

Allergic airway hyperresponsiveness, inflammation, and remodeling do not develop in phosphoinositide 3-kinase γ -deficient mice

Masahide Takeda, MD,^a Wataru Ito, MD, PhD,^a Masako Tanabe,^a Shigeharu Ueki, MD, PhD,^a Hikari Kato,^a Junko Kihara,^a Tomomi Tanigai, MD,^a Takahito Chiba, MD, PhD,^a Kazutoshi Yamaguchi, MD, PhD,^a Hiroyuki Kayaba, MD, PhD,^a Yumiko Imai, MD, PhD,^b Kaori Okuyama, PhD,^c Isao Ohno, MD, PhD,^c Takehiko Sasaki, PhD,^d and Junichi Chihara, MD, PhD^a Akita and Sendai, Japan

Background: Bronchial asthma is characterized by chronic airway inflammation caused by inflammatory cells. Phosphoinositide 3-kinases (PI3Ks) are known to play a prominent role in fundamental cellular responses of various inflammatory cells, including proliferation, differentiation, and cell migration. PI3Ks therefore are expected to have therapeutic potential for asthma. Although some investigations of the involvement between the pathogenesis of asthma and PI3K have been performed, it is unknown whether PI3K γ , a PI3K isoform, is involved in the pathogenesis of asthma.

Objective: We investigated the role of PI3K γ in allergen-induced allergic airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling with PI3K γ -deficient mice.

Methods: After ovalbumin (OVA) sensitization, wild-type (WT) and PI3K γ -deficient mice were exposed to aerosolized OVA 3 days per week for 5 weeks.

Results: In OVA-sensitized and OVA-challenged (OVA/OVA) PI3K γ -deficient mice, levels of airway inflammation, AHR, and airway remodeling were significantly decreased compared with those in OVA/OVA WT mice. On the other hand, no significant differences were detected in serum OVA-specific IgE and IgG1 levels and CD4/CD8 balance in bronchoalveolar lavage fluid between OVA/OVA WT mice and OVA/OVA PI3K γ -deficient mice. To determine in which phase of allergic responses PI3K γ plays a role, we transferred splenocytes from OVA-sensitized WT or PI3K γ -deficient mice to naive mice of either genotype. Similar increased levels of eosinophils were induced in both WT recipient mice but not in both PI3K γ -deficient recipient mice.

Conclusion: PI3K γ might be involved in allergic airway inflammation, AHR, and airway remodeling by regulating the

challenge/effector phase of allergic responses. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Airway hyperresponsiveness, airway remodeling, airway inflammation, asthma, phosphoinositide 3-kinase γ , TGF- β , eosinophil, migration, adoptive transfer

Bronchial asthma is a chronic inflammatory disorder of the airway and is associated with airway hyperresponsiveness (AHR) to nonspecific stimuli, mucus production, and alterations in structure.¹ Airway inflammation is characterized by an infiltration of inflammatory cells into the airway, and T_H2 cytokines play critical roles in orchestrating the allergic inflammatory response leading to AHR.²⁻⁵ Furthermore, persistent inflammation leads to airway structural changes termed *airway remodeling*.⁶

Phosphoinositide 3-kinases (PI3Ks) are a family of proteins that phosphorylate the 3'-OH position of the inositol ring of phosphatidylinositols, resulting in the formation of PI(3,4,5)P₃, which activates downstream signaling, such as Akt. As a result, PI3Ks play a prominent role in various inflammatory cells by regulating fundamental cellular responses, including proliferation, differentiation, and cell migration.⁷ PI3Ks can be divided into 3 classes: class I, II, and III.⁸ In particular, class I PI3Ks have commanded attention because these enzymes are activated on receptor stimulation. The class IA PI3Ks are activated by stimulation of tyrosine kinase-based receptors and consist of a heterodimeric complex composed of a 110-kd (p110 α , β , δ) catalytic subunit and adaptor proteins (p85 α , β and p55 α , β , γ). PI3K γ , the only member of class IB, consists of a heterodimer composed of p110 γ and p101 adaptor proteins and is activated by stimulation of the $\beta\gamma$ subunits of the G protein-coupled receptor. Recently, it has been reported that the acute asthmatic response in mice was diminished by administering LY294002, a pharmacologic inhibitor of PI3K.^{9,10} In addition to LY294002 being a known inhibitor of all isoforms of p110, it has been revealed that LY294002 also inhibits activation of the serine/threonine kinase Pim-1.¹¹ Thus it is important to clarify the role of PI3Ks and their isoforms in allergic responses. Lee et al¹² showed that IC87114, a selective p110 δ inhibitor, significantly attenuated allergic airway inflammation and hyperresponsiveness in a murine model of asthma. Furthermore, it has been demonstrated that these allergic responses were decreased in p110 δ mutant mice.¹³ It also has been reported that PI3K γ is involved in leukocyte migration, including neutrophils and eosinophils.^{14,15} Accordingly, PI3K γ is thought to be involved in the pathogenesis of asthma; however, the role of

From the Departments of ^aClinical and Laboratory Medicine, ^bPharmacology, and ^cPathology and Immunology, Akita University School of Medicine, and ^dthe Department of Pathophysiology, Tohoku Pharmaceutical University, Sendai.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication March 27, 2008; revised November 7, 2008; accepted for publication November 11, 2008.

Reprint requests: Junichi Chihara, MD, PhD, Department of Clinical and Laboratory Medicine, Akita University School of Medicine, 1-1-1, Hondo, Akita 010-8543 Japan.

E-mail: chihara@hos.akita-u.ac.jp.

0091-6749/\$36.00

© 2009 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2008.11.047

Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
MT: Masson trichrome
NIH: National Institutes of Health
OVA: Ovalbumin
PAS: Periodic acid–Schiff
PDGF: Platelet-derived growth factor
PI3K: Phosphoinositide 3-kinase
 α -SMA: α -Smooth muscle actin
WT: Wild-type

PI3K γ in bronchial asthma, especially airway remodeling, is poorly reported. Therefore in this study we investigated the role of PI3K γ in bronchial asthma using PI3K γ -deficient mice.

METHODS**Animals**

Female C57BL/6 mice (8–10 weeks of age) were purchased from Charles River Japan, Inc (Yokohama, Japan). Mice deficient in PI3K γ were generated by using homologous recombination in embryonic stem cells, as described previously.¹⁴ The mice were maintained on a diet free of ovalbumin (OVA). All experimental animals used in this study were housed under constant temperature and light cycles and under a protocol approved by the Institutional Animal Care and Use Committee of Akita University Medical School.

Sensitization and airway challenge

Mice (6 per group per experiment) receiving the following treatments were studied: (1) nonsensitized and nonchallenged mice (Non/Non) and (2) OVA-sensitized and OVA airway-challenged mice (OVA/OVA). OVA/OVA mice were immunized by means of intraperitoneal injection of 20 μ g of OVA (Grade V; Sigma-Aldrich, St Louis, Mo) emulsified in 2.25 mg of alum (AlumImject; Pierce, Rockford, Ill) in a total volume of 100 μ L on days 0 and 14. Mice were challenged through the airway with OVA (1% in saline) for 20 minutes on days 28, 29, and 30 by means of ultrasonic nebulization and further exposed to aerosolized challenge 3 days per week for 4 additional weeks. AHR and immunologic examinations were assessed 48 hours after the last challenge.^{16–18}

Determination of airway responsiveness

Lung resistance to methacholine (Sigma-Aldrich) was measured by using an invasive system, as previously described.¹⁹ Briefly, mice were deeply anesthetized, and tracheotomies were performed. The mice were then placed in a whole-body plethysmograph chamber (Buxco Electronics, Sharon, Conn) and ventilated mechanically. Once baseline data were established, saline and an increasing dose of nebulized methacholine were administered. In each dose air-flow changes in the sealed chamber and pressure changes in the airway were analyzed with BioSystem XA software (Buxco Electronics) for 3 minutes. Lung resistance for each methacholine dose was expressed as a percentage change from baseline level.

Bronchoalveolar lavage and measurement of bronchoalveolar lavage fluid cytokines

After AHR measurement, the lungs were lavaged through the tracheal tube with saline (2×1 mL, 37°C). The volume of collected bronchoalveolar lavage (BAL) fluid was measured in each sample, and the number of BAL cells was counted. Cytospin slides were stained with May-Giemsa, and at least 300 cells were differentiated in a blinded fashion under light microscopy. Cytokine concentrations in the BAL fluid supernatants were measured by means of ELISA. Colorimetric measurement was performed according to the

manufacturer's instructions. The limit of detection was 4 pg/mL for IL-4, IL-5, IL-13, IFN- γ , and platelet-derived growth factor (PDGF; R&D Systems, Minneapolis, Minn). The limit of detection was 30 pg/mL for total TGF- β (R&D Systems).

Measurement of serum OVA-specific antibody

OVA-specific IgE and IgG1 antibody levels were measured by means of ELISA 48 hours after the last airway challenge.^{12,20}

Histologic and immunohistochemistry studies

Lungs were inflated through a tracheal tube with 2 mL of air and fixed in 10% formalin, and lung tissue was embedded in paraffin. Tissue sections 4 μ m thick were affixed to microscope slides and deparaffinized. The slides were stained with hematoxylin and eosin to detect inflammatory cell infiltration, periodic acid–Schiff (PAS) for identification of mucus-containing cells (goblet cells), Masson trichrome (MT) stain for the peribronchiolar collagen layer, and immunostain for α -smooth muscle actin (α -SMA; DAKO, Glostrup, Denmark), and they were examined under light microscopy.^{20,21} In hematoxylin and eosin–stained lung sections, the numbers of inflammatory cells per square millimeter in the peribronchial tissue were analyzed by using the National Institutes of Health (NIH) Image Analysis system.²² The numbers of goblet cells were counted in 10 or more distal bronchioles in 10 high-power fields per animal by measuring the length of epithelium defined along the basement membrane and luminal area with the NIH Image Analysis system. The area of the MT-positive peribronchiolar collagen layer or α -SMA–positive peribronchiolar smooth muscle was measured also with the NIH Image Analysis system.²³

Phenotypic analysis

Cells in blood and BAL fluid of each mouse were incubated with the following mAbs: phycoerythrin-labeled anti-mouse CD8a (BioLegend, San Diego, Calif), fluorescein isothiocyanate–labeled anti-mouse CD4 (BioLegend), and peridinin-chlorophyll-protein complex-labeled anti-mouse CD3e (BD Biosciences, San Jose, Calif) for 30 minutes at 4°C. After staining, the cells were incubated with diluted lysing solution for 30 minutes at 4°C to hemolyze red blood cells. Then the cells were washed with PBS and analyzed with a FACSscan flow cytometer (BD Biosciences). An analysis gate was set on lymphocytes by scatter properties and CD3. CD4⁺CD8[–] or CD4[–]CD8⁺ cells in CD3⁺ cells were analyzed.

Adoptive transfer assay

The adoptive splenocyte transfer assay was performed as described previously.^{13,24} Briefly, splenocytes from each OVA-sensitized mouse were harvested at day 17 and restimulated *in vitro* with 200 μ g/mL OVA for 3 days and transferred to a nonirradiated naive recipient by means of intraperitoneal injection (wild-type [WT] or PI3K γ -deficient mice, 5×10^6 cells per recipient). At 3 days after transfer, mice were challenged intranasally twice with 0.5% OVA in 60 minutes. Five days after challenge, BAL fluid was harvested, and inflammatory cells were counted.

Statistical analysis

Two groups of data were compared by using the Student *t* test. The *P* value for significance was set at less than .05. All results were expressed as the mean $\pm$ SEM.

RESULTS**AHR and inflammatory cell accumulation in PI3K γ -deficient mice**

There were no significant differences in baseline pulmonary resistance among all groups. Additionally, there were no

differences in the increase of AHR in response to increased doses of inhaled methacholine between Non/Non WT mice and Non/Non PI3K γ -deficient mice. In OVA/OVA WT mice AHR significantly increased in a dose-dependent manner compared with that seen in Non/Non mice. In contrast, in OVA/OVA PI3K γ -deficient mice AHR was decreased significantly compared with that seen in OVA/OVA WT mice (Fig 1, A). Very few inflammatory cells other than macrophages were detected in Non/Non WT mice and Non/Non PI3K γ -deficient mice, and there were no significant differences between them. A marked increase in the number of eosinophils and lymphocytes, as well as an increase in total cell numbers in BAL fluid, was detected in OVA/OVA WT mice. In contrast, the number of eosinophils, lymphocytes, and total cells decreased in OVA/OVA PI3K γ -deficient mice compared with numbers seen in OVA/OVA WT mice (Fig 1, B).

Inflammatory cell infiltration was investigated by means of histologic examination of hematoxylin and eosin-stained slides. The number of inflammatory cells in the peribronchial and perivascular areas of OVA/OVA WT mice was significantly increased compared with the number seen in OVA/OVA PI3K γ -deficient mice (Fig 2, A and C).

Airway remodeling in PI3K γ -deficient mice

It has been reported that airway remodeling is associated with histologic changes.⁶ PAS, MT, and α -SMA staining was performed to investigate the influence of PI3K γ on airway remodeling. A significant decrease in the number of PAS-positive cells was found in OVA/OVA PI3K γ -deficient mice compared with the numbers seen in OVA/OVA WT mice (Fig 2, B and D). In addition, there was a significant decrease in the peribronchiolar collagen layer in MT stain and smooth muscle layer in α -SMA stain in OVA/OVA PI3K γ -deficient mice compared with values seen in OVA/OVA WT mice (Fig 3).

Cytokine and growth factor levels in BAL fluid of PI3K γ -deficient mice

In OVA/OVA PI3K γ -deficient mice the levels of IL-4, IL-5, and IL-13 in BAL fluid were significantly reduced compared with levels seen in OVA/OVA WT mice. The levels of PDGF and TGF- β were similarly decreased in OVA/OVA PI3K γ -deficient mice. There was no significant difference between OVA/OVA WT and OVA/OVA PI3K γ -deficient mice in the levels of IFN- γ (Fig 4).

Serum OVA-specific IgE and IgG1 levels in PI3K γ -deficient mice

In OVA/OVA WT mice and OVA/OVA PI3K γ -deficient mice the levels of OVA-specific IgE and IgG1 were significantly increased compared with those seen in control mice; however, there was no significant difference between OVA/OVA WT mice and OVA/OVA PI3K γ -deficient mice (Fig 5, A and B).

T-cell phenotype in PI3K γ -deficient mice

The percentage of CD3⁺CD4⁺ cells in BAL fluid was increased in OVA/OVA WT mice (79.5% $\pm$ 5.4%) compared with that seen in Non/Non WT mice (58.5% $\pm$ 7.2%). No significant changes were detected in the percentage of CD3⁺CD4⁺ cells between OVA/OVA WT mice and OVA/OVA PI3K γ -deficient mice

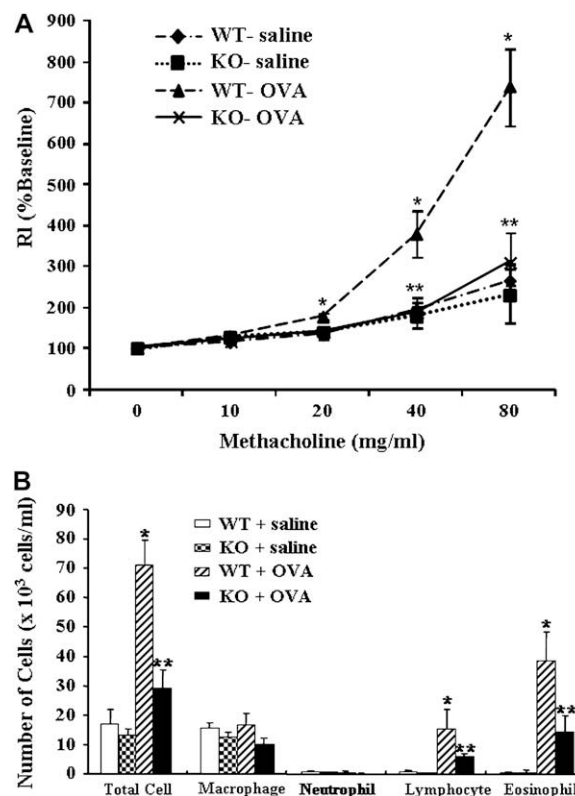


FIG 1. A, Lung resistance (RI) for increasing methacholine dose. B, Inflammatory cell accumulation in BAL fluid. Results for each group are expressed as the mean $\pm$ SEM. *Significant differences ($P < .05$) between Non/Non and OVA/OVA WT mice. **Significant differences ($P < .05$) between OVA/OVA WT and PI3K γ -deficient mice. KO, Knockout.

(79.6% $\pm$ 13.3%). In contrast, the percentage of CD3⁺CD8⁺ cells was significantly decreased in OVA/OVA WT mice (11.0% $\pm$ 4.6%) compared with that seen in Non/Non WT mice (15.4% $\pm$ 13.4%), and no significant changes were detected in the percentage of CD3⁺CD8⁺ cells between OVA/OVA WT mice and OVA/OVA PI3K γ -deficient mice (5.8% $\pm$ 3.3%). Furthermore, similar results were detected in the blood of each group.

Adaptive transfer of p110 γ -deficient splenocytes

To determine in which phase of development of allergic inflammation PI3K γ plays an important role, we transferred splenocytes from OVA-sensitized WT or PI3K γ -deficient mice to naive mice of either genotype. The results showed that similar levels of eosinophils were induced in both WT recipient mice when prime splenocytes of WT or PI3K γ -deficient mice were adoptively transferred. Eosinophil infiltration into the airway was not induced in either of the PI3K γ -deficient recipient mice (Fig 5, C). Similarly, lung resistance and dynamic compliance were facilitated in both WT recipients compared with that seen in PI3K γ -deficient recipients (data not shown).

DISCUSSION

The pathologic mechanism of asthma is complex, with allergens triggering infiltration and interactions of various inflammatory

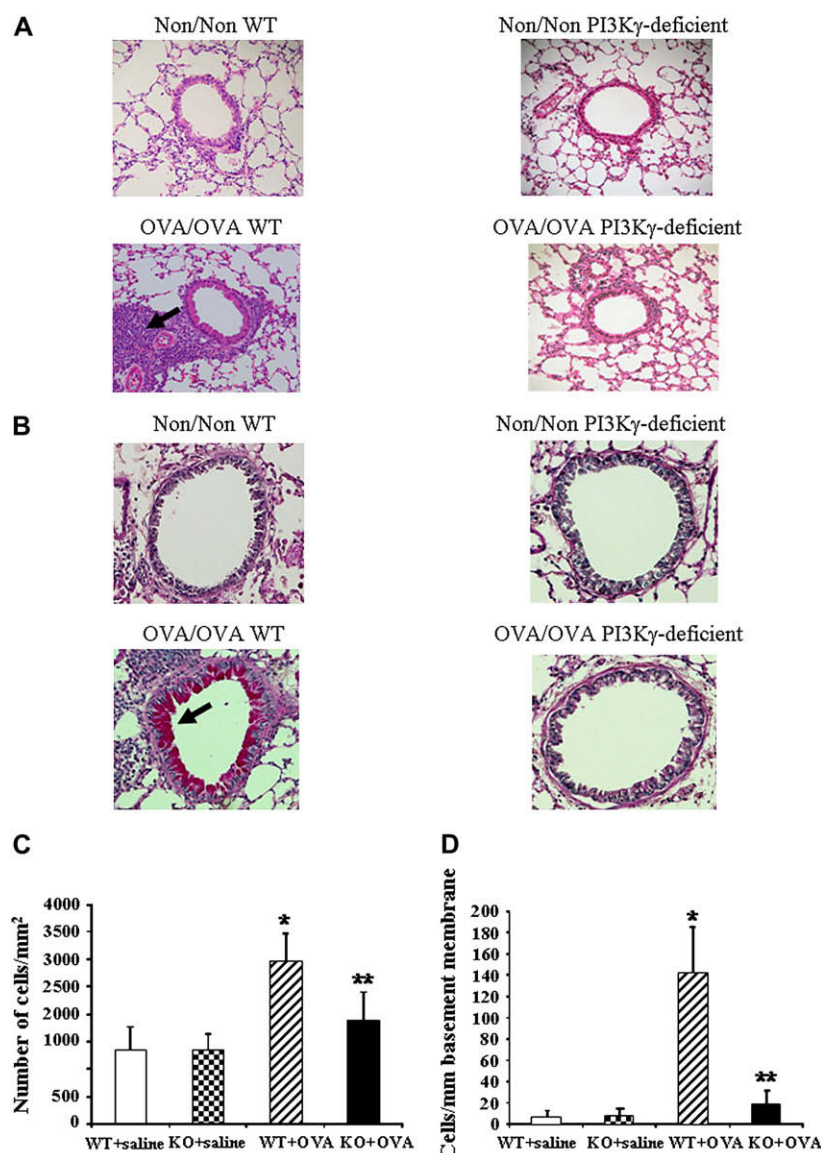


FIG 2. **A** and **C**, Hematoxylin and eosin stain and quantified data (final magnification $\times 200$). **B** and **D**, PAS stain and quantified data (final magnification $\times 400$). Striking infiltration of inflammatory cells and goblet cell hyperplasia (arrow) are present near the airway in OVA/OVA WT mice. Data represent the mean $\pm$ SEM. *Significant differences ($P < .05$) between Non/Non and OVA/OVA WT mice. **Significant differences ($P < .05$) between OVA/OVA WT and PI3K γ -deficient mice. KO, Knockout.

cells and the release of cytokines and growth factors involved in airway inflammation, AHR, and airway remodeling.

In this study we show for the first time that AHR, lung inflammatory cells, airway remodeling, and the levels of T_H2 cytokines and growth factors in BAL fluid were significantly decreased in OVA/OVA PI3K γ -deficient mice compared with those seen in OVA/OVA WT mice.

Although airway remodeling is difficult to define, subepithelial fibrosis, goblet cell hyperplasia, and myofibroblast hypertrophy have been identified as components of this response.⁶ Numerous studies have reported that airway remodeling is formed by cytokines, such as IL-13, and growth factors, such as TGF- β and PDGF. TGF- β activates downstream signaling and causes myofibroblast differentiation, leading to tissue fibrosis.²⁵ In addition, TGF- β and PDGF are involved in migration and proliferation

of airway smooth muscle cells, which are thought to contribute to airway remodeling in asthma.²⁶⁻²⁸ In this study the level of TGF- β and PDGF in BAL fluid was reduced in OVA/OVA PI3K γ -deficient mice compared with that seen in OVA/OVA WT mice. Therefore the observed reduction in tissue fibrosis and airway smooth muscle thickness in OVA/OVA PI3K γ -deficient mice might be due to the decrease of TGF- β and PDGF levels in BAL fluid.

Both TGF- β and PDGF are mainly released from eosinophils and lymphocytes.^{29,30} Therefore eosinophils and lymphocytes are also considered to be involved in airway remodeling.³¹ In this study the number of eosinophils and lymphocytes in BAL fluid was significantly decreased in OVA/OVA PI3K γ -deficient mice compared with that seen in OVA/OVA-WT mice. These findings might indicate that in PI3K γ -deficient mice

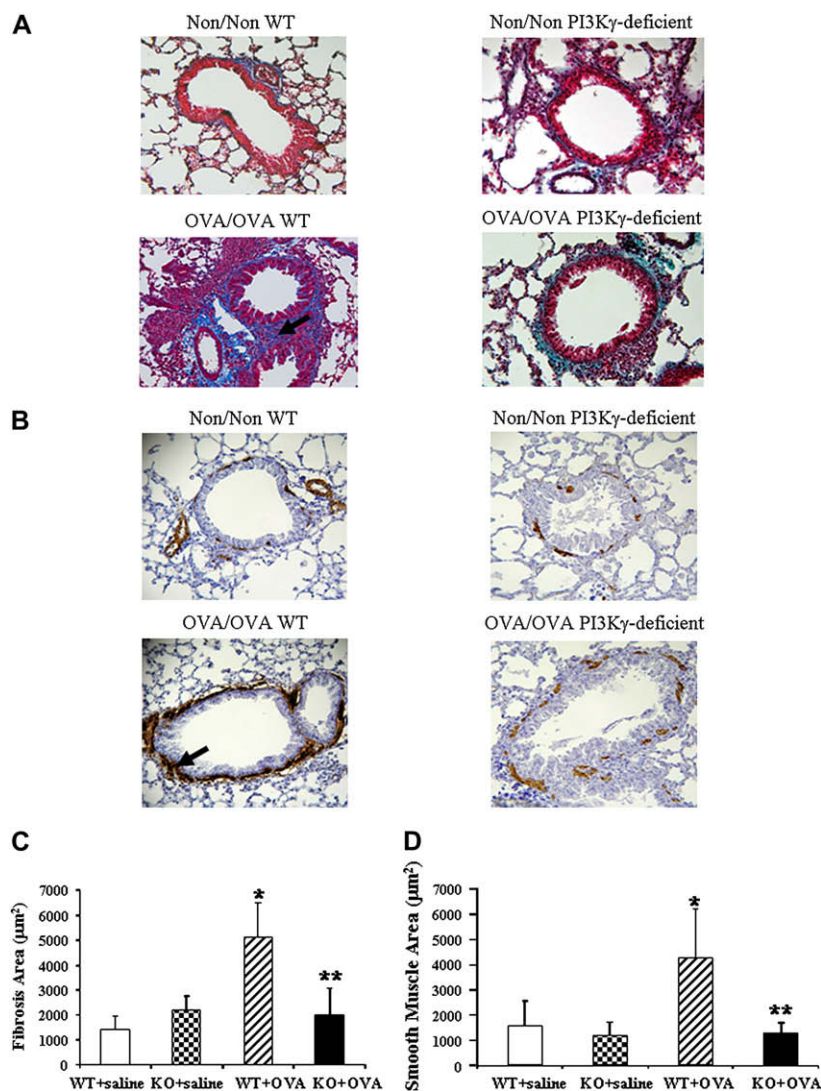


FIG 3. MT stain and quantified data (**A** and **C**) and immunostaining for α -SMA and quantified data (**B** and **D**; final magnification $\times 200$). Striking peribronchiolar collagen layer and smooth muscle layer (arrow) are present in OVA/OVA WT mice but not in OVA/OVA PI3K γ -deficient mice. *Significant differences ($P < .05$) between Non/Non and OVA/OVA WT mice. **Significant differences ($P < .05$) between OVA/OVA WT and PI3K γ -deficient mice. KO, Knockout.

suppression of eosinophil migration into lung tissue leads to a decrease in TGF- β and PDGF production, which in turn causes a reduction in airway remodeling. IL-13 has also been shown to play a vital role in airway remodeling by regulating goblet cell hyperplasia.^{32,33} In this study the levels of IL-13 detected in BAL fluid were reduced in OVA/OVA PI3K γ -deficient mice. Therefore the observed reduction of goblet cell hyperplasia in OVA/OVA PI3K γ -deficient mice might be due to the decrease of IL-13 levels in BAL fluid through the suppression of lymphocytes.

In the mechanism of AHR, a large number of factors interact with each other. IL-5 induces eosinophil proliferation, differentiation, and migration from bone marrow, and IL-5-activated eosinophils release granular proteins, leading to tissue damage and AHR.^{34,35} IL-4 and IL-13 also contribute to AHR.³⁶ Not only eosinophils and lymphocytes but also mast cells and dendritic cells are involved in AHR. In this study the levels of T_H2

cytokines in BAL fluid were decreased in OVA/OVA PI3K γ -deficient mice compared with those seen in OVA/OVA WT mice. It has been reported that PI3K γ regulates dendritic cell migration and mast cell degranulation in PI3K γ -deficient mice.^{37,38} Structural and functional changes in airway remodeling have also been shown to be involved in the mechanism of AHR.³⁹ Therefore our results showing that pulmonary resistance and dynamic compliance were not exacerbated in PI3K γ -deficient mice might be due to the deficit of PI3K γ .

It is known that immunologic responses, such as IgE production, alterations in T-cell phenotype, and the shift in T_H1/T_H2 balance, occur in bronchial asthma.^{40,41} In this study we found that there was no significant difference in serum OVA-specific IgE and IgG1 levels or the percentage of CD3⁺CD4⁺ T cells in BAL fluid between OVA/OVA WT mice and OVA/OVA PI3K γ -deficient mice. Thus PI3K γ might have no effect on T-cell phenotype and IgE production in bronchial asthma. The

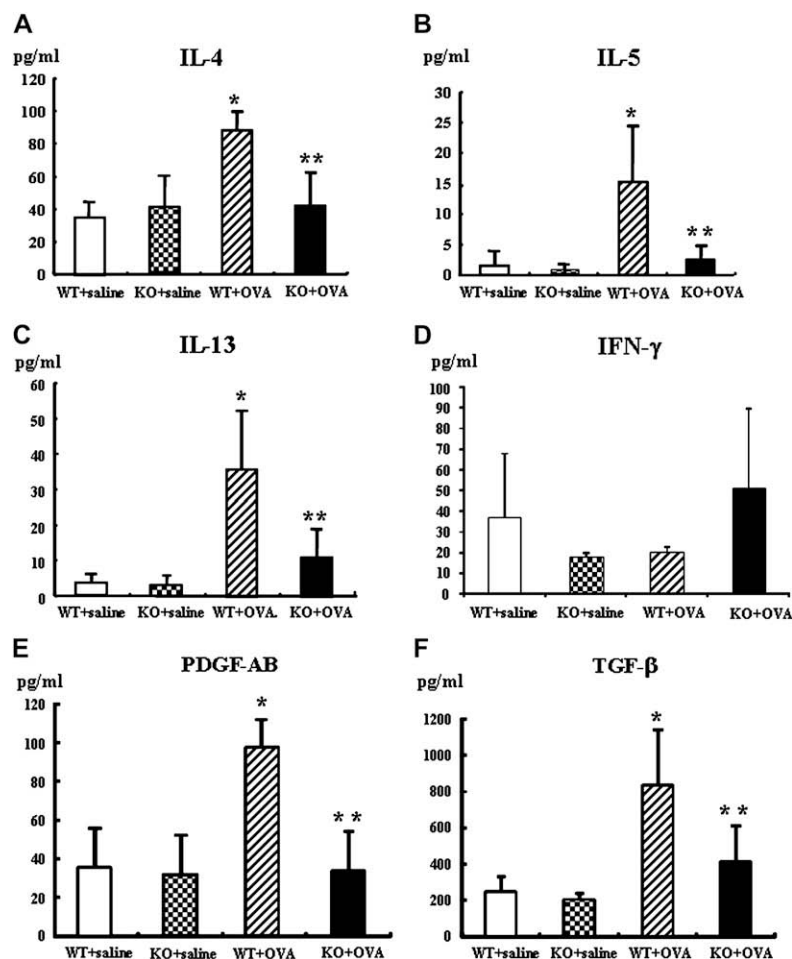


FIG 4. The levels of IL-4 (A), IL-5 (B), IL-13 (C), IFN- γ (D), PDGF (E), and TGF- β (F) in BAL fluid of mice were measured by means of ELISA. The results are expressed as the mean $\pm$ SEM. *Significant differences ($P < .05$) between Non/Non and OVA/OVA WT mice. **Significant differences ($P < .05$) between OVA/OVA WT and PI3K γ -deficient mice. KO, Knockout.

result in this study might indicate that the observed reduction in AHR, airway inflammation, and airway remodeling in OVA/OVA PI3K γ -deficient mice might be caused by regulation in the challenge/effector phase of asthma but not in the sensitization phase.

To determine in which phase of allergic responses PI3K γ plays a vital role, we transferred splenocytes from OVA-sensitized WT or PI3K γ -deficient mice to respective naive mice. The results demonstrate that the suppression of airway inflammation might be caused by regulation of the challenge/effector phase but not the sensitization phase because an increase in eosinophil counts in BAL fluid was detected only in both WT recipient mice. In this result there is the possibility that the deficit of PI3K γ in parenchymal cells, such as epithelial cells and endothelial cells or leukocytes, is involved in this phenomenon. Leukocyte PI3K γ is thought to play a critical role in its migration.^{14,15} On the other hand, it is also known that parenchymal PI3K γ is involved in leukocyte infiltration into tissue.⁴² Thus the involvement of parenchymal PI3K γ is not ruled out. Further studies are necessary to elucidate which cells are responsible for inflammatory cell infiltration into tissue in the challenge/effector phase.

As for the association between asthma and other PI3K isoforms, it has already been reported that allergic airway inflammation and AHR were suppressed by inhibiting PI3K δ or antagonizing p85 δ , which is consistent with our data.^{12,43} However, reported serum IgE levels were different from our data. Lee et al¹² reported that OVA-specific IgE was attenuated by a PI3K δ inhibitor. Moreover, it has been reported that class IA PI3Ks are implicated in early B-cell development and B-cell function.^{44,45} In contrast, no difference was seen between OVA/OVA WT and OVA/OVA PI3K γ -deficient mice in terms of the levels of OVA-specific IgE in our study. It has been demonstrated that PI3K γ plays no role in the development or function of B cells.¹⁴ These findings indicate that PI3Ks might have different immunologic function, depending on the isoform. However, the mechanism of this difference is still unclear.

In summary, our study found that PI3K γ might regulate allergen-induced AHR, airway infiltration, and airway remodeling through modulation of the challenge/effector phase but not the sensitization phase. These findings indicate that PI3K γ plays a critical role in the pathologic mechanism of asthma and that PI3K γ could be a therapeutic target for bronchial asthma, especially prevention of airway remodeling.

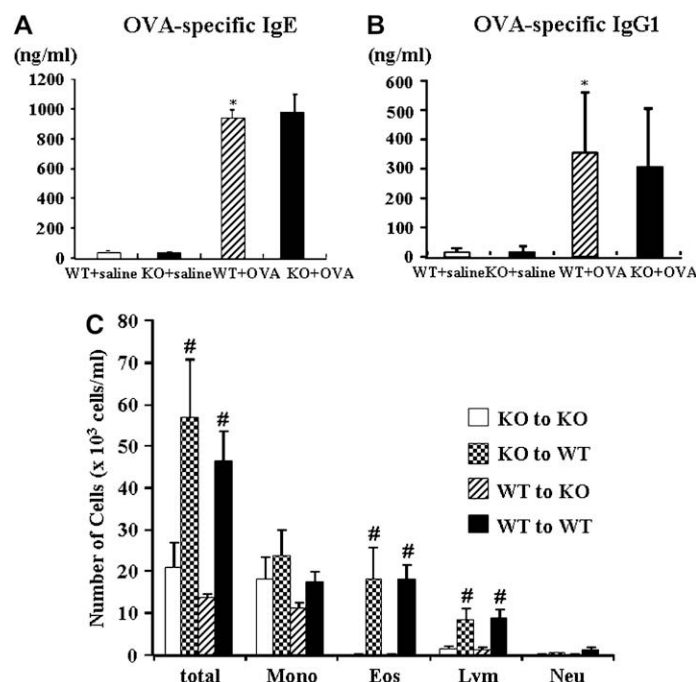


FIG 5. The levels of serum OVA-specific IgE (**A**) and IgG1 (**B**) were decreased in OVA/OVA PI3K γ -deficient mice. **C**, Inflammatory cell accumulation in BAL fluid of mice adoptively transferred with splenocytes. Results are expressed as the mean $\pm$ SEM. *Significant differences ($P < .05$) between Non/Non and OVA/OVA WT mice. #Significant differences ($P < .05$) compared with the PI3K γ -deficient recipient group.

Key message

- Through modulation of challenge/effector phase, PI3K γ might play an important role in the regulation of allergic airway inflammation, AHR, and airway remodeling. Therefore PI3K γ could be a therapeutic target for bronchial asthma.

REFERENCES

- Holgate ST. Airway inflammation and remodeling in asthma: current concepts. *Mol Biochem* 2002;22:179-89.
- Rosenberg HF, Phipps S, Foster PS. Eosinophil trafficking in allergy and asthma. *J Allergy Clin Immunol* 2007;119:1303-10.
- Larché M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;111:450-63.
- Kips JC. Cytokine in asthma. *Eur Respir J Suppl* 2001;34:24-33.
- Chihara J. Molecular mechanisms of eosinophil activation in allergic diseases. *Clin Exp Allergy* 2005;5:46-9.
- Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. *J Allergy Clin Immunol* 2003;111:215-25.
- Toker A, Cantley LC. Signalling through the lipid products of phosphoinositide-3-OH kinase. *Nature* 1997;387:673-6.
- Wymann MP, Björklöf K, Calvez R, Finan P, Thomast M, Trifilieff A, et al. Phosphoinositide 3-kinase gamma: a key modulator in inflammation and allergy. *Biochem Soc Trans* 2003;31:275-80.
- Duan W, Aguinaldo Datiles AM, Leung BP, Vlahos CJ, Wong WS. An anti-inflammatory role for a phosphoinositide 3-kinase inhibitor LY294002 in a mouse asthma model. *Int Immunopharmacol* 2005;5:495-502.
- Kwak YG, Song CH, Yi HK, Hwang PH, Kim JS, Lee KS, et al. Involvement of PTEN in airway hyperresponsiveness and inflammation in bronchial asthma. *J Clin Invest* 2003;111:1083-92.
- Jacobs MD, Black J, Futer O, Swenson L, Hare B, Fleming M, et al. Pim-1 ligand-bound structures reveal the mechanism of serine/threonine kinase inhibition by LY294002. *J Biol Chem* 2005;280:13728-34.
- Lee KS, Lee HK, Hayflick JS, Lee YC, Puri KD. Inhibition of phosphoinositide 3-kinase delta attenuates allergic airway inflammation and hyperresponsiveness in murine asthma model. *FASEB J* 2006;20:455-65.
- Nashed BF, Zhang T, Al-Alwan M, Srinivasan G, Halayko AJ, Okkenhaug K, et al. Role of the phosphoinositide 3-kinase p110delta in generation of type 2 cytokine responses and allergic airway inflammation. *Eur J Immunol* 2007;37:416-24.
- Sasaki T, Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, et al. Function of PI3K γ in thymocyte development, t cell activation, and neutrophil migration. *Science* 2000;287:1040-6.
- Pinho V, Souza DG, Barsante MM, Hamer FP, De Freitas MS, Rossi AG, et al. Phosphoinositide-3 kinases critically regulate the recruitment and survival of eosinophils in vivo: importance for the resolution of allergic inflammation. *J Leukoc Biol* 2005;77:800-10.
- Kanehiro A, Takeda K, Joetham A, Tomkinson A, Ikemura T, Irvin CG, et al. Timing of administration of anti-VLA-4 differentiates airway hyperresponsiveness in the central and peripheral airways in mice. *Am J Respir Crit Care Med* 2000;162:1132-9.
- Ito W, Kanehiro A, Matsumoto K, Hirano A, Ono K, Maruyama H, et al. Hepatocyte growth factor attenuates airway hyperresponsiveness, inflammation, and remodeling. *Am J Respir Cell Mol Biol* 2005;32:268-80.
- Ito W, Tanimoto M, Ono K, Mizuno S, Yoshida A, Koga H, et al. Growth factors temporally associate with airway responsiveness and inflammation in allergen-exposed mice. *Int Arch Allergy Immunol* 2007;145:324-39.
- Lai WQ, Goh HH, Bao Z, Wong WS, Melendez AJ, Leung BP. The role of sphingosine kinase in a murine model of allergic asthma. *J Immunol* 2008;180:4323-9.
- Temann UA, Prasad B, Gallup MW, Basbaum C, Ho SB, Flavell RA, et al. A novel role for murine IL-4 in vivo: induction of MUC5AC gene expression and mucin hypersecretion. *Am J Respir Cell Mol Biol* 1997;16:471-8.
- Henderson WR Jr, Tang LO, Chu SJ, Tsao SM, Chiang GK, Jones F, et al. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 2002;165:108-16.
- Kuhn C III, Homer RJ, Zhu Z, Ward N, Flavell RA, Geba GP, et al. Airway hyperresponsiveness and airway obstruction in transgenic mice: morphologic correlates in mice overexpressing interleukin (IL)-11 and IL-6 in the lung. *Am J Respir Cell Mol Biol* 2000;22:289-95.
- Bonner JC, Rice AB, Moomaw CR, Morgan DL. Airway fibrosis in rats induced by vanadium pentoxide. *Am J Physiol Lung Cell Mol Physiol* 2000;278:209-16.
- Okuyama K, Wada K, Chihara J, Takayanagi M, Ohno I. Sex-related splenocyte function in a murine model of allergic asthma. *Clin Exp Allergy* 2008;38:1212-9.

25. Jeffrey CH, David SR, Vishal S, Ragni V, Eric SW, Zongbin C, et al. Combinatorial activation of FAK and AKT by transforming growth factor- β 1 confers an anoikis-resistant phenotype to myofibroblasts. *Cell Signal* 2007;19:761-71.
26. Shaoping X, Maria BS, Razao I, Nadia MK, Kian C. Mechanisms of induction of airway smooth muscle hyperplasia transforming growth factor- β . *Am J Physiol Lung Cell Mol Physiol* 2007;293:245-53.
27. Gang C, Nasreen K. TGF- β 1 increases proliferation of airway smooth muscle cells by phosphorylation of map kinases. *Respir Res* 2006;7:2.
28. Stephen MC, Michael R, Judith LB. Urokinase potentiates PDGF-induced chemotaxis of human airway muscle cell. *Am J Physiol Lung Cell Mol Physiol* 2003;284:1020-6.
29. Elovic A, Wong DT, Weller PF, Matossian K, Galli SJ. Expression of transforming growth factors-alpha and beta1 messenger RNA and product by eosinophils in nasal polyps. *J Allergy Clin Immunol* 1994;93:864-9.
30. Kohler N, Lipton A. Platelets as a source of fibroblast growth-promoting activity. *Exp Cell Res* 1974;87:297-301.
31. Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR, et al. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004;305:1773-6.
32. Tyner JW, Kim EY, Ide K, Pelletier MR, Roswit WT, Morton JD, et al. Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 trans-differentiation signals. *J Clin Invest* 2006;116:309-21.
33. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities and eotaxin production. *J Clin Invest* 1999;103:779-88.
34. Nakagome K, Dohi M, Okunishi K, Tanaka R, Kouro T, Kano MR, et al. IL-5-induced hypereosinophilia suppresses the antigen-induced immune response via a TGF- β -dependent mechanism. *J Immunol* 2007;179:284-94.
35. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin-5 deficiency abolishes eosinophilia, airway hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195-201.
36. Vargaftig BB, Singer M. Leukotrienes mediate murine bronchopulmonary, hyper-reactivity, inflammation, and part of mucosal metaplasia and tissue injury induced by recombinant murine interleukin-13. *Am J Respir Cell Mol Biol* 2003;28:410-9.
37. Del Prete A, Vermi W, Dander E, Otero K, Barberis L, Luini W, et al. Defective dendritic cell migration and activation of adaptive immunity in PI3Kgamma-deficient mice. *EMBO J* 2004;23:3505-15.
38. Laffargue M, Calvez R, Finan P, Trifilieff A, Barbier M, Altruda F, et al. Phospho-inositide 3-kinase gamma is an essential amplifier of mast cell function. *Immunity* 2002;16:441-51.
39. Cockcroft DW, Davis BE. Mechanism of airway hyperresponsiveness. *J Allergy Clin Immunol* 2006;118:551-9.
40. Georas SN, Guo J, De Fanis U, Casolaro V. T-helper cell type-2 regulation in allergic disease. *Eur Respir J* 2005;26:1119-37.
41. Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med* 2001;164:1-5.
42. Puri KD, Doggett TA, Huang CY, Douangpanya J, Hayflick JS, Turner M, et al. The role of endothelial PI3Kgamma activity in neutrophil trafficking. *Blood* 2005;106:150-7.
43. Myou S, Leff AR, Myo S, Boetticher E, Tong J, Meliton AY, et al. Blockade of inflammation and airway hyperresponsiveness in immune-sensitized mice by dominant-negative phosphoinositide 3-kinase-TAT. *J Exp Med* 2003;198:1573-82.
44. Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW, et al. Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha. *Science* 1999;283:393-7.
45. Suzuki H, Terauchi Y, Fujiwara M, Aizawa S, Yazaki Y, Kadowaki T, et al. SXid-like immunodeficiency in mice with disruption of the p85alpha subunit of phospho-inositide 3-kinase. *Science* 1999;283:390-2.