PI 3-kinase inhibition: a therapeutic target for respiratory disease

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Abstract
Asthma and COPD (chronic obstructive pulmonary disease) are a growing major health burden, which, despite improvements in disease management, still require new effective treatments. As our understanding of the cellular and molecular processes which govern respiratory diseases improves, the range of potential therapeutic targets increase. PI 3-kinases (phosphoinositide 3-kinases) are a family of closely related enzymes, which play pivotal roles in a diverse array of cellular mechanisms. In the present paper, we review the evidence for PI 3-kinase involvement in various cellular processes underlying asthma and COPD generated through inhibitor studies and gene-targeting approaches, and discuss the prospects for PI 3-kinase inhibition as a future therapeutic strategy for the treatment of respiratory disease.

Introduction
The activation of cells by a wide variety of stimuli leads to rapid changes in 3-phosphorylated inositol lipids through the action of a family of enzymes known as PI 3-kinases (phosphoinositide 3-kinases). PI 3-kinase activation is central to the co-ordinated control of multiple cell-signalling pathways leading to cell growth, cell proliferation, cell survival and cell migration. However, in recent years it has become apparent that individual PI 3-kinase isoforms regulate distinct cellular events, opening up the therapeutic opportunities for PI 3-kinase inhibition. The PI 3-kinases have been classified into three groups according to their primary sequence and domain structure, mode of regulation and substrate specificity in vitro [1]. The class IA PI 3-kinase subgroup consist of three catalytic subunits, p110α, β and δ, which form heterodimers with one of five SH2 (Src homology 2)-domain-containing regulatory subunits, p85α, p85β, p55γ, p50δ and p50ε. These heterodimers can be recruited either directly to cell-surface receptors, e.g. growth factor receptors, or indirectly by adaptor molecules such as Shc, Grb2 or IRS-1 [2]. Class IB consists of one member, a heterodimer of p110γ and a regulatory subunit termed p101, and is activated by G-protein βγ subunits following the stimulation of GPCRs (G-protein coupled receptors), e.g. chemokine receptors. Both class IA and class IB catalyse the formation of the lipid PtdIns(3,4,5)3 in vitro, which can be converted back into PtdIns(4,5)2 by the 3′ phosphatase, PTEN (phosphatase and tensin homologue deleted on chromosome 10) or be metabolized to PtdIns(3,4)2 by 5′ phosphatases such as SHIP (SH2-containing inositol 5′-phosphatase). Both PtdIns (3,4,5)3 and PtdIns (3,4)2 act as second messengers via the recruitment of proteins containing phosphoinositide-binding modules termed pleckstrin homology (PH) domains [3].

PI 3-kinase inhibitors – from pharmacological tools to drug leads
The dissection of PI 3-kinase signalling pathways has been aided greatly by the availability of two pharmacological tools, wortmannin and LY294002 (Figure 1). The fungal metabolite wortmannin inhibits PI 3-kinase in the low nanomolar range [4] and binds covalently to a conserved lysine residue in the ATP-binding site of the enzyme [5]. LY294002 is a reversible, ATP-competitive inhibitor with an IC50 for recombinant PI 3-kinase in the low micromolar range [6]. The binding mode of wortmannin, LY294002 and other non-selective kinase inhibitors has been refined further through the elucidation of several X-ray crystal structures of p110γ–inhibitor complexes [7]. It is important to point out that neither inhibitor displays selectivity for different members of the class I PI 3-kinases. LY294002 also inhibits casein kinase 2 with a similar potency to PI 3-kinase and has been shown to block Kv currents directly [8]. At high concentrations, wortmannin inhibits PI 3-kinase–related enzymes [e.g. mTOR (mammalian target of rapamycin), ATM (ataxia-telangiectasia mutated)], PI 4-kinase β and myosin light chain kinase. In addition, methylxanthines, such as caffeine and theophylline (Figure 1, structures 3 and 4) inhibit p110γ, although their activity is rather weak [9]. Recently, a number of patent specifications have been published, which describe inhibitors of PI 3-kinase including compounds that exhibit some selectivity for individual.
Isoforms. Piramed have described several imidazopyridine derivatives (e.g. compounds of general structure 5, Figure 1), which are claimed to exhibit excellent PI 3-kinase inhibitory activities, especially against p110\(\gamma\), although no isoform-selectivity data is provided. Kinacia have disclosed a series of morpholino-substituted compounds related to LY294002 that showed isoform selectivity. They describe quinoline and pyridopyrimidine compounds (Figure 1, structures 6 and 7) that are approx. 100-fold more potent against p110\(\alpha\) compared with the p110\(\gamma\) isoform. ICOS Corporation have recently described IC87114, which is a selective p110\(\delta\) inhibitor containing a quinazoline core structure (Figure 1, structure 8). This compound has an IC\(_{50}\) of 0.5 \(\mu\)M for p110\(\delta\) inhibition and >50-fold selectivity over the other class I PI 3-kinase isoforms [10]. Finally, a patient specification from Novartis describes 5-phenylthiazole derivatives as PI 3-kinase inhibitors. The diverse chemical scaffolds reported in these patents represent promising lead compounds for the future generation of PI 3-kinase-isoform-selective inhibitors.

Control of neutrophil migration and activation
Pharmacological inhibition of PI 3-kinases by wortmannin and LY294002 have shed considerable light on the involvement of these enzymes in neutrophil function. Both inhibitors block superoxide production by neutrophils in response to various ligands [4,11,12] and chemotaxis towards fMLP (N-formylmethionyl-leucylphenylalanine) and IL (interleukin)-8 [13]. Neutrophils from p110\(\gamma\)-knockout (p110\(\gamma\)-/+) mice fail to produce PtdIns(3,4,5)\(P_3\) in response to GPCR stimulation by a variety of chemoattractants, and chemotaxis of p110\(\gamma\)-/+ neutrophils is greatly reduced, but not totally abolished [14–16]. Movement along a chemoattractant gradient requires the rearrangement of the actin cytoskeleton, leading to cell elongation and the formation of lamellipodia at the front edge of the cell. Recent studies in the neutrophilic cell line HL60 have shown recruitment of a fluorescently tagged PKB (protein kinase B) PH domain to the edge of the cell exposed to the highest concentration of chemoattractant. This has led to the suggestion that PI 3-kinase and the localization of PtdIns(3,4,5)\(P_3\) act as a ‘molecular compass’, guiding the direction of neutrophils towards the source of chemoattractant [17]. Recent data support a role for p110\(\gamma\) in this direction-finding process. Neutrophils from p110\(\gamma\)-/+ mice fail to migrate along a chemoattractant gradient (i.e. chemotaxis) but random cell movement (chemokinesis) is not impaired [18]. In human neutrophils, the p110\(\delta\)-selective inhibitor IC87114 blocks fMLP-stimulated PtdIns(3,4,5)\(P_3\) production and directional cell movement [10]. Therefore the relative contributions of p110\(\gamma\) and p110\(\delta\) towards neutrophil function need to be addressed further. In addition to a vital role of PI 3-kinase in neutrophil chemotaxis and activation, protection from apoptosis in neutrophils is also a PI 3-kinase-dependent event. Cytokines, including GM-CSF (granulocyte/macrophage-colony-stimulating factor) and TNF\(\alpha\) (tumour necrosis factor-\(\alpha\)), reduce the rate of neutrophil apoptosis through changes in the phosphorylation and expression of the pro-apoptotic Bcl-2 (B-cell leukaemia/lymphoma 2) family member, Bad (Bcl-2/X1-antagonist, causing cell death). Incubation of neutrophils with LY294002 inhibited cytokine-induced changes in Bad phosphorylation and blocked the survival effect of GM-CSF and TNF\(\alpha\) [19].

**Figure 1** | PI 3-kinase inhibitor structures
Chemical structures of known PI 3-kinase inhibitors from the scientific and patent literature (patent numbers are in parentheses).
the treatment of Th2-dominant respiratory diseases, such as asthma.

**PI 3-kinase and control of mast-cell function**

Mast cells are key regulators of the allergic response and the role of PI 3-kinase activation in the control of mast-cell function has been established using pharmacological tools and gene targeting in mice [25,26]. Activation of the receptor for IgE (FceRI) culminates in the activation of a tyrosine kinase signalling cascade, phosphoinositide turnover and an increase in intracellular calcium levels. Cross-linking of the FceRIα subunit leads to the phosphorylation of ITAMs (immunoreceptor tyrosine-based activation motifs) in the FceRIβ and FceRIγ subunits and the recruitment of the tyrosine kinase, Syk. Docking sites for class IA PI 3-kinases are created in the ITAMs, and the resultant PtdIns(3,4,5)P_3 levels by the gene-targeting in vivo approach [25], a role for PI 3-kinase in the control of mast-cell signalling in response to various GPCR ligands, such as adenosine and chemokines, and it is suggested that the increase in PtdIns(3,4,5)P_3 levels in response to these stimuli can circumvent the inhibitory role of SHIP. Furthermore, p110γ mice were protected from anaphylaxis following injection of IgE and allergen.

**Effects of PI 3-kinase inhibition in animal models of respiratory disease**

Although LY294002 and wortmannin have been available for the last decade, their use in in vivo models of airway disease has been rather limited. The lack of responsiveness of p110γ−-knockout mice to adenosine is particularly interesting as a role for this mediator in asthma has long been advocated [39]. Levels of adenosine are increased in the bronchoalveolar lavage of asthmatics and local delivery of AMP induces bronchoconstriction in asthmatics, but not normal individuals. In actively sensitized Brown Norway rats, bronchoconstrictor responses to adenosine are blocked following local delivery of wortmannin [40]. In addition, allergen-induced inflammation, measured by leucocyte influx and EPO (eosinophil peroxidase) release, is also inhibited by wortmannin at higher concentrations [41]. In guinea-pigs, EPO release is also blocked by wortmannin, but little effect on eosinophilia is observed [42]. In a murine model of bronchial inflammation and airway hyperresponsiveness, allergen challenge has been shown to increase PI 3-kinase activity in p-Tyr immunoprecipitates from lung tissue [43]. This was accompanied by decreased levels of PTEN, the 3′ phosphatase responsible for the breakdown of PtdIns(3,4,5)P_3. Phosphorylation of PKB in lung tissues was increased following allergen challenge, and could be reduced by pre-treatment with wortmannin, LY294002 or transfection of adenosival vectors that expressed PTEN. The inhibitors and adenosival PTEN also significantly reduced allergen-induced eosinophilia in bronchoalveolar lavage, reduced thickening of the airway epithelium, cytokine release and airway hyperresponsiveness.

Acute lung injury/ARDS (acute respiratory distress syndrome) and COPD (chronic obstructive pulmonary disease) are associated with underlying inflammatory processes, which include the accumulation of neutrophils in the lung [46,48]. As discussed above, PI 3-kinase plays a vital role in the control of neutrophil chemotaxis and activation. In p110γ−-deficient mice, LPS (lipopolysaccharide)-induced lung oedema, neutrophil recruitment and pro-inflammatory cytokine production are all reduced in vivo when compared...
The development of asthma is dependent on the generation of a Th2 response upon allergen presentation to a naive T-cell by a dendritic antigen-presenting cell (APC), a process which can be skewed towards a negative regulatory Th1 phenotype by inhibition of class IA PI 3-kinase signalling (i.e. through lack of the p85α subunit) (A). The cytokine help provided to B-cells facilitates IgE production which can be suppressed by targeting p110δ (B). IL-5 produced from Th2 cells leads to eosinophil maturation and activation, which, in concert with IgE-allergen-stimulated mast cells, release numerous inflammatory mediators, thus causing the pathology associated with asthma. The degranulation of both effector cell types can be blocked by inhibition of p110γ (C–D). COPD-associated pathology begins with airway epithelium and macrophage release of chemokines and leukotrienes in response to tobacco smoke. The chemotactic effect of these molecules on circulating neutrophils can be disrupted by targeting p110γ (E), as can the subsequent elastase release and superoxide production (F).

with wild-type mice [44]. Mechanical ventilation of ARDS patients contribute to pulmonary inflammation and tissue destruction. In a recent study, over-ventilation of mouse lungs led to NF-κB (nuclear factor κB) translocation and release of pro-inflammatory cytokines, such as IL-6 and the chemokine MIP2α (macrophage inflammatory protein 2α) [45]. These effects could be blocked by pre-treatment with LY294002. These studies suggest that the inhibition of p110γ may prove beneficial for the treatment of acute lung injury and ARDS. However, it should noted that many anti-inflammatory strategies have failed in this indication [46].

**PI 3-kinase inhibition – prospects for drug development**

The prospects for the use of PI 3-kinase inhibitors in the treatment of various diseases, including respiratory disease, but also cancer, other proliferative disorders and cardiovascular disease, look promising [2,47]. However, a limited number of in vivo studies (including side-effect and toxicological assessment) have been reported with the two well-characterized inhibitors, wortmannin and LY294002. Consequently, a number of questions regarding the merits of PI 3-kinase inhibition remain unanswered. The PI 3-kinase family of enzymes control a wide variety of cellular processes thereby questioning whether an acceptable therapeutic index can be achieved from targeting this pathway. The advantage for treatment of respiratory disease is the potential to deliver PI 3-kinase inhibitors locally and optimize properties, which will minimize systemic exposure of the drugs. In the last few years, it has become apparent that individual PI 3-kinase isoforms regulate distinct cellular events e.g. chemokine stimulated neutrophil migration is controlled by p110γ, mast-cell degranulation is amplified by p110γ, B-cell receptor signalling is mediated by p110δ. Therefore it is reassuring that isoform-selective inhibitors can be generated despite the similarities in the ATP binding sites of the class I PI 3-kinases. PI 3-kinase activation occurs in response to many different ligands and appears to be vital for a number of cellular processes which underlie acute and chronic respiratory diseases. Therefore inhibition of PI 3-kinase may lead to the blockade of multiple components of disease pathophysiology (Figure 2). The co-ordinated inhibition of cell signalling in response to multiple chemokines by targeting of p110γ, in particular, may be advantageous over the blockade of single mediators, such as IL-8 or eotxin. The outcome of
drug-discovery efforts focused towards the development of PI 3-kinase inhibitors is eagerly awaited.

References


