ABSTRACT

The phosphoinositide 3-kinase(s) (PI3K) are a family of proteins that catalyze the phosphorylation of the 3-OH position of phosphoinositides and generate lipids that control a wide variety of intracellular signaling pathways. They are classified into three families according to their structure and substrate specificity and are thought to have distinct biological roles. Recent studies suggested that numerous components of the PI3K pathway play a crucial role in the expression and activation of inflammatory mediators, inflammatory cell recruitment, immune cell function, airway remodeling, and corticosteroid insensitivity in chronic inflammatory respiratory disease. Selective PI3K inhibitors have been developed that reduce inflammation and some characteristics of disease in experimental animal models. Targeting specific PI3K isoforms that may be overexpressed or overactive in disease should allow for selective treatment of respiratory diseases. Encouraging data from animal models, primary cells and clinical studies in other diseases suggest that inhibitors of PI3K/Akt may prove to be useful novel therapies in the treatment of asthma and chronic obstructive pulmonary disease.

Chronic inflammatory airway diseases, such as bronchial asthma and chronic obstructive pulmonary disease (COPD), represent a profound and growing public health problem worldwide. Bronchial asthma, in addition to placing a considerable burden in terms of direct medical costs, has enormous indirect costs and is one of the leading causes of work or school absenteeism. Most patients with asthma respond well to current corticosteroid-based therapies; however, a small percentage (10%) fails to respond well and this poor control of symptoms is a major issue that can result in adverse clinical and economic outcomes (accounting for >50% of the total asthma health care costs). COPD (smoking lung) is a chronic inflammatory disease of the lower airways and lung, and this progressive and relentless loss of lung function is caused by emphysema due to destruction of lung parenchyma and by narrowing of small airways as a result of chronic inflammation (Barnes et al., 2003; Barnes and Kleinert, 2004). COPD is predicted to become the third most common cause of death and the fifth most common cause of disability in the world by 2020 (Barnes and Kleinert, 2004). Cystic fibrosis is also recognized as an inflammatory disease with increased neutrophilia, inflammatory gene expression, and enhanced activation of several transcription factors. Unfortunately, there are no effective treatments for these diseases (severe asthma and COPD and cystic fibrosis), and the molecular mechanisms underlying their pathogenesis are not fully understood. Recent research suggests that many kinases are involved in chronic airway inflammation and its associated pathology.

ABBREVIATIONS: COPD, chronic obstructive pulmonary disease; MAPK, mitogen-activated protein kinase; GR, glucocorticoid receptor; DC, dendritic cell(s); PTEN, phosphatase and tensin homolog deleted on chromosome 10 protein; GPCRs, G-protein-coupled receptors; PI3K, phosphoinositide 3-kinase(s); PI(4)P, phosphatidylinositol 4-phosphate; PI(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate; HO-1, heme oxygenase-1; NF-κB, nuclear factor κB; ROS, reactive oxygen species; MMP, matrix metalloproteinase; PKC, protein kinase C; SHIP, src homology 2-containing inositol 5-phosphatase; RANTES, regulated on activation normal T cell expressed and secreted; HDAC, histone deacetylase; IL, interleukin; OVA, ovalbumin; LY294002, 2-(4-morpholino)-8-phenyl-4H-1-benzopyran-4-one; IC87114, quinolone pyrrolopyrimidine; MCP, monocyte chemotactic protein; ZSTK474, 2-(2-difluoromethylbenzimidazol-1-yl)4,6-dimorpholino-1,3,5-triazine; SF1126; 2-[2-methoxyethylamino]-8-phenyl-4H-1-benzopyran-4-one;
exacerbation (Adcock et al., 2006). Here we focus on phosphoinositide 3-kinase (PI3K) family, which are expected to be involved in the airway inflammatory response, particularly under conditions of oxidative stress, and discuss the potential of PI3K inhibitors for the treatment of these diseases.

**Inflammatory Component of Asthma and COPD**

Lower airways inflammation is a central feature of many lung diseases, including asthma and COPD. This involves recruitment and activation of inflammatory cells and changes in the structural cells of the lung, although the specific characteristics of the inflammatory response and the site of inflammation differ between one disease to another. Inflammation in asthma is associated with increased airway hyper-responsiveness, leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These conditions are characterized by an increased expression of components of the inflammatory cascade, including chemokines, cytokines, growth factors, enzymes, noxious gas, reactive oxygen, receptors, and adhesion molecules.

This inflammation is present even in those with very mild asthma, and T-lymphocytes of the T-helper (Th) type 2 phenotype, eosinophils, macrophages/monocytes, and mast cells, infiltrate into airway wall. Airway inflammation is also amplified during exacerbation, with an increase in eosinophils and sometimes neutrophils. Chronic inflammation may also lead to structural changes in the airway, including increased thickness of airway smooth muscle, increased number of mucus-secreting cells, subepithelial fibrosis, and increased numbers of blood vessels (angiogenesis), which are referred to as airway remodeling. These changes may not be fully reversible with current treatments.

COPD is a chronic inflammatory disease of the lower airways and lung, which is enhanced during exacerbations (Barnes, 2003). The pathological characteristics of COPD are destruction of the lung parenchyma (emphysema) and inflammation of the peripheral airways and the central airways. Most patients with COPD show chronic obstructive bronchitis, emphysema, and/or mucus plugging. There is a marked increase in macrophages and neutrophils in bronchoalveolar-lavage fluid and induced sputum as well as T lymphocytes and B lymphocytes in lung parenchyma. Our recent study suggested that activity and expression of histone deacetylase (HDAC), a transcriptional corepressor, was decreased in COPD due to oxidative stress, and consequently, cytokine transcription was increased (Ito et al., 2005).

Another important feature of severe asthma and COPD is corticosteroid resistance (Ito, 2005). Several large studies suggest that long-term treatment with corticosteroids did not stop the inexorable decline of lung function in COPD patients. This is consistent with the demonstration that inhaled or oral corticosteroids fail to reduce inflammatory cell numbers, cytokines, chemokines, or proteases in induced sputum or bronchial biopsies of patients with COPD. The molecular mechanisms for corticosteroid insensitivity are not fully elucidated but may include overexpression of transcriptional factors to trap glucocorticoid receptor (GR), GR degradation by oxidative stress, and/or decay GR (GRβ) overexpression. Recently, we found that HDAC2 reduction in COPD and severe asthma are involved in corticosteroid resistance possibly via hyperacetylation of GR (Ito et al., 2005, 2006), although kinases such as mitogen-activated protein kinase (MAPK) may also be important in corticosteroid insensitivity in severe asthma (Irusen et al., 2002).

**PI3K Isoforms and Signaling**

PI3Ks have been divided into three classes according to their structure and lipid substrate specificity (Table 1) (Ward and Finan, 2003; Hennessy et al., 2005; Vanhaesebroeck et al., 2005). The most extensively investigated are class I PI3Ks. Type I PI3Ks are activated by cell surface receptors, such as growth factors, insulin, and G-protein-coupled receptors (GPCRs). Upon activation, class I PI3Ks convert phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3], a ubiquitous second messenger (Fig. 1). PI(3,4,5)P3 then acts as a targeting site for downstream signaling molecules with pleckstrin homology domains, such as protein serine/threonine kinases (protein kinase B/Akt) and phosphoinositide-dependent kinase 1 (PDK1) or protein kinase C (PKCα and PKCδ) and MAPK signaling pathway. These signaling proteins are actively involved in the modulation of cell growth, proliferation and shape, apoptosis (prevent/enhance), cell movement, and activation of cells.

Class II PI3Ks comprised α, β, and γ isoforms, which are characterized by the presence of a C2 domain at the C terminus. They predominantly use phosphatidylinositol and phosphatidylinositol 4-phosphate [PI(4)P] as substrates (Table 1). The class II PI3Ks only use phosphatidylinositol as a substrate. This class of PI3K is reported to be involved in macroautophagy, which is a multistep process responsible for the degradation of long-lived proteins and organelle renewal, and starts with the formation of an autophagosome, which ultimately fuses with the endosomal/lysosomal compartment. This path-

<table>
<thead>
<tr>
<th>Characteristics of the PI3K family</th>
<th>Catalytic Molecule</th>
<th>Regulatory Molecule</th>
<th>In Vitro Substrate</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ia</strong></td>
<td>PI3Kα</td>
<td>p110α</td>
<td>p85α, p55β</td>
<td>PtdIns Ubiquitous</td>
</tr>
<tr>
<td><strong>Ib</strong></td>
<td>PI3Kβ</td>
<td>p110β</td>
<td>p55γ</td>
<td>PtdIns(4)P Ubiquitous</td>
</tr>
<tr>
<td><strong>II</strong></td>
<td>PI3Kδ</td>
<td>p110δ</td>
<td>p55α, p55β</td>
<td>PtdIns(4,5)P2 Whole blood, thymus</td>
</tr>
<tr>
<td><strong>III</strong></td>
<td>PI3Kγ</td>
<td>p110γ</td>
<td>p84/p87PIKAP</td>
<td>PtdIns(4,5)P2 Whole blood, thymus</td>
</tr>
<tr>
<td></td>
<td>C2α</td>
<td>Clathrin</td>
<td></td>
<td>PtdIns Widely expressed</td>
</tr>
<tr>
<td></td>
<td>C2B</td>
<td>Clathrin</td>
<td></td>
<td>PtdIns Widely expressed</td>
</tr>
<tr>
<td></td>
<td>C2B</td>
<td>Clathrin</td>
<td></td>
<td>PtdIns Constitutive</td>
</tr>
<tr>
<td></td>
<td>Vps34p</td>
<td>Vps15p (p150)</td>
<td>Beclin 1</td>
<td>PtdIns Ubiquitous, Prostate, Liver, Breast</td>
</tr>
</tbody>
</table>

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[Table 1](#)
way is known to be important in the maintenance of cell function during periods of nutrient deprivation.

Class I PI3Ks are further divided into class IA and class IB PI3Ks. Structurally, PI3Ks IA exist as heterodimeric complexes in which a catalytic p110 subunit (designated as α, β, or γ) is in association with a particular regulatory subunit (designated p85, p55, and p50). There are five regulatory isoforms that are responsible for protein-protein interactions via the Src homology 2 domain and phosphotyrosine residues of proteins [p85α, p85β, and p55γ], which are encoded by specific genes, and alternate splicing of the p85α gene, p55α and p50α] and three catalytic isoforms, p110α, p110β, and p110γ (Table 1). p110α and p110β isoforms are ubiquitously expressed, and genetic knockout leads to early embryonic death. By contrast, expression of the p110γ and p110γ isoforms are largely restricted to the hematopoietic systems, and mice lacking expression of PI3Kγ and γ do not show any overt adverse phenotype. Importantly, PI3K IA signals downstream of receptor tyrosine kinase and Ras (Fig. 1). The single class PI3K IB consists of the p110γ catalytic subunit complexed to the p101 regulatory subunit and signals downstream of GPCRs and Ras, which is activated by βγ subunits from GPCRs, such as the receptors for chemokines (Fig. 1).

**PI3K and Airway Inflammation/Corticosteroid Sensitivity**

Despite limitations in selectivity, the two commercially available PI3K inhibitors, wortmannin and LY294002, have contributed greatly to our understanding of the biological role of PI3K in lung inflammation. Duan et al. (2005) reported that intratracheal administration of LY294002 significantly inhibited ovalbumin (OVA)-induced increases in total cell counts, eosinophil counts, and interleukin (IL)-5, IL-13, and CCL11 (eotaxin) levels in bronchoalveolar lavage fluid and dramatically inhibited OVA-induced tissue eosinophilia and airway mucus production. This was associated with a significant suppression of OVA-induced airway hyper-responsiveness to inhaled methacholine. Importantly, this study confirmed that LY294002 markedly attenuated OVA-induced serine phosphorylation of Akt, a direct downstream substrate of PI3K. In addition, ex vivo experiments examin-
ing antigen-induced anaphylactic contraction of bronchial rings and the release of histamine and peptide leukotrienes from chopped lung preparations from sensitized guinea pigs were also blocked by LY294002. These findings were also supported by other studies, which showed that LY294002 and wortmannin attenuated eosinophilic airway inflammation and airway hyper-responsiveness in a murine asthma model (Ezeamuzie et al., 2001; Kwak et al., 2003; Lee et al., 2006b). Thus, PI3K inhibition was indicated to have therapeutic potential for the treatment of asthmatic airway inflammation.

However, these inhibitors do not distinguish among the four class I PI3Ks and also broadly affect multiple cell types that express these kinases. IC87114, a selective p110δ inhibitor, has recently been used to investigate the role of p110δ in allergic airway inflammation and hyper-responsiveness using a mouse asthma model (Lee et al., 2006b). IC87114 significantly reduced the serum levels of total immunoglobulin (IgE) and OVA-specific IgE and leukotriene C4 release into the airspace, OVA-induced lung tissue eosinophilia, airway mucus production, and inflammation score, and importantly, OVA-induced increase in expression of IL-4, IL-5, IL-13, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, CCL5 (RANTES), and CCL11. Furthermore, IC87114 significantly suppressed OVA-induced airway hyper-responsiveness to inhaled methacholine, and this corresponded to a reduction in OVA-induced Akt serine phosphorylation. These results were supported by the in vitro findings that p110δ is involved in B and T-cell antigen receptor signaling and activation and allergen-IgE-induced mast cell degranulation. Mutation of p110δ also leads to defects in mast cells and possibly neutrophils (Ali et al., 2004; Puri et al., 2004).

There is also evidence using knockout mice that PI3Kγ is also important component in the pathogenesis of asthma. Wymann et al. (2003) has demonstrated that murine mast cell responses are exacerbated in vitro and in vivo by autocrine signals and require functional PI3Kγ. Adenosine, acting through the A1 adenosine receptor, as well as other agonists of Gαi-coupled receptors, transiently increased PI(3,4,5)P3 exclusively via PI3Kγ. Furthermore, mice that lacked PI3Kγ did not form edema when challenged by passive systemic anaphylaxis. Thus, PI3Kγ relays inflammatory signals through various GPCRs and is thus central to mast cell function. Eosinophil accumulation was also reported to be inhibited at 48 h in these PI3Kγ-deficient mice compared with wild-type mice but not at earlier time points (6 and 24 h), suggesting that PI3Kγ plays a role in the maintenance of eosinophilic inflammation in vivo.

There are no published reports on the effect of PI3K inhibitors on experimental models of COPD, but the data suggesting a potential role of PI3K in the pathogenesis of COPD is now accumulating. Matrix metalloproteinase (MMP) 9 degrades extracellular matrix components (particularly elastin) and is related to the pathogenesis of pulmonary emphysema. MMP9 is present in low quantities in the healthy adult lung but much more abundant in COPD, and the inappropriate expression of MMP9 is thought to contribute to the pathogenesis of COPD (Barnes et al., 2003). MMP9 expression, whether stimulated by PAF or fibronectin, probably through an action on NF-κB, is also regulated by PI3K signaling pathways (Ko et al., 2005). Furthermore, several lines of evidence point to the importance of PI3K in the activation of macrophage and neutrophils, which are key players in COPD inflammation (Thomas et al., 2005).

As discussed above, patients with COPD and severe asthma do not respond well to corticosteroids, although corticosteroids are very effective in controlling mild to moderate asthma. This is consistent with the demonstration that inhaled or oral steroids fail to reduce inflammatory cell numbers, cytokines, chemokines, or proteases in induced sputum or airway biopsies of patients with COPD and severe asthma. Previously, we have reported a reduction in co-repressor HDAC2 expression and total HDAC activity in COPD patients (Ito et al., 2005). By overexpression and knockdown of HDAC2, we have shown that HDAC2 is a prerequisite molecule for corticosteroid action in airway macrophages and that reduction of HDAC2 is one of the causes of corticosteroid insensitivity in COPD (Ito et al., 2006). In vitro experiments have also shown that oxidative stress raised by hydrogen peroxide reduced HDAC2 expression, and in preliminary experiments, LY294002 and Akt inhibitor SH-5 (Kozikowski et al., 2003) restored defective HDAC2 expression and activity in these cells. This suggests that PI3K may be involved in corticosteroid sensitivity through reducing HDAC activity. Further studies are in progress to elucidate this aspect.

PI3K and Cell Migration

A hallmark of inflammation is the migration of leukocytes (e.g., eosinophils, neutrophils, macrophages, and T cells) to the inflammatory lesion in response to chemokines and other chemoattractants (Curnock et al., 2002). This migration has been shown to be dependent upon the activation of PI3K. Control of cell polarity is essential for neutrophil chemotaxis and also for other cells and is dependent on GPCR-mediated myosin assembly at the tailing edge of the cell and F-actin polymerization and phosphor-protein kinase B/Akt colocalization at the leading edge of the cell. Studies in mice lacking PI3Kγ have shown that this isoform is essential for PI3Kγ PIP3 production, Akt/ protein kinase B activation, and superoxide production in neutrophils exposed to chemoattractants, such as N-formyl-Met-Leu-Phe, C5a, and IL-8, as well as neutrophil chemotactic events (rather than chemokinetic events) (Thomas et al., 2005). The chemotaxis of cells involved in mounting an effective immune response to a pathogen or foreign body (e.g., neutrophils, macrophages, and T lymphocytes) was also impaired in the absence of PI3Kγ, both in vitro and in vivo.

There is evidence to suggest that other PI3K isoforms are also activated by chemokines in PI3Kγ−/− mice. There is incomplete (e.g., 50–70%) reduction in the capacity of neutrophils to migrate to a range of chemoattractants, and PI3Kγ knockout does not prevent chemoattractant-induced actin polymerization. Certainly, in vitro assays of immunoprecipitated p85 subunits of PI3K indicate that the p85/p110 heterodimer is activated by stromal cell-derived factor-1 and RANTES in T cells and by MCP-1 in THP-1 cells. Thus, GPCR stimulation can activate p85/p110 PI3K as well as PI3Kγ through G-protein βγ subunits and/or Gαq subunit (Fig. 1).

Dendritic cells (DC) are also a target of PI3Kγ, and DC obtained from PI3Kγ−/− mice showed a reduced ability to respond to chemokines in vitro and ex vivo and to travel to draining lymph nodes under inflammatory conditions (Del et al., 2004). In addition, PI3Kγ−/− mice had a selective defect in the number of skin Langerhans cells and a reduced capacity to
PI3K in Airway Inflammation

mount contact hypersensitivity and delayed-type hypersensitivity reactions. Thus, PI3Kγ plays a nonredundant role in DC trafficking and in the activation of specific immunity.

The contribution of PI3Kγ to macrophage responses to chemoattractants has also been investigated. They observed that early membrane ruffling induced by MCP-1, which activates a GPCR, or by colony-stimulating factor-1, which activates a tyrosine kinase receptor, is unaltered in PI3Kγ−/− mice compared with wild-type macrophages. Furthermore, macrophages from PI3Kγ−/− mice showed reduced migration speed and translocation and no chemotaxis to MCP-1. This study also indicated that the initial actin reorganization induced by either a GPCR or tyrosine kinase receptor agonist is not dependent on PI3Kγ, whereas PI3Kγ is needed for optimal migration of macrophages to either agonist. Chemotaxis of airway epithelial cells is also controlled by PI3K. Shahabuddin et al. (2006) showed that wortmannin concentration-dependently inhibited the chemotactic response of epithelial cells to interferon-inducible T-cell α chemoattractant (CXCR3 ligand).

In addition to type I PI3K, activation by MCP-1 of a novel PI3K-C2α is also reported to be involved in cell (THP-1) migration, and this activation exhibits the same resistance to wortmannin and sensitivity to pertussis toxin as MCP-1-stimulated increases in 3’-phosphoinositide lipid generation. Recent work using RNA interference suggested that a class II PI3K (PI3K-C2δ) regulates lysophosphatidic acid-stimulated HeLa cell (Maffucci et al., 2005) and human embryonic kidney 293 cell (Domin et al., 2005) migration. Thus, type II PI3K might be involved in growth factor-mediated cell migration, but its role in inflammatory cell migration has not been firmly established.

PI3K and Oxidative Stress

The inflammation in COPD is an amplification of the normal inflammatory response to inhaled noxious agents (cigarette smoke or other irritants) and oxidative stress (Rahman and Adcock, 2006). Reactive oxygen species (ROS), such as H2O2 and O2•−, have emerged as key mediators of intracellular signaling, which is elevated by various types of extracellular stimuli, including growth factors, cytokines, and environmental stresses. In COPD or severe asthma, a high level of ROS from exposure to cigarette smoke or other irritants or endogenously produced from inflammatory cells, such as neutrophils and macrophages, is thought to be an important component of amplification of inflammation in the lung (Rahman and Adcock, 2006). It is widely accepted that ROS can modulate cell functions by activating MAPKs, phospholipase C, protein kinase C, and various other types of signaling components.

ROS induction is often accompanied by activation of PI3K. For example, LY294002, a specific inhibitor for PI3K, was shown to abolish chemokine-induced ROS generation in phagocytes, which was further confirmed by studies using PI3K knockout mice. It was similarly reported that the ROS accumulation induced by tumor necrosis factor α, platelet-derived growth factor, or vascular endothelial growth factor in various other cell types was suppressed when PI3K activity/activation was blocked by pharmacological or molecular means. Therefore, PI3K seems to be commonly involved in the ROS accumulation induced by cytokines and growth factors (Qin and Chock, 2003). It was also reported that serum withdrawal (SW) killed human U937 blood cells by elevating cellular ROS levels, which occurred through PI3K activation (Lee et al., 2005).

In addition to the role of PI3K in ROS induction, evidence that supports the opposite hierarchical relationship exists between ROS and PI3K. PI3K in various cell types was activated in response to the exogenous application of hydrogen peroxide (H2O2). Consistent with the ability of H2O2 to activate PI3K, the PI3K activation induced by UV irradiation or Zn2+ treatment was blocked by the addition of antioxidants. Exogenous H2O2 can activate an array of nonreceptor-type protein tyrosine kinases. H2O2 stimulation leads to the initiation of downstream signaling events, such as activation of PLCγ2, MAPK, and activation of PI3K. This activation of PI3K is selective as H2O2 induced tyrosine phosphorylation of the p110 but not the p85 subunit of PI3K in DT40 cells (chicken B cell line) (Qin and Chock, 2003). In addition, hydrogen peroxide treatment caused an increase in the amount of p85 PI3K associated with the particulate fraction. Collectively, these results indicate that the hydrogen peroxide-induced PI3K and Akt activation was achieved through PI3K membrane recruitment to its substrate site, thereby enabling PI3K to maximize its catalytic efficiency.

In contrast, PI3K is also involved in expression of antioxidant molecules. The antioxidant cell defense represented by heme oxygenase-1 (HO-1) at the level of a newly identified Sp1 site in the human HO-1 proximal promoter (Rojo et al., 2006). Interestingly, PKCζ, but not Akt-1, is a downstream effector of PI3K for the regulation of HO-1 expression at the Sp1 site. PKCζ then acts on the canonical MAPK mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathway that is responsible for PI3K-induced up-regulation of HO-1 expression at this promoter region, and NF-κB, which is a downstream signal of Akt-1, is not relevant at this promoter. However, in a human aortic smooth muscle cell system, HO-1 is reported to be up-regulated through the Akt pathway, and this is dependent on the activation of the HO-1 promoter by NF-E2-related factor-2 (Nrf2) transcriptional factor (Brunt et al., 2006). Importantly, increased induction of HO-1 has been reported in asthma and COPD (Mo et al., 2005; Tsoumakidou et al., 2005). This overexpression of HO-1 might be a compensatory mechanism for the regulation of airway inflammation through PI3K activation. To elucidate whether PI3K plays a role in the inflammatory or anti-inflammatory pathway under oxidative stress in COPD and asthma, more translational research will be required.

PI3K and Th1/Th2 Balance

In asthma, Th2-type cytokine (IL-4, IL-5, and IL-13) production is dominant; however, in COPD and perhaps in severe asthma, Th1 cytokines (e.g., interferon-γ) are reported to be produced more than Th2 cytokines, suggesting that the Th1/Th2 balance might affect the pathogenesis of these diseases. Several papers using both pharmacological tools and gene-modified mice have implicated PI3K in T and B lymphocyte activation/function (Koyasu, 2003; Ward and Finan, 2003; Vanhaesebroeck et al., 2005). Recent studies into the role of PI3Ks in innate immunity have also highlighted their involvement in the control of cellular responses to pathogens.
In this regard, the amount of IL-12 produced by stimulation through Toll-like receptors is critical in the balance between Th1 and Th2 responses. Interestingly, mice lacking the p85\(\alpha\) regulatory subunit (single knockout) show impaired immunity against the intestinal nematode Strongyloides venezuelensis as a result of impaired Th2 responses. In contrast, p85\(\alpha^{−/−}\) mice demonstrate enhanced Th1 responses and, unlike wild-type mice, are resistant to Leishmania major infection. These observations indicate that class IA PI3Ks are important in the Th1 versus Th2 balance in vivo and that they control induction of the Th2 response and/or suppression of the Th1 response. p85\(\alpha^{−/−}\) splenic and bone marrow-derived DCs produce more IL-12 than wild-type DCs, suggesting that PI3K is one of the key regulators in the Th1 versus Th2 balance through control of IL-12 production. Overproduction of IL-12 by DCs might cause the skewed Th1 response in p85\(\alpha^{−/−}\) mice. These observations indicate that PI3K plays a critical negative regulatory role during induction of the Th1 immune response by suppressing the production of IL-12 from DCs, although it is unclear which of the three class IA catalytic isoforms is involved (Fukau and Koyasu, 2003). In addition to Toll-like receptor, CD40L and RANKL (ligand to receptor activator of NF-κB) also induce IL-12 via activation of class 1A PI3K.

### TABLE 2

PI3K inhibitors and their potency

<table>
<thead>
<tr>
<th>Compound</th>
<th>Company</th>
<th>Specificity</th>
<th>Activity (\mu M)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wortmannin</td>
<td>Nonspecific</td>
<td></td>
<td>0.001 (α)</td>
<td>Myosin light chain kinase inhibitor</td>
</tr>
<tr>
<td>LY29002</td>
<td>Lilly</td>
<td>Nonspecific</td>
<td>0.014 (β)</td>
<td>Casein kinase 2 inhibitor</td>
</tr>
<tr>
<td>PX-866</td>
<td>ProlIX</td>
<td>Nonspecific</td>
<td>0.72 (α)</td>
<td></td>
</tr>
<tr>
<td>SF1126</td>
<td>Semafor</td>
<td>Nonspecific</td>
<td>0.31 (β)</td>
<td></td>
</tr>
<tr>
<td>ZSTK474</td>
<td>Zenyaku Kagyo</td>
<td>Nonspecific</td>
<td>1.3 (δ)</td>
<td></td>
</tr>
<tr>
<td>YM-024</td>
<td>Yamanouchi</td>
<td>p110(\alpha)</td>
<td>0.005 (γ)</td>
<td></td>
</tr>
<tr>
<td>TGX-221</td>
<td>Kinacia Pty Ltd.</td>
<td>p110(\beta)</td>
<td>&gt;0.3 (β)</td>
<td></td>
</tr>
<tr>
<td>CBL1309 (KN309)</td>
<td>Cerylid</td>
<td>p110(\beta)</td>
<td>0.0027 (δ)</td>
<td></td>
</tr>
<tr>
<td>IC87114</td>
<td>ICOS</td>
<td>p110(\delta)</td>
<td>0.009 (γ)</td>
<td></td>
</tr>
<tr>
<td>Theophylline</td>
<td></td>
<td>p110(\delta)</td>
<td>9.1 (γ)</td>
<td>Phase I prodrug</td>
</tr>
<tr>
<td>AS-604850</td>
<td>Serono</td>
<td>p110(\gamma)</td>
<td>0.1 (δ)</td>
<td></td>
</tr>
</tbody>
</table>

**Phosphatase**

Termination of PI3K signaling by degradation of PI(3,4,5)\(P_3\) can be mediated by at least two different types of phosphatases, namely Src homology 2-containing inositol 5-phosphatase (SHIP) and phosphatase and tensin homolog deleted on chromosome 10 protein (PTEN) (Koyasu, 2003). PTEN removes the 3-phosphate of PI(3,4,5)\(P_3\) and, thus, directly counteracts all types of PI3K by catalyzing the opposite reaction. PTEN knockout in mice is embryonic lethal; however, PTEN\(^{−/−}\) mice showed tumorigenesis, T-lymphocyte activation, and increased T-cell chemotaxis. PTEN is reported to play a pivotal role in Th2-mediated airway inflammation and airway responsiveness (Kwak et al., 2003), and also PTEN overexpression reduced airway hyper-responsiveness and vascular endothelial growth factor expression in a murine model of asthma (Lee et al., 2006a). In contrast, SHIP removes the 5-phosphate from the inositol ring of PI(3,4,5)\(P_3\) to generate PI(3,4)\(P_2\), and this dephosphorylation can be mediated by at least two different types of PI3K inhibitors and their potency.

**CLB1309 (KN309), (±)-2-[[1-(7-methyl-2-(morpholin-4-yl)-4-oxo-pyrido[1,2-c]pyrimidin-9-yl)ethyl]amino]benzoic acid; TGX-221, 7-methyl-2-(4-morpholinyl)-9-[1-(phenylamino)ethyl]-4H-pyrido[1,2-a]pyrimidin-4-one; PX-866, acetic acid (1S,4E,10R,11R,13S,14R)-1,4-diallylaminomethylene-6-hydroxy-1-methoxymethyl-10,13-dimethyl-3,7,17-trioxo-1,3,4,7,10,11,12,13,14,15,16,17-dodecahydro-2-oxacyclohepta[α]phenanthrene-11-yl ester.**

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and increased chemotaxis. These mice also suffer from a lethal accumulation of macrophages and neutrophils into the lungs; therefore, persistent high levels of PI(3,4,5)P3 and subsequent activation of its downstream effectors might lead to excessive inflammation (Helgason et al., 1998; Rauh et al., 2003).

**Isoform Specific Inhibitors**

The availability of two PI3K inhibitors wortmannin, an irreversible inhibitor (isolated from *Penicillium wortmannii*), and the morpholino derivative LY294002, a reversible inhibitor that is derived from the broad-spectrum kinase inhibitor quercetin, has contributed greatly to our understanding of the biological role of PI3K and its effector proteins (Ward and Finan, 2003). However, wortmannin and LY294002 have no selectivity for individual PI3K isoforms, poor stability, solubility, toxicity, and absorption. In addition, wortmannin and LY294002 exhibit some compound-specific toxicity and possess off-target effects (wortmannin, myosin light chain kinase inhibition; LY294002, casein kinase-2 inhibition). Efforts are underway to develop new nonspecific inhibitors, such as ZSTK474 (Ward et al., 2003), polyethylene glycol-wortmannin, LY294002-stable derivative, and LY294002 prodrug, and one of these compounds, named SF1126 (Semaphore Pharmaceuticals, Indianapolis, IN), is about to undergo clinical evaluation (Table 2).

As discussed above, selective PI3Kδ inhibitors have the potential to treat chronic airway disease. A number of patent specifications have been published that describe isoform-specific inhibitors of PI3K (Powis et al., 2006). ICOS Corporation has described several p110δ inhibitors, including IC87114, which contains a quinazoline core structure. Methylxanthines, such as caffeine and theophylline, were also reported as selective inhibitors for p110δ isoforms, although their activity is rather low (Foukas et al., 2002), and also possess several off-target effects, such as phosphodiesterase inhibition and adenosine A receptor antagonism. Pomel et al. (2006) also reported furan-2-ylmethenyl thiazolidinediones as novel, potent, and selective inhibitors of PI3K by structure based design and X-ray crystallography of complexes formed by inhibitors bound to PI3K. AS-604850 and related compounds are selective PI3Kγ inhibitors that show efficacy in a murine model of rheumatoid arthritis (Camps et al., 2005). In addition, a number of companies have declared active programs in PI3Kγ inhibitor development (Novartis, Boehringer, Pfizer, Bayer, etc.) (Pomel et al., 2006) for cancer and chronic inflammatory disease, but no published re-
suits are available for these compounds indicating anti-inflammatory efficacy in respiratory disease models.

Conclusions
PI3K family plays a prominent role in various inflammatory cells by controlling cell growth, differentiation, survival, proliferation, migration, and mediator production (such as cytokines) through its downstream components. As shown in Fig. 2, most inflammatory cells relevant to asthma and COPD are controlled by type I PI3Ks, especially PI3Kδ and γ. Several PI3K inhibitors are under development for the treatment of asthma and COPD, as well as cancer, thrombosis, cardiacc contractility during heart failure, hypertension, rheumatoid arthritis, and inflammatory bowel disease. It is most likely that PI3K inhibitors will be more efficacious in more severe steroid-insensitive asthma and in COPD where corticosteroids are of limited effectiveness and no alternative therapy is available. In addition, it is possible that specific PI3K inhibitors will be more efficient in augmenting current therapies, particularly corticosteroids rather than monotherapy because PI3Kδ inhibitors have the potential to restore corticosteroid sensitivity in vitro as discussed above. The success of selective PI3K inhibitors in reaching the clinic will depend upon the specific isoform activated in each disease as a group and, importantly, in each individual patient.

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References

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