Biochimica et Biophysica Acta 1851 (2015) 882–897

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Review

PI3K signalling in inflammation☆

P.T. Hawkins *, L.R. Stephens *

Signalling Programme, Babraham Institute, Babraham Research Campus, Cambridgeshire, CB22 3AT, UK

ARTICLE INFO

Article history:
Received 5 September 2014
Received in revised form 24 November 2014
Accepted 9 December 2014
Available online 13 December 2014

Keywords:
PI3K
Inflammation

ABSTRACT

PI3Ks regulate several key events in the inflammatory response to damage and infection. There are four Class I PI3K isoforms (PI3Kα,β,γ,δ), three Class II PI3K isoforms (PI3KC2α, C2β, C2γ) and a single Class III PI3K. The four Class I isoforms synthesise the phospholipid 'PIP3'. PIP3 is a 'second messenger' used by many different cell surface receptors to control cell movement, growth, survival and differentiation. These four isoforms have overlapping functions but each is adapted to receive efficient stimulation by particular receptor sub-types. PI3Kγ is highly expressed in leukocytes and plays a particularly important role in chemokine-mediated recruitment and activation of innate immune cells at sites of inflammation. PI3Kδ is also highly expressed in leukocytes and plays a key role in antigen receptor and cytokine-mediated B and T cell development, differentiation and function. Class III PI3K synthesises the phospholipid PI3P, which regulates endosome-lysosome trafficking and the induction of autophagy, pathways involved in pathogen killing, antigen processing and immune cell survival. Much less is known about the function of Class II PI3Ks, but emerging evidence indicates they can synthesise PI3P and PI34P2 and are involved in the regulation of endocytosis.
The creation of genetically-modified mice with altered PI3K signalling, together with the development of isoform-selective, small-molecule PI3K inhibitors, has allowed the evaluation of the individual roles of Class I PI3K isoforms in several mouse models of chronic inflammation. Selective inhibition of PI3Kδ, γ or β has each been shown to reduce the severity of inflammation in one or more models of autoimmune disease, respiratory disease or allergic inflammation, with dual γ/δ or β/δ inhibition generally proving more effective. The inhibition of Class I PI3Ks may therefore offer a therapeutic opportunity to treat non-resolving inflammatory pathologies in humans. This article is part of a Special Issue entitled Phosphoinositides.

1. Overview of the inflammatory response

There have been several excellent reviews detailing the role of PI3Ks in various immune cells [1–5]. The aim here is to place some of this knowledge in the broader context of the inflammatory response and to focus on the roles played by individual PI3K isoforms, particularly isoforms of the Class I family.

Inflammation is generally defined as the body's response to infection and damage [6]. A successful inflammatory response removes the initial irritant and repairs any tissue damage that has occurred. Unfortunately, several pathological conditions are sustained by a non-resolving inflammation that leads to chronic pain and disability e.g., rheumatoid arthritis (RA); systemic lupus erythematosus (SLE); chronic obstructive pulmonary disease (COPD); Crohn's disease; ulcerative colitis. Even tumours are considered by some to represent 'open wounds' that are fuelled by an inflammatory response. It is therefore important to understand the causes of non-resolving inflammation and how they might be treated [7,8].

2. The role of innate immune cells

The course of an inflammatory response depends on the precise nature of the initial stimulus and where in the body it is located. Although this involves a highly co-ordinated response from many different types of immune and non-immune cells [9], several common principles can be discerned (Fig. 1). The initial phase of acute inflammation is co-ordinated by the innate immune system. This phase generally lasts from minutes to hours and involves the detection of pathogens/damage by resident immune cells (mostly macrophages, dendritic cells, mast cells and "innate lymphoid" cells – lymphoid cells without rearranged antigen receptors [10]), activation of platelets, activation of the complement cascade and the release of signals from damaged or infected host cells. This creates a complex mixture of soluble inflammatory mediators that communicate the source of infection to neighbouring capillaries. Here they perform overlapping and individual roles to alter vascular tone and permeability and also locally to re-model the endothelium to drive the first wave of recruitment of further

☆ This article is part of a Special Issue entitled Phosphoinositides.

* Corresponding authors.
E-mail addresses: phillip.hawkins@babraham.ac.uk (P.T. Hawkins), len.stephens@babraham.ac.uk (L.R. Stephens).

http://dx.doi.org/10.1016/j.bbalip.2014.12.006

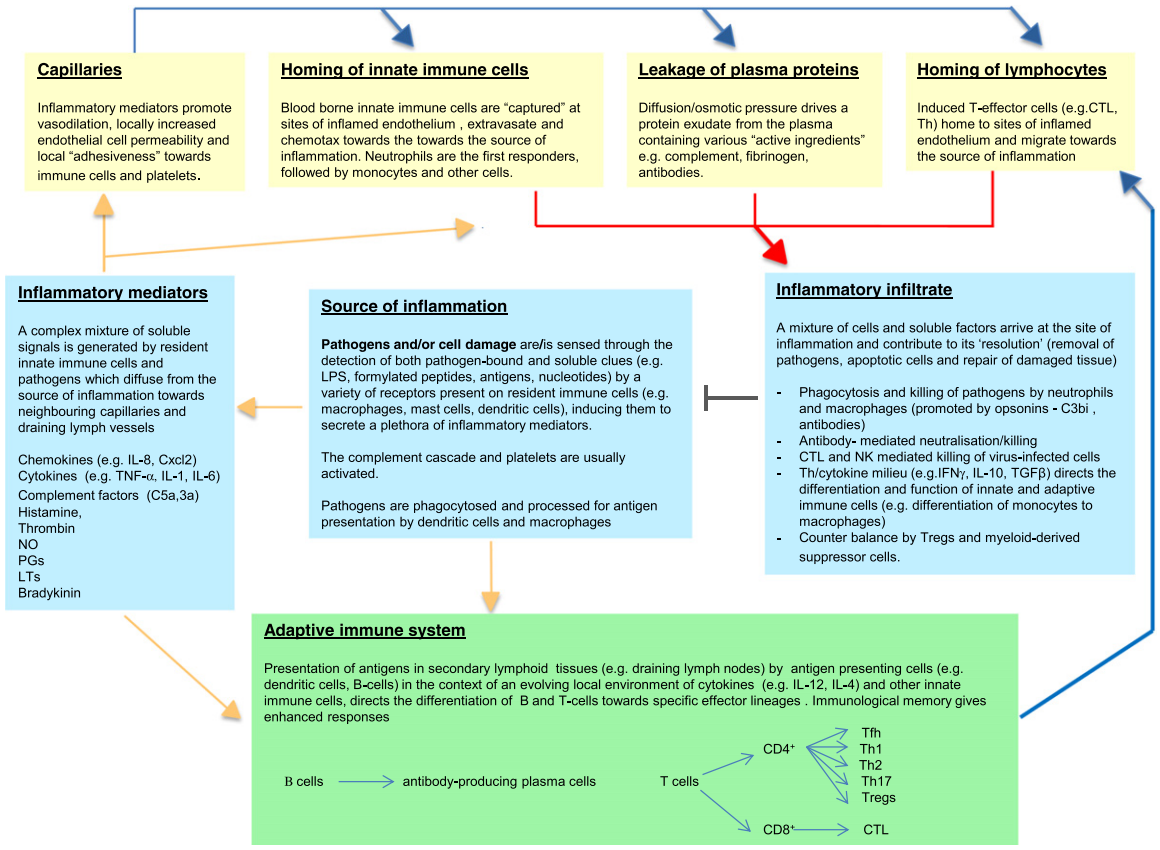

Fig. 1. An overview of the inflammatory response.

immune cells (mostly neutrophils) [11]. Of particular relevance here, key mediators in this initial recruitment are chemoattractants and chemokines which stimulate leukocyte attachment, extravasation and chemotaxis towards the source of inflammation [12–16]. Ideally, resident and infiltrating immune cells are stimulated in this inflammatory milieu to eliminate the foreign invaders by deploying a battery of microbicidal weapons and the inflammation proceeds towards a rapid resolution. Macrophages then arrive to mop up apoptosed cells and other debris. Less ideally, the innate immune response at least buys time for the adaptive immune response to be brought into play.

3. The development of B and T-lymphocytes

The adaptive immune response operates on a time scale of days to weeks and essentially attempts to create a tailored response that depends on the specific nature of the invading pathogen. The chief clue to mounting an appropriate response is the context in which antigens are presented to the main components of the adaptive immune system, the B and T lymphocytes. B-lymphocyte development in the bone marrow creates a repertoire of naïve B-cells each of which expresses a different functional antigen receptor (BCR) capable of recognising foreign molecules in their 'native' state e.g., proteins or polysaccharides [17]. T-lymphocyte development is completed in the thymus and creates analogous repertoires of either $CD4^+$ T-helper cells (Th) or $CD8^+$ cytotoxic T-cells (CTLs) which also possess antigen receptors (TCRs) [18,19], but these receptors are adapted to recognise foreign peptides only when bound to either MHC-I ($CD8^+$-CTLs) or MHC-II ($CD4^+$-Th) molecules [20]. Mature lymphocytes then circulate through the various vessels of the vascular and lymphatic systems and through secondary lymphoid tissues (lymph nodes and spleen). A major task of the adaptive immune system and its anatomical structure is to promote effective encounters between foreign antigens and the small numbers of lymphocytes capable of recognising them.

4. The T cell response

Professional antigen-presenting cells (APCs; dendritic cells, B-cells and macrophages) phagocytose extracellular pathogens/proteins, creating small, foreign peptides (via phagolysosomal digestion), some of which are then presented on their cell surface bound to MHC-II [20,21]. Dendritic cells play the major role in carrying antigen/MHC-II complexes created at an initial site of infection, through the lymph vessels, to secondary lymphoid tissues, where they are engaged by naïve $CD4^+$ T-helper cells. If the combination of peptide/MHC-II and other surface molecules on the activated APCs are recognised by the TCR and its co-receptors on the $CD4^+$ T cell with appropriate affinity, and the correct local cytokines are present, a sequence of T cell differentiation and clonal expansion is initiated towards various effector subsets (eg Th1, Th2, Tfh, Th17) [22]. The type of effector cells produced is guided by the precise nature of the cytokine cues available (e.g., IL-12 promotes differentiation towards Th1; IL-4 promotes differentiation towards Th2; IL-6 promotes differentiation towards Th-17) which is in turn determined by the initial experience of the APCs themselves (i.e. the nature of the substance or pathogen encountered). The response rapidly evolves to include other autocrine and paracrine contributions (e.g. IL-2) and a characteristic feature is that paracrine signals generated from one lineage characteristically inhibit differentiation towards another (e.g. IFN-γ produced by Th1 cells inhibits differentiation towards Th2). These different effector subsets then shape the evolving adaptive immune response both locally, in lymphoid tissues, and at sites of inflammation (through their release into the vasculature and homing to sites of inflamed endothelium). Differentiation towards Th1 is a characteristic response to intracellular parasites (e.g., viruses and some types of bacteria and protozoa), its main functions being to help recruit and prime CTLs (see below) and to generate cytokines (eg IFN-γ) which promote the development of phagocytic macrophages [23]. Differentiation towards Th2 is a typical response to extracellular parasites and

plays a crucial role in helping activated B cells develop into antibody-producing plasma cells (eg through the production of IL-4 and CD40-mediated cell contact) and also leads to the production of cytokines (e.g., IL-10, IL-13) which are major factors in the recruitment and activation of eosinophils and basophils [23]. Tfh cells are also specialised to provide help to activated B cells and play an important role in supporting antibody class-switching and affinity-maturation during the germinal centre reaction (see below) [24]. Th-17 cells, which are characteristically seen as pro-inflammatory cells produced in response to extracellular bacteria and fungi, generate cytokines (eg IL-17) which contribute to the recruitment and activation of neutrophils at sites of inflammation, usually close to epithelial barriers [25].

Cells infected with intracellular pathogens (e.g. viruses) present foreign peptides (created through proteosomal digestion) bound to MHC-I [20]. These peptides can be recognised as antigens by the TCR/co-receptors on appropriately primed CTLs, which are then able to kill the presenting cell though the release of pore-forming proteins and digestive enzymes [26]. All cells possess MHC-I molecules and are potential targets for attack by CTLs. Infected APCs (e.g. dendritic cells) can present antigen/MHC-I in parallel with antigen/MHC-II and help polarise Th cells towards Th1 cells, which in turn recruit CTLs, leading to their priming and proliferation through antigen/MHC-I interactions and the generation of supporting cytokines (IFN-γ and IL-2).

5. The B cell response

Appropriate encounters between B cells and native antigens lead to their differentiation into antibody-producing plasma cells, usually termed the 'humoral response'. If the encounter between the BCR and cognate antigen is sufficiently strong to induce extensive BCR crosslinking, or is supported by TLR engagement, then differentiation towards plasma cells can occur without T-cell help ('T-cell independent response') [27]. T-cell independent antibodies are often generated against the polysaccharide coat of certain bacteria, with both marginal zone B cells in the spleen (MZ-B cells) and B1 cells in pleural and peritoneal cavities playing a prominent role. In most cases, however, an effective antibody response requires T-cell help ('T-cell dependent response') [27]. Follicular B cells that have encountered antigen (for example when presented on the surface of follicular dendritic cells) can endocytose the BCR-antigen conjugates and present foreign peptides bound to MHC-II. These activated B cells can then recruit Th cells (mostly Th2 or Tfh) which possess a TCR recognising the particular peptide/MHC-II complex to form an 'immunological synapse' [20]. Cell-cell interactions and cytokines produced by the Th cells then shape the differentiation of the B cells to plasma cells in specialised niches of lymphoid follicles called germinal centres, which are designed to maximally promote clonal B cell expansion and selection [24]. These are the sites at which cytokine-driven antibody class switching (e.g., IgM to IgG, IgA and IgE) and affinity maturation occur.

Successful T-cell dependent immune responses generate memory B cells and memory T cells that allow much faster and more effective responses if and when the same antigens are encountered subsequently [24,28,29]. Previously generated IgE also coats FcRs on long-lived mast cells [30], basophils [31] and also neutrophils [32], supporting a rapid response to a further encounter with cross-linking antigen.

6. Suppression of the immune response

There is an increasing realisation of the important role that so-called 'suppressor' cells play in maintaining an appropriately poised immune response ('peripheral tolerance'). Although the development and differentiation of both B and T cell lineages is remarkably effective at removing cells that recognise self-antigens ('central tolerance'), some autoreactive cells escape deletion and represent a potential threat to the host [33]. The activation of these cells is countered by suppressor cells, which effectively raise the threshold at which activation of B- and T-cells occurs. The most clearly understood of these sub-sets are the so-called Tregs, which are produced centrally in the thymus (nTregs) and can also be induced during an immune response (iTregs) [34]. These cells can inhibit the proliferation and function of effector cells (eg CTLs) by sequestering critical cytokines required for clonal expansion (e.g. IL-2), producing suppressive cytokines (eg IL-10) or killing cells directly through the release of granzyme or perforin [34]. There is also increasing evidence for important suppressor cell populations in the B [35] and myeloid cell lineages (MDSCs) [36], though these are currently less well understood.

7. Autoimmunity

Whilst the immune system is generally extremely effective in combating a huge variety of pathogens and challenges to the body's integrity, it does also carry significant capacity for 'self-harm'. Although remarkably effective, mechanisms of central and peripheral tolerance can fail, leading to the generation of auto-reactive B and T-cells [33], the generation of auto-antibodies and/or inappropriate activation of innate immune cells [37,38]. The ensuing chronic inflammation does not resolve properly because the source of the inflammation is the host itself. This process is thought to be at the core of several major autoimmune diseases, such as SLE, RA and type 1-diabetes. There are also several common and debilitating conditions where the inflammatory response appears to 'over react', causing more damage than appears necessary to remove the initial insult, classic cases being allergic asthma (predominantly Th2-driven, with involvement of mast cells, eosinophils and neutrophils) and chronic obstructive pulmonary disease (COPD; predominantly a Th1 response, with activation of CTLs, macrophages and neutrophils). In most of these diseases, the underlying causes are extremely complex, involving multiple interactions between genes and the environment [37,38].

8. PI3K signalling

Phosphoinositide 3-kinases (PI3Ks) are enzymes which catalyse the phosphorylation of one or more inositol phospholipids in the 3-position of the inositol ring (see Fig. 2). Both the substrates and products of these reactions are membrane-captive phospholipids and the action of PI3Ks is to generate specific molecular messengers in the membranes upon which they act. There are eight PI3Ks in mammalian cells that are classified into three families; four Class I isoforms (PI3Kα,β,γ,δ), three Class II isoforms (PI3KC2α,β,γ) and a single Class III isoform [3,39].

9. Class I PI3K signalling pathways

Class I PI3Ks selectively phosphorylate phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) to form phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3, usually referred to as 'PIP3') (Fig. 2). Class I PI3Ks are regulated directly or indirectly by cell surface receptors and play important roles in the early signal transduction events that flow from the activation of these receptors [39,40]. PIP3 is synthesised at the plasma membrane and in very early endocytic structures and regulates the localisation and activity of numerous effector proteins through binding to conserved domains, the best characterised of which are a sub-family of PH-domains [41] (Fig. 3). PIP3 is dephosphorylated at the 3-position to re-form the substrate of Class I PI3K, PI(4,5)P2, by the enzyme PTEN [42]. PIP3 can also be de-phosphorylated at the 5-position by the SHIP family of phosphatases (SHIP1/2) to form PI(3,4)P2 [43,44]. PTEN and SHIP2 are ubiquitously expressed but SHIP1 is preferentially expressed in cells of the haematopoetic lineage and is thought to play an important role in limiting PIP3 levels when recruited to inhibitory receptors [45]. PI(3,4)P2 can be dephosphorylated at the 4-position by InsPP4A/B to form PI3P [46]. The rapid appearance of PIP3 and PI(3,4)P2 are characteristic signs of Class I PI3K activation, with little accumulation of PI3P (presumably because active 3-phosphatases remove this phospholipid

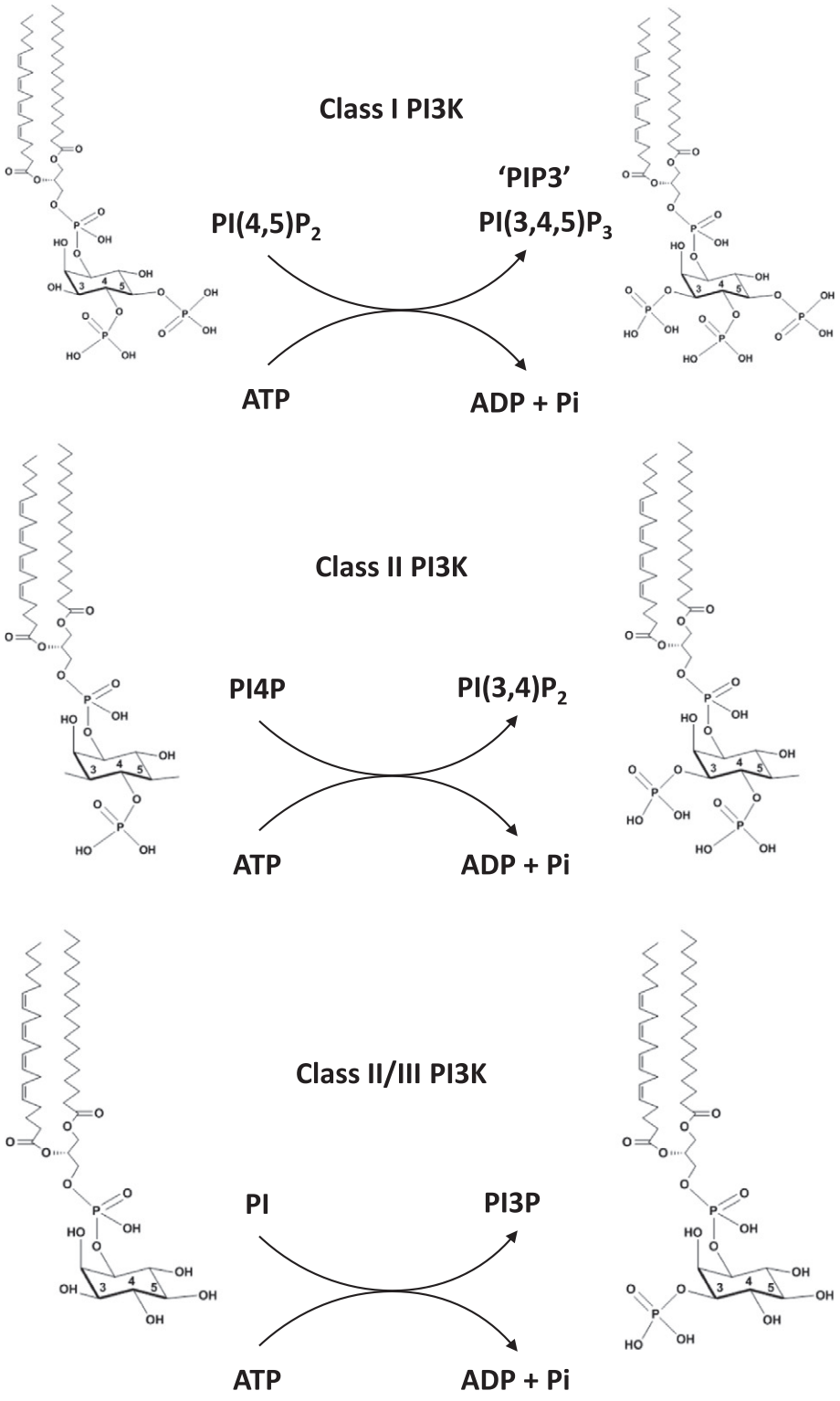

Fig. 2. Reactions catalysed by PI3Ks. PI3Ks transfer the γ-phosphate of ATP to the 3-position on the inositol ring. Class I PI3Ks selectively phosphorylate PI(4,5)P2. Class II PI3Ks phosphorylate both PI and PI4P. Class III PI3K is specific for phosphorylation of PI. Phospholipids are shown with C18:0 (stearoyl) and C20:4 (arachidonoyl) acyl chains, which are the most abundant chains found in these lipids in mammals.

at the plasma membrane) [47]. However, due to technical difficulties in accurately measuring these lipids, there are still relatively few studies that describe changes in their levels on cell activation. This is particularly true in differentiated subsets of primary immune cells, particularly in the B and T cell lineage, where it is difficult to isolate sufficient cells for analysis.

The activation of Class I PI3Ks creates a complex signalling web based on multiple interactions between PIP3 and PI(3,4)P2 and their effector proteins (Fig. 3) [39,40]; there are probably 10-30 PH-domain containing effector proteins that respond to the generation of these two lipids in a given target cell [48]. Many of these effectors bind both PIP3 and PI(3,4)P2 (e.g., PKB, PRex), some prefer PIP3 only (e.g. Grp1, BTK) and a few

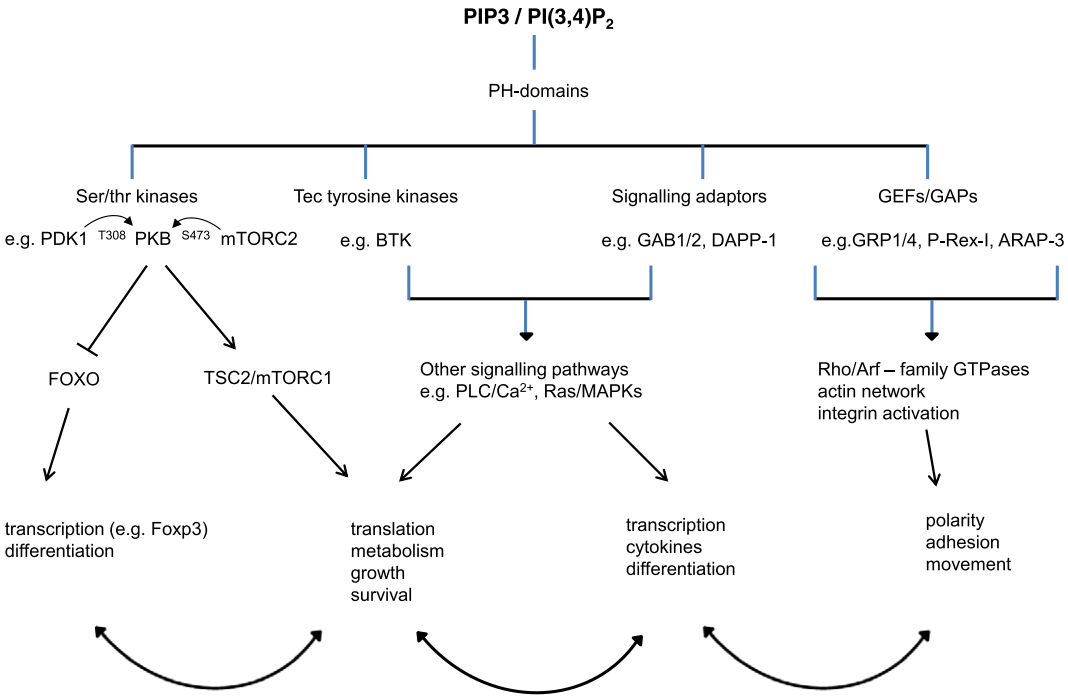

Fig. 3. The Class I PI3Ks signalling network.

prefer PI(3,4)P2 (e.g. TAPP1), suggesting PIP3 and PI(3,4)P2 have differential functions [49]. This idea is obviously important in evaluating the role of PIP3 5-phosphatases, such as SHIP1, which might be viewed either as terminating a PIP3-specific response or directing a PI(3,4)P2-specific response [50]. However, the precise role of PI(3,4)P2 in Class I PI3Ks responses still remains largely undefined.

Some of the key cellular processes regulated by Class I PI3K signalling include growth, movement and differentiation. Phosphorylation and activation of the serine kinase PKB (also known as AKT) appears to be a ubiquitous response to activation of Class I PI3K (and is often used as a surrogate read out of this pathway) [51]. One of the many substrates of PKB is the Rheb-GAP, TSC1/2, which PKB can phosphorylate and inhibit, leading to activation of the central regulator of cell growth, mTORC1. Mutations in and around the PI3K/PKB/mTORC axis are amongst the most prevalent in human cancers, illustrating its importance in growth factor signalling [52]. It should be noted however, that PI3K/PKB is not always the critical regulator of mTORC1 activity, for example mTORC1 regulates the switch to aerobic glycolysis during CTL activation and clonal expansion independently of PI3K/PKB [53].

PKB can also phosphorylate and inhibit the nuclear localisation of FOXO transcription factors, which are critical regulators of lymphocyte development and differentiation [54]. Members of the Tec kinase family (e.g. BTK in B cells and ITK in T cells) also play prominent roles in leukocytes through regulating the PLC/DAG/Ca^{2+}-driven transcription of key cytokines [55]. PIP3/PI(3,4)P2 also regulates many of the elements of actin polymerisation and the accompanying changes in cell shape that underlie cell movement and phagocytosis, including several GEFs and GAPs for small GTPases of the Rac and Arf families [56–58].

In any specific context of cell regulation, Class I PI3Ks are always activated alongside other intracellular signalling pathways and interact with them at multiple levels to create a specific cellular response. Partly because of this, PI3K signalling is often observed to influence other cellular pathways variably, depending on the cell type and receptors activated, leading to apparently 'conflicting' results. For instance within immune cells it is particularly complicating that other central signalling pathways (e.g., Erk, p38MAPK), key downstream responses (eg NFκ-B-dependent transcription) or even upstream activating receptors (e.g. TLRs) appear to be variably associated with Class I PI3K activation.

10. Class I PI3K isoforms

The Class I PI3Ks themselves are heterodimers containing one type of regulatory subunit tightly bound to one catalytic subunit (Fig. 4). There are four isoforms, PI3Kα,β,γ and δ, that are named after the catalytic subunit they contain. They are usually sub-classified into two further groups, Class IA and Class IB, based on their structure and general mode of regulation [39].

Class IA PI3Ks contain one of five homologous regulatory subunits, p85α/p55α/p50α, p85β or p55γ, bound to a single p110α, p110β, or p110δ catalytic subunit, to form five potential heterodimers for each catalytic subunit, known as 'PI3Kα', 'PI3Kβ' or 'PI3Kδ', respectively, i.e. fifteen potential heterodimers in total. Thus far, no clear subunit preferences have been described between the regulatory and catalytic subunits of the Class 1A PI3Ks, and this is an underexplored concept.

Class I B PI3Ks contain one of two homologous subunits, p101 or p84 (also known as $p87^{PIKAP}$). p101 or p84 specifically bind to a single p110γ catalytic subunit to form two potential heterodimers, p101/p110γ or p84/p110γ, both known as 'PI3Kγ'.

Each of the Class I PI3K isoforms catalyses the same reaction and, though there may be significant differences in their kinetic parameters, the main distinction between them appears to be in their adaptation to upstream regulation by receptor transduction pathways [39,59]. The Class I PI3Ks are soluble enzymes and are activated by a combination of factors that both recruit the enzyme to the membrane in which their substrate resides (which is usually envisaged to be the inner leaflet of the plasma membrane) and trigger conformational changes that make them more efficient catalysts. Key factors are the binding of the regulatory subunits to specific signals generated by cell-surface receptors, the conformational changes that ensue from these interactions and the binding of membrane-captive small GTPases to the Ras Binding Domains (RBDs) of the catalytic subunits [60].

The Class I B isoform, PI3Kγ, is differentially expressed amongst cells and tissues, with highest levels of expression in the myeloid lineage (eg neutrophils, macrophages and mast cells), though important

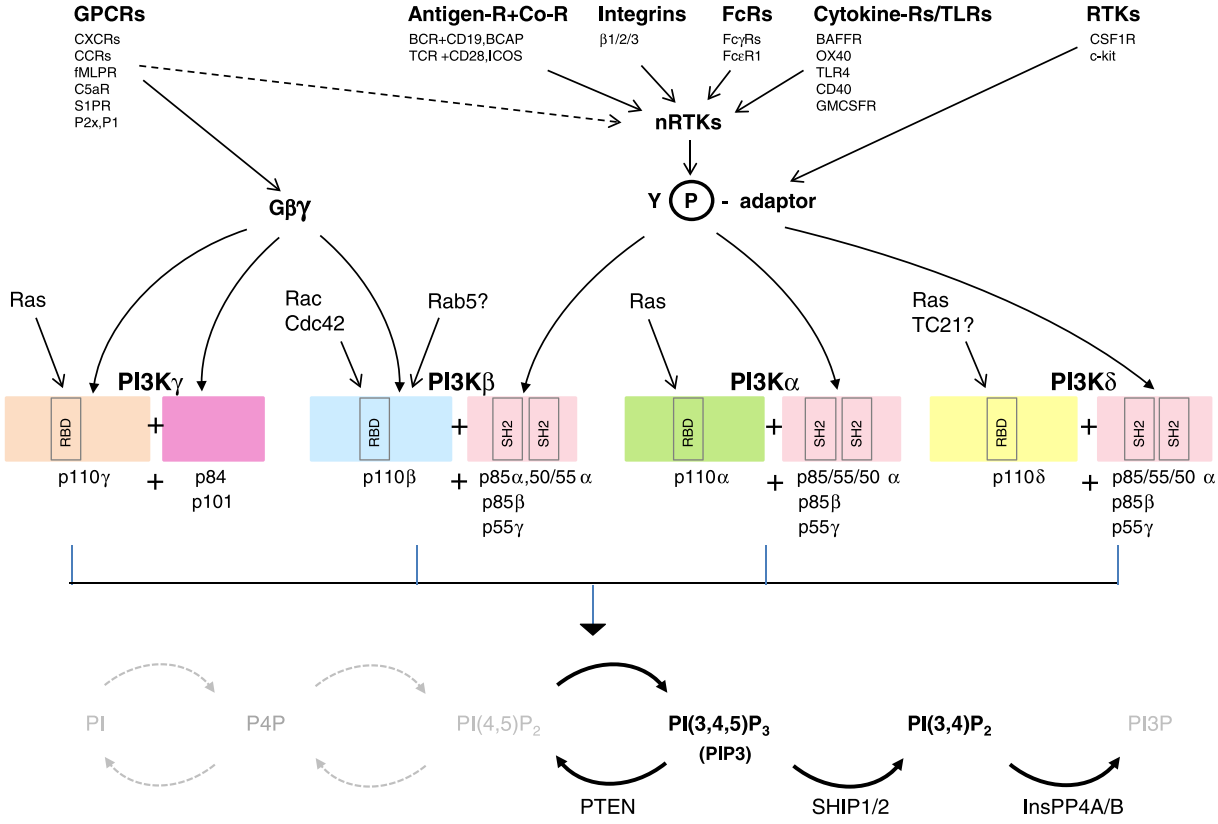

Fig. 4. Regulation of Class I PI3K isoforms.

functions have also been attributed to this isoform in other cells, such as T-cells, endothelial cells and cardiac myocytes [61]. PI3Kγ is adapted to regulation by GPCRs that signal through Gi-family heterotrimeric G-proteins; the important regulatory elements here are direct binding of Gβγ subunits to the heterodimer (the precise interaction sites have yet to be mapped) and synergistic activation by GTP-Ras (Fig. 4) [62]. Interestingly, p84 and p101 appear to be differentially expressed, with p84 being the dominant regulatory subunit in mast cells [63]. Both *in vitro* and model cell studies have suggested that p84/p110γ dimers may be more sensitive to activation by GTP-Ras while p101/p110γ dimers may be more sensitive to activation by Gβγ, implying differential adaptation to upstream inputs [64,65]. Further, both reconstitution studies in mast cells [63] and a recent comparison of the phenotypes of mouse neutrophils lacking either p84 or p101 (Deladeriere et al, Science Signaling in press), suggest p101/p110γ and p84/p110γ are designed to regulate different cell responses, even in the same cell. The molecular explanation for this remarkable specificity is unknown but it seems likely that p84- and p101-specific interactions must generate signalling complexes with distinct properties, possibly involving spatially segregated 'nano-domains' of PIP3. An additional level of regulation of the p84/p110γ dimer has also been described recently in mast cells, where PKCβ has been shown to phosphorylate p110γ downstream of the FcεR, decoupling the regulatory and catalytic subunits [66].

The Class IA isoforms PI3Kα and PI3Kβ are relatively ubiquitous in their expression but PI3Kδ is mostly confined to expression in cells of the myeloid and lymphoid lineages [3,5]. The presence of high levels of PI3Kδ is thus a characteristic of most immune cells. All Class IA PI3Ks are adapted to regulation by receptors that directly or indirectly signal via protein tyrosine kinases, with one or both SH2 domains in the regulatory subunits binding to phosphotyrosines within specific recognition motifs (eg 'YXXM') [60]. Early work defined important PI3K-recruitment motifs in the cytoplasmic tails of growth factor receptors with intrinsic protein tyrosine kinase activity (e.g. PDGF-R, CSF1-R). It has however, been surprisingly difficult to identify the analogous sites in signalling adaptors downstream of receptors which indirectly employ protein tyrosine kinases (e.g. Src, Tec, Syk) in their signalling networks e,g, FcγRs and antigen receptors on immune cells. This is probably because there is significant redundancy in these networks (which means multiple 'knock-in' mutations need to be created to fully define Class IA PI3K recruitment and activation).

Phosphotyrosine-SH2-domain mediated interactions serve to both recruit PI3Ks to the membrane and to relieve regulatory subunit-mediated inhibition of the catalytic domain [60]. Each Class IA regulatory subunit contains two functional SH2 domains but the shorter versions (p55α, p50α and p55γ) lack a potential small GTPase regulatory domain (the BCR homology domain) and N-terminal SH3 domain. The functional differences between these different regulatory subunits are unclear but are likely to involve differential recruitment to upstream signalling complexes, possibly mediated through BCR and SH3 domain-mediated contacts. Notwithstanding differences between the regulatory subunits, each of the Class IA PI3K catalytic subunits is thought to be able to bind to each of the p85/55/50 regulatory subunits, apparently limiting the potential for catalytic isoform-selective activation. However, some important distinctions have emerged recently that start to explain the observed differential sensitivities of some of these heterodimers to upstream regulation.

The RBD domain of p110α is selective for GTP-Ras and this interaction is important for efficient activation of PI3Kα heterodimers [67]. By contrast, the RBD of p110β is selective for GTP-Rac or GTP-Cdc42 and this is important for activation of PI3Kβ [67]. The selectivity or, indeed, necessity of small GTPase interaction with the RBD of p110δ is currently unclear and warrants further investigation. Elegant structural studies have recently revealed that the same regulatory subunit makes different contacts with each catalytic subunit, creating differences in their sensitivity to SH2-phosphotyrosine-mediated release from regulatory subunit inhibition [60,68]. PI3Kβ can also be directly activated by

Gβγ subunits and the site of interaction on p110β has recently been mapped [69]. The sensitivity and scale of activation of PI3Kβ by Gβγ is much lower than the analogous activation of PI3Kγ, but contributes significantly to a very substantial and synergistic activation of PI3Kβ by both Gβγ and phosphotyrosine peptides both *in vitro* and in response to GPCRs in cellular models [70]. The extent to which Gβγs alone may be important for physiological activation of PI3Kβ is difficult to establish, however, because of the difficulty imposed by the requirement to interfere specifically with SH2 domain-directed input in only this isoform. There is also good evidence that GTP-Rab5 can bind p110β via a novel interaction that has yet to be precisely defined [71].

Thus, one can envisage that, upon the appearance of appropriate activating phosphotyrosines, each of the Class IA enzymes undergoes SH2-domain engagement in proportion to their relative levels of expression (favouring PI3Kδ in immune cells), but specific regulatory subunit-catalytic subunit interactions (perhaps dual engagement of SH2 domains being required for maximal activation of PI3Kα and δ), and the simultaneous presence of GTP-Ras (favouring PI3Kα) or GTP-Rac/Cdc42 (favouring PI3Kβ) and/or the presence of GPCR-liberated Gβγ subunits (favouring PI3Kβ) will result in a differential contribution of each isoform to the generation of PIP3.

Although the above discussion has focussed on differences between Class I PI3K isoforms, it should be noted that in many, perhaps most, contexts of cell regulation more than one PI3K contributes to the 'PIP3 response' e.g. [72–74]. Even in scenarios where it is claimed a particular PI3K isoform plays a 'non-redundant' role in a particular cell response, usually on the basis of the differential impact of gene deletion or isoform selective inhibitors, there is insufficient detail to delineate the precise contribution that each isoform makes at different doses of agonist. This is especially the case when surrogate readouts of Class I PI3K activity are measured, which may have a non-linear relationship to PI3K activity (eg PKB phosphorylation). Further, the precise expression levels of each isoform are almost never known in terms of molecular equivalents, precluding a 'true' assessment of isoform-selective engagement by upstream receptors. Layered upon these considerations, Gi-coupled GPCRs characteristically stimulate protein tyrosine kinases of the Src/Tec families in their signalling networks, thus engaging Class IA as well as Class IB PI3Ks e.g. [75]. Further, there are now several examples where receptor tyrosine kinase signalling stimulates autocrine loops which engage GPCRs, again entangling both Class IA and IB PI3Ks [76,77]. When each receptor/cell combination is then considered in the context of an inflammatory response, involving a huge array of mediators and cell types, it is clear that we are far from being able to build a convincing understanding of the role of individual PI3Ks in inflammation from a consideration of the elemental properties of the enzymes and pathways alone.

11. Class II and Class III PI3Ks

Class III PI3K phosphorylates phosphatidylinositol (PI) to form phosphatidylinositol 3-phosphate (PI3P) at specific intracellular locations (Fig. 5), such as endosomes and early autophagocytic structures, and regulates the fate and function of these structures through binding to distinct effector domains (eg FYVE and PX domains)(Fig. 6) [78,79]. There is only a single Class III PI3K catalytic subunit (also known as Vps34 by relation to its initial discovery in *S. cerevisiae*) that binds to a putative protein kinase (p150 or Vps15), which is involved in its regulation. Class III PI3K/p150 can be isolated from cells in distinct complexes with other proteins (beclin, UVRAG, ATG14) that determine its precise location and function. Complexes containing ATG14 are involved in the induction of autophagosomes [80,81], whilst complexes containing UVRAG are involved in endo-lysosomal transport [82]. The molecular details of how these complexes function and indeed how Class III PI3K activity is regulated are still poorly understood (largely because of the significant problems involved in recreating these complexes from recombinant proteins *in vitro*) but are likely to involve multiple

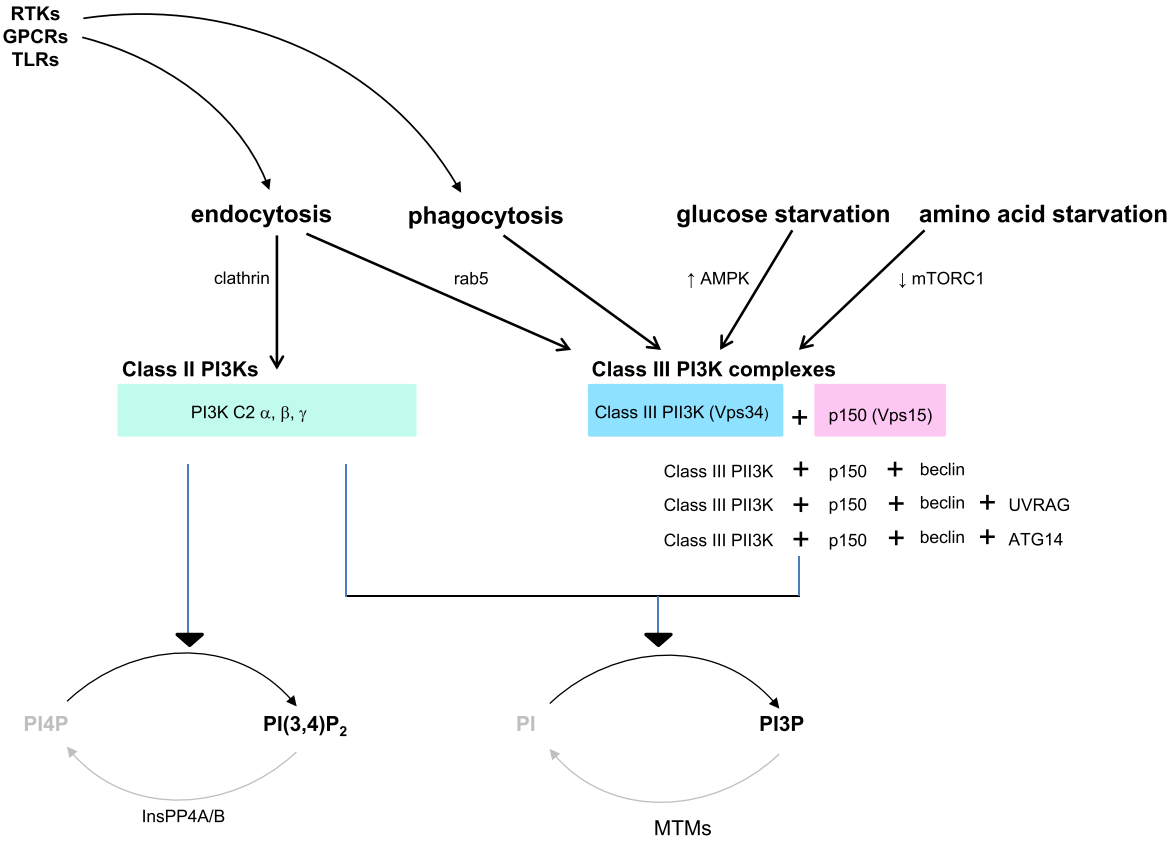

Fig. 5. Regulation of Class II/III PI3Ks.

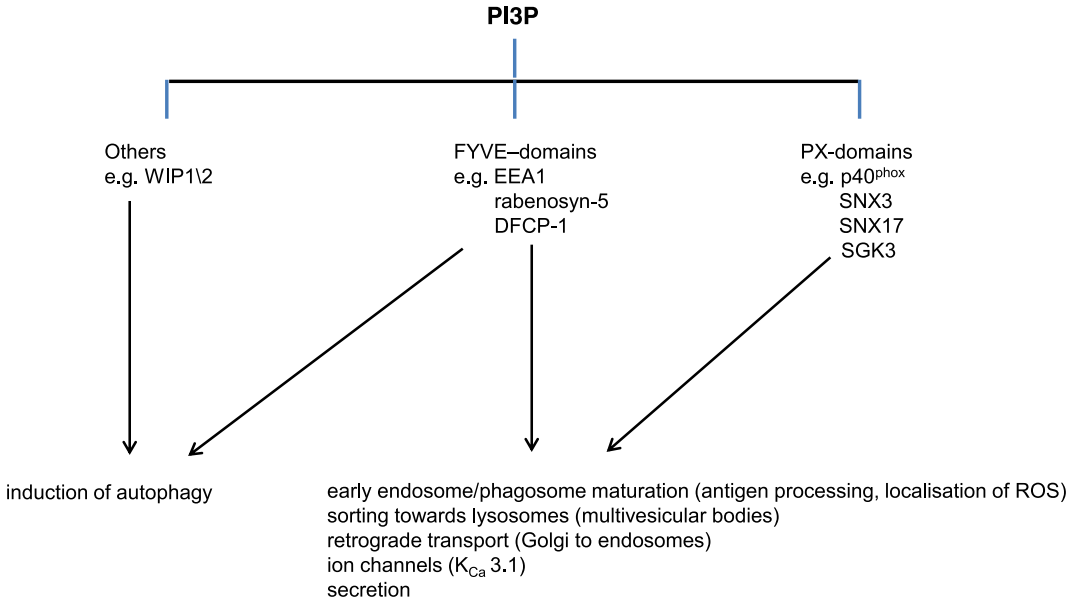

Fig. 6. Signalling through Class II/III PI3Ks.

phosphorylation events catalysed by upstream kinases (eg AMPK, mTORC1) [82]. It also seems highly likely that further components of Class III PI3K complexes have yet to be discovered.

Class II PI3Ks are even less well understood but accumulating evidence suggests they are able to synthesise either PI3P or PI(3,4)P2 at the plasma membrane or endosomes, at least in some circumstances in response to receptor stimulation (Fig. 5) [83–86]. Presumably the generation of these lipids can trigger regulatory events analogous to those which occur when the same lipids are synthesised by Class III/I PI3Ks.

12. Major roles for specific PI3Ks in the inflammatory response

Most of our information concerning PI3K isoform-specific roles stems from the use of p110δ, p110γ and, more recently, p110β, 'knock-out', 'conditional knock-out' and 'kinase-dead knock-in' mice, together with highly selective p110δ, p110β and p110γ inhibitors (Table 1) [1,3–5,87], though the p110γ-selective reagents generally show poor cell-penetrance, making judgements over 'selective' doses difficult. Mice lacking a functional version of PI3Kγ [88–90] or PI3Kδ [91,92], are viable and 'healthy' when bred under animal house conditions and early studies indicated they had significant defects in aspects of both innate and adaptive immunity, driving a great deal of the work in this field. Mice lacking functional PI3Kβ are viable, but are small [76]. The lack of functional PI3Kα is embryonic lethal and therefore tissue-specific ('conditional') deletion or the use of heterozygotes is necessary to delineate function [5]; further, p110α-selective inhibitors with good cell-penetrance have generally been unavailable in the public domain, meaning the specific function of PI3Kα in immune cells is generally much less well understood. Mice lacking functional Class III PI3K also die in the early embryo

Table 1
PI3K-selective inhibitors and mouse genetic models useful for evaluating PI3K isoform involvement in pathophysiological processes.

Compound	IC 50 (μM)								
	Class I PI3K				Class II PI3K			Class III PI3K	mTOR
	p110α	p110β	p110γ	p110δ	C2α	C2β	C2γ		
BYL719	0.004	>1	0.25	0.29				>1	>1
A66	0.04	>12	>3	>1		0.45			
TGX 221	≅5	0.005	>10	0.1	>10				
IC 87114	>100	≅5	≅1	0.1				>100	>100
AS 605240	0.06	0.27	0.008	0.3					
VPS34-IN1	8.04	21.44	2.649	1.90	>10	>10	>10	0.025	>1
	Genetic model								
Mouse	+/− [221] +/KD [222] flox/flox [223]	KD/KD [76,116] −/− [76,116] flox/flox [224]	KD/KD [225] −/− [88–90]	KD/KD [92] −/− [91] flox/flox [173]	+/− [84] flox/flox [84]	−/− [97]		flox/flox [226] +/−[93]	+/− [227] flox/flox

The inhibitor data is drawn from several sources, see [4,5,87,205]. PI3Kα-selective inhibitors: the BYL719 and A66 have significant (0.58–0.25 μM) IC50s on PI4Kβ; INK1117 looks a promising reagent but less has been published on its properties. PI3Kβ-selective inhibitors: TGX 221, although it has poor drug-like properties, remains an excellent tool; in contrast, AZD6482 has improved drug-like properties but is less PI3K-selective. The PI3Kδ inhibitors: IC87114 remains an excellent tool. The PI3Kγ inhibitors: AS 605240 remains a widely used tool although it has a relatively low selectivity over PI3Kα, particularly *in vivo*; AS252424 is more PI3Kγ-selective although more difficult to synthesise and less widely available; because p110γ has a lower Km for ATP than the other p110s, the practice, that is sometimes described in the literature, of assaying an inhibitor with different p110s at ATP concentrations equal to their individual Kms for ATP, although technically sound, tends to make p110γ-selective inhibitors appear more selective than they may be in more cell-like environments. VPS34 inhibitors; VPS34-IN1 is the first highly selective inhibitor that has been reported (WO patent 2012085815 A1; Cornella Taracdo, I., Harrington, E.M., Honda, A. and Keaney E.) and extensively characterized [94].

Genetic manipulation of PI3Ks in mice: kinase dead alleles are abbreviated to KD; +/− indicates that homozygous loss results in mice not surviving to adulthood; flox/flox indicates a Cre-conditional allele has been derived. Mouse Class II PI3K C2α has been targeted by the Sanger/EUCOMM consortium and mice are available (PIK3C2a$^{tmla(EUCOM)Hmgu}$). Mice expressing a floxed mTor allele are available from Jackson labs.

[3,93], though the very recent development of good Class III PI3K-selective catalytic site inhibitors should allow rapid progress in this area [94,95]. Mice lacking PI3KC2α develop chronic renal failure [96] and mice lacking PI3KC2β have not yet revealed phenotypes in the immune system [97]; good selective inhibitors of these enzymes are also not yet available.

The use of these genetic and pharmacological tools has allowed rapid progress to be made in identifying key roles for PI3Ks in immune cells [1,3–5,87].

13. Recruitment of innate immune cells

There is now a substantial body of evidence from both *in vitro* and *in vivo* studies that suggests PI3Kγ plays an important role in the initial signal transduction events downstream of Gi-coupled chemoattractant and chemokine receptors (eg receptors for fMLP, C5a, IL-8, LTB4) that lead to the extravasation and migration of innate immune cells (eg neutrophils, monocytes, eosinophils) to sites of inflammation [56,57,88–90,98–102]. Important cellular responses underlying this effect include the regulation of selectin- and integrin-mediated adhesion and actin dependent cell polarisation and chemokinesis [14,15,57]. Some of these studies suggest different extents of PI3Kγ involvement and several also implicate the co-involvement of PI3Kδ [103,104], presumably via indirect activation of tyrosine kinases (e.g. src family kinases), possibly mediated through engagement of adhesion receptors, such as integrins, which allow the migrating cell to gain traction [57]. Studies *in vivo* also suggest roles for PI3Kγ and δ in the inflamed endothelium [104,105]. The net result appears to be that loss of PI3Kγ blunts the initial recruitment of innate immune cells to the site of inflammation but that loss of both PI3Kγ and δ is more effective [105]. An interesting recent study has also linked high Class I PI3K activity in neutrophils from COPD patients with dysregulated chemotaxis [106].

14. Activation of innate immune cells

PI3Ksβ and δ play important roles in regulating the spreading and activation of neutrophils and macrophages when they attach to various surfaces, such as extracellular matrix, or large pathogens (e.g. fungi) [76,107–109]. This activation characteristically leads to the secretion of ROS and proteases downstream of protein tyrosine kinase signalling induced by ligation of integrins and FcγRs. These signalling pathways are similar to those downstream of antigen receptors, involving Src-family tyrosine kinase phosphorylation of ITAM motifs in the Fc γ-chain, the recruitment of Syk and Tec tyrosine kinases and the tyrosine phosphorylation of multiple adaptors, though the specific phosphotyrosines directly recruiting the SH2-domains in Class IA PI3Ks are still unclear [110]. These Class I PI3Ks are also required for efficient phagocytosis of large antibody-opsonised particles but, interestingly, they appear to be less important for phagocytosis and killing of smaller, complement-opsonised bacteria [111–113]. Both PI3Kβ and δ are also required for a maximal ROS response of neutrophils spreading on immobilised immune complexes (a model of auto-antibody mediated host damage), but PI3Kβ is much more important at lower antibody densities, possibly because it can more efficiently integrate GPCR-induced Gβγ inputs arising from an autocrine LTB4 loop [76]. PI3Kγ plays an essential role in chemoattractant and chemokine-mediated ROS production and granule secretion in neutrophils and macrophages in response to diverse receptors, including GPCRs, (eg C5a or formylated peptides) [102,114,115] with significant contributions also from PI3Kβ in macrophages [116] and PI3Kδ in neutrophils [75]. A key feature of Class I PI3K involvement in all of these responses is the activation of Rac, which plays an essential role in assembling an active NADPH oxidase complex and organising the actin cytoskeleton [114,117].

Neutrophil apoptosis and subsequent phagocytosis by macrophages is an important component of the resolution of inflammation. All of the Class I PI3Ks appear to play 'redundant' roles in the regulation of cytokine-mediated neutrophil survival, possibly via the regulation of PKB [73].

Evidence on the role of Class I PI3Ks in the signalling pathways downstream of TLRs on macrophages and dendritic cells is at present confusing, with different publications suggesting this pathway can play either positive or negative roles in the production of pro-inflammatory cytokines [118]. In macrophages, TLRs can induce the recruitment of Class IA PI3Ks to phosphorylated YXXM motifs in BCAP (previously identified as a major signalling adaptor in B cells, see below) and this plays an important role in down-regulating NF-κB driven transcription of pro-inflammatory cytokines [118]. Further, in dendritic cells, loss of functional PI3Kδ reduces TLR4 internalisation and relocation to the endosomes in response to LPS, thus promoting the early-phase secretion of pro-inflammatory cytokines (IL-6 and IL-12) and reducing the later-phase secretion of anti-inflammatory cytokines (IL-10, IFN-β) [119]. PI3Kγ also plays a significant role *in vivo* in the chemokine-driven migration of dendritic cells to draining lymph nodes and hence antigen presentation to $CD4^+$ T cells [120,121]. In other cell contexts, however, TLRs appear to play a more important role as 'primers' of subsequent responses to activating ligands, cf, TNFα-priming of GPCR-stimulated PI3Kδ-driven PIP3 responses in human neutrophils [75,122].

PI3Kδ plays an important role in the protein tyrosine kinase-driven signal transduction pathways stimulated by antigen-dependent cross-linking of the FcεR1 on mast cells, leading to calcium-stimulated granule secretion and the release of important pro-inflammatory mediators, such as histamine and adenosine [123]. Clustering of IgE-bound FcεRs by antigen initiates a complex network of tyrosine kinase activities, including Src family kinases and Syk, leading to the phosphorylation of YXXM motifs on the adaptor Gab2 and the recruitment and activation of Class IA PI3Ks [124]. The release of adenosine also contributes to sustained activation via purinergic (A_3AR) receptor-stimulation of PI3Kγ [77]. The use of transgenic mice and pharmacological inhibitors has also implicated both PI3Kδ and γ isoforms in the migration and activation of mast cells *in vivo* [74,77,123,125–129].

Natural killer lymphocytes (NK cells) are innate lymphoid cells (see above). NK cells possess a collection of activatory and inhibitory receptors which allow them to specifically engage and destroy cells with low levels of MHC-I, which is a characteristic of foreign, unhealthy, infected or cancerous cells. The role of both PI3Kγ and δ in NK cell mediated homing and killing of target cells is currently confusing, with data *in vitro* suggesting a more prominent role (eg in response to CXCL12, CCL3, S1P) than appears *in vivo* [130,131].

The relative roles of PTEN, SHIP1/2 or other PIP3 phosphatases in regulating the action of Class I PI3K responses in innate immune cells are still mostly unexplored [3,4,132]. However, SHIP1 clearly plays an important role in regulating Class I PI3K activation in mast cells and macrophages via recruitment to inhibitory receptors [133,134]. SHIP1 has also been implicated in generating an appropriate PIP3 signal for effective neutrophil polarisation and chemotaxis ('too much PIP3' can induce strong adhesion and inhibit effective front-back polarisation) [135]. A further mechanism for the regulation of PIP3 signalling in neutrophils has recently been suggested by the apparent competition between PIP3 and soluble inositol pyrophosphates for common PH-domain effectors [136].

In nearly all of the above contexts of PI3K regulation it has been extremely difficult to tease apart the contributions of individual elements in an *in vivo* context, because they are all interdependent e.g. activation and recruitment are nearly always part of a self-regulated cycle.

15. Phagosome maturation

PI3P is generated on the cytoplasmic surface of endocytic structures in all cells and appears just after severance from the plasma membrane in the phagosomes of neutrophils, macrophages and dendritic cells, irrespective of the size or content of the particle internalised or the initial receptors engaged [78,137–139]. Where studied, it appears this PI3P is generated by the recruitment of a Class III PI3K complex on the

phagosome, though in most cases the involvement of Class II PI3Ks or sequential dephosphorylation of the products of Class I PI3Ks cannot be excluded. At least in one clear example, a Class IIIPI3K/p150/beclin complex is recruited to the phagosome by the SLAM receptor, which recognises components of the bacterial outer membrane [140].

This rise in PI3P acts to recruit and regulate the function of several effectors important in phagosome maturation [78,137,141]. One of the best studied of these is the p40phox subunit of the NADPH oxidase, which is responsible for the efficient recruitment of other oxidase subunits, leading to the generation of intra-phagosomal ROS [142,143]. This ROS plays an important role in the killing of some pathogens (particularly bacteria and fungi) [144] and also contributes to appropriate protease digestion of foreign proteins, yielding antigenic peptides that can be loaded onto MHC-I ('cross-presentation') or MHC-II molecules [145,146]. The *in vivo* significance of this interaction is clear from the phenotype of mice carrying an engineered mutation in the PI3P-binding PX domain of p40phox [147] and also the discovery of a human immunodeficiency patient who carries an analogous mutation in their p40phox PX domain [148].

16. Autophagy

Autophagy is a major catabolic pathway mediating the encapsulation of cytoplasmic components in a specialised double membrane structure and their delivery to lysosomes. The initiation and course of autophagy is regulated by Class III PI3K-containing complexes. In particular, the recruitment of an ATG14-containing Class III PI3K complex to specific sites in the endoplasmic reticulum plays a critical role in the induction of autophagy during starvation, a process in which the PI3P effectors WIPI1/2 are thought to play a major role [81]. Whilst there are still very few experiments that directly link Class III PI3K activity to specific roles for autophagy in the immune response, there is an increasing appreciation that autophagy does indeed play important roles in several aspects of immune defence and inflammation.

There is a significant and confusing literature that argues for an important role for autophagy in the activation and clonal expansion of lymphocytes after antigen recognition. This is at apparent odds with the mTORC1-regulated increase in anabolic pathways that accompanies this process (mTORC1 is normally a powerful inhibitor of autophagy), though recent evidence suggests that the induction of autophagy may actually be more closely correlated to the cessation of clonal expansion and the generation of memory cells [149].

It is also becoming increasingly apparent that autophagy may be an important mechanism that is used by many different types of cells to recognise and deliver viruses and intracellular bacteria to lysosomes, resulting in their removal. This process is driven by the recognition of specific 'eat me' signals that allow foreign molecules to be specifically targeted to the autophagosome compartment, possibly an ancient layer in the innate immune response [150].

As a consequence of delivering material for lysosomal degradation, autophagy can also generate antigenic peptides for loading onto MHC-II. Thus autophagy may play a significant role in shaping the $CD4^+$ T cell repertoire, both during the creation of self-tolerance and during the induction of an effective T cell response against pathogens or tumours (through the presentation of tumour-specific antigens) [151–153].

One of the major hurdles to progress in defining specific roles for Class III or indeed Class II PI3Ks in immune-specific aspects of autophagy is the lack of tools to interrogate their specific function in only one type of complex, in isolation from other complexes in which they reside (eg those involved in endosome regulation).

17. B cell functions

Class I PI3Ks are involved in the regulation of B cell development, survival and activation by antigen [3,5,154]. Furthermore, switching PI3K activity off appears to be as important as switching it on [3]. Development of B cells to the pro-B cell stage requires IL-7R-dependent signalling through Class I PI3Ks, though direct recruitment of Class IA PI3Ks to the phosphorylated YXXM motif in the IL-7R is redundant [155]. At this point, Class I PI3Ks are switched off to allow FOXO-dependent transcription of the Rag genes and Ig heavy chain rearrangement to form the pre-BCR [156,157]. Class I PI3K signalling through the pre-BCR is then needed, in co-operation with the IL-7R, to drive proliferation and growth of cells at the large pre-B cell stage [158]. Class I PI3K activity is then switched off again, this time to allow FOXO-dependent rearrangement of the light chains, forming the fully rearranged BCR [156,157]. The strength of BCR-dependent PI3K signalling then contributes to both positive ('clonal deletion') and negative ('anergy') selection strategies used to remove self-reactive clones and to generate functional B cells destined for the periphery [159]. Immature B cells then leave the bone marrow and differentiate into various mature B cell subsets (follicular B cells, MZ- B cells and B1 cells) in the lymph nodes, spleen and body cavities. Class I PI3Ks contribute to tonic BCR survival signals that keep these naïve B cells alive as they search for cognate antigen [158,160,161]. A patient with a homozygous premature stop codon in the p85α gene has a complete lack of mature B cells, illustrating the central importance of this pathway in B cell development [162]. Mice lacking both PI3Kα and δ in their B cell lineage exhibit a severe defect in B cell development, with substantially reduced populations of all peripheral B cells [161]. Mice lacking only PI3Kδ exhibit highly reduced MZ-B and B1 cell populations and a smaller reduction in follicular B cells, suggesting PI3Kδ plays a particularly important role in the final differentiation to MZ-B and B1 lineages or to their homing/survival in their specialised niches [161]. Mice lacking functional PI3Kδ produce substantially fewer natural antibodies, as would be expected from their decreased populations of MZ-B and B1 cells [91,92,163].

The activation of naïve B cells by antigen induces clonal expansion and differentiation into antibody-producing plasma cells and there is now a substantial body of data indicating Class I PI3Ks can play a positive role in the signalling induced by the BCR and its co-receptors driving both T cell-independent and T-cell dependent differentiation [3,5,154,158]. Further, Class I PI3Ks play a role in suppressing AID-dependent class switch recombination and affinity maturation during the germinal centre response [164].

The immediate signalling pathways downstream of the pre-BCR, the fully rearranged BCR, and their various co-receptors (e.g. CD19, CD40) are driven by several protein tyrosine kinases [154,158]. Key amongst these are Src family kinases, which phosphorylate ITAM motifs in receptor chains, driving the recruitment and activation of Syk, and, indirectly, BTK. These tyrosine kinases and their substrates create complex, self-organising structures that are designed to read out the level, context and duration of stimulation [158]. The individual details of these signalling complexes at any particular moment in time are difficult to ascertain but some key features have emerged. BCR activation drives Class IA PI3K recruitment to phosphorylated YXXM motifs on CD19 and BCAP [165] and the PIP3 produced plays a significant role in promoting BTK-dependent phosphorylation and activation of PLCγ, thus regulating Ins(1,4,5)P3/Ca^{2+} and DAG/PKCβ signals important for cell proliferation and cytokine synthesis [158,166]. Class I PI3K activation of PKB, the activation of mTORC1 and the inhibition of GSK3 also play prominent roles in antigen-stimulation of B cell clonal expansion [158]. The signalling adaptor BLNK (SLP-65) co-ordinates signalling complex formation downstream of the pre-BCR but is also responsible for limiting the activation of PKB; this may be an important element in allowing FOXO-mediated transcription of Rag1/2, IL-7R, Bcl-6 and BLNK itself during B cell development [158,167,168]. PTEN plays an important role in limiting the scale of PI3K signalling throughout the B cell lineage e.g. [159] and the direct recruitment of SHIP1 to ITIM motifs in the inhibitory FcγRIIB receptor plays an important role in setting the threshold of B cell activation [169].

A substantial amount of data indicates PI3Kδ is the predominant Class IA PI3K isoform in BCR-driven signalling [3,5,91,92,170]. It is, therefore, perhaps surprising that T cell-dependent antibody responses are only modestly reduced in mice lacking functional PI3Kδ [91,92,163,171,172]. Further, the main role for PI3Kδ seems to be in providing Th cell help to B cells, rather than in the B cells themselves [173]. It seems likely that redundant pathways of activation involving other receptors (e.g. IL-4R, CD40, TLRs) are able to support antigen-induced BCR signalling and induce an effective germinal centre response *in vivo* in the absence of PI3Kδ [5]. It is also possible that the lack of PI3Kδ activity may suppress Treg function, counterbalancing a reduction in the activation of follicular B cells. PI3Kδ is also the major PI3K isoform inhibiting class switch recombination during the germinal response, meaning T-cell dependent antibody responses are reduced in PI3Kδ-deficient mice and skewed towards IgE [174].

The significant role of PI3Kδ in regulating B cell survival and proliferation guided the development of the PI3Kδ-selective inhibitor idelalisib (also known as CAL-101 or GS-1101), which has recently received FDA approval for the treatment of chronic lymphocytic leukaemia (CLL) and non-Hodgkin's lymphoma (iNHL) [175]. The results of early clinical trials have been very promising but further work has suggested a surprising mechanism of action, indicating idelalisib not only inhibits BCR-dependent survival pathways but also both the production and response to chemokines that serve to retain CLL cells in their protective lymph node microenvironments [175,176].

18. T cell-dependent functions

Class I PI3Ks play important roles in T cell development, survival, proliferation and differentiation that are in many ways analogous to those in B cells, though they differ in significant detail. PI3Kδ is again the main Class IA PI3K engaged by complex protein tyrosine pathways downstream of antigen receptor (TCR) signalling but important roles for PI3Kγ have also been identified downstream of Gi-coupled chemokine receptors [3,5]. PI3K-regulated outputs include PKB-dependent activation of mTORC1/2 and CARMA-Bcl10, the up-regulation of nutrient receptors, ITK-dependent regulation of PLCγ, activation of STAT and NFκ-B and the inhibition of FOXOs [3,5,177–180]. An interesting feedback loop has also been described based on PLCγ-catalysed synthesis of Ins(145)P3, iptk-dependent phosphorylation to Ins(1345)P4 and the binding of Ins(1345)P4 to the PH domain of ITK, promoting PIP3-driven recruitment of ITK oligomers to the membrane [181].

Mice lacking either functional PI3Kδ or PI3Kγ have only minor defects in T cell development, while mice lacking both isoforms have significantly blocked transition through the $CD4^-CD8^-$ stage (rearrangement of the TCR β chain) and reduced survival of $CD4^+CD8^+$ cells (rearrangement of the TCR α chain) [3,5,182,183]. In this process PI3Kδ acts downstream of the pre-TCR and PI3Kγ acts downstream of the Gi-coupled CXCR4 receptor (requiring inputs from both p101 and the RBD of 110γ) [184,185]. Class III PI3K has also be shown to have an important role in T cell development through regulating the surface expression of the IL-7R [186].

Class I PI3Ks feature in the signalling pathways stimulated by the engagement of naive $CD4^+$ cells by antigen/MHC-II loaded APCs and several lines of evidence suggest they are required to drive normal proliferation and differentiation towards the different Th effector subsets [3,5]. Thus, inhibition of PI3K signalling is variably associated with reduced Th1, Th2, Th17 and Tfh responses [187–189]. PI3Kδ appears to play the most prominent role [187,190,191], with additional roles for PI3Kγ [192]. CD28 is an important co-receptor for the TCR and possesses a YXXM motif, but this does not appear to be a non-redundant signal for Class IA PI3K recruitment [191]. PI3Kδ is required to produce a maximal germinal centre response [173], and here there appears to be a clear role for the co-receptor ICOS in driving direct recruitment of Class IA PI3Ks to its phosphorylated YXXM motif and stimulating sufficient PI3K activation to support Tfh function [193]. PI3Kγ is involved in supporting chemokine-induced homing and trafficking of $CD4^+$ effector cells to sites of inflammation [194].

Class I PI3Ks play a less important role in the activation and proliferation of $CD8^+$ cells after encounters with antigen/MHC-I [195]. However, PI3K/PKB signalling is important for initiating the transcription programme required for mature CTL function (including the expression of granzyme and perforin) and the inhibition of FOXO-mediated transcription of genes that is required for retention of $CD8^+$ cells in lymph nodes (CD62L, KLF2, CCR7 and S1P1) [195,196]. There is evidence that the PI3Kγ isoform is required for CTL chemotaxis and trafficking to sites of infection [197].

A role for Class I PI3Ks in the development and function of Tregs has also emerged [198]. Mice lacking functional PI3Kδ have increased numbers of Tregs in the thymus but reduced numbers in the periphery [199]. This may be a consequence of enhanced FOXO-mediated transcription of Foxp3, a critical transcription factor in defining Treg identity and survival [200,201]. Here, high PTEN expression may be required to limit Class I PI3K signalling and allow Foxp3 to drive naïve T-cells towards the Treg lineage [201,202]. Importantly however, Tregs lacking PI3Kδ do not escape and survive in the periphery and are inefficient suppressors of T-cell proliferation, suggesting PI3Kδ is required for their homing, survival and function [199].

So, the net effect of PI3Kδ inhibition on the adaptive immune system is confusing with several conflicting aspects. Overall, PI3Kδ is required for maximally effective humoral and cell-mediated immune responses but it is also required for effective Treg function. The potential importance of PI3Kδ in Tregs is nicely illustrated by a recent study showing that mice lacking functional PI3Kδ mount an enhanced immune attack on solid tumours; here the reduction in Treg function and enhanced CTL-mediated tumour killing is suggested to outweigh a reduction in the cell-autonomous function of the CTLs themselves [203]. Another example is that mice lacking functional PI3Kδ show enhanced clearance of *Leishmania major*, despite reduced Th1 responses, also possibly due to reduced Treg function [204]. Further, mice lacking functional PI3Kδ seem susceptible to autoimmune colitis when housed under animal house conditions [92]. Thus, the overall effect of PI3Kδ inhibition on T cell responses is likely to be highly context dependent.

19. Potential therapeutic opportunities

Given the central role of a dysregulated and over-active inflammatory response in many different human pathologies, and the important roles uncovered for PI3Ks in promoting inflammation, many studies have now investigated the effects of genetic or pharmacological inhibition of PI3K isoforms in mouse models of inflammatory disease (Table 2) [1,2,4,5,87,205,206]. There are difficulties in interpreting the results from these models, partly because in most cases the cause of the human immune condition is still unclear and hence the validity of the mouse model can be questioned, partly because mouse and human immune systems sometimes appear to behave differently (for example, in several of the mouse models of RA, neutrophilic infiltration is much more prominent than in the human disease) and partly because genetic ablation can create developmental defects which compromise an assessment of mature cell function. Nonetheless, they do represent opportunities to test the idea that single or combined inhibition of Class I PI3K isoforms might confer resistance to different types of chronic inflammation. This is particularly the case with the so called 'effector-phase' models, where the effects of the 'passive' transfer of disease-inducing serum are modelled, rather than complex underlying causes of the disease itself (such as loss of tolerance). On the whole, the results have been promising (Table 2): mice lacking PI3Kγ activity are resistant to models of SLE [207], RA [208,209], EAE [210], allergic asthma [129], systemic anaphylaxis [77] and cardiovascular disease [115,211]; mice lacking PI3Kδ activity are resistant to models of RA [212], EAE [213], allergic asthma [127,128,214] and cutaneous hypersensitivity [123]; mice lacking PI3Kβ activity are resistant to a model

Table 2
Examples of mouse models of chronic inflammatory disease in which the involvement of Class I PI3K isoforms has been evaluated.

Mouse Model	PI3K	Reference
SLE	PI3Kγ	[207]
	PI3Kδ	[228]
RA	PI3Kγ	[208,209,229]
	PI3Kδ	[212]
	PI3Kγ + δ	[212]
	PI3Kβ + δ	[76,230]
EAE/MS	PI3Kγ	[121,210,231]
	PI3Kδ	[213]
EBA	PI3Kβ	[76]
Asthma	PI3Kγ	[129]
	PI3Kδ	[127,128]
	PI3Kγ + δ	[126]
COPD	PI3Kδ	[232]
	PI3Kγ + δ	[126]
Allergy	PI3Kγ	[77]
	PI3Kδ	[123]
ALI/ARDS	PI3Kγ	[233]
Type-I diabetes	PI3Kγ	[234]
Atherosclerosis	PI3Kγ	[115]
Ischemic stroke	PI3Kδ	[235]
Colitis	PI3Kγ	[236]
Dermatitis	PI3Kγ	[237]
	PI3Kδ	[237]

of the autoimmune skin blistering disease epidermolysis bullosa acquisita (EBA) [76]. As might be predicted from their overlapping functions in inflammatory processes, mice deficient in both PI3Kγ and δ [212,215] or both PI3Kβ and δ [76] are more protected in a model of RA than mice lacking the individual PI3K isoforms. It should be noted that these mouse models are studied under 'clean' animal house conditions and hence the extent to which PI3K inhibition might lead to reduced resistance to infection is difficult to predict. In this regard mice lacking PI3Kγ can show increased susceptibility to some infections e.g. impaired resolution to pneumococcal infection [216] or impaired resistance to vaccinia virus [197]. Further, as described above, mice lacking functional PI3Kδ can show increased IgE-mediated eosinophilia and inflammation [172,174,190], but the extent to which this might be transferred to humans is unclear.

The recent discovery of activating mutations in the p110δ subunit in a group of primary immune deficiency patients both illustrates the importance of this isoform in the immune system and indicates some major gaps in our understanding [217,218]. These patients suffer recurring infections of the respiratory tract. They possess dominant mutations in p110δ that are analogous to oncogenic mutations in the kinase domain of p110α. These mutations result in enhanced binding of p110δ to the membrane and enhanced activity. In agreement, PIP3 levels in primary T cells from these patients were found to be significantly elevated. These patients exhibit distorted B and T cell differentiation and modestly reduced and skewed antibody responses, but a coherent explanation of their phenotype is still some distance away. Whatever the mechanism however, these patients are clearly candidates for treatment with PI3Kδ-selective inhibitors, such as those currently in development for the treatment of B-cell lymphomas (see above) [219].

20. Concluding remarks

A huge amount of work still remains to be done to reconcile information gained in studying PI3K signalling in simple *in vitro* models of immune cell function (i.e. simple regimens of agonist stimulation, often involving non-primary cells) with complex *in vivo* models of inflammation (i.e. involving multiple cell types with multiple stimuli involved). This is particularly so in humans, where the capacity for experimentation is necessarily more limited, though recent developments in high throughput sequencing promise to yield further insights from linking polymorphisms and mutations in PI3K pathway components to inflammatory disease.

However, we already know that for most of the *in vivo* processes involved, the effects of selective Class I PI3K isoform inhibition are usually partial. Specifically, we know that inhibition of PI3Kγ can blunt recruitment and activation of innate immune cells, but this is not complete; inhibition of PI3Kδ prevents a normal antibody response, but some antibodies are made; inhibition of PI3Kδ and β can inhibit antibody-dependent activation of neutrophils and macrophages, but bacterial uptake and killing is relatively unscathed. Thus, the robust and redundant processes that underlie the inflammatory response may allow an opportunity to inhibit Class I PI3K-dependent processes to a level where significant alleviation of the pathology is possible but sufficient capacity in the immune system still remains. Moreover, the tissue selective expression of PI3Kγ and δ in leukocytes offers the opportunity to inhibit Class I PI3Ks in these cells without necessarily incurring widespread toxicity and organ damage. Thus far, initial studies with mouse models of chronic inflammation appear to support this.

Further, the development of isoform-selective PI3K inhibitors by academic and commercial laboratories has proceeded at pace, driven largely by the promise of inhibiting cancer cell growth [87,205]. Several potential drugs are now in clinical trials and the results from these studies, particularly the development of the PI3Kδ-inhibitor idelalisib (CAL-101), suggest ATP-site inhibitors do indeed have the potential to turn into effective drugs, with little 'off-target' toxicity. The key question then becomes: what singly- or multiply-selective PI3K inhibitors are likely to prove most useful to treat which chronic inflammatory conditions [61,87,215,220]? Arguments can be made in favour of δ, γ, γ/δ or β/δ but in the end there is sufficient uncertainty in extrapolation from mouse models to human disease that a significant effort to trial various combinations in the best pre-clinical and clinical settings available seems unavoidable.

Acknowledgements

We would like to thank Geoff Butcher and Heidi Welch for critical reading of the manuscript and Anna Kielkowska and Mara Hawkins for help in the construction of some of the figures. Our laboratory receives long-term core support from the Biotechnology and Biological Sciences Research Council (ISPG BB/J004456/1).

References

[1] A. Ghigo, F. Damilano, L. Braccini, E. Hirsch, PI3K inhibition in inflammation: Toward tailored therapies for specific diseases, Bioessays 32 (2010) 185–196.
[2] E. Banham-Hall, M.R. Clatworthy, K. Okkenhaug, The therapeutic potential for PI3K inhibitors in autoimmune rheumatic diseases, Open Rheumatol. J. 6 (2012) 245–258.
[3] K. Okkenhaug, Signaling by the phosphoinositide 3-kinase family in immune cells, Annu. Rev. Immunol. 31 (2013) 675–704.
[4] J.G. Foster, M.D. Blunt, E. Carter, S.G. Ward, Inhibition of PI3K signaling spurs new therapeutic opportunities in inflammatory/autoimmune diseases and hematological malignancies, Pharmacol. Rev. 64 (2012) 1027–1054.
[5] L. So, D.A. Fruman, PI3K signalling in B- and T-lymphocytes: New developments and therapeutic advances, Biochem. J. 442 (2012) 465–481.
[6] C. Nathan, Points of control in inflammation, Nature 420 (2002) 846–852.
[7] C. Nathan, A. Ding, Nonresolving inflammation, Cell 140 (2010) 871–882.
[8] M. Karin, T. Lawrence, V. Nizet, Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer, Cell 124 (2006) 823–835.
[9] R. Medzhitov, Origin and physiological roles of inflammation, Nature 454 (2008) 428–435.
[10] H. Spits, D. Artis, M. Colonna, A. Diefenbach, J.P. Di Santo, et al., Innate lymphoid cells – a proposal for uniform nomenclature, Nat. Rev. Immunol. 13 (2013) 145–149.
[11] M. Phillipson, P. Kubes, The neutrophil in vascular inflammation, Nat. Med. 17 (2011) 1381–1390.
[12] M.A. Shelef, S. Tauzin, A. Huttenlocher, Neutrophil migration: moving from zebrafish models to human autoimmunity, Immunol. Rev. 256 (2013) 269–281.
[13] K. Newton, V.M. Dixit, Signaling in innate immunity and inflammation, Cold Spring Harb. Perspect. Biol. 4 (2012).
[14] E. Kolaczkowska, P. Kubes, Neutrophil recruitment and function in health and inflammation, Nat. Rev. Immunol. 13 (2013) 159–175.

[15] K. Ley, C. Laudanna, M.I. Cybulsky, S. Nourshargh, Getting to the site of inflammation: The leukocyte adhesion cascade updated, Nat. Rev. Immunol. 7 (2007) 678–689.
[16] C.N. Morrell, A.A. Aggrey, L.M. Chapman, K.L. Modjeski, Emerging roles for platelets as immune and inflammatory cells, Blood 123 (2014) 2759–2767.
[17] R.R. Hardy, P.W. Kincade, K. Dorshkind, The protean nature of cells in the B lymphocyte lineage, Immunity 26 (2007) 703–714.
[18] C. Linnemann, R. Mezzadra, T.N. Schumacher, TCR repertoires of intratumoral T-cell subsets, Immunol. Rev. 257 (2014) 72–82.
[19] A. Singer, S. Adoro, J.H. Park, Lineage fate and intense debate: myths, models and mechanisms of CD4- versus CD8-lineage choice, Nat. Rev. Immunol. 8 (2008) 788–801.
[20] J. Neefjes, M.L. Jongsma, P. Paul, O. Bakke, Towards a systems understanding of MHC class I and MHC class II antigen presentation, Nat. Rev. Immunol. 11 (2011) 823–836.
[21] J.M. Vyas, A.G. Van der Veen, H.L. Ploegh, The known unknowns of antigen processing and presentation, Nat. Rev. Immunol. 8 (2008) 607–618.
[22] K.M. Murphy, B. Stockinger, Effector T cell plasticity: flexibility in the face of changing circumstances, Nat. Immunol. 11 (2010) 674–680.
[23] P. Kidd, Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease, Altern. Med. Rev. 8 (2003) 223–246.
[24] G.D. Victora, M.C. Nussenzweig, Germinal centers, Annu. Rev. Immunol. 30 (2012) 429–457.
[25] K. Hirota, H. Ahlfors, J.H. Duarte, B. Stockinger, Regulation and function of innate and adaptive interleukin-17-producing cells, EMBO Rep. 13 (2012) 113–120.
[26] M. Barry, R.C. Bleackley, Cytotoxic T lymphocytes: all roads lead to death, Nat. Rev. Immunol. 2 (2002) 401–409.
[27] S.A. Oracki, J.A. Walker, M.L. Hibbs, L.M. Corcoran, D.M. Tarlinton, Plasma cell development and survival, Immunol. Rev. 237 (2010) 140–159.
[28] N. Schmitt, S.E. Bentebibel, H. Ueno, Phenotype and functions of memory Tfh cells in human blood, Trends Immunol. 35 (2014) 436–442.
[29] N.D. Bushar, D.L. Farber, Recalling the year in memory T cells, Ann. N. Y. Acad. Sci. 1143 (2008) 212–225.
[30] S.C. Bischoff, Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data, Nat. Rev. Immunol. 7 (2007) 93–104.
[31] H. Karasuyama, K. Mukai, Y. Tsujimura, K. Obata, Newly discovered roles for basophils: a neglected minority gains new respect, Nat. Rev. Immunol. 9 (2009) 9–13.
[32] Y. Bordon, Allergy: shocking behaviour, Nat. Rev. Immunol. 11 (2011) 302–303.
[33] H. von Boehmer, F. Melchers, Checkpoints in lymphocyte development and autoimmune disease, Nat. Immunol. 11 (2010) 14–20.
[34] S.Z. Josefowicz, A. Rudensky, Control of regulatory T cell lineage commitment and maintenance, Immunity 30 (2009) 616–625.
[35] E.C. Rosser, P.A. Blair, C. Mauri, Cellular targets of regulatory B cell-mediated suppression, Mol. Immunol. 62 (2014) 296–304.
[36] S. Nagaraj, J.I. Youn, D.I. Gabrilovich, Reciprocal relationship between myeloid-derived suppressor cells and T cells, J. Immunol. 191 (2013) 17–23.
[37] E. Rai, E.K. Wakeland, Genetic predisposition to autoimmunity – what have we learned? Semin. Immunol. 23 (2011) 67–83.
[38] J. Ermann, C.G. Fathman, Autoimmune diseases: genes, bugs and failed regulation, Nat. Immunol. 2 (2001) 759–761.
[39] B. Vanhaesebroeck, J. Guillermet-Guibert, M. Graupera, B. Bilanges, The emerging mechanisms of isoform-specific PI3K signalling, Nat. Rev. Mol. Cell Biol. 11 (2010) 329–341.
[40] P.T. Hawkins, K.E. Anderson, K. Davidson, L.R. Stephens, Signalling through Class I PI3Ks in mammalian cells, Biochem. Soc. Trans. 34 (2006) 647–662.
[41] M.A. Lemmon, Membrane recognition by phospholipid-binding domains, Nat. Rev. Mol. Cell Biol. 9 (2008) 99–111.
[42] M.S. Song, L. Salmena, P.P. Pandolfi, The functions and regulation of the PTEN tumour suppressor, Nat. Rev. Mol. Cell Biol. 13 (2012) 283–296.
[43] M.J. Hamilton, V.W. Ho, E. Kuroda, J. Ruschmann, F. Antignano, et al., Role of SHIP in cancer, Exp. Hematol. 39 (2011) 2–13.
[44] D. Blero, B. Payrastre, S. Schurmans, C. Erneux, Phosphoinositide phosphatases in a network of signalling reactions, Pflugers Arch. 455 (2007) 31–44.
[45] M. Huber, B.F. Gibbs, SHIP1 and the negative control of mast cell/basophil activation by supra-optimal antigen concentrations, Mol. Immunol. 63 (2015) 32–37.
[46] J.M. Dyson, C.G. Fedele, E.M. Davies, J. Becanovic, C.A. Mitchell, Phosphoinositide phosphatases: just as important as the kinases, Subcell. Biochem. 58 (2012) 215–279.
[47] L.R. Stephens, K.T. Hughes, R.F. Irvine, Pathway of phosphatidylinositol(3,4,5)-trisphosphate synthesis in activated neutrophils, Nature 351 (1991) 33–39.
[48] S. Krugmann, K.E. Anderson, S.H. Ridley, N. Risso, A. McGregor, et al., Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices, Mol. Cell 9 (2002) 95–108.
[49] A. Allam, A.J. Marshall, Role of the adaptor proteins Bam32, TAPP1 and TAPP2 in lymphocyte activation, Immunol. Lett. 97 (2005) 7–17.
[50] M.D. Blunt, S.G. Ward, Targeting PI3K isoforms and SHIP in the immune system: new therapeutics for inflammation and leukemia, Curr. Opin. Pharmacol. 12 (2012) 444–451.
[51] B.D. Manning, L.C. Cantley, AKT/PKB signaling: navigating downstream, Cell 129 (2007) 1261–1274.
[52] J.A. Engelman, J. Luo, L.C. Cantley, The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism, Nat. Rev. Genet. 7 (2006) 606–619.
[53] D.K. Finlay, mTORC1 regulates CD8+ T-cell glucose metabolism and function independently of PI3K and PKB, Biochem. Soc. Trans. 41 (2013) 681–686.
[54] K.U. Birkenkamp, P.J. Coffer, FOXO transcription factors as regulators of immune homeostasis: molecules to die for? J. Immunol. 171 (2003) 1623–1629.
[55] J.M. Bradshaw, The Src, Syk, and Tec family kinases: distinct types of molecular switches, Cell. Signal. 22 (2010) 1175–1184.
[56] Q. Deng, A. Huttenlocher, Leukocyte migration from a fish eye's view, J. Cell Sci. 125 (2012) 3949–3956.
[57] L. Stephens, L. Milne, P. Hawkins, Moving towards a better understanding of chemotaxis, Curr. Biol. 18 (2008) R485–R494.
[58] Y. Mazaki, Y. Nishimura, H. Sabe, GBF1 bears a novel phosphatidylinositol-phosphate binding module, BP3K, to link PI3Kgamma activity with Arf1 activation involved in GPCR-mediated neutrophil chemotaxis and superoxide production, Mol. Biol. Cell 23 (2012) 2457–2467.
[59] R. Fritsch, J. Downward, SnapShot: Class I PI3K isoform signaling, Cell 154 (2013) 940–940.e1.
[60] O. Vadas, J.E. Burke, X. Zhang, A. Berndt, R.L. Williams, Structural basis for activation and inhibition of class I phosphoinositide 3-kinases, Sci. Signal. 4 (2011) re2.
[61] C. Costa, E.L. Martin-Conte, E. Hirsch, Phosphoinositide 3-kinase p110gamma in immunity, IUBMB Life 63 (2011) 707–713.
[62] S. Suire, A.M. Condliffe, G.J. Ferguson, C.D. Ellson, H. Guillou, et al., Gbetagammas and the Ras binding domain of p110gamma are both important regulators of PI(3)Kgamma signalling in neutrophils, Nat. Cell Biol. 8 (2006) 1303–1309.
[63] T. Bohnacker, R. Marone, E. Collmann, R. Calvez, E. Hirsch, et al., PI3Kgamma adaptor subunits define coupling to degranulation and cell motility by distinct PtdIns(3,4,5)P3 pools in mast cells, Sci. Signal. 2 (2009) ra27.
[64] B. Kurig, A. Shymanets, T. Bohnacker, Brock C. Prajwal, et al., Ras is an indispensable coregulator of the class IB phosphoinositide 3-kinase p87/p110gamma, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 20312–20317.
[65] S. Suire, J. Coadwell, G.J. Ferguson, K. Davidson, P. Hawkins, et al., p84, a new Gbetagamma-activated regulatory subunit of the type IB phosphoinositide 3-kinase p110gamma, Curr. Biol. 15 (2005) 566–570.
[66] R. Walser, J.E. Burke, E. Gogvadze, T. Bohnacker, X. Zhang, et al., PKCbeta phosphorylates PI3Kgamma to activate it and release it from GPCR control, PLoS Biol. 11 (2013) e1001587.
[67] R. Fritsch, I. de Krijger, K. Fritsch, R. George, B. Reason, et al., RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms, Cell 153 (2013) 1050–1063.
[68] J.M. Backer, The regulation of class IA PI 3-kinases by inter-subunit interactions, Curr. Top. Microbiol. Immunol. 346 (2010) 87–114.
[69] O. Vadas, H.A. Dbouk, A. Shymanets, O. Perisic, J.E. Burke, et al., Molecular determinants of PI3Kgamma-mediated activation downstream of G-protein-coupled receptors (GPCRs), Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 18862–18867.
[70] H.A. Dbouk, O. Vadas, A. Shymanets, J.E. Burke, R.S. Salamon, et al., G protein-coupled receptor-mediated activation of p110beta by Gbetagamma is required for cellular transformation and invasiveness, Sci. Signal. 5 (2012) ra89.
[71] Z. Dou, J.A. Pan, H.A. Dbouk, L.M. Ballou, J.L. DeLeon, et al., Class IA PI3K p110beta subunit promotes autophagy through Rab5 small GTPase in response to growth factor limitation, Mol. Cell 50 (2013) 29–42.
[72] L. So, S.S. Yea, J.S. Oak, M. Lu, A. Manmadhan, et al., Selective inhibition of phosphoinositide 3-kinase p110alpha preserves lymphocyte function, J. Biol. Chem. 288 (2013) 5718–5731.
[73] J.K. Juss, R.P. Hayhoe, C.E. Owen, I. Bruce, S.R. Walmsley, et al., Functional redundancy of class I phosphoinositide 3-kinase (PI3K) isoforms in signaling growth factor-mediated human neutrophil survival, PLoS ONE 7 (2012) e45933.
[74] G. Takayama, M. Ohtani, A. Minowa, S. Matsuda, S. Koyasu, Class I PI3K-mediated Akt and ERK signals play a critical role in FcepsilonRI-induced degranulation in mast cells, Int. Immunol. 25 (2013) 215–220.
[75] A.M. Condliffe, K. Davidson, K.E. Anderson, C.D. Ellson, T. Crabbe, et al., Sequential activation of class IB and class IA PI3K is important for the primed respiratory burst of human but not murine neutrophils, Blood 106 (2005) 1432–1440.
[76] S. Kulkarni, C. Sitaru, Z. Jakus, K.E. Anderson, G. Damoulakis, et al., PI3Kbeta plays a critical role in neutrophil activation by immune complexes, Sci. Signal. 4 (2011) ra23.
[77] M. Laffargue, R. Calvez, P. Finan, A. Trifilieff, M. Barbier, et al., Phosphoinositide 3-kinase gamma is an essential amplifier of mast cell function, Immunity 16 (2002) 441–451.
[78] K.O. Schink, C. Raiborg, H. Stenmark, Phosphatidylinositol 3-phosphate, a lipid that regulates membrane dynamics, protein sorting and cell signalling, Bioessays 35 (2013) 900–912.
[79] J.M. Backer, The regulation and function of Class III PI3Ks: novel roles for Vps34, Biochem. J. 410 (2008) 1–17.
[80] F. O' Farrell, T.E. Rusten, H. Stenmark, Phosphoinositide 3-kinases as accelerators and brakes of autophagy, FEBS J. 280 (2013) 6322–6337.
[81] R. Roberts, N.T. Ktistakis, Omegasomes: PI3P platforms that manufacture autophagosomes, Essays Biochem. 55 (2013) 17–27.
[82] J. Kim, Y.C. Kim, C. Fang, R.C. Russell, J.H. Kim, et al., Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy, Cell 152 (2013) 290–303.
[83] M. Falasca, T. Maffucci, Regulation and cellular functions of class II phosphoinositide 3-kinases, Biochem. J. 443 (2012) 587–601.
[84] K. Yoshioka, K. Yoshida, H. Cui, T. Wakayama, N. Takuwa, et al., Endothelial PI3K-C2alpha, a class II PI3K, has an essential role in angiogenesis and vascular barrier function, Nat. Med. 18 (2012) 1560–1569.
[85] I. Franco, F. Gulluni, C.C. Campa, C. Costa, J.P. Margaria, et al., PI3K class II alpha controls spatially restricted endosomal PtdIns3P and Rab11 activation to promote primary cilium function, Dev. Cell 28 (2014) 647–658.
[86] Y. Posor, M. Eichhorn-Gruenig, D. Puchkov, J. Schoneberg, A. Ullrich, et al., Spatiotemporal control of endocytosis by phosphatidylinositol-3,4-bisphosphate, Nature 499 (2013) 233–237.

[87] T.D. Cushing, D.P. Metz, D.A. Whittington, L.R. McGee, PI3Kdelta and PI3Kgamma as targets for autoimmune and inflammatory diseases, J. Med. Chem. 55 (2012) 8559–8581.
[88] E. Hirsch, V.L. Katanaev, C. Garlanda, O. Azzolino, L. Pirola, et al., Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation, Science 287 (2000) 1049–1053.
[89] Z. Li, H. Jiang, W. Xie, Z. Zhang, A.V. Smrcka, et al., Roles of PLC-beta2 and -beta3 and PI3Kgamma in chemoattractant-mediated signal transduction, Science 287 (2000) 1046–1049.
[90] T. Sasaki, J. Irie-Sasaki, R.G. Jones, A.J. Oliveira-dos-Santos, W.L. Stanford, et al., Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration, Science 287 (2000) 1040–1046.
[91] E. Clayton, G. Bardi, S.E. Bell, D. Chantry, C.P. Downes, et al., A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase in B cell development and activation, J. Exp. Med. 196 (2002) 753–763.
[92] K. Okkenhaug, A. Bilancio, G. Farjot, H. Priddle, S. Sancho, et al., Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice, Science 297 (2002) 1031–1034.
[93] X. Zhou, J. Takatoh, F. Wang, The mammalian class 3 PI3K (PIK3C3) is required for early embryogenesis and cell proliferation, PLoS ONE 6 (2011) e16358.
[94] R. Bago, N. Malik, M.J. Munson, A.R. Prescott, P. Davies, et al., Characterization of VPS34-IN1, a selective inhibitor of Vps34, reveals that the phosphatidylinositol 3-phosphate-binding SGK3 protein kinase is a downstream target of class III phosphoinositide 3-kinase, Biochem. J. 463 (2014) 413–427.
[95] B. Ronan, O. Flamand, L. Vescovi, C. Dureuil, L. Durand, et al., A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy, Nat. Chem. Biol. 10 (2014) 1013–1019.
[96] D.P. Harris, P. Vogel, M. Wims, K. Moberg, J. Humphries, et al., Requirement for class II phosphoinositide 3-kinase C2alpha in maintenance of glomerular structure and function, Mol. Cell. Biol. 31 (2011) 63–80.
[97] K. Harada, A.B. Truong, T. Cai, P.A. Khavari, The class II phosphoinositide 3-kinase C2beta is not essential for epidermal differentiation, Mol. Cell. Biol. 25 (2005) 11122–11130.
[98] G.J. Ferguson, L. Milne, S. Kulkarni, T. Sasaki, S. Walker, et al., PI(3)Kgamma has an important context-dependent role in neutrophil chemokinesis, Nat. Cell Biol. 9 (2007) 86–91.
[99] D.F. Smith, T.L. Deem, A.C. Bruce, J. Reutershan, D. Wu, et al., Leukocyte phosphoinositide-3 kinase {gamma} is required for chemokine-induced, sustained adhesion under flow in vivo, J. Leukoc. Biol. 80 (2006) 1491–1499.
[100] L. Barberis, C. Pasquali, D. Bertschy-Meier, A. Cuccurullo, C. Costa, et al., Leukocyte transmigration is modulated by chemokine-mediated PI3Kgamma-dependent phosphorylation of vimentin, Eur. J. Immunol. 39 (2009) 1136–1146.
[101] D.H. Lim, J.Y. Cho, D.J. Song, S.Y. Lee, M. Miller, et al., PI3K gamma-deficient mice have reduced levels of allergen-induced eosinophilic inflammation and airway remodeling, Am. J. Physiol. Lung Cell. Mol. Physiol. 296 (2009) L210–L219.
[102] G.E. Jones, E. Prigmore, R. Calvez, C. Hogan, G.A. Dunn, et al., Requirement for PI 3-kinase gamma in macrophage migration to MCP-1 and CSF-1, Exp. Cell Res. 290 (2003) 120–131.
[103] C. Sadhu, B. Masinovsky, K. Dick, C.G. Sowell, D.E. Staunton, Essential role of phosphoinositide 3-kinase delta in neutrophil directional movement, J. Immunol. 170 (2003) 2647–2654.
[104] K.D. Puri, T.A. Doggett, J. Douangpanya, Y. Hou, W.T. Tino, et al., Mechanisms and implications of phosphoinositide 3-kinase delta in promoting neutrophil trafficking into inflamed tissue, Blood 103 (2004) 3448–3456.
[105] K.D. Puri, T.A. Doggett, C.Y. Huang, J. Douangpanya, J.S. Hayflick, et al., The role of endothelial PI3Kgamma activity in neutrophil trafficking, Blood 106 (2005) 150–157.
[106] E. Sapey, J.A. Stockley, H. Greenwood, A. Ahmad, D. Bayley, et al., Behavioral and structural differences in migrating peripheral neutrophils from patients with chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 183 (2011) 1176–1186.
[107] R. Shimizu-Hirota, W. Xiong, B.T. Baxter, S.L. Kunkel, I. Maillard, et al., MT1-MMP regulates the PI3Kdelta.Mi-2/NuRD-dependent control of macrophage immune function, Genes Dev. 26 (2012) 395–413.
[108] Y. Leverrier, K. Okkenhaug, C. Sawyer, A. Bilancio, B. Vanhaesebroeck, et al., Class I phosphoinositide 3-kinase p110beta is required for apoptotic cell and Fcgamma receptor-mediated phagocytosis by macrophages, J. Biol. Chem. 278 (2003) 38437–38442.
[109] K.B. Boyle, D. Gyori, A. Sindrilaru, K. Scharffetter-Kochanek, P.R. Taylor, et al., Class IA phosphoinositide 3-kinase beta and delta regulate neutrophil oxidase activation in response to Aspergillus fumigatus hyphae, J. Immunol. 186 (2011) 2978–2989.
[110] A. Mocsai, J. Ruland, V.L. Tybulewicz, The SYK tyrosine kinase: a crucial player in diverse biological functions, Nat. Rev. Immunol. 10 (2010) 387–402.
[111] P. Beemiller, Y. Zhang, S. Mohan, E. Levinsohn, I. Gaeta, et al., A Cdc42 activation cycle coordinated by PI 3-kinase during Fc receptor-mediated phagocytosis, Mol. Biol. Cell 21 (2010) 470–480.
[112] D. Cox, C.C. Tseng, G. Bjekic, S. Greenberg, A requirement for phosphatidylinositol 3-kinase in pseudopod extension, J. Biol. Chem. 274 (1999) 1240–1247.
[113] K.E. Anderson, K.B. Boyle, K. Davidson, T.A. Chessa, S. Kulkarni, et al., CD18-dependent activation of the neutrophil NADPH oxidase during phagocytosis of Escherichia coli or Staphylococcus aureus is regulated by class III but not class I or II PI3Ks, Blood 112 (2008) 5202–5211.
[114] P.T. Hawkins, L.R. Stephens, S. Suire, M. Wilson, PI3K signaling in neutrophils, Curr. Top. Microbiol. Immunol. 346 (2010) 183–202.
[115] J.D. Chang, G.K. Sukhova, P. Libby, E. Schvartz, A.H. Lichtenstein, et al., Deletion of the phosphoinositide 3-kinase p110gamma gene attenuates murine atherosclerosis, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 8077–8082.
[116] J. Guillermet-Guibert, K. Bjorklof, A. Salpekar, C. Gonella, F. Ramadani, et al., The p110beta isoform of phosphoinositide 3-kinase signals downstream of G protein-coupled receptors and is functionally redundant with p110gamma, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 8292–8297.
[117] J.A. Swanson, Shaping cups into phagosomes and macropinosomes, Nat. Rev. Mol. Cell Biol. 9 (2008) 639–649.
[118] T.D. Troutman, J.F. Bazan, C. Pasare, Toll-like receptors, signaling adapters and regulation of the pro-inflammatory response by PI3K, Cell Cycle 11 (2012) 3559–3567.
[119] E. Aksoy, S. Taboubi, D. Torres, S. Delbauve, A. Hachani, et al., The p110delta isoform of the kinase PI(3)K controls the subcellular compartmentalization of TLR4 signaling and protects from endotoxic shock, Nat. Immunol. 13 (2012) 1045–1054.
[120] A. Del Prete, W. Vermi, E. Dander, K. Otero, L. Barberis, et al., Defective dendritic cell migration and activation of adaptive immunity in PI3Kgamma-deficient mice, EMBO J. 23 (2004) 3505–3515.
[121] I. Comerford, W. Litchfield, E. Kara, S.R. McColl, PI3Kgamma drives priming and survival of autoreactive CD4(+) T cells during experimental autoimmune encephalomyelitis, PLoS ONE 7 (2012) e45095.
[122] A.M. Condliffe, P.T. Hawkins, L.R. Stephens, C. Haslett, E.R. Chilvers, Priming of human neutrophil superoxide generation by tumour necrosis factor-alpha is signalled by enhanced phosphatidylinositol 3,4,5-trisphosphate but not inositol 1,4,5-trisphosphate accumulation, FEBS Lett. 439 (1998) 147–151.
[123] K. Ali, M. Camps, W.P. Pearce, H. Ji, T. Ruckle, et al., Isoform-specific functions of phosphoinositide 3-kinases: p110 delta but not p110 gamma promotes optimal allergic responses in vivo, J. Immunol. 180 (2008) 2538–2544.
[124] H. Gu, K. Saito, L.D. Klaman, J. Shen, T. Fleming, et al., Essential role for Gab2 in the allergic response, Nature 412 (2001) 186–190.
[125] E. Collmann, T. Bohnacker, R. Marone, J. Dawson, M. Rehberg, et al., Transient targeting of phosphoinositide 3-kinase acts as a roadblock in mast cells' route to allergy, J. Allergy Clin. Immunol. 132 (2013) 959–968.
[126] J. Doukas, L. Eide, K. Stebbins, A. Racanelli-Layton, L. Dellamary, et al., Aerosolized phosphoinositide 3-kinase gamma/delta inhibitor TG100-115 [3-[2,4-diamino-6-(3-hydroxyphenyl)pteridin-7-yl]phenol] as a therapeutic candidate for asthma and chronic obstructive pulmonary disease, J. Pharmacol. Exp. Ther. 328 (2009) 758–765.
[127] B.F. Nashed, T. Zhang, M. Al-Alwan, G. Srinivasan, A.J. Halayko, et al., Role of the phosphoinositide 3-kinase p110delta in generation of type 2 cytokine responses and allergic airway inflammation, Eur. J. Immunol. 37 (2007) 416–424.
[128] S.J. Park, K.S. Lee, S.R. Kim, K.H. Min, H. Moon, et al., Phosphoinositide 3-kinase delta inhibitor suppresses interleukin-17 expression in a murine asthma model, Eur. Respir. J. 36 (2010) 1448–1459.
[129] M. Takeda, W. Ito, M. Tanabe, S. Ueki, H. Kato, et al., Allergic airway hyperresponsiveness, inflammation, and remodeling do not develop in phosphoinositide 3-kinase gamma-deficient mice, J. Allergy Clin. Immunol. 123 (2009) 805–812.
[130] N. Kim, A. Saudemont, L. Webb, M. Camps, T. Ruckle, et al., The p110delta catalytic isoform of PI3K is a key player in NK-cell development and cytokine secretion, Blood 110 (2007) 3202–3208.
[131] W.G. Kerr, F. Colucci, Inositol phospholipid signaling and the biology of natural killer cells, J. Innate Immun. 3 (2011) 249–257.
[132] G. Krystal, Lipid phosphatases in the immune system, Semin. Immunol. 12 (2000) 397–403.
[133] J. Ruschmann, F. Antignano, V. Lam, K. Snyder, C. Kim, et al., The role of SHIP in the development and activation of mouse mucosal and connective tissue mast cells, J. Immunol. 188 (2012) 3839–3850.
[134] M.J. Rauh, J. Kalesnikoff, M. Hughes, L. Sly, V. Lam, et al., Role of Src homology 2-containing-inositol 5'-phosphatase (SHIP) in mast cells and macrophages, Biochem. Soc. Trans. 31 (2003) 286–291.
[135] M. Nishio, K. Watanabe, J. Sasaki, C. Taya, S. Takasuga, et al., Control of cell polarity and motility by the PtdIns(3,4,5)P3 phosphatase SHIP1, Nat. Cell Biol. 9 (2007) 36–44.
[136] A. Prasad, Y. Jia, A. Chakraborty, Y. Li, S.K. Jain, et al., Inositol hexakisphosphate kinase 1 regulates neutrophil function in innate immunity by inhibiting phosphatidylinositol-(3,4,5)-trisphosphate signaling, Nat. Immunol. 12 (2011) 752–760.
[137] M. Bohdanowicz, S. Grinstein, Role of phospholipids in endocytosis, phagocytosis, and macropinocytosis, Physiol. Rev. 93 (2013) 69–106.
[138] O.V. Vieira, R.J. Botelho, L. Rameh, S.M. Brachmann, T. Matsuo, et al., Distinct roles of class I and class III phosphatidylinositol 3-kinases in phagosome formation and maturation, J. Cell Biol. 155 (2001) 19–25.
[139] C.D. Ellson, K.E. Anderson, G. Morgan, E.R. Chilvers, P. Lipp, et al., Phosphatidylinositol 3-phosphate is generated in phagosomal membranes, Curr. Biol. 11 (2001) 1631–1635.
[140] S.B. Berger, X. Romero, C. Ma, G. Wang, W.A. Faubion, et al., SLAM is a microbial sensor that regulates bacterial phagosome functions in macrophages, Nat. Immunol. 11 (2010) 920–927.
[141] O.V. Vieira, R.J. Botelho, S. Grinstein, Phagosome maturation: aging gracefully, Biochem. J. 366 (2002) 689–704.
[142] C.I. Suh, N.D. Stull, X.J. Li, W. Tian, M.O. Price, et al., The phosphoinositide-binding protein p40phox activates the NADPH oxidase during FcgammaIIA receptor-induced phagocytosis, J. Exp. Med. 203 (2006) 1915–1925.
[143] C.D. Ellson, K. Davidson, G.J. Ferguson, R. O'Connor, L.R. Stephens, et al., Neutrophils from p40phox −/− mice exhibit severe defects in NADPH oxidase regulation and oxidant-dependent bacterial killing, J. Exp. Med. 203 (2006) 1927–1937.
[144] M.C. Dinauer, Chronic granulomatous disease and other disorders of phagocyte function, Hematology Am. Soc. Hematol. Educ. Program (2005) 89–95.

[145] E.R. Allan, P. Tailor, D.R. Balce, P. Pirzadeh, N.T. McKenna, et al., NADPH oxidase modifies patterns of MHC class II-restricted epitopic repertoires through redox control of antigen processing, J. Immunol. 192 (2014) 4989–5001.
[146] A. Savina, C. Jancic, S. Hugues, P. Guermonprez, P. Vargas, et al., NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells, Cell 126 (2006) 205–218.
[147] C. Ellson, K. Davidson, K. Anderson, L.R. Stephens, P.T. Hawkins, PtdIns3P binding to the PX domain of p40phox is a physiological signal in NADPH oxidase activation, EMBO J. 25 (2006) 4468–4478.
[148] J.D. Matute, A.A. Arias, N.A. Wright, I. Wrobel, C.C. Waterhouse, et al., A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40 phox and selective defects in neutrophil NADPH oxidase activity, Blood 114 (2009) 3309–3315.
[149] X. Xu, K. Araki, S. Li, J.H. Han, L. Ye, et al., Autophagy is essential for effector CD8(+) T cell survival and memory formation, Nat. Immunol. 15 (2014) 1152–1161.
[150] K.B. Boyle, F. Randow, The role of 'eat-me' signals and autophagy cargo receptors in innate immunity, Curr. Opin. Microbiol. 16 (2013) 339–348.
[151] B. Levine, N. Mizushima, H.W. Virgin, Autophagy in immunity and inflammation, Nature 469 (2011) 323–335.
[152] B. Yordy, A. Iwasaki, Autophagy in the control and pathogenesis of viral infection, Curr. Opin. Virol. 1 (2011) 196–203.
[153] C. Munz, Enhancing immunity through autophagy, Annu. Rev. Immunol. 27 (2009) 423–449.
[154] K.D. Puri, M.R. Gold, Selective inhibitors of phosphoinositide 3-kinase delta: modulators of B-cell function with potential for treating autoimmune inflammatory diseases and B-cell malignancies, Front. Immunol. 3 (2012) 256.
[155] L.C. Osborne, K.A. Duthie, J.H. Seo, R.D. Gascoyne, N. Abraham, Selective ablation of the YxxM motif of IL-7Ralpha suppresses lymphomagenesis but maintains lymphocyte development, Oncogene 29 (2010) 3854–3864.
[156] R.H. Amin, M.S. Schlissel, Foxo1 directly regulates the transcription of recombination-activating genes during B cell development, Nat. Immunol. 9 (2008) 613–622.
[157] H.S. Dengler, G.V. Baracho, S.A. Omori, S. Bruckner, K.C. Arden, et al., Distinct functions for the transcription factor Foxo1 at various stages of B cell differentiation, Nat. Immunol. 9 (2008) 1388–1398.
[158] R.C. Rickert, New insights into pre-BCR and BCR signalling with relevance to B cell malignancies, Nat. Rev. Immunol. 13 (2013) 578–591.
[159] C.D. Browne, C.J. Del Nagro, M.H. Cato, H.S. Dengler, R.C. Rickert, Suppression of phosphatidylinositol 3,4,5-trisphosphate production is a key determinant of B cell anergy, Immunity 31 (2009) 749–760.
[160] L. Srinivasan, Y. Sasaki, D.P. Calado, B. Zhang, J.H. Paik, et al., PI3 kinase signals BCR-dependent mature B cell survival, Cell 139 (2009) 573–586.
[161] F. Ramadani, D.J. Bolland, F. Garcon, J.L. Emery, B. Vanhaesebroeck, et al., The PI3K isoforms p110alpha and p110delta are essential for pre-B cell receptor signaling and B cell development, Sci. Signal. 3 (2010) ra60.
[162] M.E. Conley, A.K. Dobbs, A.M. Quintana, A. Bosompem, Y.D. Wang, et al., Agammaglobulinemia and absent B lineage cells in a patient lacking the p85alpha subunit of PI3K, J. Exp. Med. 209 (2012) 463–470.
[163] C.A. Durand, K. Hartvigsen, L. Fogelstrand, S. Kim, S. Iritani, et al., Phosphoinositide 3-kinase p110 delta regulates natural antibody production, marginal zone and B-1 B cell function, and autoantibody responses, J. Immunol. 183 (2009) 5673–5684.
[164] S.A. Omori, M.H. Cato, A. Anzelon-Mills, K.D. Puri, M. Shapiro-Shelef, et al., Regulation of class-switch recombination and plasma cell differentiation by phosphatidylinositol 3-kinase signaling, Immunity 25 (2006) 545–557.
[165] Y. Aiba, M. Kameyama, T. Yamazaki, T.F. Tedder, T. Kurosaki, Regulation of B-cell development by BCAP and CD19 through their binding to phosphoinositide 3-kinase, Blood 111 (2008) 1497–1503.
[166] D.A. Fruman, G. Bismuth, Fine tuning the immune response with PI3K, Immunol. Rev. 228 (2009) 253–272.
[167] A. Alkhatib, M. Werner, E. Hug, S. Herzog, C. Eschbach, et al., FoxO1 induces Ikaros splicing to promote immunoglobulin gene recombination, J. Exp. Med. 209 (2012) 395–406.
[168] M. Werner, E. Hobeika, H. Jumaa, Role of PI3K in the generation and survival of B cells, Immunol. Rev. 237 (2010) 55–71.
[169] I. Isnardi, P. Bruhns, G. Bismuth, W.H. Fridman, M. Daeron, The SH2 domain-containing inositol 5-phosphatase SHIP1 is recruited to the intracytoplasmic domain of human FcgammaRIIB and is mandatory for negative regulation of B cell activation, Immunol. Lett. 104 (2006) 156–165.
[170] S.T. Jou, N. Carpino, Y. Takahashi, R. Piekorz, J.R. Chao, et al., Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the B-cell receptor complex, Mol. Cell. Biol. 22 (2002) 8580–8591.
[171] A.C. Donahue, D.A. Fruman, Proliferation and survival of activated B cells requires sustained antigen receptor engagement and phosphoinositide 3-kinase activation, J. Immunol. 170 (2003) 5851–5860.
[172] T.T. Zhang, K. Okkenhaug, B.F. Nashed, K.D. Puri, Z.A. Knight, et al., Genetic or pharmaceutical blockade of p110delta phosphoinositide 3-kinase enhances IgE production, J. Allergy Clin. Immunol. 122 (2008) 811–812.e2.
[173] J. Rolf, S.E. Bell, D. Kovesdi, M.L. Janas, D.R. Soond, et al., Phosphoinositide 3-kinase activity in T cells regulates the magnitude of the germinal center reaction, J. Immunol. 185 (2010) 4042–4052.
[174] T.T. Zhang, K.J. Makondo, A.J. Marshall, p110delta phosphoinositide 3-kinase represses IgE switch by potentiating BCL6 expression, J. Immunol. 188 (2012) 3700–3708.
[175] D.A. Fruman, L.C. Cantley, Idelalisib–a PI3Kdelta inhibitor for B-cell cancers, N. Engl. J. Med. 370 (2014) 1061–1062.
[176] J.A. Burger, K. Okkenhaug, Haematological cancer: idelalisib-targeting PI3Kdelta in patients with B-cell malignancies, Nat. Rev. Clin. Oncol. 11 (2014) 184–186.
[177] A.P. Kelly, D.K. Finlay, H.J. Hinton, R.G. Clarke, E. Fiorini, et al., Notch-induced T cell development requires phosphoinositide-dependent kinase 1, EMBO J. 26 (2007) 3441–3450.
[178] G.M. Delgoffe, T.P. Kole, Y. Zheng, P.E. Zarek, K.L. Matthews, et al., The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment, Immunity 30 (2009) 832–844.
[179] G.M. Delgoffe, K.N. Pollizzi, A.T. Waickman, E. Heikamp, D.J. Meyers, et al., The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2, Nat. Immunol. 12 (2011) 295–303.
[180] J. Cheng, B. Phong, D.C. Wilson, R. Hirsch, L.P. Kane, Akt fine-tunes NF-kappaB-dependent gene expression during T cell activation, J. Biol. Chem. 286 (2011) 36076–36085.
[181] Y.H. Huang, K. Sauer, Lipid signaling in T-cell development and function, Cold Spring Harb. Perspect. Biol. 2 (2010) a002428.
[182] L.M. Webb, E. Vigorito, M.P. Wymann, E. Hirsch, M. Turner, Cutting edge: T cell development requires the combined activities of the p110gamma and p110delta catalytic isoforms of phosphatidylinositol 3-kinase, J. Immunol. 175 (2005) 2783–2787.
[183] W. Swat, V. Montgrain, T.A. Doggett, J. Douangpanya, K. Puri, et al., Essential role of PI3Kdelta and PI3Kgamma in thymocyte survival, Blood 107 (2006) 2415–2422.
[184] M.L. Janas, G. Varano, K. Gudmundsson, M. Noda, T. Nagasawa, et al., Thymic development beyond beta-selection requires phosphatidylinositol 3-kinase activation by CXCR4, J. Exp. Med. 207 (2010) 247–261.
[185] M.L. Janas, M. Turner, Interaction of Ras with p110gamma is required for thymic beta-selection in the mouse, J. Immunol. 187 (2011) 4667–4675.
[186] I.X. McLeod, X. Zhou, Q.J. Li, F. Wang, Y.W. He, The class III kinase Vps34 promotes T lymphocyte survival through regulating IL-7Ralpha surface expression, J. Immunol. 187 (2011) 5051–5061.
[187] K. Okkenhaug, D.T. Patton, A. Bilancio, F. Garcon, W.C. Rowan, et al., The p110delta isoform of phosphoinositide 3-kinase controls clonal expansion and differentiation of Th cells, J. Immunol. 177 (2006) 5122–5128.
[188] D.R. Soond, E. Bjorgo, K. Moltu, V.Q. Dale, D.T. Patton, et al., PI3K p110delta regulates T-cell cytokine production during primary and secondary immune responses in mice and humans, Blood 115 (2010) 2203–2213.
[189] Q. Wan, L. Kozhaya, A. ElHed, R. Ramesh, T.J. Carlson, et al., Cytokine signals through PI-3 kinase pathway modulate Th17 cytokine production by CCR6+ human memory T cells, J. Exp. Med. 208 (2011) 1875–1887.
[190] H. Ji, F. Rintelen, C. Waltzinger, D. Bertschy Meier, A. Bilancio, et al., Inactivation of PI3Kgamma and PI3Kdelta distorts T-cell development and causes multiple organ inflammation, Blood 110 (2007) 2940–2947.
[191] F. Garcon, D.T. Patton, J.L. Emery, E. Hirsch, R. Rottapel, et al., CD28 provides T-cell costimulation and enhances PI3K activity at the immune synapse independently of its capacity to interact with the p85/p110 heterodimer, Blood 111 (2008) 1464–1471.
[192] N. Ladygina, S. Gottipati, K. Ngo, G. Castro, J.Y. Ma, et al., PI3Kgamma kinase activity is required for optimal T-cell activation and differentiation, Eur. J. Immunol. 43 (2013) 3183–3196.
[193] M. Gigoux, J. Shang, Y. Pak, M. Xu, J. Choe, et al., Inducible costimulator promotes helper T-cell differentiation through phosphoinositide 3-kinase, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 20371–20376.
[194] M.S. Thomas, J.S. Mitchell, C.C. DeNucci, A.L. Martin, Y. Shimizu, The p110gamma isoform of phosphatidylinositol 3-kinase regulates migration of effector CD4 T lymphocytes into peripheral inflammatory sites, J. Leukoc. Biol. 84 (2008) 814–823.
[195] A.N. Macintyre, D. Finlay, G. Preston, L.V. Sinclair, C.M. Waugh, et al., Protein kinase B controls transcriptional programs that direct cytotoxic T cell fate but is dispensable for T cell metabolism, Immunity 34 (2011) 224–236.
[196] E.M. Putz, M. Prchal-Murphy, O.A. Simma, F. Forster, X. Koenig, et al., PI3Kdelta is essential for tumor clearance mediated by cytotoxic T lymphocytes, PLoS ONE 7 (2012) e40852.
[197] A.L. Martin, M.D. Schwartz, S.C. Jameson, Y. Shimizu, Selective regulation of CD8 effector T cell migration by the p110 gamma isoform of phosphatidylinositol 3-kinase, J. Immunol. 180 (2008) 2081–2088.
[198] D.R. Soond, E.C. Slack, O.A. Garden, D.T. Patton, K. Okkenhaug, Does the PI3K pathway promote or antagonize regulatory T cell development and function? Front. Immunol. 3 (2012) 244.
[199] D.T. Patton, O.A. Garden, W.P. Pearce, L.E. Clough, C.R. Monk, et al., Cutting edge: the phosphoinositide 3-kinase p110 delta is critical for the function of CD4 + CD25 + Foxp3+ regulatory T cells, J. Immunol. 177 (2006) 6598–6602.
[200] Y.M. Kerdiles, E.L. Stone, D.R. Beisner, M.A. McGargill, I.L. Ch'en, et al., Foxo transcription factors control regulatory T cell development and function, Immunity 33 (2010) 890–904.
[201] W. Ouyang, O. Beckett, Q. Ma, J.H. Paik, R.A. DePinho, et al., Foxo proteins cooperatively control the differentiation of Foxp3+ regulatory T cells, Nat. Immunol. 11 (2010) 618–627.
[202] M.G. Strainic, E.M. Shevach, F. An, F. Lin, M.E. Medof, Absence of signaling into CD4(+) cells via C3aR and C5aR enables autoinductive TGF-beta1 signaling and induction of Foxp3(+) regulatory T cells, Nat. Immunol. 14 (2013) 162–171.
[203] K. Ali, D.R. Soond, R. Pineiro, T. Hagemann, W. Pearce, et al., Inactivation of PI(3)K p110delta breaks regulatory T-cell-mediated immune tolerance to cancer, Nature 510 (2014) 407–411.
[204] D. Liu, T. Zhang, A.J. Marshall, K. Okkenhaug, B. Vanhaesebroeck, et al., The p110delta isoform of phosphatidylinositol 3-kinase controls susceptibility to Leishmania major by regulating expansion and tissue homing of regulatory T cells, J. Immunol. 183 (2009) 1921–1933.

[205] S.J. Shuttleworth, F.A. Silva, A.R. Cecil, C.D. Tomassi, T.J. Hill, et al., Progress in the preclinical discovery and clinical development of class I and dual class I/IV phosphoinositide 3-kinase (PI3K) inhibitors, Curr. Med. Chem. 18 (2011) 2686–2714.
[206] M.P. Wymann, M. Zvelebil, M. Laffargue, Phosphoinositide 3-kinase signalling–which way to target? Trends Pharmacol. Sci. 24 (2003) 366–376.
[207] D.F. Barber, A. Bartolome, C. Hernandez, J.M. Flores, C. Redondo, et al., PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus, Nat. Med. 11 (2005) 933–935.
[208] M. Camps, T. Ruckle, H. Ji, V. Ardissone, F. Rintelen, et al., Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis, Nat. Med. 11 (2005) 936–943.
[209] S. Hayer, N. Pundt, M.A. Peters, C. Wunrau, I. Kuhnel, et al., PI3Kgamma regulates cartilage damage in chronic inflammatory arthritis, FASEB J. 23 (2009) 4288–4298.
[210] L. Berod, C. Heinemann, S. Heink, A. Escher, C. Stadelmann, et al., PI3Kgamma deficiency delays the onset of experimental autoimmune encephalomyelitis and ameliorates its clinical outcome, Eur. J. Immunol. 41 (2011) 833–844.
[211] A. Fougerat, S. Gayral, P. Gourdy, A. Schambourg, T. Ruckle, et al., Genetic and pharmacological targeting of phosphoinositide 3-kinase-gamma reduces atherosclerosis and favors plaque stability by modulating inflammatory processes, Circulation 117 (2008) 1310–1317.
[212] T.M. Randis, K.D. Puri, H. Zhou, T.G. Diacovo, Role of PI3Kdelta and PI3Kgamma in inflammatory arthritis and tissue localization of neutrophils, Eur. J. Immunol. 38 (2008) 1215–1224.
[213] S. Haylock-Jacobs, I. Comerford, M. Bunting, E. Kara, S. Townley, et al., PI3Kdelta drives the pathogenesis of experimental autoimmune encephalomyelitis by inhibiting effector T cell apoptosis and promoting Th17 differentiation, J. Autoimmun. 36 (2011) 278–287.
[214] W.C. Rowan, J.L. Smith, K. Affleck, A. Amour, Targeting phosphoinositide 3-kinase delta for allergic asthma, Biochem. Soc. Trans. 40 (2012) 240–245.
[215] D.G. Winkler, K.L. Faia, J.P. DiNitto, J.A. Ali, K.F. White, et al., PI3K-delta and PI3K-gamma inhibition by IPI-145 abrogates immune responses and suppresses activity in autoimmune and inflammatory disease models, Chem. Biol. 20 (2013) 1364–1374.
[216] U.A. Maus, M. Backi, C. Winter, M. Srivastava, M.K. Schwarz, et al., Importance of phosphoinositide 3-kinase gamma in the host defense against pneumococcal infection, Am. J. Respir. Crit. Care Med. 175 (2007) 958–966.
[217] C.L. Lucas, H.S. Kuehn, F. Zhao, J.E. Niemela, E.K. Deenick, et al., Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency, Nat. Immunol. 15 (2014) 88–97.
[218] I. Angulo, O. Vadas, F. Garcon, E. Banham-Hall, V. Plagnol, et al., Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage, Science 342 (2013) 866–871.
[219] M.E. Conley, D.A. Fruman, Genetics. Can cancer drugs treat immunodeficiency? Science 342 (2013) 814–815.
[220] C. Rommel, M. Camps, H. Ji, PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? Nat. Rev. Immunol. 7 (2007) 191–201.
[221] L. Bi, I. Okabe, D.J. Bernard, A. Wynshaw-Boris, R.L. Nussbaum, Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of phosphoinositide 3-kinase, J. Biol. Chem. 274 (1999) 10963–10968.
[222] L.C. Foukas, M. Claret, W. Pearce, K. Okkenhaug, S. Meek, et al., Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation, Nature 441 (2006) 366–370.
[223] M. Graupera, J. Guillermet-Guibert, L.C. Foukas, L.K. Phng, R.J. Cain, et al., Angiogenesis selectively requires the p110alpha isoform of PI3K to control endothelial cell migration, Nature 453 (2008) 662–666.
[224] H. Al-Qassab, M.A. Smith, E.E. Irvine, J. Guillermet-Guibert, M. Claret, et al., Dominant role of the p110beta isoform of PI3K over p110alpha in energy homeostasis regulation by POMC and AgRP neurons, Cell Metab. 10 (2009) 343–354.
[225] E. Patrucco, A. Notte, L. Barberis, G. Selvetella, A. Maffei, et al., PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects, Cell 118 (2004) 375–387.
[226] T. Willinger, R.A. Flavell, Canonical autophagy dependent on the class III phosphoinositide-3 kinase Vps34 is required for naive T-cell homeostasis, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 8670–8675.
[227] M. Murakami, T. Ichisaka, M. Maeda, N. Oshiro, K. Hara, et al., mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells, Mol. Cell. Biol. 24 (2004) 6710–6718.
[228] Y. Wang, L. Zhang, P. Wei, H. Zhang, C. Liu, Inhibition of PI3Kdelta improves systemic lupus in mice, Inflammation 37 (2014) 978–983.
[229] Y. Oka, T. Yabuuchi, T. Oi, S. Kuroda, Y. Fujii, et al., Discovery of N-{5-[3-(3-hydroxypiperidin-1-yl)-1,2,4-oxadiazol-5-yl]-4-methyl-1,3-thiazol-2-yl}acetamide (TASP0415914) as an orally potent phosphoinositide 3-kinase gamma inhibitor for the treatment of inflammatory diseases, Bioorg. Med. Chem. 21 (2013) 7578–7583.
[230] F. Gonzalez-Lopez de Turiso, Y. Shin, M. Brown, M. Cardozo, Y. Chen, et al., Discovery and in vivo evaluation of dual PI3Kbeta/delta inhibitors, J. Med. Chem. 55 (2012) 7667–7685.
[231] H. Li, D. Park, P.M. Abdul-Muneer, B. Xu, H. Wang, et al., PI3Kgamma inhibition alleviates symptoms and increases axon number in experimental autoimmune encephalomyelitis mice, Neuroscience 253 (2013) 89–99.
[232] J.A. Marwick, G. Caramori, C.S. Stevenson, P. Casolari, E. Jazrawi, et al., Inhibition of PI3Kdelta restores glucocorticoid function in smoking-induced airway inflammation in mice, Am. J. Respir. Crit. Care Med. 179 (2009) 542–548.
[233] D.I. Kim, S.R. Kim, H.J. Kim, S.J. Lee, H.B. Lee, et al., PI3K-gamma inhibition ameliorates acute lung injury through regulation of IkappaBalpha/NF-kappaB pathway and innate immune responses, J. Clin. Immunol. 32 (2012) 340–351.
[234] J. Azzi, R.F. Moore, W. Elyaman, M. Mounayar, N. El Haddad, et al., The novel therapeutic effect of phosphoinositide 3-kinase-gamma inhibitor AS605240 in autoimmune diabetes, Diabetes 61 (2012) 1509–1518.
[235] P.C. Low, S. Manzanero, N. Mohannak, V.K. Narayana, T.H. Nguyen, et al., PI3Kdelta inhibition reduces TNF secretion and neuroinflammation in a mouse cerebral stroke model, Nat. Commun. 5 (2014) 3450.
[236] X. Sun, B. Liu, R.B. Sartor, C. Jobin, Phosphatidylinositol 3-kinase-gamma signaling promotes Campylobacter jejuni-induced colitis through neutrophil recruitment in mice, J. Immunol. 190 (2013) 357–365.
[237] A. Roller, A. Perino, P. Dapavo, E. Soro, K. Okkenhaug, et al., Blockade of phosphatidylinositol 3-kinase PI3Kdelta or PI3Kgamma reduces IL-17 and ameliorates imiquimod-induced psoriasis-like dermatitis, J. Immunol. 189 (2012) 4612–4620.