Targeting phosphoinositide 3-kinase δ for allergic asthma

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Abstract

Chronic inflammation in the lung has long been linked to the pathogenesis of asthma. Central to this airway inflammation is a T-cell response to allergens, with Th2 cytokines driving the differentiation, survival and function of the major inflammatory cells involved in the allergic cascade. PI3Kδ (phosphoinositide 3-kinase δ) is a lipid kinase, expressed predominantly in leucocytes, where it plays a critical role in immune receptor signalling. A selective PI3Kδ inhibitor is predicted to block T-cell activation in the lung, reducing the production of pro-inflammatory Th2 cytokines. PI3Kδ is also involved in B-cell and mast cell activation. Therefore the inhibition of PI3Kδ should dampen down the inflammatory cascade involved in the asthmatic response through a wide breadth of pharmacology. Current anti-inflammatory therapies, which are based on corticosteroids, are effective in controlling inflammation in mild asthmatics, but moderate/severe asthmatic patients remain poorly controlled, experiencing recurrent exacerbations. Corticosteroids have no effect on mast cell degranulation and do not act directly on B-cells, so, overall, a PI3Kδ inhibitor has the potential to deliver improvements in onset of action, efficacy and reduced exacerbations in moderate/severe asthmatics. Additionally, PI3Kδ inhibition is expected to block effects of Th17 cells, which are increasingly implicated in steroid-insensitive asthma.

Introduction

Asthma is a common respiratory disease. It has been estimated that between 7 and 10% of the world’s population are affected [1]. The number of sufferers has increased significantly over the last 50 years, with 300 million people recorded with asthmatic symptoms reported in 2010. Asthma is characterized by chronic airway inflammation, increased mucus production in the lungs and airway hyperreactivity, causing shortness of breath through narrowing of the airway lumen. On the basis of symptoms, asthmatics can be subdivided into mild, moderate and severe. In the majority of mild and moderate asthmatics, the symptoms are controlled by a combination of inhaled steroid-insensitive asthma.

Key words: allergic asthma, B-cell, cytokine, mast cell, phosphoinositide 3-kinase (PI3K), T-cell

Abbreviations used: BCR, B-cell receptor; BTK, Bruton’s tyrosine kinase; FcεRI, high-affinity IgE receptor; Fos, forkhead box D; GF, guanine-nucleotide-exchange factor; GPAR, G-protein-coupled receptor; IL, interleukin; ITK, IL-2-inducible T-cell kinase; NF-κB, nuclear factor κB; PI3K, phosphoinositide-dependent kinase 1; PH, pleckstrin homology; PI4K, phosphoinositide 3-kinase; TCR, T-cell receptor; Treg, regulatory T-cell.

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**Figure 1 | Key effector cells in allergic asthma**

In the allergic inflammation, allergen is processed by a professional antigen-presenting cell also known as a dendritic cell (DC). A peptide epitope from the allergen is presented to T-cells, leading to the activation of allergen-specific Th2 cells. These T-cells produce Th2 cytokines such as the eosinophilic factor IL-5 or IL-4, which promotes IgE synthesis in B-cells. IgE binds to receptors on the surface of mast cells which respond by releasing mediators such as histamines, prostaglandins and leukotrienes, leading to tissue inflammation and the symptoms characteristic of allergic asthma. Treg cells are a subset of T-cells with an important function in regulating helper T-cells such as Th2, but also Th17, a recently discovered subset of effector T-cells producing IL-17 cytokines and increasingly thought to play a critical role in neutrophilic steroid-resistant asthma. In allergen-induced asthma, a PI3Kδ inhibitor will probably inhibit the activation of Th2 and Th17 cells, the activation of IgE-producing B-cells and the degranulation of inflammatory mediators from mast cells. However, it remains to be determined whether PI3Kδ inhibition will also prevent important immune-regulatory function of Tregs.

IL (interleukin)-4, IL-5, IL-9 and IL-13. These cytokines drive the differentiation, survival and function of other key effector cells, notably B-cells and mast cells. B-cells, like T-cells, bear a surface receptor [BCR (B-cell receptor)] which recognizes foreign antigen. Ligation of the BCR and additional co-stimulatory signals provided by T-cells, promotes B-cell differentiation, proliferation and antibody secretion. T-cell cytokines, including IL-4, promote antibody isotype switching, which leads to the secretion of IgE. IgE binds to receptors on the surface of mast cells which respond by releasing mediators such as histamines, prostaglandins and leukotrienes, leading to tissue inflammation. IL-5 stimulates B-cell growth and increases Ig secretion and is a key mediator of eosinophil activation. IL-9 and IL-13 have both been shown to be important mediators of airway hyperresponsiveness in studies in animal models. The key cell types which feature in allergic asthma are illustrated in Figure 1.

Clinical studies support a model in which inhibition of T-cell, B-cell and mast cell function would ameliorate asthma. Cyclosporin A is a cyclic peptide which inhibits calcineurin phosphatase activity, downstream of the TCR. Calcineurin is required for activation of T-cells by NFAT (nuclear factor of activated T-cells), a key T-cell transcription factor. Chronic severe asthma has been successfully treated with cyclosporin A [5], but there are adverse side effects, which has limited its usage. Omalizumab is a humanized antibody which binds IgE in serum [6]. It is effective in 60–80% of patients, but at a cost of £6000–18000 per patient per year, it is expensive and its prescription is restricted to patients with severe persistent asthma which cannot be controlled even with high doses of corticosteroids. Mepolizumab is another humanized antibody which binds to IL-5 and has been used to treat a form of allergic asthma termed eosinophilic asthma. In eosinophilic asthma, eosinophils are present in large numbers in the lungs; these were reduced following mepolizumab treatment [7]. Montelukast is a cysteinyl leukotriene receptor antagonist which reduces bronchoconstriction and associated inflammation caused by leukotrienes [8]; like steroids, it has a delayed onset of action and has associated side effects, including gastrointestinal problems, hypersensitivity reactions and sleep disorders. All of the above, taken together, highlights the need for a cheaper safer drug.

PI3Kδ is a member of the PI3K family of lipid kinases that specifically phosphorylate phosphoinositides at the D3 position of the inositol ring. Eight mammalian PI3Ks have been identified, divided into three classes (I, II and III) on the basis of sequence homology, structure, binding partners, mode of activation and substrate preference [9]. Class I is further subdivided into Class IA, which includes PI3Kα, PI3Kβ and PI3Kδ, and Class IB, which is represented by PI3Kγ. Class I PI3Ks exist as heterodimers consisting of a regulatory and a catalytic subunit. Class IA PI3Ks...
Class I PI3Ks are subdivided into Class IA, which includes PI3Kα, PI3Kβ, and PI3Kδ, and Class IB, which is represented by the unique member PI3Kγ. Class IA PI3Ks exist as heterodimers consisting of a regulatory and a catalytic subunit. Class IA PI3Ks are activated by the binding of the regulatory subunit, specifically their SH2 (Src homology 2) domain, to phosphorylated tyrosine (pTyr) residues on YXXM motifs, linking the PI3K signalling pathways with the tyrosine kinase pathways. PI3Kγ differs from Class IA PI3Ks by being activated when the regulatory subunit p101 and the kinase subunit p110γ bind to the Gβγ subunit of an activated seven-transmembrane GPCR. The oncology targets PI3Kα and PI3Kβ are ubiquitously expressed; in contrast, both PI3Kδ and PI3Kγ are expressed predominantly in cells of the haemopoietic lineage and are targeted for immune-inflammatory conditions.

Class IA PI3Ks convert the membrane phospholipid PtdIns(4,5)P2 into PtdIns(3,4,5)P3. This allows recruitment of effector proteins to the plasma membrane which bind to PtdIns(3,4,5)P3 via PH (pleckstrin homology) domains and include the serine/threonine kinases PDK1 (phosphoinositide-dependent kinase 1) and Akt, and Tec tyrosine kinase family members. As a consequence of co-localization, PDK1 is able to phosphorylate Akt, and a second phosphorylation by the mTOR (mammalian target of rapamycin) complex completes the activation of Akt, creating a vast signalling network regulating cellular functions including glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. In T-cells, Akt activation ultimately leads to the activation of the nuclear transcription factor for the activation of immune cells, NF-κB, and to the down regulation of the Foxo (forkhead box O) transcription factors that regulate cell growth and promote apoptosis [10]. In B- and T-cells, PI3K signalling also leads to activation of the Tec family of protein tyrosine kinases which include BTK (Bruton’s tyrosine kinase) in B-cells and ITK (IL-2-inducible T-cell kinase) in T-cells. Upon PI3K activation, Tec family members translocate to the plasma membrane where they are phosphorylated by Src kinases. One of the major targets of activated Tec family kinases is phospholipase Cγ, which hydrolyses PtdIns(4,5)P2 into Ins(3,4,5)P3, leading to an increase in intracellular calcium levels and production of DAG (diacylglycerol), which is required for activation of protein kinase C family members [11]. Other PH-domain-containing proteins which become activated downstream of PI3K signalling include Rac/Arf GEFs (guanine-nucleotide-exchange factors). These proteins bind to PtdIns(3,4,5)P3 and, as a result, are able to bind to GTP and become activated. Following activation, Rac/Arf GEFs regulate signalling pathways, leading to proliferation, differentiation and apoptosis [11].

The importance of PI3Kδ in immune cell signalling has been demonstrated in a number of different studies. For example, confocal microscopy studies investigating signalling in the immune synapse (the interface between the T-cell and an interacting antigen-presenting cell) have shown that PtdIns(3,4,5)P3 accumulation is one of the earliest detectable signals to occur at the T-cell plasma membrane. The signal is sustained for as long as the association between the antigen-presenting cell and the T-cell is maintained [12].
PI3Kδ is activated downstream of the BCR, TCR and FcεRI in mast cells. This leads to the activation of PH-domain-containing effector proteins including the serine/threonine kinase Akt, the tyrosine kinase ITK/BTK and Rac GEFs. Akt connects PI3Kδ to the activation of the nuclear transcription factor for the activation of immune cells, NF-κB, and to the down-regulation of the Foxo transcription factors that regulate cell growth and promote apoptosis. PI3Kδ, through the membrane recruitment of Tec kinases such as ITK or BTK, contributes to calcium signalling. Rac GTPases are activated by PH-domain-containing GEFs, leading to proliferation and differentiation. Ag, antigen; PLCγ1, phospholipase Cγ1.

A key role for PI3Kδ in immune cell function is supported by investigations which have followed the generation of p110δ kinase-dead knockin mice. Unlike PI3Kα and PI3Kβ knockins, p110δ kinase-dead knockin mice are viable and their phenotype is restricted to defects in immune signalling [13]. Studies in the mice have demonstrated that PI3Kδ is required for cell activation downstream of the TCR and BCR and the mast cell high-affinity IgE receptor FcεRI. [12]. Functionally, PI3Kδ is required for cytokine production and proliferation in T-cells, for antibody production, including IgE, in B-cells, and for proliferation and migration as well as allergen-IgE-induced degranulation in mast cells [14]. Signalling pathways downstream of the TCR, BCR and IgE receptor are illustrated in Figure 2.

In addition to the PI3Kδ-deficient mice, tool compounds selective for PI3Kδ over other Class I PI3Ks have been reported in the patent WO01/81346 filed by ICOS Corporation (Bothell, WA, U.S.A.) [15]. It is believed that this class of compound is highly selective for PI3Kδ and to a lesser extent for PI3Kγ, owing to its unusual binding mode within the ATP pocket [16]. IC87114 (IC50 values of 0.5, 1.3, 3.2 and 10 μM against PI3Kδ, PI3Kγ, PI3Kβ and PI3Kα respectively) has been used to demonstrate a role for PI3Kδ in neutrophil activation and migration [17], vascular-mediated adhesion of neutrophils [18], VEGF (vascular endothelial growth factor)-dependent vascular permeability [19], B-cell antigen and IL-4 receptor signalling [20], B-cell antigen-presenting function [21] and mast cell proliferation, migration and degranulation [22]. IC87114 has also been shown to be effective in an acute allergic airway inflammation and hyperresponsiveness model with reduction of cell infiltrates in the lung, mucus hypersecretion, cytokine and chemokine levels, ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1) expression and airway hyperresponsiveness. Th2 cytokines IL-4, IL-5 and IL-13 and serum levels of IgE (including total and antigen-specific IgE) were inhibited [23].

To validate the promise of PI3Kδ inhibition as a treatment for asthma, we have undertaken further translational studies looking at the effects of PI3Kδ inhibitors in human-derived immune cell subsets, including T-cells, B-cells and mast cells, using cells derived from both normal and atopic donors (blood taken with full donor consent). In T-cells,
the role of PI3Kδ signalling downstream of the TCR has been confirmed by showing PI3Kδ-dependent inhibition of Akt phosphorylation. We have investigated effects on T-cell function and have shown that Th2 cytokines, including IL-4, IL-5 and IL-13, are inhibited in allergen-stimulated peripheral blood mononuclear cells. In addition to Th2 cytokines, we have shown inhibition of cytokine production from Th1 and Th17 T-cell subsets; at present, it is unclear whether the consequences of impairing the function of other effector T-cell subsets would result in added benefits or risks. In tonsil B-cells, we have shown inhibition of anti-IgM-stimulated proliferation and the early activation marker CD69. In mast cells derived from nasal polyp tissue, stimulated with anti-IgE antibody in order to mimic cross-linking of the receptor by antigen bound to IgE, inhibition of PI3Kδ blocked PGD2 (prostaglandin D2) and cytokine release. In the same assay, FP (fluticasone propionate) inhibited cytokine release, but had no effect blocking release. In the same assay, FP (fluticasone propionate) inhibited cytokine release, but had no effect blocking release.

In conclusion, there is mounting evidence to support PI3Kδ as a novel drug target for the treatment of allergic asthma. PI3Kδ is expressed in key cell subsets which have been shown to play a role in allergic asthma, including T-cells, B-cells and mast cells and is a key signalling molecule in inflammation. The relatively restricted tissue distribution of PI3Kδ and the fact that, for a respiratory disease, drugs can be administered via an inhaled route, limiting exposure to body tissue, suggest that a PI3Kδ inhibitor would have an acceptable safety profile. This is supported further by the observation that PI3Kδ-deficient mice are viable and the defect in the mice is restricted to immune cells. A PI3Kδ drug may convey additional benefits: inhibition of the PI3K pathway in T-cells has been shown to enhance differentiation and function of a subset designated as Treg (regulatory T-cells) [24]. Treg are associated with immune tolerance and, as these observations were repeated in a clinical setting, it would allow for testing the hypothesis that a PI3Kδ inhibitor may deliver long-term disease modification in allergic indications by the induction of allergen-specific tolerance. It should be noted that there are reports in which PI3Kδ inhibition is suggested as detrimental to Treg development and function [25]. This may reflect the stage of differentiation of the Treg population studied, and, on a positive note, we have shown that the suppression function of isolated Treg was not impaired in the presence of a PI3Kδ compound (W.C. Rowan, unpublished work). Inhibition of PI3Kδ may also increase corticosteroid sensitivity [26], which would benefit severe asthmatics, dependent on high doses of steroids. The true value of a new drug in terms of additional benefits over existing therapies will not become apparent until the drug is tested in patients in the clinic. On the basis of present evidence, PI3Kδ shows considerable promise as a new drug for the treatment of allergic asthma.

References


Received 26 July 2011
doi:10.1042/BST20110665

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