ear swelling in a contact hypersensitivity model.

Genetic or pharmacological inhibition of p110δ does not fully block proliferation of naive CD4 T-cells or differentiation of Th1 or Th2 subsets [98,101]. Therefore other class IA isoforms might contribute to PI3K signalling that drives Th expansion and differentiation (Figure 3). Supporting this idea, total class IA PI3K activity measured by an in vitro kinase assay using immunoprecipitated p85 is reduced by only 50% in p110δKO T-cells suggesting that p110α and/or p110β activity might also

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Role of PI3K and downstream effectors in the differentiation of activated CD4 T-lymphocytes

Antigen encounter triggers clonal expansion of naïve CD4 T-cells that is mainly dependent on p110δ. Depending on the immune microenvironment (cytokines, etc.), primed CD4 T-cells differentiate into different CD4 T-cell subsets (Th1, Th2, Th17, Tfh and iTregs). TORC2-dependent Akt activation promotes Th1 differentiation through TORC1 and NF-κB, and TORC2-dependent PKCθ activation promotes Th2 differentiation. PDK-1 also triggers NF-κB activation through modulation of FOXP3 and its target genes including KLF2. ICOS stimulation triggers Th differentiation through a p110δ-dependent pathway, with a speculative role for p110α binding to ICOS. The total PI3K output acts as a rheostat (grey circle) with decreased PI3K/Akt activity favouring iTreg induction. Isoforms other than p110δ including p110α probably suppress iTreg induction in this context. PI3K/Akt blockade results in the induction of FOXO-mediated FoxP3 expression and low TORC1 activity leads to decreased metabolic activity via S6K and HIF-1α. Established FoxP3+ Tregs also depend on p110δ activity for proper suppressive function, such as IL-10 secretion. Due to space constraints, the nucleus is not depicted where all of the genes are regulated. IL-2R, IL-2 receptor.

PI3K influences Th1 and Th2 differentiation through complex mechanisms. One key signalling protein linking PI3K activation to Th differentiation is TOR (often referred to as mTOR). Originally identified in searches for rapamycin targets in yeast and mammalian cells, this serine/threonine kinase is now known to have multiple functions and only some of these are rapamycin-sensitive [102,103]. TOR is encoded by a single gene in mammals (MTOR), but is the catalytic subunit of two distinct multi-protein assemblies known as TORC1 and TORC2. TORC1 contains the protein raptor (regulatory associated protein of mTOR) and is nutrient-sensitive; TORC2 contains the distinct protein rictor and is nutrient-insensitive. The activation of both TORC1 and TORC2 is at least partially PI3K-dependent [103–105]. TORC1 phosphorylates many substrates, but the best studied are two protein families that control mRNA translation: the S6Ks (ribosomal S6 kinases) and the 4EBPs (eIF4E-binding proteins). TORC2 phosphorylates the hydrophobic motif of Akt and other AGC (protein kinase A/protein kinase G/PKC) family kinases including SGK (serum- and glucocorticoid-inducible kinase) and certain PKC isoforms. In mouse T-cells, conditional knockout of Mtor [the gene was previously known as Frap1 (FKBP-rapamycin-associated protein 1)] blunts clonal expansion and completely blocks differentiation into Th1, Th2 or Th17 subsets [106]. In contrast, the induction of FoxP3 expression, the hallmark transcription factor driving a Treg programme, is increased in TOR-deficient T-cells [106]. This observation fits with abundant literature that rapamycin promotes Treg formation [107,108].

Both TORC1 and TORC2 contribute to Th differentiation (Figure 3). Deletion of the small GTPase Rheb1 (Ras homologue enriched in brain) to block TORC1 activation prevents Th1 and Th17, but not Th2, differentiation [109]. Deletion of rictor, an essential component of TORC2, impairs Th2, but not Th17, differentiation [109,110]. Boothby and colleagues reported that rictor deletion also impairs Th1 differentiation [110], but this was not observed by Powell and colleagues [109]. Inhibition of both TORC1 and TORC2 is required for efficient induction of Tregs. Rheb1- or rictor-deficient T-cells show only a small increase in Treg generation [109]. Low concentrations of rapamycin, which inhibit TORC1, but not TORC2, achieve weak Treg induction unless TORC2 is genetically inactivated by rictor knockout [109].

The mechanisms that modulate Th differentiation downstream of TORC1 and TORC2 are beginning to be defined. Powell and colleagues provided evidence that TORC1 and TORC2 control differentiation programmes through distinct effects on STAT (signal transducer and activator of transcription) phosphorylation and SOCS (suppressor of cytokine signalling) protein expression [109]. The signalling components linking TOR complexes to the STAT pathway are not yet assigned. Boothby and colleagues showed that a constitutively active PKCθ restored...
PI3K signalling in lymphocytes

473

The Tfh subset of CD4 T-cells, discovered in 2003, is developmentally and functionally distinct from Th1 or Th2 cells [125a]. These cells produce a different set of cytokines (IL-17A, IL-17F, IL-21 and IL-22) and are specialized to protect against microbial pathogens at epithelial surfaces. Th17 cells have raised major interest due to their primary role in autoimmune diseases such as colitis and EAE (experimental autoimmune encephalomyelitis), a mouse model for multiple sclerosis. The role of PI3K in Th17 differentiation has been explored recently. However, data from three different groups show apparently conflicting results. SHIP-knockout T-cells, which presumably have elevated PtdIns(3,4,5)P3 levels, fail to differentiate into Th17 cells both in vitro and in vivo [126]. T-cells expressing myr-Akt (a myristoylated Akt transgene) likewise cannot differentiate into Th17 cells in vitro [127]. The simplest interpretation of these results is that PI3K signalling suppresses Th17 differentiation. However, PI3K inhibition also blocks Th17 differentiation. p110δK1 T-cells cannot differentiate into Th17 cells in vitro and p110δK1 mice are less susceptible to EAE due to a Th17 defect in vivo [128]. (Figure 3) It is possible to reconcile these observations by considering that PI3K lipid production and hydrolysis is not a simple on/off switch; each of these genetic models has qualitatively distinct effects on the PI3K signalling network. SHIP dephosphorylates the 5-position of PtdIns(3,4,5)P3 to produce PtdIns(3,4)P2. Since both PIP3 and PtdIns(3,4)P2 can promote PDK-1-mediated Akt phosphorylation, loss of SHIP should not necessarily affect Akt activation. Indeed, activation of Akt through TCR engagement in SHIP-knockout T-cells is unchanged [129]. It is possible that loss of SHIP prevents the recruitment of effector proteins that specifically bind to PtdIns(3,4)P2, and that these proteins transmit signals to induce Th17 differentiation. This model is consistent with the data from p110δK1 T-cells, which should also have reduced PtdIns(3,4)P2 levels. Both p110δK1 and SHIP-knockout T-cells also have reduced Th2 differentiation [98,129]. The observation that myr-Akt blocks Th17 differentiation could result from the atypical localization or activation kinetics of this Akt variant. The increased Treg differentiation observed in this myr-Akt strain [127] is also at odds with other approaches to modulate PI3K/Akt activity (see below). An important future question to address is whether PI3K isoforms other than p110δ have a distinct role in Th17 differentiation. It will also be interesting to identify PtdIns(3,4)P2 effectors that function in T-cells; PtdIns(3,4)P2-selective binding proteins Bem32, TAPP1 and TAPP2 have important roles in B-cells [78,130]. In addition, it is worth considering whether SHIP and potential PtdIns(3,4)P2 effectors function mainly in signalling from the TCR or from cytokine receptors that influence Th17 differentiation.

A positive role for PI3K/Akt signalling in Th17 is supported by studies of T-cells lacking TORC1 function. Mice with T-cell-specific deletion of Mtor or Rheb1 have a drastic reduction in Th17 differentiation and are protected from EAE [106,109] (Figure 3). Further supporting a role for TORC1, rapamycin treatment also suppresses Th17 differentiation and ameliorates EAE [131,132]. Other work suggests that TORC1 regulates the balance of Th17/Treg differentiation through a central regulator of cellular metabolism, HIF-1α (hypoxia-inducible factor-1α) [133,134]. HIF-1α expression is increased by TORC1 in a manner independent of hypoxia, and contributes to increased glycolysis during Th17 differentiation. The idea that TORC1 controls metabolic programmes that determine Th differentiation is supported by other recent work [135–137].

Human memory Th17 cells can be defined by surface expression of the chemokine receptor CCR6 [chemokine (C-C motif) receptor 6]. Culturing these cells in the presence of cytokines that signal through the γc (gamma-common) chain (e.g. IL-2, IL-7 and IL-15) induces production of IL-17, IL-22 and other cytokines. Under these conditions, pharmacological inhibition of PI3K or Akt suppresses IL-17/IL-22 production by human CCR6+ cells [138]. This indicates that PI3K activation is important not only for establishment of Th17 effector cells, but also for their function (Figure 3). Further mechanistic experiments suggest that PI3K/Akt signalling promotes Th17 cytokine production through suppression of FOXO and KLF (Krüppel-like factor) transcription factors [138]. The ability of PI3K/Akt signalling to suppress FOXO and KLF-dependent gene expression is also responsible for changes in homing and trafficking that accompany activation of both CD4 and CD8 T-cells in mice [139].

**Th17 cells**

The Th17 subset of CD4 T-cells, discovered in 2003, is developmentally and functionally distinct from Th1 or Th2 cells [125a]. These cells produce a different set of cytokines (IL-17A, IL-17F, IL-21 and IL-22) and are specialized to protect against microbial pathogens at epithelial surfaces. Th17 cells have raised major interest due to their primary role in autoimmune diseases such as colitis and EAE (experimental autoimmune encephalomyelitis), a mouse model for multiple sclerosis. The role of PI3K in Th17 differentiation has been explored recently. However, data from three different groups show apparently conflicting results. SHIP-knockout T-cells, which presumably have elevated PtdIns(3,4,5)P3 levels, fail to differentiate into Th17 cells both in vitro and in vivo [126]. T-cells expressing myr-Akt (a myristoylated Akt transgene) likewise cannot differentiate into Th17 cells in vitro [127]. The simplest interpretation of these results is that PI3K signalling suppresses Th17 differentiation. However, PI3K inhibition also blocks Th17 differentiation. p110δK1 T-cells cannot differentiate into Th17 cells in vitro and p110δK1 mice are less susceptible to EAE due to a Th17 defect in vivo [128] (Figure 3). It is
by the expression of the chemokine receptor CXCR5 and their specific requirement for the transcription factor BCL6 (B-cell lymphoma-6) and the ICOS transmembrane receptor. Following encounter with antigen, B-cells that initiate the GC reaction undergo somatic hypermutation and isotype switching to increase antibody affinity and diversify effector functions. By expressing CXCR5, primed T-cells can migrate into the B-cell FO along the CXCL [chemokine (C-X-C motif) ligand] 13 gradient and first receive signals from B-cells in the form of antigen-mediated TCR signalling and co-stimulatory signals to initiate the Tfh program. Many co-stimulatory signals provided by activated B-cells are crucial for Tfh signalling. Of these, PI3K signalling downstream of ICOS appears to play a non-redundant role in regulating the abundance and effector functions of Tfh cells [140].

Interruption of ICOSL (ICOS–ICOS ligand) interaction or ICOS deficiency (Icos−/− mice) leads to impaired GC responses [140]. To address the question of whether PI3K signalling downstream of ICOS is the main mediator for this defect, an Icos knockin transgenic mouse (ICOS-YF) was generated that harbours a mutation in the crucial Tryptophan 181 that is required for p85 recruitment [141]. The ability of ICOS to strongly enhance TCR-mediated Akt phosphorylation is blocked in ICOS-YF T-cells. Class-switched Ig levels, as well as the magnitude of the GC response, are severely impaired in these mice. This phenotype is mainly due to a defect in the generation of Tfh cells and the effects are very similar in Icos−/− mice. This suggests that class IA PI3K is a central player in ICOS signalling leading to Tfh differentiation (Figure 3). FOXO inhibition might play an important role in this pathway as Foxo1/Foxo3 deletion in T-cells promotes Tfh generation [118].

A subsequent study from Turner and colleagues provided evidence that p110δ is the primary catalytic isoform that couples ICOS engagement to Tfh generation [142] (Figure 3). Conditional deletion of Pik3cd (encoding p110δ) in mature T-cells (using CD4-Cre) or in primed T-cells (using OX40-Cre) prevents the expression of Tfh-selective cytokines IL-4 and IL-21. T-cell-specific deletion of p110δ reduces Th numbers with an accompanying decrease in GC B-cells and high-affinity-antibody production. Importantly, amplifying PtdIns(3,4,5)P3 through PTEN deletion in activated T-cells has the converse effect of increasing Tfh cells, GC B-cells and antibody affinity. A surprising finding from this study was that B-cell-specific ablation of p110δ using CD19-Cre does not impair the GC response or antibody production, other than an increase in IgE production. Hence the decreased T-dependent antibody response in the original ‘whole body’ p110δKI mice can be attributed to defects in T-cells rather than B-cells. Although p110δ controls many facets of B-cell development and T-cell-independent antibody production, this study suggests that the B-cell intrinsic role of p110δ in T-cell-dependent responses is mainly restricted to suppression of IgE isotype switching during the GC response.

A number of observations add complexity to the simple model that p110δ in T-cells controls Tfh differentiation and antibody responses. Acute inhibition of p110δ using IC87114 in mice reduces GC B-cell numbers, but does not reduce antigen-specific IgG1 and markedly increases the IgE response [73,79]. This indicates that extrafollicular B-cell activation and class switching can still proceed when the GC response is suppressed. It is also possible that PI3K isoforms other than p110δ might contribute to ICOS signalling. Using synthetic ICOS peptides and ICOS immunoprecipitations, a significant amount of p110α bound to ICOS can be detected after stimulation of the CD4 T-cell line D10 and primary CD4 T-cell blasts [143]. siRNA (small interfering RNA)-mediated knockdown or pharmacological inhibition of p110α partially reduces ICOS-dependent Akt phosphorylation in D10 cells [143]. The application of newer inhibitors with greater selectivity and improved pharmacological properties should further resolve the roles of PI3K isoforms in ICOS function in vitro and in vivo. With the entry of isomform-selective inhibitors and pan-class I PI3K inhibitors in clinical trials, we will also begin to learn how different PI3K target profiles affect antibody isotypes in humans.

## Tregs

The discussion thus far makes it clear that PI3K activation plays a generally positive role in signalling to generate different effector CD4 T-cell subsets (Figure 3). The major catalytic isoform responsible in most contexts seems to be p110δ, although other isoforms contribute. This function for PI3K in CD4 T-cell expansion and differentiation is not surprising given the central role of PI3K in the growth factor responses of many other cell types. In this respect, it was initially perplexing that reduced PI3K function in immune cells could lead to autoimmunity in mice. p110δKI mice develop colitis [9] and r1ΔTfr2n mice (a strain with T-cell-specific deletion of Pik3r2, encoding p85α, and germine deletion of Pik3r2, encoding p85β) develop an exocrinopathy that resembles primary SJÖGREN’s syndrome [144]. It now seems likely that both of these phenotypes can be attributed to reduced numbers and/or function of Tregs ([144,145] and unpublished work). Accumulating data suggest that PI3K activity is intimately linked to both development and function of Tregs.

Tregs are essential for peripheral tolerance induction. Both nTregs and iTregs can suppress proliferation of potentially autoreactive T-cells, through direct contact with effector T-cells and dendritic cells as well as through secreted factors. Thymic development of nTregs seems to be constrained by PI3K activity as p110δKI mice have increased thymic Treg numbers [145]. Recent studies suggest a critical role for FOXO transcription factors in linking PI3K/Akt signalling to the thymic development of nTregs. Although a single allele of Foxo1 or Foxo3 is enough to maintain nTreg development, combined deletion of both genes leads to a severe defect in Foxo3+ nTregs in the thymus [118,120]. FOXO factors directly activate FoxP3 expression through direct binding to FOXO consensus sequences in the Foxp3 promoter [120,121]. To circumvent this, when PI3K activity is reduced FOXO factors can relocate to the nucleus to turn on a Treg transcriptional programme that includes Foxp3 expression (Figure 3). On the other hand, elevated PI3K/Akt signalling does not necessarily suppress nTreg development as thymic nTreg development is unaltered in a mouse model where PTEN is deleted specifically in T-cells (CD4-Cre) [146]. Conflicting results have been obtained using activated Akt transgenes in two different models of nTreg development in thymocytes [127,147].

PI3K/Akt signalling has complex roles in mature Tregs. Despite the marked decrease in thymic nTregs in the T-cell-specific Foxo1/Foxo3 double knockouts, peripheral (spleen and lymph node) Tregs are only modestly reduced [118,120]. This suggests that PI3K/Akt signalling promotes, and FOXO factors suppress, homoeostatic maintenance of peripheral Tregs. In agreement, peripheral Treg numbers are decreased in both p110δKI and r1ΔTfr2n mice [144,145]. Adding further complexity, PI3K/Akt signalling is attenuated in mature Tregs. Compared with conventional CD4 T-cells, isolated Tregs show reduced activation of Akt in response to TCR engagement or IL-2 [126,146,148]. This phenotype correlates with elevated PTEN expression in Tregs, and PTEN-deletion promotes IL-2-dependent proliferation.
of Tregs [120]. It is conceivable that a low level of PI3K/Akt signalling is required to prevent FOXO-dependent cell-cycle arrest and apoptosis, but that higher levels could suppress the Treg phenotype.

PI3K activity is also required for the function of peripheral Tregs. Purified Tregs from p110δKI mice have reduced suppressive activity and severely impaired secretion of IL-10, an anti-inflammatory cytokine [145] (Figure 3). Interestingly, this defect in Treg function provides protection of mice from Leishmania major infection [149]. Despite having impaired Th1 responses, p110δKI mice are able to contain the parasite and this phenotype is reversed by adoptive transfer of wild-type Tregs. This opens up the possibility of developing p110δ-selective inhibitors for Leishmania treatment.

An extensive focus in Treg biology has been the study of ex vivo expansion of naïve conventional CD4 T-cells to iTregs. This process is relevant to peripheral tolerance induction and has therapeutic implications with respect to adoptive transfer of Tregs. There is strong evidence that limiting PI3K/Akt/TOR activity during T-cell priming promotes the conversion of conventional CD4 T-cells into iTregs. Merkenschlager and colleagues showed that withdrawal of TCR stimulus after 18 h triggers iTreg conversion, and used small molecule inhibitors to probe the signalling pathways responsible for this effect [122]. Inhibition of PI3K and/or TOR, or Akt inhibition, led to greatly increased FoxP3+ iTregs in this system. Although the p110δ inhibitor IC87114 modestly increased iTregs, the effect of a p110α inhibitor with some activity against p110γ and p110δ (PIK-90; see Table 1) was more profound. This result further supports involvement of diverse p110 isoforms in different immune contexts. Other work supports a role for PI3K/Akt signalling to oppose iTreg conversion through suppression of FOXO activity (Figure 3). In CD4 T-cells lacking the ubiquitin ligase Cbl-b, phosphorylation of Akt and FOXO factors are elevated and TGFβ (transforming growth factor β)-induced iTreg conversion is greatly reduced [121]. This phenotype can be rescued by overexpressing Foxo1 or Foxo3. Conversely, iTreg induction is abolished in Foxo1/Foxo3 double-knockout T-cells or in cells expressing constitutively active Akt [79,120,147]. Taken together these data indicate that, similar to thymic nTregs, FOXO-driven FoxP3 expression is also important for iTreg generation in peripheral T-cells.

As discussed above, TOR activity also opposes iTreg induction. It is interesting that inhibition of both TORC1 and TORC2 are required for conversion of conventional CD4 T-cells into Tregs [132,138]. It makes sense that TORC2 inhibition should augment FOXO-dependent Treg programming through Akt suppression (Figure 3). What is the role of TORC1? We have obtained evidence that S6K activity downstream of TORC1 opposes iTreg differentiation. Genetic or pharmacological suppression of S6K increases iTreg induction in vitro and increases Treg numbers in vivo [135]. A search of the patent literature reveals S6K inhibition as a strategy for ex vivo expansion of Tregs [149a]. TORC1 also opposes the iTreg fate through HIF-1α, which binds to FoxP3 and promotes its degradation [133,134]. Whether PI3K inhibitors promote iTreg conversion primarily through the Akt/FOXO axis or also through TORC1 inhibition remains to be determined.

CD8 T-cells

Upon antigen encounter, resting CD8 T-cells differentiate into effector CTLs with a fundamental role in anti-viral and anti-tumour responses. Cantrell and colleagues have reported key roles for PI3K/Akt/TOR signalling in CD8 T-cells, with interesting distinctions from PI3K function in CD4 T-cells [150]. Although PI3K inhibition suppresses antigen-driven clonal expansion of CD4 T-cells, PI3K is not required for CD8 T-cell proliferation or associated metabolic changes, such as increased glucose uptake. Clonal expansion of antigen-primed CD8 T-cells cultured in the presence of IL-2 are not affected by the p110δ inhibitor IC87114, a specific Akt inhibitor, nor in CD8 T-cells from p110δKI mice. Proliferation and glucose uptake are impaired in PDK1-null cells, implying that AGC kinases regulated by PDK-1 can compensate for Akt in this context. However, PI3K/Akt signalling is required in activated CD8 T-cells to initiate the transcriptional programme for CTL effector function. Expression of cytotoxic effector molecules including granymes and perforin, and production of the cytokine IFNγ, correlate with Akt activity and FOXO inactivation. Akt activation also co-ordinates changes in expression of homing and trafficking receptors to allow effector CTLs to leave lymph nodes and traffic to sites of infection. Specifically, Akt inhibition or genetic interference with PDK-1-mediated Akt activation prevents the down-regulation of FOXO target genes encoding proteins important for naïve CD8 T-cell trafficking: L-selectin (CD62L), KLF2, CCR7 and S1P1 (sphingosine-1 phosphate receptor 1). An earlier study from this group reported that rapamycin treatment of antigen-primed CD8 T-cells also prevents the down-regulation of L-selectin (CD62L), KLF2, CCR7 and S1P1 [151]. Whether rapamycin acts in this system through inhibition of TORC2/Akt or TORC1 signalling remains to be resolved [139].

The role of PI3K signalling in cytotoxicity of mature CTLs has not been studied in detail. However, there is evidence that the p110γ isoform is required for CTL chemotaxis and trafficking to sites of infection [152]. Similarly, the p110γ isoform has a significant role in trafficking and survival of effector CD4 cells [153]. This raises the possibility that drugs in development that target p110γ, either alone or with other class I isoforms, will suppress effector T-cell trafficking and compromise host defence. Although NK (natural killer) cells are traditionally classified as part of the innate immune system, they are lymphocytes and display some features of adaptive immune cells [154]. Both p110δ and p110γ appear to play important roles in NK cell maturation and function, including cytokine production and cytotoxicity. The functions of p110δ and p110γ in NK cells and the discrepancies among experimental systems were reviewed recently by Kerr and Colucci [155].

**CAL-101 AND THE POTENTIAL OF PI3K INHIBITORS IN LYMPHOID MALIGNANCIES**

Starting from the initial description of wortmannin as a PI3K inhibitor that suppresses neutrophil responses [156,157], there has been interest in developing PI3K inhibitors for inflammatory and autoimmune diseases. Most efforts have focused on inhibitors of p110δ and/or p110γ based on the leucocyte-restricted expression of these isoforms and the immune phenotypes of gene-targeted mice [158]. The p110γ isoform has received particular attention for inflammatory diseases, as p110γ plays an integral role in chemotaxis and respiratory burst of neutrophils during inflammation [159], and amplifies mast cell degranulation and allergic responses [160]. p110γ has a limited role in T-cells and is largely dispensable in B-cells. p110δ has been considered a more promising target for suppression of adaptive immune responses, based on the fundamental role of this isoform in both B- and T-cells [46]. The discovery of compounds with high selectivity for p110δ has proceeded more rapidly than for selective p110γ...
CAL-101 inhibits p110δ when profiled against other protein and lipid kinases. In cell isoforms [170,171]. The compound also shows great selectivity at concentrations 40–300-fold lower than other class I PI3K inhibitors and selective TOR inhibitors for cancer [161–167]. Notably, the most exciting success in the clinic so far has been the efficacy of the p110δ inhibitor CAL-101 in a subset of cancers of B-cell origin. Although at the time of writing the present review the results of CAL-101 clinical trials have not been published in peer-reviewed journals, some of the data have been presented at professional meetings and conferences [168,169]. Remarkably, treatment with CAL-101 as a single agent provided durable remissions to a significant percentage of patients with CLL (chronic lymphocytic leukaemia), iNHL (indolent non-Hodgkin’s lymphoma) or MCL (mantle cell lymphoma). Most of these patients had relapsed from multiple other treatment regimens, yet responded to the p110δ inhibitor. CAL-101 also showed impressive efficacy when combined with standard-of-care agents in CLL and iNHL (rituximab and bendamustine).

CAL-101 was discovered by Calistoga and is a chemical derivative of IC87114. CAL-101 inhibits p110δ in vitro at a concentration 40–300-fold lower than other class I PI3K isoforms [170,171]. The compound also shows great selectivity when profiled against other protein and lipid kinases. In cell-based assays, CAL-101 inhibits p110δ-dependent responses at low nanomolar concentrations. At concentrations above 1 μM, CAL-101 significantly inhibits cellular responses mediated by p110β and p110γ. These concentration ranges are important to remember when considering the mechanism of action of CAL-101 in cellular model systems as well as in patients.

The actions of CAL-101 have been studied in cell lines and primary patient samples representing diverse B-cell malignancies including CLL, DLBCL (diffuse large B-cell lymphoma) and MM (multiple myeloma) [170–173]. In general, CAL-101 has low nanomolar concentrations. At concentrations above 1 μM, it shows impressive efficacy when combined with standard-of-care agents in CLL and iNHL (rituximab and bendamustine). CAL-101 also showed impressive efficacy when combined with standard-of-care agents in CLL and iNHL (rituximab and bendamustine).

In parallel with studies of PI3K inhibitors in inflammation, there has been a tremendous effort to develop pan-class I PI3K inhibitors, isoform-selective PI3K inhibitors, dual PI3K/TOR inhibitors and selective TOR inhibitors for cancer [161–167]. Notably, the most exciting success in the clinic so far has been the efficacy of the p110δ inhibitor CAL-101 in a subset of cancers of B-cell origin. Although at the time of writing the present review the results of CAL-101 clinical trials have not been published in peer-reviewed journals, some of the data have been presented at professional meetings and conferences [168,169]. Remarkably, treatment with CAL-101 as a single agent provided durable remissions to a significant percentage of patients with CLL (chronic lymphocytic leukaemia), iNHL (indolent non-Hodgkin’s lymphoma) or MCL (mantle cell lymphoma). Most of these patients had relapsed from multiple other treatment regimens, yet responded to the p110δ inhibitor. CAL-101 also showed impressive efficacy when combined with standard-of-care agents in CLL and iNHL (rituximab and bendamustine).

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The actions of CAL-101 have been studied in cell lines and primary patient samples representing diverse B-cell malignancies including CLL, DLBCL (diffuse large B-cell lymphoma) and MM (multiple myeloma) [170–173]. In general, CAL-101 has weak cytotoxic activity on isolated cancer cells at submicromolar concentrations, and variable cytotoxicity in the range 1–10 μM. This suggests that p110δ is not required for intrinsic or serum-dependent survival signalling in culture. However, lower concentrations of CAL-101 (0.1–0.5 μM) do effectively suppress the survival signals provided by stromal cells and extracellular factors including TNFα, BAFF, fibronectin and soluble CD40L. Importantly, submicromolar concentrations of CAL-101 effectively suppress chemotaxis of CLL cells to CXCL12 and CXCL13 [173], chemokines that are commonly present in B-cell follicles of lymph nodes. This suggests that in malignant B-cells, similar to normal B-cells, chemokine receptors signal via the class IA isoform p110δ. In addition, p110δ may serve important functions in stromal cells as CAL-101 suppresses the secretion of cytokines by monocyte-derived ‘nurse-like’ cells in co-culture with CLL cells [173]. Together these findings indicate that CAL-101 disrupts the microenvironment and might block access of tumour cells to niches that provide extrinsic survival signals (Figure 4).

This model is strongly supported by clinical data. In a phase I trial of CAL-101 in CLL (clinical trials identifier NCT00710528), a large majority of patients (>80%) across all dose levels showed significant shrinkage of lymph nodes, but this was accompanied by an elevation of circulating lymphocytes. This is consistent with release of CLL cells from lymphoid tissue and/or failure of circulating cells to home to lymph nodes. Patients treated with CAL-101 showed markedly reduced amounts of chemokines CCL2, CCL3 and CXCL13 [173], in accordance with the in vitro experiments mentioned above. Importantly, the peripheral lymphocytosis resolved over time suggesting that tumour cells died out when prevented from accessing lymph nodes (Figure 4). It remains unclear whether direct cytotoxic effects of CAL-101 contribute to efficacy in CLL patients. The peak plasma levels of CAL-101 are greater than 2 μM suggesting that partial blockade of p110β and/or p110γ together with complete p110δ inhibition might cause direct tumour-cell killing. Nevertheless, the lymphocytosis indicates that many tumour cells do not die immediately. In patients treated with a combination of CAL-101 and either rituximab or bendamustine, lymphocytosis was not observed. This suggests that release from the protective niche by CAL-101 sensitizes leukaemia/lymphoma cells to the companion agent.
Calistoga is now part of the company Gilead, which has plans to rapidly develop CAL-101 (now GS-1101) for oncology applications. When representatives of these companies have presented clinical data on CAL-101, the excitement among the audience members has been palpable. The emerging success of CAL-101 validates more than a decade of intense PI3K drug-discovery efforts across academia and industry. The CAL-101 results also raise many questions, some of which are discussed here (also see [161]).

One question is whether inhibition of microenvironmental signals is also the basis for CAL-101 efficacy in NHL and MCL. When the clinical data become available it will be interesting to learn whether CAL-101 causes transient lymphocytosis and reduced plasma chemokine levels in these patient populations. A related issue is whether CAL-101 fails to block microenvironmental signals in malignancies where the drug has not shown efficacy, such as DLBCL, AML and MM. A more general point is that CAL-101 studies have underscored the importance of modelling cancer cell interactions with the microenvironment even at early stages of development of PI3K-targeted therapies. It is likely that the efficacy of PI3K inhibitors in cancer patients does not correlate linearly with the IC₅₀ value for cytotoxic effects in vitro. In other words, the potential of a certain inhibitor can be either underestimated or overestimated by studies of isolated tumour cell lines.

Another question is whether greater efficacy can be achieved using less selective PI3K inhibitors. Some tumours arising from mature B-cells depend on chronic BCR signalling [174]; if these signals require both p110α and p110δ then a dual inhibitor of these two isoforms might provide a potent cytotoxic effect. Perhaps pan-PI3K inhibitors would have even broader activity in leukaemia and lymphoma subtypes that have been resistant to CAL-101 in early clinical studies. The potentially greater efficacy might be counterbalanced by reduced tolerability and general immunosuppression. It is even possible that broader PI3K inhibition will promote tumour survival through increased production of Tregs.

CONCLUSIONS AND FUTURE DIRECTIONS

Thus far, a p110δ inhibitor in B-cell malignancies is the biggest success story in the world of PI3K drug development. Where does this leave the community of inflammation and autoimmunity investigators? In some ways the barriers are greater for development of drugs in this arena. Most of the target diseases are not terminal, so there is a higher standard for safety. There are usually other treatment options with reasonable efficacy. Furthermore, animal models of inflammation and autoimmunity are generally of limited predictive value for efficacy in humans. Nevertheless, there remains a medical need and large potential market for improved anti-inflammatory and immunosuppressive drugs. Also, PI3K inhibitors seem to cause more manageable side effects in humans than initially feared. Considering these issues, it makes sense to move PI3K inhibitors forward quickly, establishing safe doses first in healthy volunteers and then testing in patient populations. This is where the promise of PI3K inhibitors will truly be tested: in humans.

If one accepts this premise, then the question is which isoform(s) to target. Both p110α and p110γ are preferentially expressed in leucocytes and are central mediators of immune responses. Mice lacking either protein are healthy, suggesting that compounds selective for one of these isoforms will be well tolerated. Yet given the overlap in function between these isoforms, and possible roles of p110α and/or p110δ, it might not be possible to shut down pathological immune responses or cancer cells by inhibiting just one isoform. Compounds that inhibit both p110α and p110γ (e.g. IPI-145, Table 1) might offer better control of autoimmunity and inflammation, but what will be the effect on host defence? Again, the best choice might be to test different target profiles in various disease states to establish which profile provides the best therapeutic window. It is important to keep in mind that compounds with ‘partial’ selectivity might provide the best balance between efficacy and tolerability. For example, a p110δ inhibitor that partially blocks p110γ at the effective dose might suppress effector T-cell function without interfering with the myriad functions of p110γ in innate immunity. An equally important consideration is that each compound has its own pharmacological profile. Two experimental agents with similar isoform selectivity might have distinct efficacy in humans based on their pharmacokinetics. Relating such findings back to the biology of PI3K in different immune cells will be a challenge.

One research question that deserves more detailed investigation is the role of different PI3K isoforms in memory lymphocytes. Unlike laboratory mice maintained in pathogen-free conditions, which have mostly naive T- and B-cells, humans are constantly exposed to microbes and the majority of peripheral lymphocytes are memory or effector cells. A typical autoimmune disease patient will have an even greater fraction of such cells. There has been some progress in studying PI3K signalling in human memory lymphocytes. As noted above, memory Th17 cells stimulated with cytokines produce IL-17 through a PI3K-dependent pathway [138], and p110δ inhibition reduces proliferation and cytokine production by effector/memory T-cells from humans with inflammatory diseases [101]. Similarly, human lupus patients have increased PI3K activity in peripheral lymphocytes, with a direct correlation between p110δ activity and the fraction of activated/memory T-cells [175]. We need to learn more about how different PI3K inhibitors affect the function and trafficking of pathogenic lymphocytes in different immune disease states. At the same time it will be important to consider whether PI3K inhibitors that effectively suppress autoimmunity also interfere with immunological memory to pathogens. Although the road ahead is certain to be filled with challenges, the fundamental premise that PI3K inhibition will be useful for human disease therapy seems more and more solid.

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SUPPLEMENTARY ONLINE DATA

PI3K signalling in B- and T-lymphocytes: new developments and therapeutic advances

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Table S1 Gene-targeted mouse strains with null, conditional or knockin mutations in class I PI3K genes

<table>
<thead>
<tr>
<th>Targeted isoform</th>
<th>Approach</th>
<th>Studied cell types</th>
<th>References</th>
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<td>p85α</td>
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<td>[1]</td>
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<td>[2–6]</td>
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<td>B-cell</td>
<td>[7]</td>
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<tr>
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<td>T-cell</td>
<td>[8]</td>
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<td>p85β</td>
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<td>B-cell</td>
<td>[9]</td>
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<td>T- and B-cell</td>
<td>[11,12]</td>
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<td>KI knockin (p110αKI)</td>
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<td>[13,14]</td>
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<td>[15,16]</td>
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