Phosphoinositide 3-kinase signalling in lung disease: leucocytes and beyond

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Summary

The family of lipid kinases termed phosphoinositide-3-kinase (PI3K) is known to contribute at multiple levels to innate and adaptive immune responses, and is hence an attractive target for drug discovery in inflammatory and autoimmune disease, including respiratory diseases. The development of isoform-selective pharmacological inhibitors, targeted gene manipulation and short interfering RNA (siRNA) target validation have facilitated a better understanding of the role that each member of this family of kinases plays in the physiology and pathology of the respiratory system. In this review, we will evaluate the evidence for the roles of specific PI3K isoforms in the lung and airways, and discuss their potential as targets for novel drug therapies.

Keywords: PI3K; lung disease; asthma; inflammation

The defining characteristics of chronic respiratory diseases include inflammatory cell recruitment, inflammatory mediator expression, tissue remodelling, and altered airway smooth muscle contraction. Central to these events is a complex interplay between receptor signalling and downstream lipid and protein kinases. One of the main families of kinases involved in these processes is phosphoinositide 3-kinase (PI3K). As respiratory diseases are very common[1,2] and novel drugs are required to cover the unmet needs of patients, recent advances point to the modulation of PI3K as a possible therapeutic target. In this article, we review the role that PI3K plays in lung diseases and the possible beneficial effects of targeting PI3K activity.

The PI3K signalling pathway: an overview

Phosphatidylinositol 3-kinase was initially postulated as a cancer target because it was initially co-purified with oncoproteins in 1987.[3] Since then it has been implicated as a potential drug target in other disease settings, reflecting its essential roles in many of our basic homeostatic mechanisms, including cell differentiation, growth, metabolism and immune function.

The term PI3K is applied to a family of kinases that phosphorylates the D-3 position of the inositol ring of target lipids; Class I PI3Ks phosphorylate $PI(4,5)P_2$ to produce $PI(3,4,5)P_3$, a critical second messenger that propagates intracellular signals through a host of downstream protein mediators.[4] $PI(3,4,5)P_3$ acts as a docking site at the plasma membrane, recruiting and activating proteins containing phospholipid-binding domains. These downstream PI3K effectors include protein kinases that promote cell growth, survival and proliferation (such as Akt, PDK1, and the Tec family kinases); GAPs and GEFs (such as P-Rex[5] and Swap70[6]) that regulate the GTPases mediating cell motility and membrane trafficking; and scaffolding proteins (Gab2, etc.) that nucleate the assembly of key signalling complexes.[7,8] As highlighted in Fig. 1, PI3K is therefore a hub through which many signals are relayed before being specialized into secondary streams of signalling, and thus the therapeutic challenge is to achieve control of pathological activation without detrimental off-target effects. Specific targeting of individual PI3K isoforms may provide the key to understanding and controlling PI3K in disease.

As seen on Table 1, the PI3K family is divided into three classes based on their different isoform structure and substrate specificity. These differences, coupled with differences in their distribution, allow PI3K isoforms to mediate distinct functions (a list of which also appears in Table 1). Class I PI3K is the most studied of the PI3K classes and the majority of research focuses on these isoforms. Comparatively little is known about Class II PI3Ks, aside from their suspected role in clathrin-associated

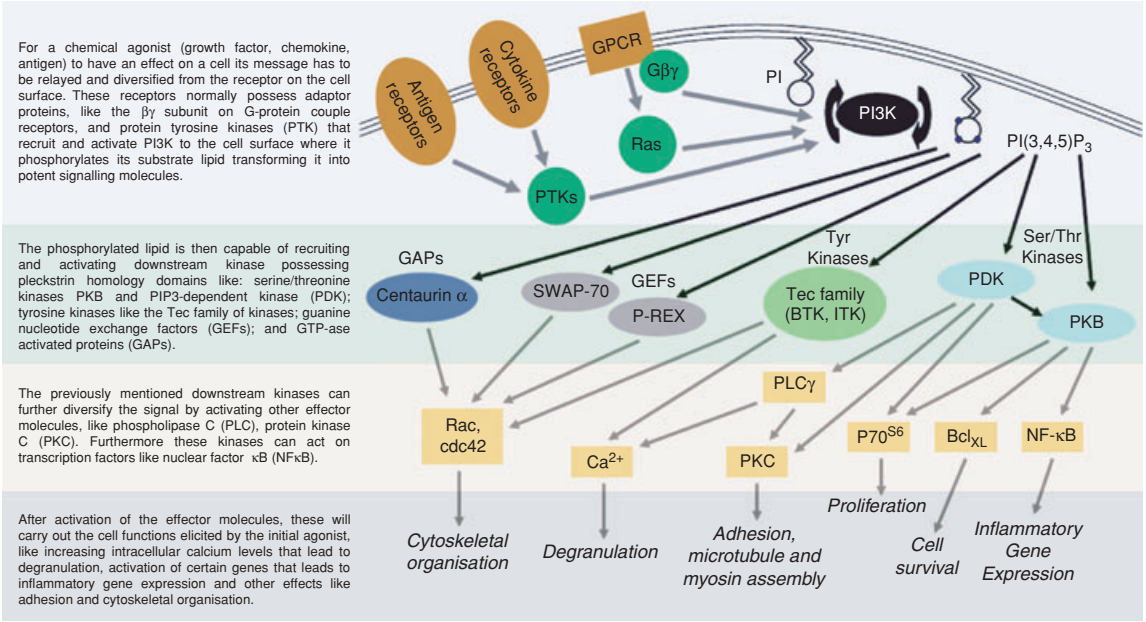

Figure 1. PI3K as a central hub in signalling in inflammation and homeostasis. This figure aims to be representative of the main kinases and proteins involved in different cell functions.

vesicle trafficking,[9] partly because Class II PI3K-C2α is refractory to inhibition by wortmannin and LY294002,[10] the most widely used broad-spectrum inhibitors of PI3K. Therefore, use of these compounds (LY294002, wortmannin) has probably underestimated the potential roles for this isoform. Finally, class III PI3K produce an apparently non-signalling inositol lipid, although class III PI3K has also been recently suggested to be important in Toll-like receptor (TLR) signalling.[11]

Class I PI3K functions

Elucidation of the precise functions of different classes of PI3K has been a difficult process. The primary pharmacological tools to determine PI3K function, the inhibitors wortmannin and LY294002, have broad specificity across PI3K isoforms.[12] These compounds also inhibit enzymes that are functionally associated with PI3K, like the mammalian target of rapamycin and myosin light-chain kinase.[13] With high interest within the pharmaceutical industry, second-generation isoform-specific inhibitors are now being generated (Table 1). The use of these inhibitors, as well as molecular and gene-targeting technology, have begun to give us a more profound insight into the different roles of each one of the members of the PI3K family. The ubiquitously expressed p110α and p110β catalytic subunits have been particularly difficult to study individually, because knockout mice have proven to be lethal at an embryonic stage, suggesting a role for these isoforms in cell proliferation during development.[14,15] Nevertheless, studies using heterozygous animals and isoform-specific antibodies have yielded some results, as seen in Table 1. Most progress has been made regarding the p110γ and p110δ isoforms as outlined below.

p110γ and p110δ are key signals in the innate and adaptive immune responses

Both p110γ and p110δ are expressed predominantly (but not exclusively) in leucocytes, leading to speculation that these isoforms are the dominant isoforms involved in PI3K-mediated signalling of both the innate and adaptive immune responses.[16] Indeed, PI3K is activated by different TLRs in eosinophils,[17] macrophages,[18] neutrophils[19] and dendritic cells.[20] However, PI3Ks are implicated in the negative regulation of TLR-induced interleukin-12 (IL-12) and interferon-γ production.[20,21] Hence, gene targeting of class IA isoforms results in defective clearance of intestinal nematodes [a T helper type 2 (Th2) response] but improved resistance to *Leishmania* infection (a Th1-dependent response).[20,22]

For adaptive immunity, both p110δ$^{-/-}$ mice[23] and mice expressing an inactive form of the p110δ catalytic subunit[24] display impaired B-cell and T-cell antigen receptor signalling. Of specific importance to respiratory disease, it has been shown recently that B-cell receptor-mediated antigen presentation is dependent on p110δ activity.[25] Furthermore, analysis of mice expressing a catalytically inactive form of the p110δ isoform has revealed a pivotal role for this isoform in CD28-costimulated T-cell clonal

Table 1. Main characteristics of the members of the PI3K family

Class	Subclass	Catalytic subunit	Adaptor/ Regulatory subunits	Regulated by	Distribution	*In vitro* lipid products	Functions for selective inhibitors	Organization with licences
I	A	p110α	p85α, p85β, p55α, p55γ, p50α	RTK[a]	Ubiquitous	PI(3)P[b], PI(3,4)P2[d], PI(3,4,5)P3[f]	Development[c 14], Human cancer[e 116], Myocardial contractility[117]	Eli Lilly Iconix Echelon Astra Zeneca Wyeth PIramed More[g]
		p110β	p85α, p85β, p55α, p55γ, p50α	RTK[a], GPCR[h]	Ubiquitous	PI(3)P[b], PI(3,4)P2[d] PI(3,4,5)P3[f]	Development[c 15], Insulin signalling[118], Motility[119], Phagocytosis[120], Thrombus formation[121]	Kinacia
		p110δ	p85α, p85β, p55α, p55γ, p50α	RTK[a]	Leucocytes[i]	PI(3)P[b], PI(3,4)P2[d], PI(3,4,5)P3[f]	Immunity[24], Cytokine receptor signalling[122], B-cell development[24], B-cell migration[38], T-cell development[123], Cell proliferation[124], B- and T-cell antigen receptor signalling[24], Smooth muscle tone and hypertension[125], Neutrophil migration and burst[40]	ICOS PIramed
	B	p110γ	p101, p87[PIKAP]	GPCR[h]	Myeloid[i]	PI(3)P[b], PI(3,4)P2[d], PI(3,4,5)P3[f]	Thymocyte development[126], T cell development[123], T cell migration[38], Neutrophil migration[126], Macrophage and dendritic cell migration[37], Mast cell degranulation[29], Neutrophil burst[40], Insulin secretion[127], Myocardial contractility[117]	Novartis Bayer Pfizer Serono Targegen Calbiochem
II		C2α	Clathrin	RTK[a] GPCR[h], IR[j]	Widespread	PI(3)P[b], PI(3,4)P2[d]	Vesicle trafficking[9], Insulin signalling[128], Cell survival[129], Vascular smooth muscle contraction[130]	
		C2β	Clathrin	RTK[a], GPCR[h], IR[j]	Widespread	PI(3)P[b], PI(3,4)P2[d]	Vesicle trafficking[131], Cell migration[132,133], Liver growth[134]	
		C2γ	Clathrin	?	Hepatic	PI(3)P[b], PI(3,4)P2[d]	Liver regeneration[135]	
III		Vps34p	Vps15p	TLR[k], constitutive	Ubiquitous	PI(3)P[b]	Toll-like receptor signalling[136], Receptor-independent membrane trafficking[137]	

[a]Receptor tyrosine kinase; [b]phosphatidyl inositol-3-monophosphate; [c]deletion associated with defective mouse embryonic proliferation and death; [d]phosphatidyl inositol-3,4-bisphosphate; [e]mutated/amplified in human cancer; [f]phosphatidyl inositol 3,4,5-trisphosphate; [g]due to the high frequency of *PIK3CA* mutations in human cancer, extensive research into selective inhibitors for this isoform have been carried out by many other institutions (Yamanouchi, Xinxiang Medical College, Semafore, Chiron, Zenyaku Kogyo, Boehringer, University of British Columbia); [h]G-protein coupled receptors; [i]mainly, but also expressed in endothelial cells and others; [j]integrin receptors; [k]Toll-like receptors.

expansion and differentiation[26] as well as function of $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells.[27] Finally, PI3K isoforms have also been demonstrated to play key roles in mast cell function, which are linked to both innate and adaptive responses and will be discussed in further detail later.[28,29]

p110γ and p110δ are the predominant isoforms involved in leucocyte migration

The ordered directional migration of leucocytes is a key process fundamental to constitutive immune surveillance and immune responses. Macrophages and neutrophils from p110γ$^{-/-}$ mice display reduced migratory capacity in response to several chemoattractants including chemokines.[30] The p110γ isoform also mediates T-cell migration both *in vitro* and *in vivo* although its involvement is context-dependent and often depends on the activation/differentiation status of the T cell.[31–34] More recent evidence indicates that while 3′-phosphoinositide lipids accumulate in a p110γ-dependent fashion at the up-gradient leading edge, this signal is not required for efficient gradient sensing or gradient-biased movement.[35,36] Rather, p110γ appears to be a critical determinant of overall cell motility rather than directional aspects of migration. Possibly as a consequence of impaired migratory capacity, p110γ$^{-/-}$ mice exhibit defects in T-cell development and activation. These mice also show a defective capacity to mount contact hypersensitivity and delayed-type hypersensitivity reactions.[37] T-cell migration is largely unaffected by p110δ mutation,[38] suggesting that the p110γ isoform is the predominant isoform regulating migration in this cell type.

In contrast with the T-cell effects described above, B-cell migration to chemokines is not significantly affected by p110γ deficiency, but is strikingly impaired in mice expressing a catalytically inactive form of the p110δ isoform.[19,20] In this regard, analysis of p110δ-deficient B cells showed a defect in B-cell chemotaxis to CXCL13, while responses to CCR7 and CXCR4 ligands were less affected. Similarly, *in vivo* administration of the novel p110δ-specific inhibitor IC87114 reduces neutrophil tethering and increases rolling velocities on cytokine-activated microvessels.[39]

The p110δ–p110γ partnership

As p110δ and p110γ share similar patterns of distribution within immune cells, they interact to accomplish certain functions within these cells. *N*-formyl-methionyl-leucyl-phenylalanine stimulation of tumour necrosis factor-α (TNF-α)-primed human neutrophils results in a biphasic activation of PI3K. The first phase depends on p110γ, while the second phase is largely dependent on p110δ. The second phase of PI3K activation is increased by TNF-α priming and it regulates the activation of reactivate oxygen species production. However, this p110δ-dependent second-phase PI3K activity requires the p110γ-dependent first phase.[40] Similarly, in p110δ/p110γ$^{-/-}$ mice, the activity of both these isoforms has been found to be essential for thymocyte survival as well as T-cell development and production.[41,42] Furthermore, as reviewed further on, this p110δ–p110γ partnership also exists in parenchymal cells (i.e. specialized tissue cells of an organ, like lung epithelial and endothelial cells), where these isoforms interact to express adhesion molecules.[43] In short, both p110δ and p110γ represent exciting therapeutic targets in specific inflammatory conditions because blockade of their activity is likely to influence immune function at multiple levels.

Importance of PI3K in immune cell function and relevance to lung disease

Within chronic respiratory diseases, asthma and chronic obstructive pulmonary disease (COPD) are the most common clinical entities; while acute respiratory distress syndrome and lung fibrotic diseases represent a significant therapeutic challenge. A summary of PI3K research into the pathophysiology of lung disease can be found in Table 2.

Acute lung injury (ALI) and adult respiratory distress syndrome (ARDS)

The importance of ALI-ARDS has recently been highlighted by a European multicentre epidemiological survey, in which the occurrence in intensive-care units approximates 7% of all admissions and 15% of patients receiving more than 24 hr of mechanical ventilation. Overall, the hospital mortality of patients diagnosed with ARDS was close to 55%.[44] ALI-ARDS is characterized by increased expression of proinflammatory cytokines, development of interstitial pulmonary oedema and neutrophil accumulation in the lungs[45,46] that cannot be explained by, but may coexist with, left atrial or pulmonary capillary hypertension.

The distinction between ALI and ARDS is the degree of hypoxaemia; ARDS represents that subset of patients at the severe end of the spectrum of ALI[47] who are characterized by a diffuse inflammation of their lung parenchyma. ALI-ARDS is caused by an uncontrolled systemic inflammatory response resulting from clinical events including major surgery, trauma, multiple transfusions, severe burns, pancreatitis, sepsis and prolonged mechanical ventilation.[48] The major animal models for ALI-ARDS replicate more closely the events of these latter two causes, as intratracheal instillation of lipopolysaccharide (LPS) or mechanical overventilation of the lungs is commonly used.[49,50] The initial insult on the lung tissue results in the release of inflammatory mediators by local macrophages, epithelial and endothelial cells. This array of chemical agents, in conjunction with surface expression of adhesion molecules by parenchymal cells, attracts neutrophils and T lymphocytes into the lungs, further amplifying the inflammation, creating a positive feedback loop in which inflammation leads to more inflammation, leading inexorably to tissue destruction and fibrosis.[50]

Table 2. PI3K, and its individual isoforms, as targets in respiratory disease research

Target	Disease	Model	Research tool	Targeting impact	Refs
PI3K	ALI-ARDS	Endotoxin-induced (*in vitro*)	Inhibitor	Reduced expression of IL-1β and TNF-α in cultured neutrophils	46
		Endotoxin-Induced (*in vivo*)	Inhibitor	No reduction in IL-6 or CXCL2 levels	54
		Endotoxin-Induced (*in vivo*)	Inhibitor	Decreased oedema, neutrophil infiltration and inflammatory cytokine expression	46
		Overventilation (*ex vivo*)	Inhibitor	Attenuated filtration coefficients in isolated lungs	55
		Overventilation (*in vivo*)	Inhibitor	Diminished IL-6 and CXCL2 release	54
		HCl lung instillation (*in vivo*)	Inhibitor	Reduced neutrophil recruitment to lungs	60
	COPD	IL-1β-induced mucin secretion (*in vitro*)	Inhibitor	Attenuated mucin secretion in cultured airway epithelial cells	95
		Chemotaxis assay (*in vitro*)	Inhibitor	Inhibited chemotactic responses to a CXCR3 ligand in cultured airway epithelial cells	138
		Nicotine-induced survival and transformed phenotype (*in vitro*)	Inhibitor	Prevented transformed phenotype and enhanced survival in human airway epithelial cells	87
	Asthma	IL-13-induced mucus secretory phenotype (*in vitro*)	Inhibitor	Reduced mucus hypersecretory phenotype and goblet cell metaplasia in bronchial epithelial cell cultures	88
		Thrombin-, EGF-induced and PDGF-induced (*in vitro*)	Inhibitor	Reduced proliferation of cultured airway smooth muscle cells	101, 102, 104
		Long-term serum deprivation (*in vitro*)	Inhibitor	Inhibited the transformation of cultured airway myocytes into a contractile phenotype	96
		Methacoline-induced (*in vitro*)	Inhibitor	Partially attenuated contraction in cultured airway smooth muscle strips	99
		Ovalbumin-induced (*in vitro*)	Inhibitor	Decreased bronchial contraction in isolated bronchial tissue	98
		Ovalbumin-induced (*in vivo*)	Inhibitor	Diminished levels of interleukins (4, 5 and 13), lung tissue eosinophilia, airway mucus production and AHR	71, 72
		Ovalbumin-induced (*in vivo*)	Inhibitor	No improvement in lung eosinophilia or in AHR to acetylcholine, attenuated eosinophil levels and degranulation in BAL fluid, and AHR to histamine	78, 80
p85α	ALI-ARDS	TNF-α-induced (*in vitro*)	Blocking protein (TAT construct)	Attenuated reactive oxygen species production in primary granulocytes	59
		TNF-α-induced (*in vivo*)	Blocking protein (TAT construct)	Reduced microvascular injury and granulocyte recruitment to the lung	59
	Asthma	Ovalbumin-induced (*in vivo*)	Gene disruption	Decreased secretion of interleukins (4 and 5), eosinophil and lymphocyte lung infiltration, and AHR	73
p110	Asthma	Thrombin-, EGF-induced (*in vitro*)	Gene disruption	Attenuated proliferation of cultured airway smooth muscle cells	106
p110δ	ALI-ARDS	i.p. *E. coli* challenge (*in vivo*)	Gene disruption	No protection against disease model	61
		Endotoxin-Induced (*in vivo*)	Inhibitor	Reduced lung neutrophil accumulation	39, 46
		Endotoxin-Induced (*in vivo*)	Gene disruption	Diminished neutrophils recruitment, pulmonary levels of IL-1β and TNF-α	46
		Endotoxin-Induced (*in vivo*)	Gene disruption	Reduced neutrophil recruitment	43
	COPD	Intranasal chemokine instillation (*in vivo*)	Gene disruption	No protection against disease model	67

Table 2. (*Continued*)

Target	Disease	Model	Research tool	Targeting impact	Refs
	Asthma	Allergen-IgE-induced (*in vitro*)	Gene disruption and inhibitor	Inhibited degranulation and cytokine release in cultured mast cells	28
		Ovalbumin-induced (*in vivo*)	Inhibitor	Diminished levels of interleukins (4, 5 and 13), leucocyte infiltration to the lungs, and IgE and LTC4 release	74
		Ovalbumin-induced (*in vivo*)	Haemopoietic cell-restricted gene disruption	Reduced levels of type 2 cytokines, eosinophil recruitment to the lung, airway inflammation and mucus production	75
p110γ	ALI-ARDS	Overventilation (*ex vivo*)	Gene disruption	Attenuated levels of histological indices and levels of PKB phosphorylation in isolated lungs	56
		Endotoxin-induced (*in vivo*)	Gene disruption	Decreased neutrophil recruitment to the lung	43
	COPD	Chemotaxis assay (*in vitro*)	Gene disruption	Reduced chemotaxis to CXCR2 ligands in neutrophils	67
		Intranasal chemokine instillation (*in vivo*)	Gene disruption	Diminished neutrophil accumulation in the lung	67
	Asthma	Allergen-IgE-adenosine-induced (*in vitro*)	Gene disruption	Partially attenuated degranulation and cytokine release in cultured mast cells	29

AHR, airway hyperresponsiveness; ALI-ARDS, acute lung injury–adult respiratory distress syndrome; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; EGF, epidermal growth factor; IgE, immunoglobulin E; IL-6, interleukin-6; LTC4, leukotriene C4; PDGF, platelet-derived growth factor; TNF-α, tumour necrosis factor-α.

PI3K and ALI-ARDS

In isolated lungs, ventilation with increased volumes or pressures, termed overventilation, elicits local and systemic concentrations of proinflammatory mediators[51] to an extent that is comparable with that achieved by bacterial endotoxins.[52] Various studies have identified ventilation with high distending volumes/pressures as a potent physical force that activates PI3K[53,54] with overventilation causing a much stronger activation of protein kinase B (PKB) in lung homogenates than that seen with LPS instillation.[54]

Recently two publications have emphasized the role of PI3K in ALI-ARDS models. Miyahara *et al.* showed that LY294002-pretreatment in isolated lungs attenuated ventilation-induced lung injury, as measured by filtration coefficients.[55] Moreover, Lioetti *et al.* demonstrated that overventilated isolated lungs from $p110\gamma^{-/-}$ mice possessed diminished levels of histological indices of lung injury and of PKB phosphorylation compared to wild-type[56] suggesting this PI3K isoform as a possible drug target within ventilation-induced ALI-ARDS.

As is the case for most inflammatory diseases, the main focus of research in ALI-ARDS is maintained in immune cells. Prolonged exposure to inflammatory mediators, for example in sepsis, is considered one of the main underlying mechanisms in ARDS.[57] As a consequence of an overreactive immunological response to infection, circulating leucocytes become activated with an increased fraction moving into the pulmonary vessels. As the inflammation continues, leucocytes migrate into the lung tissue and increase endothelial permeability, generating pulmonary oedema and impaired gas exchange function, hence the beginning of ALI-ARDS.[58] In the case of endotoxin-induced ARDS models, Yum *et al.* found that *in vitro* exposure of neutrophils to endotoxin resulted in phosphorylation of PKB, activation of nuclear factor-κB (NF-κB) and expression of the proinflammatory cytokines IL-1β and TNF-α through PI3K-dependent pathways. *In vivo*, endotoxin administration to mice resulted in the activation of PI3K and PKB in neutrophils that accumulated in the lungs.[46] Furthermore, inhibition of PI3K leading to a blockade of NF-κB activation, decreased endotoxin-induced oedema, neutrophil infiltration and proinflammatory cytokine expression in the lungs.[46] In a study using a TNF-α-induced model of lung inflammation, mice expressing a dominant negative form of the PI3K p85 regulatory protein showed reduced microvascular injury and decreased recruitment of granulocytes to the lungs.[59] Moreover, they demonstrated that granulocytes treated with LY294002 or expressing the dominant negative p85 construct were prevented from oxidant production in response to TNF-α.[59] In an *in vivo* mouse model of gastric acid aspiration-induced ALI, treatment with presqualene diphosphate (a novel structural mimetic of an anti-inflammatory membrane lipid) inhibits PI3K

activity and reduces lung neutrophil recruitment compared with the results from control groups.[60]

There are few studies looking at the specific roles of each of the PI3K isoforms in ALI-ARDS models. Puri *et al.* using the Class IA p110δ-selective inhibitor IC87114, showed a significant reduction in neutrophil accumulation in an endotoxin-induced model of acute lung injury.[39] Moreover, the severity of endotoxaemia-induced ALI was significantly diminished in p110$\gamma^{-/-}$ mice.[46] The extent of injury (quantified by lung oedema, nuclear translocation of NF-κB, neutrophil recruitment, and pulmonary levels of IL-1β and TNF-α) was significantly reduced in p110$\gamma^{-/-}$ mice compared with wild-type mice.[46] However, in another model, where lung neutrophil recruitment and vascular injury was bacteraemia-induced (i.e. by intraperitoneal *Escherichia coli* challenge) instead of endotoxin-induced, p110$\gamma^{-/-}$ mice presented higher levels of leucocyte accumulation in the lung, as well as greater microvascular permeability, resulting in lung oedema. These changes were only significant for the first hour after *E. coli* injection, thereafter pathological events were similar in both groups (p110$\gamma^{-/-}$ and wild-type) mice.[61] Although a possible explanation for this increased infiltration of leucocytes could be the neutrophilia previously observed in p110$\gamma^{-/-}$ mice[62] the authors associated these unfavourable events in lung inflammation with increased expression of CD47 and integrin β_3 by p110$\gamma^{-/-}$ leucocytes.[61] These results show that the role of PI3K, particularly p110γ, in septicaemia-related neutrophil activation (including that involved in ALI-ARDS) is still unclear.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease is one of the most important causes of impaired respiratory function. It is defined by irreversible, progressive airflow limitation associated with an abnormal inflammatory response of the lungs to certain particles or gases, primarily those found in tobacco smoke. COPD includes different clinical entities, most prominently chronic bronchitis and emphysema.[63] The cell types that are characteristically involved within the pathogenesis of COPD are $CD8^+$ T cells, macrophages and neutrophils with certain lung parenchymal cells (such as epithelial cells) also activated.[64] There are many different animal models for COPD, the most common being elastase instillation and cigarette-smoke exposure, with gene-targeting techniques quickly gaining widespread acceptance and usage.[65]

PI3K and COPD

Unfortunately, no studies have reported the responses of PI3K gene-targeted animals in COPD models. Nevertheless, there are certain studies that evaluate the potential importance of PI3K within this pathology. Neutrophil recruitment into sites of inflammation is central to the pathology of several disease states, including COPD.[66] Thomas *et al.* observed that p110$\gamma^{-/-}$ neutrophils *in vitro* presented reduced chemotaxis towards keratinocyte-derived chemokine (KC, the mouse orthologue of human CXCL1) and macrophage inflammatory protein-2 (MIP-2 or CXCL2), both chemokines signalling through the CXCR2 receptor. Furthermore, p110$\gamma^{-/-}$ mice showed reduced accumulation of neutrophils following intranasal instillation *in vivo*; whereas mice possessing a p110δ kinase-dead mutation showed no inhibition in either scenario.[67]

Asthma

Asthma, a chronic disease characterized by airway hyperreactivity, inflammation and remodelling, occurs in 5–8% of the US population and is an extraordinarily common cause of pulmonary impairment worldwide.[68] Bronchial asthma is characterized by airway eosinophilia, goblet cell hyperplasia with mucus hypersecretion, and hyperresponsiveness to inhaled allergens and to non-specific stimuli[69]. In atopic individuals, T cells mature preferentially towards the Th2 subtype after exposure to allergen on the surface of antigen-presenting cells. These cells, through the production of a diverse array of inflammatory mediators, recruit and activate mast cells, granulocytes, B cells and local cells (primarily airway epithelial and smooth muscle cells); leading to the pathological events that define this disease.[70]

PI3K and asthma

There is an important therapeutic effect of PI3K inhibitors in asthma models of disease. Initial reports established that the greatly increased levels of IL-4 and IL-5 achieved 72 hr after OVA inhalation could be significantly reduced by the intratracheal administration of PI3K inhibitors (wortmannin or LY294002).[71] Subsequently, it was shown that intratracheal administration of LY294002 significantly inhibited most of the pathological characteristics of the mouse asthma model, like eosinophil counts, eotaxin levels, IL-5 and IL-13 in bronchoalveolar lavage fluid (BAL). Furthermore, lung tissue eosinophilia, airway mucus production and airway hyperresponsiveness to inhaled methacholine were all significantly suppressed.[72]

Accordingly, both PI3K and, consequently, PKB activities are increased significantly after allergen challenge in murine models of asthma. Although the activity of PKB was shown to be sensitive to pretreatment with broad-spectrum PI3K inhibitors[71] these studies could not distinguish among the various forms of PI3K. It was not until Myou *et al.* examined the effects of dysfunctional p85 in the context of an asthma animal model that it was clearly demonstrated that Class IA PI3K play key roles in pulmonary infiltration of lymphocytes and eosinophils,

antigen-induced airway inflammation and hyperresponsiveness (including increase in mucus-containing epithelial cells), and Th2 cytokine production (IL-5 and IL-4) in BAL. Although p85 blockage reduced the secretion of Th2 cytokines, it had no effect on the BAL secretion of the Th1 cytokine, interferon-γ. These data suggest that PI3K Class IA regulates, in part, the balance between Th1 and Th2 responses.[73] A study looking specifically at the role of p110δ in Th1 and Th2 differentiation, found that T cells obtained from p110δ kinase-dead mice had impaired differentiation towards both of these subtypes.[26]

Class IA p110δ has been implicated as a key mediator in the asthmatic response. p110δ silencing *in vitro*, either genetically or pharmacologically, has been shown to inhibit mast cell allergen-immunoglobulin E (IgE)-induced degranulation and cytokine release.[28] Recently, Lee *et al.* using intratracheal administration of the p110δ-specific inhibitor, IC87114, in a mouse asthma model, were able to diminish leucocyte, eosinophil, neutrophil and lymphocyte accumulation in the lungs, as well as attenuate Th2 cytokine levels (IL-4, IL-5 and IL-13). This compound also reduced IgE and leukotriene C4 release into the airways.[74] A recent paper, where a haemopoietic cell-restricted p110δ gene disruption was carried out, showed that p110δ-inactivated mice when challenged with ovalbumin had lower levels of type 2 cytokines, attenuated airway inflammation and reduced mucus production and eosinophil recruitment to the lung. Furthermore, the response of the p110δ-inactivated mice to inhaled methacholine was also reduced.[75] These studies highlight the therapeutic potential of targeting p110δ in the context of asthma.

With regard to Class IB PI3K, p110γ knockout mice have demonstrated partial protection from anaphylaxis following intradermal injection of IgE, adenosine and allergen.[29] The lack of responsiveness of p110γ-knockout mice to adenosine is particularly interesting because a role for this mediator in asthma and COPD has long been advocated. Several different G-protein-coupled receptors for adenosine have been found; the main receptor involved in adenosine-induced mast cell degranulation, and consequent bronchoconstriction, is the A2B receptor.[76] Levels of adenosine are increased in the BAL of asthmatics and local delivery of AMP induces bronchoconstriction in asthmatics, but not normal individuals.[77]

The eosinophil response appears to be a critical feature in asthma. Many inflammatory mediators activate eosinophils via signal transduction pathways involving the enzyme PI3K.[78] Furthermore, PI3K has also been shown to be essential in the migration of eosinophils caused by a number of chemoattractants. Specifically, Palframan *et al.* reported that wortmannin inhibited IL-5-induced release of eosinophils from perfused bone marrow, as well as eosinophil chemokinesis *in vitro*.[79] Similarly, Tigani *et al.* showed that wortmannin given at high concentrations inhibits the increased number of eosinophils and eosinophil peroxidase activity in the BAL of ovalbumin-challenged animals.[80] Considerable controversy remains regarding the relationship between bronchial eosinophilic inflammation and airway hyperresponsiveness, but it is believed that eosinophils degranulate to release toxic granule proteins and that these products can cause airway hyperresponsiveness.[81] In allergen-induced lung inflammation, LY294002 completely failed to prevent the increase in eosinophil influx into the lung, but significantly attenuated the increase in the BAL index of bronchial eosinophil degranulation and airway hyperresponsiveness to histamine, but not to acetylcholine. This might suggest that it is the degranulation of eosinophils in the lungs, rather than their mere accumulation, that is important for the development of allergen-induced airway hyperresponsiveness to histamine.[78] Others have reported a similar dissociation between airway hyperresponsiveness and pulmonary eosinophilia in allergen-sensitized and challenged guinea pigs.[82–84] However, there is some evidence to suggest that BAL eosinophil count in asthmatic patients has often positively correlated with the magnitude of airway hyperresponsiveness.[85,86]

Evidence that PI3K isoforms expressed in lung parenchymal cells contribute to airway disease

Acute lung injury and adult respiratory distress syndrome

Several studies highlight the importance of parenchymal cell PI3K in lung disease. It was initially demonstrated that overventilation elicits PI3K-dependent activation of PKB in pulmonary endothelial cells, leading to production of nitric oxide.[53] This same group later showed that in isolated perfused mouse lungs, overventilation causes, in a PI3K-dependent manner, nuclear translocation of NF-κB.[54] Consequently, expression of I-κBβ, a NF-κB inhibitor, is diminished in alveolar cells of overventilated lungs. This effect can be reversed by inhibition of PI3K, suggesting that PI3K leads to phosphorylation and degradation of I-κBβ proteins and hence the activation NF-κB. Furthermore, this LY294002-mediated PI3K blockade inhibits NF-κB nuclear translocation, impairing the production of IL-6 and CXCL2 in an overventilation (but not endotoxin-induced) ARDS model.[54] The selectivity of PI3K activation for ventilation injury suggests that it may be possible to reduce some of the side-effects of ventilation without causing severe immune suppression.

In some systems endothelial, rather than leucocyte, p110δ and p110γ may play a predominant role. Using an endotoxaemia-induced model of ALI, reconstituting $p110\gamma^{-/-}$ mice with wild-type neutrophils does not fully

restore lung neutrophil recruitment, and the resistance of neutrophil recruitment to wild-type reconstitution is even more pronounced in double (p110δ and p110γ) knockout mice.[43] *In vitro* experiments reveal that endothelial p110δ and p110γ activities are required for selectin-dependent adhesion of neutrophils to the endothelium, demonstrating that both PI3K subclasses (IA and IB) are needed in the endothelium to recruit neutrophils efficiently to the inflamed lung. This highlights the fact that PI3K in parenchymal cells, and not just in leucocytes, plays an important part in inflammation in the lung.

Chronic obstructive pulmonary disease

Nicotine, the addictive chemical found in tobacco cigarettes and a precursor of certain carcinogens, activates PI3K and leads to increased phosphorylation levels of its downstream mediators PKB, GSK-3 and others, in non-immortalized human airway epithelial cells.[87] This activation leads to enhanced survival and partially induces a transformed phenotype, reminiscent of premalignant lesions typically found in patients with smoking-associated COPD. This information highlights the fact that some of the chemicals found in cigarette smoke activate PI3K in lung parenchymal cells, and that this interaction mediates alterations in the cells that could have consequences in the establishment not only of COPD but also of lung cancer.

Furthermore, when the epithelium is persistently exposed to inflammatory mediators in COPD and asthma, it can change phenotype to a mucus-hypersecreting metaplasia, called goblet cell. This response is a basic innate host defence to enhance mucus production and, thus, the removal of harmful stimuli from the lungs.[88] Various cytokines and inflammatory mediators stimulate mucus hypersecretion, directly or indirectly.[89,90] Mucin secretion is activated by TNF-α,[91] IL-1β[92] and LPS[93] by up-regulating expression of the mucin genes. Among the cytokines that have various inflammatory functions in acute and chronic upper respiratory tract infection, IL-1β is related to the pathogenesis of respiratory tract infection in upper airway diseases such as COPD and asthma.[94] When epithelial cells were pretreated with a PI3K inhibitor, IL-1β-induced *MUC2* gene expression and mucin secretion were attenuated.[95] Furthermore, IL-13, another cytokine shown to play an important role in airway disease, was demonstrated to induce a mucus hypersecretory phenotype via the mitogen-activated protein kinase (MAPK) and PI3K pathways.[88]

Asthma

Airway smooth muscle (ASM) cells play a prominent role in the perpetuation of airway inflammation. ASM cells under chronic inflammatory conditions have the capacity to contract more intensely and for longer periods of time than normal ASM cells, further restricting airflow to the lungs.[96] When airway mast cells degranulate as a result of their interaction with allergens, toxins and other autocrine factors, they liberate chemical mediators which cause ASM to contract and further restrict airflow to the lungs.[97] Histamine and peptidoleukotrienes are the two major mast cell-derived mediators responsible for the anaphylactic contraction of airways isolated from both humans and guinea pigs. Treatment with PI3K and/or MAPK inhibitors has been shown to attenuate the anaphylactic bronchial contraction and facilitate the relaxation of constricted airways,[98] implying a role for these kinases in the activity of mast cell-derived mediators in the contraction of ASM cells. Furthermore, *in vitro* tests have shown that PI3K plays a role in regulating contraction[99] and migration[100] in ASM cells, as well as in the transformation of ASM cells into the contractile phenotype observed in asthma.[96]

ASM cells also play an important role in the induction of the chronic features of airway remodelling that occur in asthma, because when these cells grow either in size and/or number they limit the flow of air into the lungs by trapping the air already contained in the lungs.[97] Many different factors, including cytokines, growth factors, inflammatory mediators, contractile agonists, and extracellular matrix proteins induce ASM proliferation. These mitogens can be divided into two broad groups: those that activate receptors with intrinsic protein tyrosine kinase activity (e.g. epidermal growth factor, EGF) and those that activate G-protein-coupled receptors (e.g. thrombin).[101] PI3K kinase inhibitors reduce ASM proliferation induced by thrombin,[101,102] EGF,[101] transforming growth factor-β[103] and platelet-derived growth factor (PDGF)[104]. It is important to note that when the inhibitors were added 6 hr after the cells were stimulated with certain mitogens, proliferation was no longer inhibited, suggesting that PI3K may be more important in modulating the early signals that lead to proliferation in ASM.[105]

Despite PI3K playing an important role in modulating mitogen-induced smooth muscle proliferation, the repertoire of PI3K isoforms expressed and their attendant functions have not been well studied in ASM cells. It is known that human ASM cells express class IA, and class II PI3K but not the class IB isoform.[106] Transient expression of constitutively active class IA PI3K human ASM cells was associated with activation of the cyclin D1 promoter,[107] induction of DNA synthesis and generation of ASM cell growth. Interestingly, inhibition of class IA PI3K does not completely inhibit mitogen-induced DNA synthesis.[106] The incomplete inhibition of mitogen-induced proliferative responses in human ASM cells suggests that although class IA PI3K may play a major role in myocyte growth, other pathways are also likely to modulate this event. Some have suggested that mitogens act via their receptors to activate two parallel signalling

pathways in ASM cells, namely extracellular signal-regulated kinase (ERK) and the PI3K pathways, each pathway being crucial in obtaining the proliferative response,[68] although this may be mitogen-specific[108].

The downstream targets of PI3K that are associated with promoting protein synthesis and accumulation include pp70/85-kDa S6 kinases, collectively referred to as $pp70^{S6k}$.[109] In numerous cell types, PI3K has been shown to be an important activator of $pp70^{S6k}$ in response to serum and growth factors.[110] It has been demonstrated that inhibition of $pp70^{S6k}$ attenuates human ASM proliferation.[101] Recently, a critical role for PI3K-dependent $pp70^{S6k}$ activation following induction of DNA synthesis by PDGF in bovine tracheal smooth muscle cells was demonstrated.[111] Furthermore, Halayko *et al.* have provided primary evidence that PI3K activation of $pp70^{S6k}$ in airway myocytes leads to the accumulation of contractile apparatus proteins and to differentiation and growth of ASM cells.[96] Moreover, it has been shown that expression of a constitutively active p110 results in activation of $pp70^{S6k}$, and expression of a dominant negative mutant p85 blocks EGF and thrombin-induced $pp70^{S6k}$ activation and DNA synthesis,[101] clearly indicating a role for Class IA PI3K in $pp70^{S6k}$ activation and thrombin and EGF-induced ASM cell proliferation.

The way forward: making the choice between isoform specificity and global inhibition?

The recent advent of clinical trials using small molecule inhibitors against p38 MAPK for use in patients with a diverse array of inflammatory pathologies, like Crohn's disease,[112,113] and the use of RX-0201, an antisense oligonucleotide that directly blocks PKB signalling, as a treatment for cancer in a phase I clinical trial (http://www.rexahn.com), would predict that the future for small molecule inhibitors for signalling molecules is very promising, with some speculating that inhibitors targeting PI3K Class IB p110γ could be the anti-inflammatory drug of the future.[114]

Some companies, like Semafore Pharmaceuticals Inc. (Indianapolis, IN), believe that a pan-PI3K inhibitor, like their lead compound SF1126, could prove to be useful against tumour development in cancer therapy; while others, like TargeGen, Inc. (San Diego, CA), with their selective PI3Kδ/γ inhibitor TG100-115, which is about to begin clinical trials for the treatment of acute myocardial infarction (ClinicalTrials.gov Identifier: NCT00103350), believe specificity is the correct approach to delivering kinase inhibitors in the clinic. Whether PI3K inhibitors, either isoform-selective or broad-spectrum, eventually prove to be sufficiently effective as anti-inflammatory agents is a question that only time and research will answer.[115]

In respiratory diseases, a wide variety of therapeutic options are available but better drugs are required. As seen in this review, a p110δ-specific inhibitor seems to offer the most potential as a therapeutic target in respiratory disease. This inhibitor could offer the opportunity of reducing Th2 responses without severely affecting Th1-mediated immunity, which would prove highly beneficial to the allergic patient. Furthermore, the effects of p110δ silencing on mucus production, mast cell degranulation and leucocyte recruitment are all very positive effects that an inhibitor could have in the clinic. For better or for worse, in the pharmaceutical industry the race to develop therapeutic prototypes that target PI3K is well underway. Meanwhile, basic research continues to elucidate the possible advantages and disadvantages of using isoform selective versus pan-isoform inhibitors in therapeutic settings.

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