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PTEN/PI3K PATHWAY AS A THERAPEUTIC TARGET FOR ALLERGIC AIRWAYS INFLAMMATION

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CONTENTS

ABSTRACT

Asthma is a common lung disease that is characterized by chronic airways inflammation and variable airflow obstruction with airways hyperresponsiveness. Recent studies highlight the importance of the underlying inflammatory cascade, particularly signaling pathways, in the pathogenesis of asthma. Phosphatase and tensin homolog (PTEN) is a complex signaling molecule that regulates important biological processes such as the metabolism, proliferation and survival of cells. PTEN antagonizes the action of phosphoinositide 3-kinase (PI3K) by dephosphorylating phosphatidylinositol 3,4,5-trisphosphate. There is increasing evidence that the PTEN/PI3K pathway plays a major role in the expression and activation of inflammatory mediators, inflammatory cell recruitment and immune cell function, thereby contributing to the pathogenesis of asthma. Upregulation of PTEN expression or inhibition of PI3K activity leads to the attenuation of allergic airways inflammation and bronchial hyperresponsiveness by modulating multiple regulatory pathways in asthma. This review describes recent advances in understanding the role and related mechanisms of the PTEN/PI3K pathway in regulating allergic airways inflammation and the potential for targeting this pathway for the treatment of asthma.

INTRODUCTION

Asthma is a common lung disease that is characterized by chronic airways inflammation with airflow obstruction and airways hyperresponsiveness (AHR). The prevalence of asthma has increased in most countries, affecting as many as 300 million people worldwide (1-3). According to the Global Initiative for Asthma guidelines, the global prevalence of asthma is reported to range from 1% to 18% of the population in different countries (2, 3). In addition to placing a considerable burden in terms of direct medical costs, asthma has enormous indirect costs, including absence from work or school (4). Therefore, asthma represents a profound and growing public health problem worldwide.

In recent years, the introduction of effective new medications and improved formulations for the treatment of asthma has led to a relatively favorable trend in outcomes, with a reduction in annual hospitalizations for asthma attacks and asthma-related deaths (1). Therapeutic agents for asthma are divided into two classes: acute relief medications for airways obstruction and controller medications for airways inflammation. For decades, the impressive benefit seen in patients treated with bronchodilators and the concern about the adverse effects of systemic steroids led physicians and patients to rely heavily on relief medications for the rapid control of asthma symptoms (5). However, the appreciation that asthma is a chronic inflammatory disorder and the availability of inhaled steroids with a low prevalence of systemic side effects have contributed to a change in this pattern of practice, increasing the use of controller medications. Among controller medications, inhaled corticosteroids are the most potent anti-inflammatory agents used in the treatment of asthma. However, 5-25% of asthmatic patients may show a poor or no response (6, 7), and some patients can develop adverse effects to high-dose inhaled corticosteroid treatment. Therefore, novel therapeutic agents are required to cover the unmet needs of asthmatic patients.

Airways inflammation in asthma consists of the infiltration of inflammatory cells, mucus hyperplasia, vascular engorgement, smooth muscle hypertrophy and basement membrane thickening (1, 8, 9). Asthmatic inflammation is associated with AHR, which contributes to the characteristic symptoms, such as cough, shortness of breath, chest tightness and wheezing. Therefore, control of airways inflammation is important in treating asthmatic patients. Targeting signal transduction pathways is a promising strategy to treat inflammatory diseases, including asthma, because the same signaling pathway is usually involved in a variety of cell types and can regulate several coordinated inflammatory processes (10).

Many inflammatory stimuli, such as allergens, cytokines and enzymes, bind to multiple transmembrane receptors that subsequently activate signal transduction pathways, most of which involve

cascades of kinases. Kinase pathways in turn activate downstream transcription factors, which can switch on the expression of various inflammatory genes and thus amplify the inflammatory process. Some inflammatory mediators that are induced by kinase pathways can amplify kinase activation, creating a positive feedback loop. Hence, modulators of signal transduction pathways have captured the interest of researchers as an approach to switching off airways inflammation, and advances in the understanding of the signaling pathways involved in allergic airways inflammation have opened the door to a variety of new targets in asthma treatment. One of the most investigated signaling pathways in asthma is the phosphatase and tensin homolog (PTEN) and phosphoinositide 3-kinase (PI3K) pathway (10).

PI3K catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP_2), forming a lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which controls a variety of intracellular signaling pathways. PI3K and its most prominent effector, Akt, regulate a wide spectrum of cellular processes, including cell viability, metabolism, proliferation and motility (11-13). PI3K is negatively regulated by PTEN, which converts PIP_3 to PIP_2. Therefore, the imbalance between PTEN and PI3K action is a profound obstacle to cellular processes and eventually impairs the function of the relevant organ system. Recently, there is increasing evidence that the PTEN/PI3K pathway plays a major role in the expression and activation of inflammatory mediators, inflammatory cell recruitment and immune cell function in asthma (14-16). Therefore, the PTEN/PI3K pathway is suggested to be an attractive target for asthma therapy. In this review, we focus on the PTEN/PI3K signaling pathway associated with allergic airways inflammation and the therapeutic opportunities for targeting this pathway for asthma treatment.

BIOLOGICAL FUNCTIONS OF THE PTEN/PI3K PATHWAY

PTEN was first identified as a tumor suppressor gene that was mutated in human cancers (17). After its discovery, several researchers generated null mutations of the *Pten* gene in mice to assess its function in vivo. Unfortunately, homozygosity for the null mutation of the *Pten* gene in mice results in early embryonic lethality, precluding the functional analysis of PTEN in organs (18-20). Subsequently, in order to investigate the biological functions of PTEN in viable mice, transgenic mice expressing various tissue-specific *Pten* mutants were generated (19, 20). Mutations in the *Pten* gene producing PTEN protein deletion are associated with enhanced cell proliferation and transformation, thereby contributing to tumor formation (21). The PTEN protein is a dual-specificity lipid and protein phosphatase, and antagonizes PI3K by dephosphorylation of PIP_3 at the 3′ position of the inositol ring (22, 23). Thus, loss of PTEN function in cells results in accumulation of PIP_3, mimicking the effect of PI3K activation. PIP_3 recruits many proteins to the membrane by binding to the pleckstrin homology domains of proteins, including the serine/threonine-protein kinases Akt and 3-phosphoinositide-dependent protein kinase (PDK1) and the phosphatase PH domain leucine-rich repeat-containing protein phosphatase (PHLPP) (24). Membrane-bound Akt is activated following phosphorylation by PDK1 and the rapamycin-insensitive mammalian target of rapamycin (mTOR) complex, and is inactivated through its dephosphorylation by PHLPP (25, 26). Activated Akt in turn regulates the activity of a wide range of target molecules through phosphorylation. Deletion of Akt reverses the cell survival phenotype in *PTEN*-null cells (27). In addition, inactivation of Akt by dominant-negative mutants inhibits the survival advantage provided by activation of PI3K (28). These results indicated the essential role of Akt in the PTEN/PI3K pathway. Through this complex cascading pathway, the balance between PI3K and PTEN activity is important to maintain homeostasis in a wide variety of cellular responses, such as the metabolism, proliferation, growth and survival of cells (19, 20).

The role of PTEN in immunity has been investigated using PTEN-deficient mice. A recent paper has shown that PTEN-deficient ($Pten^{flox/-}$) mice exhibit elevated levels of B cells and $CD4^+$ T cells in the periphery, spontaneous activation of $CD4^+$ T cells, autoantibody production and hypergammaglobulinemia. Loss of PTEN prevents T cells from initiating apoptosis in response to several stimili. $Pten^{flox/-}$ T cells also show enhanced proliferation and increased production of both T helper type 1 (Th1) and Th2 cytokines (29). Similar phenomena have been observed in PTEN-deficient splenic B cells (30). These findings suggested that PTEN is an important regulator of T-cell and B-cell homeostasis and self-tolerance in the immune system. The PTEN/PI3K signaling pathway also underlies cell polarization and directional cell migration (4, 18, 31). Thus, migration of a variety of cell types, including fibroblasts, neutrophils, dendritic cells and macrophages, has been shown to be PI3K-dependent (4, 32). Additionally, PI3K is essential for IL-5-induced eosinophil release from bone marrow and the migration of eosinophils caused by several chemoattractants (33). With regard to endothelial cells, the PTEN/PI3K pathway is involved in angiogenesis through regulation of the expression of vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis (34).

PI3Ks are classified into three groups (classes I, II and III) based on their structural differences and substrate specificity (35). Class I PI3Ks are the most studied group and are further divided into class IA PI3Ks (PI3Kα, PI3Kβ and PI3Kδ isoforms) and class IB PI3Ks (PI3Kγ isoform) on the basis of their regulatory partners and mechanisms of action. Members of class IA PI3Ks are heterodimers of a p110 catalytic subunit (p110α, β and δ) and a regulatory subunit (p85α, p85β, p55α, p55γ and p50α) (36). Class IB members are comprised of a p110γ catalytic subunit and one of two regulatory subunits (p101 and p84) (37). PI3Kα and PI3Kβ are expressed broadly in many tissues and control fundamental biological processes such as cellular proliferation, so that genetic deletion of either isoform is lethal at the embryonic stage (38). In contrast, PI3Kδ and PI3Kγ are predominantly expressed in leukocytes and play pivotal roles in mediating inflammatory responses (39). Therefore, mice deprived of PI3Kδ or PI3Kγ are viable and merely display reduced inflammatory responses (39, 40). Hence, PI3Kδ and PI3Kγ are considered to be suitable targets of study in inflammatory diseases.

PTEN/PI3K PATHWAY IN ASTHMA

Asthma is characterized by airways obstruction which is mediated by chronic airways inflammatory responses. Th2 cells, together with other inflammatory cells such as mast cells, B cells and eosinophils, have been proposed to play critical roles in the initiation and maintenance of airways inflammation in asthma (41, 42). Activated inflammatory cells produce numerous mediators, such as Th2

cytokines (IL-4, IL-5 and IL-13) and chemokines (eotaxin and RANTES), and thereby mediate several regulatory and effector functions. These mediators induce the production of allergen-specific IgE by B cells, the development and recruitment of eosinophils, mucus secretion, smooth muscle contraction, and ultimately AHR. As described above, PTEN and PI3K constitute an important pathway that regulates the biological function and migration of inflammatory cells, such as eosinophils, lymphocytes and neutrophils, which contribute to allergic airways inflammation. Therefore, it is possible to speculate that the PTEN/PI3K pathway is involved in the pathogenesis of asthma (43).

A study in a murine model of asthma has shown that the expression of PTEN protein and PTEN activity are decreased with the increase in PI3K activity induced by ovalbumin (OVA) challenge (14). Intratracheal administration of adenoviruses carrying PTEN-complementary DNA (AdPTEN), thus recovering PTEN expression in the lungs, attenuated allergen-induced airways inflammation and AHR in a murine model of asthma (14, 16). Moreover, the OVA-induced increases in levels of Th2 cytokines (IL-4, IL-5 and IL-13) and eosinophilic cationic protein in bronchoalveolar lavage fluid (BALF) were significantly blocked by the administration of the PI3K inhibitors wortmannin and LY-294002 or AdPTEN (14, 16, 43). In addition, the administration of PI3K inhibitors or AdPTEN markedly reduced the increases in the levels of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (V-CAM 1), as well as chemokines such as RANTES and eotaxin (14, 16). A marked reduction in mucus production with less goblet cell hyperplasia has also been reported in LY-294002-treated mice (43). Transduction of the dominant negative form of PI3 regulatory subunit into mice reduces the OVA-induced increase in mucus-containing epithelial cells in the airways (44).

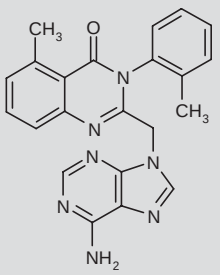
A previous in vitro study demonstrated that activation of PI3K leads to the accumulation of contractile apparatus proteins and the growth of elongated contractile airways smooth muscle cells (45). This action of PI3K in the regulation of airways-resident smooth muscle cells may contribute to an increase in airways smooth muscle mass and changes in intrinsic contractile properties associated with asthma. A significant amount of data indicate that PI3K is required for the mitogenic effect induced by numerous stimuli, such as epidermal growth factor (EGF), thrombin and transforming growth factor β (TGF-β) in airways smooth muscle cells (46-48). Additionally, PI3K signaling is necessary to mediate the proliferation and migration of pulmonary vascular smooth muscle cells, indicating that the activation of PI3K may play an important role in vascular remodeling in the lung (49). Since hypertrophy and hyperplasia of airways and vascular smooth muscle cells are characteristic changes of airways remodeling in asthma, PI3K inhibitors are suggested to have the potential for preventing and/or treating airways remodeling in asthma through their effects on smooth muscle cells (50). Taken together, these observations indicate that the PTEN/PI3K pathway may be a promising therapeutic target for asthma.

ROLE OF PI3K ISOFORMS IN ASTHMA

Among PI3K isoforms, PI3Kδ and PI3Kγ have been mainly studied to explore their role in the pathogenesis of asthma due to their effect in mediating inflammatory responses. Recent studies using a p110δ-specific inhibitor, **IC-87114**, have shown that pharmacological blockade of p110δ activity attenuates allergen-induced airways inflammation and AHR and reduces Th2 cytokine levels in a mouse asthma model (51, 52). IC-87114 also decreases the increases in the levels of total IgE and OVA-specific IgE in serum, as well as LTC_4 release in BALF (51). Moreover, a previous study revealed that genetic inactivation of the hemopoietic cell-restricted p110δ in mice reduces OVA-induced Th2 cytokine-dependent airways inflammation and AHR as compared with wild-type mice (53). Providing evidence for the important role of the p110δ isoform in mast cells, genetic or pharmacological inactivation of the p110δ isoform in mast cells has been found to induce defective stem cell factor (SCF)-mediated proliferation, adhesion and migration, as well as impaired allergen/IgE-induced degranulation and cytokine release (54, 55). These findings thus demonstrate a biological role for p110δ signaling in allergic airways inflammation, highlighting the importance of the p110δ isoform as a novel target for therapeutic intervention in asthma.

With regard to PI3Kγ, PI3Kγ-deficient mice show a significant decrease in airways inflammation, AHR and airways remodeling after OVA challenge as compared with wild-type mice. On the other hand, there are no significant differences in serum OVA-specific IgE levels and CD4/CD8 balance in BALF between PI3Kγ-deficient mice and wild-type mice (40). PI3Kγ is involved in allergic inflammation by regulating the challenge/effector phase of allergic responses. Interestingly, a more recent study has found that a dual PI3Kδ/γ inhibitor, **TG-100115**, markedly reduces eosinophilic inflammation, IL-13 levels in BALF, mucin accumulation and AHR in a murine asthma model (56).

Thus, it appears that PI3Kδ and PI3Kγ isoforms are very attractive targets for the treatment of asthma and we expect that more stud-



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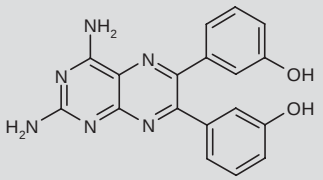

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ies will be performed to define the specific roles of each isoform and related mechanisms in allergic airways diseases, including asthma.

REGULATORY MECHANISM OF PTEN OR PI3K INHIBITORS IN ASTHMA

PI3K can activate various signaling pathways, such as PDK1, protein kinase C (PKC), extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase p38 (MAP kinase p38) (4, 34, 57). By creating a complex network with many signaling molecules, the PTEN/PI3K pathway may serve as a hub or switch point in allergic airways inflammation. Although there is a growing body of evidence suggesting the critical role of the PTEN/PI3K pathway in the pathogenesis of asthma, the data regarding the exact regulatory mechanism related to its role in allergic airways inflammation are limited. Here, we focus on peroxisome proliferator-activated receptor γ (PPARγ), VEGF, nuclear factor NF-kappa-B (NF-κB), IL-17 and TGF-β1, which are reported to be associated with the PTEN/PI3K pathway in allergic airways inflammation and airways remodeling in asthma. A schematic representation of the possible mechanisms of the PTEN/PI3K signaling pathway in asthma is shown in Figure 1.

PPARs are members of the nuclear receptor superfamily containing transcription factors regulating gene expression and are divided into PPARα, PPARβ/δ and PPARγ (58, 59). PPARγ, the most extensively studied receptor among the three PPAR subtypes, has been shown to affect cell cycle, differentiation and apoptosis in many cell types (60). In addition, PPARγ activation downregulates the synthesis and release of immunomodulatory cytokines from various cell types that participate in inflammatory processes (61). Due to its anti-inflammatory and immunomodulatory properties, PPARγ is a potential candidate for treating inflammatory diseases. The administration of various PPARγ agonists, such as rosiglitazone, pioglitazone and ciglitazone, has demonstrated positive therapeutic effects in airways inflammation, AHR and airways remodeling in animal models of asthma (62-64). A possible mechanism related to the anti-inflammatory action of PPARγ has been provided by the observation that PPARγ antagonizes PI3K-mediated signaling by upregulating PTEN expression in airways epithelial cells (65). Consistent with these observations in vitro, we have shown that the administration of PPARγ agonists or an adenovirus carrying PPARγ cDNA increases PTEN expression with a decrease in PI3K activity in a murine model of asthma (64). Therefore, PPARγ is suggested to act as an upstream regulator of the PTEN/PI3K pathway and may be a therapeutic target in asthma.

VEGF, an endothelial cell-specific mitogenic peptide, is a well-known, potent promoter of vasculogenesis and angiogenesis. Additionally, VEGF enhances microvascular permeability such that plasma proteins, including inflammatory mediators and inflammatory cells, leak into the extravascular space, leading to migration of inflammatory cells to the airways (66, 67). There is increasing evidence that VEGF is a crucial stimulator of inflammation, AHR, air-

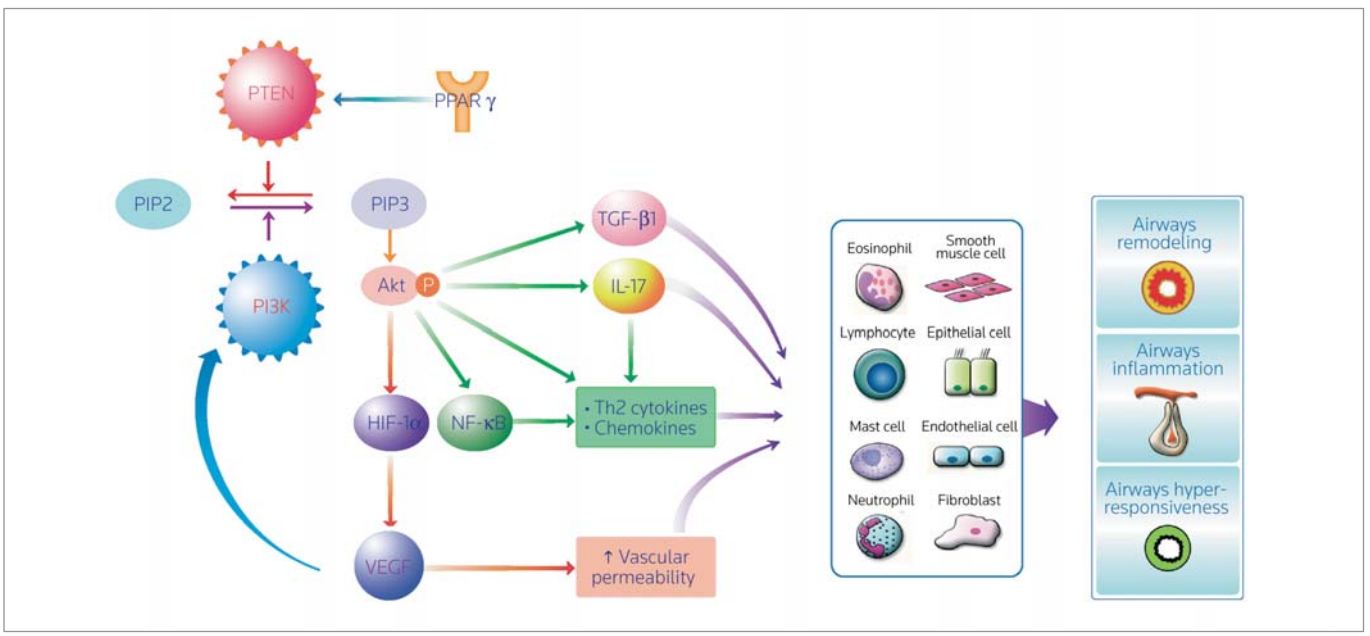

Figure 1. Schematic representation of the possible mechanisms of the PTEN/PI3K signaling pathway in asthma. PTEN negatively regulates PI3K by dephosphorylating PIP_3. PI3K phosphorylates PIP_2 to form PIP_3, which leads to activation of Akt by phosphorylation (P). Activated Akt in turn regulates the activity of various effector molecules. Stimulation of the PI3K pathway increases HIF-1α activity and resulting VEGF expression, thus enhancing vascular permeability and inducing an adaptive Th2 immune response. This pathway also leads to an increase in NF-κB activity and the subsequent release of inflammatory mediators in asthmatic airways. In addition, PTEN/PI3K signaling regulates the expression not only of IL-17, inducing the release of cytokines and chemokines, but also of TGF-β1, contributing to airways remodeling. Activation of PPARγ modulates allergic inflammation through upregulation of PTEN. The PTEN/PI3K pathway and its regulatory mechanisms influence the biological functions of various airways inflammatory and structural cells and is thus suggested to be an attractive target for asthma therapeutics.

ways remodeling and physiological dysregulation that increases antigen sensitization and the Th2 immune response in asthma (66-68). In a murine model of asthma, administration of VEGF receptor antagonists ameliorated airways inflammation and AHR (12, 55).

VEGF expression is known to be regulated by the transcriptional factor hypoxia-inducible factor 1α (HIF-1α) (69). Previous studies have demonstrated that activation of the PI3K/Akt pathway causes an increase in HIF-1α protein levels (70, 71). In keeping with these results, we have shown that administration of PI3K inhibitors reduces HIF-1α levels in nuclear protein extracts from lung tissues of OVA-sensitized and -challenged mice (16, 72, 73). As expected, VEGF expression in the lungs is also decreased by the administration of PI3K inhibitors (16). These findings imply that HIF-1α activation and the resulting VEGF expression depend on PI3K signaling in allergic airways disease. Therefore, the enhanced vascular permeability in asthmatic inflammation is suggested to be regulated by the PI3K/HIF-1α/VEGF axis (73). PI3K is known to be involved in mediating the various biological functions of VEGF (74). Moreover, inhibition of VEGF decreases Akt phosphorylation and PI3K enzyme activity in a murine model of allergic airways disease, suggesting the presence of a positive feedback loop between VEGF expression and PI3K activation (72). Thus, in asthma the PTEN/PI3K pathway may regulate vascular permeability via modulation of the PI3K/HIF-1α/VEGF axis, as well as biological functions of VEGF.

NF-κB is a transcription factor expressed in many cell types and plays a critical role in the expression of numerous inflammatory genes, such as cytokines, chemokines, adhesion molecules, growth factors and enzymes (75). Many lines of evidence indicate enhanced NF-κB activation in asthmatic lungs. The levels of NF-κB activation in bronchial biopsies, sputum cells and bronchial epithelial cells from patients with asthma are higher than in those from nonasthmatic individuals (76, 77). In murine models of asthma, a rapid NF-κB p65 nuclear translocation and enhanced NF-κB activity are seen in lung tissues (78, 79). Moreover, mice that lack the p50 subunit of NF-κB are unable to mount airways eosinophilic inflammation due to a lack of T-cell production of Th2 cytokines, highlighting the importance of NF-κB in the pathogenesis of asthma (80). Recently, both in vitro and in vivo studies have demonstrated that the administration of PI3K inhibitors or AdPTEN prevents nuclear translocation of NF-κB and the subsequent release of inflammatory cytokines in the lungs (79, 81). These findings suggest that activation of the PI3K/Akt pathway is a causative factor in the pronounced increase of NF-κB activity in asthmatic lungs.

IL-17 (also known as IL-17A), a recently discovered cytokine, is known to play a role in tissue inflammation by inducing the expression of many inflammatory mediators, such as cytokines, chemokines, adhesion molecules and growth factors (82). IL-17 mRNA and/or protein expression has been shown to increase in sputum, lung cells, BALF and peripheral blood from asthmatic patients (83-85). Consistent with these results from human studies, our recent data have shown that the expression of IL-17 protein and mRNA in lungs is upregulated in a murine model of airways inflammatory disease. Moreover, inhibition of IL-17 activity with an anti-IL-17 antibody markedly reduced antigen-induced airways infiltration of inflammatory cells and AHR in mice (79). These findings demonstrate a potential role for IL-17 in the pathogenesis of asthma. The increase in IL-17 expression is markedly reduced by treatment of OVA-sensitized and -challenged mice with PI3K inhibitors or AdPTEN (78). In addition, our unpublished data have revealed that selective inhibition of the PI3Kδ isoform markedly reduces IL-17 expression induced by an allergen challenge. Thus, the PTEN/PI3K pathway, especially the PI3Kδ isoform, appears to be involved in the regulation of IL-17 expression in asthma.

The profibrotic cytokine TGF-β1 plays a pivotal role in tissue remodeling by promoting the expression of extracellular matrix proteins and has proinflammatory effects in various settings of inflammation (86). It has been well established that TGF-β1 plays an important role in the pathogenesis of structural changes, including fibrosis, in many chronic airways inflammatory diseases. Airways remodeling, which is characterized by thickening of the lamina reticularis with deposition of collagen and other extracellular matrix proteins, leading to subepithelial fibrosis, is an important pathophysiological feature of asthma (87). Asthmatic patients show an increase in TGF-β1 expression that correlates with disease severity and the degree of subepithelial fibrosis, providing evidence that TGF-β1 is a key mediator of airways remodeling in asthma (88). TGF-β1 expression is regulated by activation of the PI3K/Akt pathway (89). In keeping with these observations, we have found that the administration of PI3K inhibitors significantly reduces the increase in TGF-β1 levels in lungs after OVA inhalation in a murine model of asthma (72). Therefore, these findings suggest that the PTEN/PI3K pathway can regulate TGF-β1 expression, thus contributing to airways remodeling in asthma.

CONCLUSION

A variety of therapeutic options have been used for asthmatic patients to control symptoms, but no therapy is specifically directed to the underlying causal pathway of asthma. Recent studies highlight the importance of regulators of the inflammatory cascade, particularly signaling pathways, as a promising therapeutic strategy for the treatment of asthma. Since PI3K is a central signaling regulator in an incredibly diverse set of cellular functions, the PTEN/PI3K pathway was expected to have an important role in the pathogenesis of asthma. In fact, extensive studies have shown that upregulation of PTEN expression or inhibition of PI3K activity leads to attenuation of airways inflammation and AHR in animal models of asthma. Furthermore, recent studies have extended our understanding of the regulatory mechanisms related to the role of the PTEN/PI3K pathway in asthma. In addition, data regarding the effect of PI3K isoform-specific inhibition of allergic airways inflammation have accumulated and provide significant insight into how to tailor therapeutic blockade to produce a satisfactory effect without other reactions. The PTEN/PI3K pathway as an attractive novel target for asthma therapeutics is now taking tentative steps from bench to bed. Further research will answer the question of whether PI3K inhibitors, either broad-spectrum or isoform-specific, are sufficiently effective as new therapeutic agents in the clinical setting.

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DISCLOSURE

The authors state no conflicts of interest.

REFERENCES

1. Fanta, C.H. *Asthma. N* Engl J Med 2009, 360(10): 1002-14.
2. Masoli, M., Fabian, D., Holt, S., Beasley, R.; Global Initiative for Asthma (GINA) Program. *The global burden of asthma: Executive summary of the GINA Dissemination Committee report*. Allergy 2004, 59(5): 469-78.
3. Beasley, R. The Global Burden of Asthma Report, Global Initiative for Asthma (GINA). Available from http://www.ginasthma.org.
4. Ito, K., Caramori, G., Adcock, I.M. *Therapeutic potential of phosphatidylinositol 3-kinase inhibitors in inflammatory respiratory disease*. J Pharmacol Exp Ther 2007, 321(1): 1-8.
5. Elias, J.A., Lee, C.G., Zheng, T., Ma, B., Homer, R.J., Zhu, Z. *New insights into the pathogenesis of asthma.* J Clin Invest 2003, 111(3): 291-7.
6. Ito, K., Chung, K.F., Adcock, I.M. *Update on glucocorticoid action and resistance*. J Allergy Clin Immunol 2006, 117(3): 522-43.
7. Chan, M.T., Leung, D.Y., Szefler, S.J., Spahn, J.D. *Difficult-to-control asthma: Clinical characteristics of steroid-insensitive asthma.* J Allergy Clin Immunol 1998, 101(5): 594-601.
8. Elias, J.A., Zhu, Z., Chupp, G., Homer, R.J. *Airway remodeling in asthma*. J Clin Invest 1999, 104(8): 1001-6.
9. James, A.L., Wenzel, S. *Clinical relevance of airway remodeling in airway diseases*. Eur Respir J 2007, 30(1): 134-55.
10. Barnes, P.J. *Novel signal transduction modulators for the treatment of airway diseases.* Pharmacol Ther 2006, 109(1-2): 238-45.
11. Ward, S.G., Finan, P. *Isoform-specific phosphoinositide 3-kinase inhibitors as therapeutic agents*. Curr Opin Pharmacol 2003, 3(4): 426-34.
12. Hennessy, B.T., Smith, D.L., Ram, P.T., Lu, Y., Mills, G.B. *Exploiting the PI3K/AKT pathway for cancer drug discovery.* Nat Rev Drug Discov 2005, 4(12): 988-1004.
13. Cantley, L.C. *The phosphoinositide 3-kinase pathway.* Science 2002, 296(5573): 1655-7.
14. Kwak, Y.G., Song, C.H., Yi, H.K., Hwang, P.H., Kim, J.S., Lee, K.S., Lee, Y.C. *Involvement of PTEN in airway hyperresponsiveness and inflammation in bronchial asthma.* J Clin Invest 2003, 111(7): 1083-92.
15. Medina-Tato, D.A., Ward, S.G., Watson, M.L. *Phosphoinositide 3-kinase signalling in lung disease: Leucocytes and beyond*. Immunology 2007, 121(4): 448-61.
16. Lee, K.S., Kim, S.R., Park, S.J. et al. *Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) reduces vascular endothelial growth factor expression in allergen-induced airway inflammation*. Mol Pharmacol 2006, 69(6): 1829-39.
17. Li, J., Yen, C., Liaw, D. et al. *PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer.* Science 1997, 275(5308): 1943-7.
18. Di Cristofano, A., Pesce, B., Cordon-Cardo, C., Pandolfi, P.P. *Pten is essential for embryonic development and tumour suppression*. Nat Genet 1998, 19(4): 348-55.
19. Kishimoto, H., Hamada, K., Saunders, M. et al. *Physiological functions of Pten in mouse tissues.* Cell Struct Funct 2003, 28(1): 11-21.
20. Yamada, K.M., Araki, M. *Tumor suppressor PTEN: Modulator of cell signaling, growth, migration and apoptosis*. J Cell Sci 2001, 114(Pt. 13): 2375-82.
21. Tamguney, T., Stokoe, D. *New insights into PTEN*. J Cell Sci 2007, 120(Pt. 23): 4071-9.
22. Maehama, T., Dixon, J.E. *The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate*. J Biol Chem 1998, 273(22): 13375-8.
23. Zhao, M. *PTEN: A promising pharmacological target to enhance epithelial wound healing.* Br J Pharmacol 2007, 152(8): 1141-4.
24. Chalhoub, N., Baker, S.J. *PTEN and the PI3-kinase pathway in cancer.* Annu Rev Pathol 2009, 4: 127-50.
25. Mora, A., Komander, D., van Aalten, D.M., Alessi, D.R. *PDK1, the master regulator of AGC kinase signal transduction*. Semin Cell Dev Biol 2004, 15(2): 161-70.
26. Sarbassov, D.D., Guertin, D.A., Ali, S.M., Sabatini, D.M. *Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex*. Science 2005, 307(5712): 1098-101.
27. Stiles, B., Gilman, V., Khanzenzon, N. et al. *Essential role of AKT-1/protein kinase B alpha in PTEN-controlled tumorigenesis.* Mol Cell Biol 2002, 22(11): 3842-51.
28. Link, W., Rosado, A., Fominaya, J., Thomas, J.E., Carnero, A. *Membrane localization of all class I PI 3-kinase isoforms suppresses c-Myc-induced apoptosis in Rat1 fibroblasts via Akt.* J Cell Biochem 2005, 95(5): 979-89.
29. Suzuki, A., Yamaguchi, M.T., Ohteki, T. et al. *T cell-specific loss of Pten leads to defects in central and peripheral tolerance.* Immunity 2001, 14(5): 523-34.
30. Suzuki, A., Kaisho, T., Ohishi, M. et al. *Critical roles of Pten in B cell homeostasis and immunoglobulin class switch recombination*. J Exp Med 2003, 197(5): 657-67.
31. Iijima, M., Huang, Y.E., Devreotes, P. *Temporal and spatial regulation of chemotaxis*. Dev Cell 2002, 3(4): 469-78.
32. Puri, K.D., Doggett, T.A., Huang, C.Y. et al. *The role of endothelial PI3Kgamma activity in neutrophil trafficking.* Blood 2005, 106(1): 150-7.
33. Dunzendorfer, S., Meierhofer, C., Wiedermann, C.J. *Signaling in neuropeptide-induced migration of human eosinophils.* J Leukoc Biol 1998, 64(6): 828-34.
34. Jiang, B.H., Zheng J.Z., Aoki, M., Vogt, P.K. *Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of vascular endothelial growth factor in endothelial cells*. Proc Natl Acad Sci USA 2000, 97(4): 1749-53.
35. Fruman, D.A., Bismuth, G. *Fine tuning the immune response with PI3K.* Immunol Rev 2009, 228(1): 253-72.
36. Dhand, R., Hara, K., Hiles, I. et al. *PI 3-kinase: Structural and functional analysis of intersubunit interactions.* EMBO J 1994, 13(3): 511-21.
37. Suire, S., Coadwell, J., Ferguson, G.J., Davidson, K., Hawkins, P., Stephens, L. *p84, a new Gbetagamma-activated regulatory subunit of the type IB phosphoinositide 3-kinase p110gamma.* Curr Biol 2005, 15(6): 566-70.
38. Bi, L., Okabe, I., Bernard, D.J., Nussbaum, R.L. *Early embryonic lethality in mice deficient in the p110beta catalytic subunit of PI 3-kinase*. Mamm Genome 2002, 13(3): 169-72.
39. Al-Alwan, M.M., Okkenhaug, K., Vanhaesebroeck, B., Hayflick, J.S., Marshall, A.J. *Requirement for phosphoinositide 3-kinase p110delta signal-*

ing in B cell antigen receptor-mediated antigen presentation. J Immunol 2007, 178(4): 2328-35.

40. Takeda, M., Ito, W., Tanabe, M. et al. *Allergic airway hyperresponsiveness, inflammation, and remodeling do not develop in phosphoinositide 3-kinase gamma-deficient mice*. J Allergy Clin Immunol 2009, 123(4): 805-12.
41. Park, S.J., Min, K.H., Lee, Y.C. *Phosphoinositide 3-kinase delta inhibitor as a novel therapeutic agent in asthma.* Respirology 2008, 13(6): 764-71.
42. Humbles, A.A., Lloyd, C.M., McMillan, S.J. et al. *A critical role for eosinophils in allergic airways remodeling.* Science 2004, 305(5691): 1776-9.
43. Duan, W., Aguinaldo Datiles, A.M., Leung, B.P., Vlahos, C.J., Wong, W.S. *An anti-inflammatory role for a phosphoinositide 3-kinase inhibitor LY294002 in a mouse asthma model.* Int Immunopharmacol 2005, 5(3): 495-502.
44. Myou, S., Leff, A.R., Myo, S. et al. *Blockade of inflammation and airway hyperresponsiveness in immune-sensitized mice by dominant-negative phosphoinositide 3-kinase-TAT.* J Exp Med 2003, 198(10): 1573-82.
45. Halayko, A.J., Kartha, S., Stelmack, G.L. et al. *Phosphatidylinositol-3 kinase/mammalian target of rapamycin/p70S6K regulates contractile protein accumulation in airway myocyte differentiation*. Am J Respir Cell Mol Biol 2004, 31(3): 266-75.
46. Krymskaya, V.P., Penn, R.B., Orsini, M.J. et al. *Phosphatidylinositol 3-kinase mediates mitogen-induced human airway smooth muscle cell proliferation*. Am J Physiol 1999, 277(1, Pt. 1): L65-78.
47. Shin, J.H., Shim, J.W., Kim, D.S., Shim, J.Y. *TGF-beta effects on airway smooth muscle cell proliferation, VEGF release and signal transduction pathways*. Respirology 2009, 14(3): 347-53.
48. Xie, S., Sukkar, M.B., Issa, R., Khorasani, N.M., Chung, K.F. *Mechanisms of induction of airway smooth muscle hyperplasia by transforming growth factor-β.* Am J Physiol Lung Cell Mol Physiol 2007, 293(1): L245-53.
49. Goncharova, E.A., Ammit, A.J., Irani, C., Carroll, R.G., Eszterhas, A.J., Panettieri, R.A., Krymskaya, V.P. *PI3K is required for proliferation and migration of human pulmonary vascular smooth muscle cells*. Am J Physiol Lung Cell Mol Physiol 2002, 283(2): L354-63.
50. Krymskaya V.P. *Targeting the phosphatidylinositol 3-kinase pathway in airway smooth muscle: Rationale and promise*. BioDrugs 2007, 21(2): 85-95.
51. Lee, K.S., Lee, H.K., Hayflick, J.S., Lee, Y.C., Puri, K.D. *Inhibition of phosphoinositide 3-kinase delta attenuates allergic airway inflammation and hyperresponsiveness in murine asthma model.* FASEB J 2006, 20(3): 455-65.
52. Lee, K.S., Park, S.J., Kim, S.R., Min, K.H., Jin, S.M., Puri, K.D., Lee, Y.C. *Phosphoinositide 3-kinase-delta inhibitor reduces vascular permeability in a murine model of asthma*. J Allergy Clin Immunol 2006, 118(2): 403-9.
53. Nashed, B.F., Zhang, T., Al-Alwan, M. et al. *Role of the phosphoinositide 3-kinase p110delta in generation of type 2 cytokine responses and allergic airway inflammation*. Eur J Immunol 2007, 37(2): 416-24.
54. Ali, K., Bilancio, A., Thomas, M. et al. *Essential role for the p110delta phosphoinositide 3-kinase in the allergic response*. Nature 2004, 431(7011): 1007-11.
55. Ali, K., Camps, M., Pearce, W.P. et al. *Isoform-specific functions of phosphoinositide 3-kinases: p110 delta but not p110 gamma promotes optimal allergic responses in vivo.* J Immunol 2008, 180(4): 2538-44.
56. Doukas, J., Eide, L., Stebbins, K. et al. *Aerosolized phosphoinositide 3-kinase gamma/delta inhibitor TG100-115 [3-[2,4-diamino-6-(3-hydroxyphenyl)pteridin-7-yl]phenol] as a therapeutic candidate for asthma and chronic obstructive pulmonary disease*. J Pharmacol Exp Ther 2009, 328(3): 758-65.
57. Jiang, B.H., Liu, L.Z. *PI3K/PTEN signaling in angiogenesis and tumorigenesis*. Adv Cancer Res 2009, 102: 19-65.
58. Gelman, L., Fruchart, J.C., Auwerx, J. *An update on the mechanisms of action of the peroxisome proliferator-activated receptors (PPARs) and their roles in inflammation and cancer.* Cell Mol Life Sci 1999, 55(6-7): 932-43.
59. Park, S.J., Lee, Y.C. *Peroxisome proliferator-activated receptor gamma as a novel therapeutic target in asthma*. J Asthma 2008, 45(1): 1-8.
60. Chinetti, G., Griglio, S., Antonucci, M. et al. *Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages.* J Biol Chem 1998, 273(40): 25573-80.
61. Jiang, C., Ting, A.T., Seed, B. *PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines.* Nature 1998, 391(6662): 82-6.
62. Woerly, G., Honda, K., Loyens, M. et al. *Peroxisome proliferator-activated receptors alpha and gamma down-regulate allergic inflammation and eosinophil activation*. J Exp Med 2003, 198(3): 411-21.
63. Honda, K., Marquillies, P., Capron, M., Dombrowicz, D. *Peroxisome proliferator-activated receptor gamma is expressed in airways and inhibits features of airway remodeling in a mouse asthma model*. J Allergy Clin Immunol 2004, 113(5): 882-8.
64. Lee, K.S., Park, S.J., Hwang, P.H. et al. *PPAR-gamma modulates allergic inflammation through up-regulation of PTEN*. FASEB J 2005, 19(8): 1033-5.
65. Lee, S.Y., Kang, E.J., Hur, G.Y. et al. *Peroxisome proliferator-activated receptor-gamma inhibits cigarette smoke solution-induced mucin production in human airway epithelial (NCI-H292) cells.* Am J Physiol Lung Cell Mol Physiol 2006, 291(1): L84-90.
66. Lee, Y.C., Kwak, Y.G., Song, C.H. *Contribution of vascular endothelial growth factor to airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma.* J Immunol 2002, 168(7): 3595-600.
67. Lee, K.S., Kim, S.R., Park, H.S., Jin, G.Y., Lee, Y.C. *Cysteinyl leukotriene receptor antagonist regulates vascular permeability by reducing vascular endothelial growth factor expression.* J Allergy Clin Immunol 2004, 114(5): 1093-9.
68. Lee, C.G., Link, H., Baluk, P. et al. *Vascular endothelial growth factor (VEGF) induces remodeling and enhances TH2-mediated sensitization and inflammation in the lung.* Nat Med 2004, 10(10): 1095-103.
69. Semenza, G.L. *Targeting HIF-1 for cancer therapy*. Nat Rev Cancer 2003, 3(10): 721-32.
70. Fukuda, R., Hirota, K., Fan, F., Jung, Y.D., Ellis, L.M., Semenza, G.L. *Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells*. J Biol Chem 2002, 277(41): 38205-11.
71. Treins, C., Giorgetti-Peraldi, S., Murdaca, J., Semenza, G.L., Van Obberghen, E. *Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signaling pathway*. J Biol Chem 2002, 277(31): 27975-81.
72. Lee, K.S., Park, S.J., Kim, S.R. et al. *Inhibition of VEGF blocks TGF-beta1 production through a PI3K/Akt signalling pathway*. Eur Respir J 2008, 31(3): 523-31.
73. Lee, K.S., Kim, S.R., Park, S.J. et al. *Mast cells can mediate vascular permeability through regulation of the PI3K-HIF-1alpha-VEGF axis*. Am J Respir Crit Care Med 2008, 178(8): 787-97.
74. Thakker, G.D., Hajjar, D.P., Muller, W.A., Rosengart, T.K. *The role of phosphatidylinositol 3-kinase in vascular endothelial growth factor signaling*. J Biol Chem 1999, 274(15): 10002-7.

75. Edwards, M.R., Bartlett, N.W., Clarke, D., Birrell, M., Belvisi, M., Johnston, S.L. *Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease.* Pharmacol Ther 2009, 121(1): 1-13.
76. Gagliardo, R., Chanez, P., Mathieu, M. et al. *Persistent activation of nuclear factor-kappaB signaling pathway in severe uncontrolled asthma*. Am J Respir Crit Care Med 2003, 168(10): 1190-8.
77. Zhao, S., Qi, Y., Liu, X., Jiang, Q., Liu, S., Jiang, Y., Jiang, Z. *Activation of NF-kappa B in bronchial epithelial cells from children with asthma*. Chin Med J (Engl) 2001, 114(9): 909-11.
78. Poynter, M.E., Irvin, C.G., Janssen-Heininger, Y.M. *Rapid activation of nuclear factor-kappaB in airway epithelium in a murine model of allergic airway inflammation.* Am J Pathol 2002, 160(4): 1325-34.
79. Kim, S.R., Lee, K.S., Park, S.J. et al. *PTEN down-regulates IL-17 expression in a murine model of toluene diisocyanate-induced airway disease*. J Immunol 2007, 179(10): 6820-9.
80. Das, J., Chen, C.H., Yang, L., Cohn, L., Ray, P., Ray, A. *A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation*. Nat Immunol 2001, 2(1): 45-50.
81. Uhlig, U., Fehrenbach, H., Lachmann, R.A., Goldmann, T., Lachmann, B., Vollmer, E., Uhlig, S. *Phosphoinositide 3-OH kinase inhibition prevents ventilation-induced lung cell activation*. Am J Respir Crit Care Med 2004, 169(2): 201-8.
82. Pappu, B.P., Angkasekwinai, P., Dong, C. *Regulatory mechanisms of helper T cell differentiation: New lessons learned from interleukin 17 family cytokines*. Pharmacol Ther 2008, 117(3): 374-84.
83. Barczyk, A., Pierzchala, W., Sozanska, E. *Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine*. Respir Med 2003, 97(6): 726-33.
84. Molet, S., Hamid, Q., Davoine, F. et al. *IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines.* J Allergy Clin Immunol 2001, 108(3): 430-8.
85. Wong, C.K., Ho, C.Y., Ko, F.W., Chan, C.H., Ho, A.S., Hui, D.S., Lam, C.W. *Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-γ, IL-4, IL-10 and IL-13) in patients with allergic asthma*. Clin Exp Immunol 2001, 125(2): 177-83.
86. Fattouh, R., Jordana, M. *TGF-beta, eosinophils and IL-13 in allergic airway remodeling: A critical appraisal with therapeutic considerations*. Inflamm Allergy Drug Targets 2008, 7(4): 224-36.
87. Roche, W.R., Beasley, R., Williams, J.H., Holgate, S.T. *Subepithelial fibrosis in the bronchi of asthmatics*. Lancet 1989, 1(8637): 520-4.
88. Minshall, E.M., Leung, D.Y., Martin, R.J., Song, Y.L., Cameron, L., Ernst, P., Hamid, Q. *Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma*. Am J Respir Cell Mol Biol 1997, 17(3): 326-33.
89. Li, Z.D., Bork, J.P., Krueger, B., Patsenker, E., Schulze-Krebs, A., Hahn, E.G., Schuppan, D. *VEGF induces proliferation, migration, and TGF-beta1 expression in mouse glomerular endothelial cells via mitogen-activated protein kinase and phosphatidylinositol 3-kinase*. Biochem Biophys Res Commun 2005, 334(4): 1049-60.