Targeting PI3K-p110α Suppresses Influenza Virus Infection in Chronic Obstructive Pulmonary Disease

Alan Chen-Yu Hsu1*, Malcolm R. Starkey1, Irwan Hanish1,2, Kristy Parsons1, Tatt Jhong Haw1, Linda J. Howland1, Ian Barr3, James B. Mahony4, Paul S. Foster1, Darryl A. Knight1,5, Peter A. Wark1,6*, and Philip M. Hansbro1*

1Priority Research Centre for Asthma and Respiratory Diseases, Hunter Medical Research Institute and The University of Newcastle, Newcastle, New South Wales, Australia; 2Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; 3World Health Organization Collaborating Centre for Reference and Research on Influenza, Melbourne, Victoria, Australia; 4Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada; 5Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada; and 6Department of Respiratory and Sleep Medicine, John Hunter Hospital, Newcastle, New South Wales, Australia

Abstract

**Rationale:** Chronic obstructive pulmonary disease (COPD) and influenza virus infections are major global health issues. Patients with COPD are more susceptible to infection, which exacerbates their condition and increases morbidity and mortality. The mechanisms of increased susceptibility remain poorly understood, and current preventions and treatments have substantial limitations.

**Objectives:** To characterize the mechanisms of increased susceptibility to influenza virus infection in COPD and the potential for therapeutic targeting.

**Methods:** We used a combination of primary bronchial epithelial cells (pBECs) from COPD and healthy control subjects, a mouse model of cigarette smoke–induced experimental COPD, and influenza infection. The role of the phosphoinositide-3-kinase (PI3K) pathway was characterized using molecular methods, and its potential for targeting assessed using inhibitors.

**Measurements and Main Results:** COPD pBECs were susceptible to increased viral entry and replication. Infected mice with experimental COPD also had more severe infection (increased viral titer and pulmonary inflammation, and compromised lung function). These processes were associated with impaired antiviral immunity, reduced retinoic acid–inducible gene-1, and IFN/cytokine and chemokine responses. Increased PI3K-p110α levels and activity in COPD pBECs and/or mice were responsible for increased infection and reduced antiviral responses. Global PI3K, specific therapeutic p110α inhibitors, or exogenous IFN-β restored protective antiviral responses, suppressed infection, and improved lung function.

**Conclusions:** The increased susceptibility of individuals with COPD to influenza likely results from impaired antiviral responses, which are mediated by increased PI3K-p110α activity. This pathway may be targeted therapeutically in COPD, or in healthy individuals, during seasonal or pandemic outbreaks to prevent and/or treat influenza.

**Keywords:** chronic obstructive pulmonary disease; influenza; innate immunity; PI3K

Chronic obstructive pulmonary disease (COPD) affects approximately 64–200 million people and is the third commonest cause of death globally (1). It is characterized by progressive airway inflammation, emphysema, and impaired lung function (2). The most significant risk factor is cigarette smoking (3).

Influenza frequently causes acute exacerbations of COPD, leading to increased mortality (4). The mechanisms underpinning the increased susceptibility...
production of type I (IFN-α/β) and III interferons (IFN-λs) (14). These IFNs induce the transcription of more than 300 IFN-stimulated genes, which inhibit viral protein synthesis (15). Infection also induces the production of other cytokines, tumor necrosis factor (TNF)−α, IFN-γ−induced protein (IP)−10, macrophage inflammatory protein (MIP)−1α, and chemokine IL−8 (KC in mice), which attract immune cells to the site of infection (16, 17).

Influenza viruses use host cell-signaling pathways during infection. The phosphoinositide-3-kinase (PI3K) pathway is critical for initial influenza virus entry (13). Inhibition of PI3K does not affect viral attachment to the surface but prevents entry into cells (13). Viral replication is also dependent on PI3K activation, and influenza virus produces nonstructural 1 protein, which further activates PI3K to promote replication (18). Nonstructural protein 1 also inhibits IFN responses by binding to host factors, such as TRIM25, promoting further replication (19, 20). Importantly hyperactivation of PI3K occurs in patients with COPD and we hypothesized that this may be a mechanism that promotes increased susceptibility to influenza. PI3K has three catalytic subunit isoforms (p110α, β, and γ); their roles in influenza infection in patients with COPD and healthy individuals are unknown.

Here, we investigated this putative mechanism using a combination of primary (p)BECs from subjects with COPD and healthy subjects, and a mouse model of cigarette smoke–induced experimental COPD, which has the hallmark features and is representative of the human disease (21–24). COPD pBECs supported increased influenza virus entry and replication and had deficient antiviral responses compared with healthy control subjects. We also observed increases in viral replication and reduced antiviral responses, with exacerbated inflammation and impaired lung function in smoke-exposed mice. We then demonstrated for the first time that increased infection in COPD results from exaggerated PI3K activity, and increased p110α levels. These effects could be reversed by global or specific inhibition of PI3K or the p110α using therapeutic agents. This study provides novel insights into the mechanisms underlying increased susceptibility to influenza virus infection in COPD. It also indicates new avenues for therapeutic intervention in these patients and in healthy people. Some of the results of these studies have been previously reported in abstract form (25, 26).

Methods

Study Approvals

All procedures were performed according to approval from the University of Newcastle Human/Animal Ethics Committees.

In Vitro

Subject recruitment, viruses, cell culture, viral infection, inhibitor treatments, flow cytometry, quantitative real-time polymerase chain reaction, immunoblotting, and ELISA. Patients with COPD (13) and healthy nonsmoking (13) and smoking (5) control subjects were recruited (Table 1). COPD was defined, influenza viruses were used, human pBECs were obtained and cultured, and analyses performed as previously described and/or as in the online supplement (15, 20, 27–30).

In Vivo

Experimental COPD, influenza infection, PI3K inhibition, pulmonary inflammation, emphysema-like alveolar enlargement, lung function, immunoblotting, and ELISA. Experimental COPD and influenza infection were induced, PI3K inhibited, numbers of inflammatory cells enumerated in bronchoalveolar lavage fluid, and alveolar diameter, lung function (forced oscillation and forced maneuver techniques), and other methods performed as previously described and/or as in online supplement (17, 21, 23, 31, 32).

Statistical Analyses

Data are expressed as mean ± SEM when normally distributed. Nonnormally distributed data were analyzed using nonparametric equivalents and summarized using the median and interquartile range. Comparisons between two groups were made using a two-tailed Mann-Whitney test. Multiple comparisons were made using one-way analysis of variance with Tukey post hoc test, or Kruskal-Wallis with Dunn post hoc test, where nonparametric analyses were appropriate. P less than 0.05 was considered significant.
Results

Influenza Virus Replication Is Increased in COPD and Is Associated with More Efficient Viral Entry but Is Independent of SA Residue Levels

We first assessed whether pBECs from 13 nonsmoking patients with moderate to severe COPD supported increased influenza virus replication, compared with those from healthy control and healthy smoker subjects. pBECs were infected with two human influenza virus subtypes (H3N2 and H1N1) and a low pathogenic avian strain (H1N9). Infection with both human subtypes, but not the avian strain, resulted in significantly higher viral titers in COPD pBECs compared with both healthy control subjects and smokers (Figure 1A).

To determine if exaggerated viral replication in COPD resulted from increased SAα2,6Gal and SAα2,3Gal residue expression, we examined their levels on uninfected pBECs. The levels of SAα2,6Gal were significantly higher than SAα2,3Gal on all pBECs (Figure 1B), but there were no differences between subjects with COPD and healthy control subjects. To assess if increased viral entry occurred in COPD pBECs, influenza virus HA protein levels inside the cells were assessed 2 hours after infection. This is a validated method of assessing influenza virus internalization (20). HA levels for all three viruses were significantly higher in COPD compared with both healthy and smoker control subjects (Figure 1C; see Figure E1 in the online supplement), indicating that increased viral entry was independent of the relative abundance of SA residues.

We then confirmed these results in vivo using our mouse model of experimental COPD (21–24). BALB/c mice were exposed to cigarette smoke for 8 weeks until the hallmark features of COPD developed, and then infected with the A/PR/8/34 H1N1 strain (Smk+VIR). Viral load was assessed at 3, 7, and 10 days postinfection (dpi) (Figure 1D). Infected smoke-exposed mice had significantly increased (twofold) viral titers at 7 dpi compared with infected normal air-exposed control subjects (Figure 1E). Infection resulted in significant weight loss in all groups (see Figure E2A). In both in vitro and in vivo experiments, ultraviolet inactivated virus did not have any significant effects compared with media control subjects (data not shown).

Influenza Virus Infection in Experimental COPD Increases Pulmonary Inflammation and Impairs Lung Function

Patients with COPD with influenza virus infection have increased inflammation and reduced lung function (33). Thus, we assessed pulmonary inflammation in vivo by quantifying the number of inflammatory leukocytes in bronchoalveolar lavage fluid. Infection of smoke-exposed mice resulted in increased inflammation and numbers of total leukocytes, macrophages, and lymphocytes at 3, 7, and 10 dpi, compared with infected air-exposed control subjects (Figure 2A). The exception was neutrophil numbers, which were elevated compared with uninfected smoke-exposed control subjects, but were reduced compared with infected air-exposed control subjects. Infection had no effect on emphysema-like alveolar enlargement (see Figures E2B and E2C). Infection of smoke-exposed mice significantly increased transpulmonary resistance and total lung capacity at 7 dpi (Figure 2B) but not 3 dpi (see Figure E2D). Our data indicate that inflammatory responses are exaggerated and lung function impaired during influenza infection in experimental COPD.

Antiviral Responses to Influenza Virus Infection Are Impaired in COPD

RIG-I and IFN-β/IFN-λ1 are crucial in protecting against influenza virus infection (14). We examined the levels of these proteins produced by pBECs following infection. All three were induced 24 hours after influenza infection in healthy control subjects and smokers but not COPD pBECs (Figure 3A; see Figure E3A). In vivo infected air-exposed but not smoke-exposed mice had increased levels of RIG-I, IFN-β, and IFN-λ3 (mouse equivalent of human IFN-λ1) compared with uninfected control subjects at 7 dpi (Figure 3B; see Figures E3B and E3C). Only IFN-β was significantly altered at 3 dpi (see Figure E3C).

Other cytokines (IFN-γ and TNF-α) and chemokines (IP-10, MIP-1α, and IL-8/KC) are important in protective antiviral responses (16, 34, 35). IL-10 is an antiinflammatory cytokine important in controlling inflammation and IL-6 is a marker of global, nonspecific inflammation (35, 36). Infected air-exposed mice had increased levels of IFN-γ, TNF-α, IP-10, KC, and IL-10 but not IL-6 compared with uninfected control subjects (see Figure E3D). In contrast, infected smoke-exposed mice had decreased IFN-γ, TNF-α, IP-10, KC, and IL-10, but increased...
IL-6 compared with infected air-exposed control subjects (see Figure E3D).

**PI3K Activity Is Exaggerated in COPD pBECs and Experimental COPD**

Influenza virus entry is dependent on PI3K activity in BECs (13, 19). To investigate this dependency in COPD, we examined the level of PI3K activation by measuring phosphorylated-Akt (pAkt-Ser473) levels 2 hours after infection. pAkt levels were significantly elevated in COPD pBECs following infection compared with healthy control subjects and smoker pBECs (Figure 4A; see Figure E4A). Unphosphorylated-Akt (protein and mRNA) was also significantly increased following infection (Figure 4B; see Figures E4A and E4B).

**In vivo** we showed that pAkt levels in lung tissue were increased but only after 6 weeks of smoke exposure when the first features of experimental COPD develop (data not shown) (21). These results indicate that increased PI3K activity and

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Figure 1. Influenza virus infection is more severe in chronic obstructive pulmonary disease (COPD) primary bronchoepithelial cells (pBECs) and mice as a result of increased viral entry, which is not dependent on differences in sialic acid (SA) residue expression. (A) pBECs from healthy control subjects, patients with COPD, and healthy smokers were infected with human H3N2 and H1N1 and avian H11N9 influenza viruses, and replication was assessed. (B) SAa2.6Gal and SAa2.3Gal residue expression on the surface of healthy, COPD, and smoker pBECs 2 hours after infection. (C) Levels of influenza virus hemagglutinin (HA) protein in pBECs 2 hours after infection. (D) BALB/c mice were exposed to cigarette smoke or normal air for 8 weeks; inoculated with H1N1 (A/PR/8/34) influenza virus (8 plaque-forming units [PFU]; Smk+VIR, VIR) or media (Smk, control) on the last day of smoke exposure; and killed at 3, 7, and 10 days postinfection (dpi). (E) Viral titers in bronchoalveolar lavage fluid of mice. For *in vitro* studies (A–C), densitometry results were calculated as HA/glyceraldehyde phosphate dehydrogenase ratio; data are mean ± SEM, n = 13 (healthy control subjects and patients with COPD) or n = 5 (healthy smokers). For A, *P < 0.05 versus the 24-hour time point, *P < 0.05 versus healthy control subjects and smokers. For B, *P < 0.05 versus SAa2.3Gal. For C, *P < 0.05 versus healthy control subjects and smokers. For *in vivo* studies (E), data are mean ± SEM, n = 6–8, *P < 0.05 versus 3 dpi, *P < 0.05 versus VIR control subjects.
influenza virus entry and infection is specific to COPD and not smoke exposure.

Inhibition of PI3K in pBECs Enhances Antiviral Responses

To confirm the importance of PI3K in viral entry, PI3K was inhibited in pBECs with the pan-PI3K inhibitor, wortmannin. Inhibition of PI3K decreased pAkt and influenza virus HA 2 hours after infection in healthy and COPD pBECs (Figure 4B; see Figure E4C). We assessed the effects of inhibiting PI3K on antiviral responses in vitro. Infection increased RIG-1 and IFN-β protein levels in healthy and COPD pBECs (Figure 4C; see Figure E4D). The increased production of these proteins was associated with significant decreases in viral replication in both groups (Figure 4D).

Influenza virus infection and administration of PI3K inhibitor wortmannin to pBECs had minimal effects on cell viability (see Figure E5A).

PI3K Activity Is Increased in Experimental COPD, and Its Inhibition Enhances Antiviral Responses, Suppresses Infection, and Improves Lung Function

We examined whether elevated PI3K activity occurs in vivo in smoke-exposed...
mice and if inhibition of PI3K restores antiviral responses, decreases viral titer, and improves lung function. To assess the generalizability of our data another pan-PI3K inhibitor LY294002 was administered for the final 2 weeks of smoke exposure and during infection (Figure 5A). LY294002 reduced pAkt levels in infected air-exposed and smoke-exposed groups and enhanced IFN-β induction (Figure 5B; see Figure E6A). Only IFN-β was assessed at 7 dpi, and not RIG-I and IFN-λ1 because these were increased only at 3 and not 7 dpi (Figure 3B; see Figures E3B and E3C). PI3K inhibition suppressed viral replication and increased levels of leukocytes, neutrophils, antiviral cytokines (IFN-γ, IP-10, and MIP-1α), and IL-10; reduced IL-6; and improved lung function (Figures 5C–5E; see Figures E6B).

Our results show that the exaggerated PI3K activity in COPD resulted in increased influenza entry and reduced antiviral responses that worsened infection and associated inflammation. Global inhibition of PI3K suppressed infection, enhanced antiviral responses, and improved lung function in vivo.

**PI3K-p110α Isoform Is Increased in COPD pBECs**

Any of the three isoforms of the PI3K catalytic subunit (p110α, β, and γ) may increase susceptibility to infection in COPD. The identification of the involvement of a specific isoform would enable selective targeting and minimize off-target effects. To determine their involvement the levels of each p110 isoform was assessed. The protein and mRNA levels of p110α were significantly increased in COPD pBECs with and without infection (Figure 6A; see Figure E7A). The levels of other isoforms were not altered. The protein level of the PI3K regulatory subunit p85 was unchanged 2 hours after infection, and was not different between these pBEC groups (Figure 6A; see Figure E7A).

These data specifically implicate the PI3K-p110α isoform in increased susceptibility of patients with COPD to influenza virus infection.

**Specific Inhibition of PI3K-p110α Attenuates Influenza Virus Infection and Enhances Antiviral Responses in COPD and Healthy pBECs**

Pan-PI3K inhibitors (wortmannin and LY294002) inhibit the PI3K pathway and the downstream factor mammalian target of rapamycin (mTOR) (37, 38). However, at high concentrations they can also affect mitogen-activated protein kinase (MAPK) pathways including p38, extracellular

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**Figure 3.** Chronic obstructive pulmonary disease (COPD) is associated with reduced antiviral responses to influenza virus infection. (A) Primary bronchoepithelial cells from healthy control subjects, patients with COPD, and healthy smokers were infected with influenza viruses, and retinoic acid-inducible gene-I (RIG-I), IFN-β, and IFN-λ1 protein levels were assessed after 24 hours. BALB/c mice were exposed to cigarette smoke or normal air for 8 weeks and inoculated with A/PR/8/34 influenza virus (8 plaque-forming units; Smk+VIR, VIR) or media (Smk, Control) on the last day of smoke exposure (Figure 1D). (B) Antiviral responses (RIG-I, IFN-β) were assessed in lung homogenates, and (IFN-λ3) bronchoalveolar lavage fluid at 3 days postinfection. For in vitro studies (A), densitometry results were calculated as RIG-I/lysyl oxidase ratio fold induction from media-treated control subjects; results for IFN-β are expressed as fold induction from control subjects. IFN-λ3 results (assessed by ELISA) are presented as median and interquartile range, data are mean ± SEM, n = 13 (healthy control subjects and patients with COPD) or n = 5 (healthy smokers), *P < 0.05 versus media-treated control subjects; results for IFN-β are expressed as fold induction from control subjects. IFN-λ3 results (assessed by ELISA) are presented as median ± SEM, n = 6–8, **P < 0.05 versus Control, *P < 0.05 versus VIR control subjects.
signal-regulated kinases (Erk), and c-Jun N-terminal kinases (Jnk). The PI3K pathway interacts with Jnk in the MAPK pathway. The concentration of PI3K inhibitors used is known to specifically inhibit PI3K activity but not the MAPK pathways (37, 38). Nevertheless, to confirm the potential for selective inhibition of PI3K-p110α we specifically inhibited it during influenza virus infection of pBECs with small interfering RNA (siRNA). We compared the effects with treatment with siRNA against Akt. p110α siRNA reduced the level of pAkt to the same level as Akt siRNA (Figure 6B). Treatment with siRNA against PI3K-p110α or Akt reduced the protein levels of mTOR and Jnk, but not p38 or Erk that were induced in response to infection (see Figure E7C). Wortmannin did not affect p38 or Erk (data not shown). siRNA inhibition of PI3K-p110α or Akt reduced the protein levels of influenza HA. In contrast, inhibition of mTOR or MAPK (p38, Erk, and Jnk) pathways had no effect (see Figure E7D). These effects occurred with treatment of either healthy or COPD pBECs. Administration of siRNA did not affect the cell viability (see Figure E5B).

**Specific Inhibition of PI3K-p110α Attenuates Influenza Virus Infection and Enhances Antiviral Responses in COPD and Healthy pBECs**

We investigated if inhibition of PI3K-p110α using a therapeutic agent PI-103 suppresses influenza virus infection. We treated healthy and COPD pBECs with PI-103 3 hours before infection. Treatment significantly reduced the levels of pAkt and influenza virus HA (at 2 h) (Figure 6C; see Figure E8), and increased IFN-β (at 24 h) (Figure 6D; see Figure E8) in both healthy and COPD pBECs.

Collectively our results provide strong evidence that enhanced influenza virus entry into COPD pBECs is dependent on PI3K. Increased PI3K-p110α levels in COPD leads to exaggerated viral entry, and its specific targeting attenuates infection in both healthy and COPD pBECs, and does not affect alternate pathways.

**Increased PI3K Activity Is Associated with Impaired RIG-I Signaling in COPD pBECs**

We confirmed that exaggerated PI3K activity is associated with a defect in antiviral signaling in COPD pBECs. We assessed whether polyinosinic-polycytidylic acid (Poly I:C), a known agonist of RIG-I, does not affect alternate pathways.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Exaggerated phosphoinositide-3-kinase (PI3K) activity in chronic obstructive pulmonary disease (COPD) primary bronchoepithelial cells (pBECs) is responsible for increased influenza virus entry and reduced antiviral responses. pBECs from healthy control subjects, patients with COPD, and healthy smokers were infected with influenza viruses, and PI3K activation assessed by detection of the levels of phosphorylation of (A) phosphorylated Akt (pAkt) (S473) and unphosphorylated Akt. The effects of pan-inhibition of PI3K with wortmannin (100 nM) 2 hours after infection of pBECs from healthy subjects and subjects with COPD on (B) pAkt activation and influenza hemagglutinin (HA) levels, (C) retinoic acid-inducible gene-I (RIG-I) and IFN-β protein induction in pBECs, and (D) viral...
and innate IFNs (39), could enhance IFN-β responses in COPD pBECs. Poly IC treatment in healthy pBECs up-regulated RIG-I and IFN-β protein levels (see Figure E9A), whereas these responses were impaired in COPD pBECs.

**IFN-β-mediated Antiviral Pathways Are Functional in COPD pBECs**

To determine if the IFN-β-mediated signaling pathway was functional in COPD pBECs, pBECs were treated with exogenous recombinant IFN-β protein before infection. Treatment increased RIG-I and IFN-β production following infection compared with untreated cells (see Figure E9B). Viral replication was also significantly reduced in both healthy and COPD pBECs (see Figure E9C).

These results indicate that IFN-β– but not RIG-I–mediated antiviral pathways are partially functional in COPD. Furthermore, treatment with IFN-β may be an effective prophylactic or therapeutic strategy for influenza in COPD.

**Discussion**

Additional discussion points are in the online supplement.

Patients with COPD are more susceptible to influenza virus infection, which induces severe symptoms and declining lung function (33). The mechanisms leading to increased susceptibility in COPD are poorly understood. Current therapeutic strategies have limited efficacy, and new approaches are urgently required. To address this we used established in vitro and in vivo models of increased influenza virus infection in COPD pBECs and an experimental mouse model of COPD. We discovered novel PI3K-p110α–mediated mechanisms of increased viral entry and infection with deficient antiviral responses to influenza virus infection in COPD (Figure 7). We also identified potential new therapeutic

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**Figure 4.** (Continued). replication 24 hours after infection. Densitometry results were calculated as pAkt, Akt, HA, and RIG-I to glyceraldehyde phosphate dehydrogenase ratio and expressed as fold induction from media-treated control subjects. Data are mean ± SEM, n = 13 (healthy control subjects and patients with COPD) or n = 5 (healthy smokers). For A, *P < 0.05 versus media-treated control subjects, **P < 0.05 versus healthy control subjects. For B–D, *P < 0.05 versus media-treated nonwortmannin-treated control subjects, **P < 0.05 versus wortmannin treatment. PFU = plaque-forming units.

**Figure 5.** Exaggerated phosphoinositide-3-kinase activity in chronically cigarette smoke–exposed mice is responsible for reduced antiviral responses, increased influenza virus infection, and impaired neutrophil responses and lung function compared with control mice with influenza infection (VIR). (A) BALB/c mice were exposed to cigarette smoke or normal air for 8 weeks and infected with A/PR/8/34 influenza virus (8 plaque-forming units [PFU]; Smk+VIR, VIR) on the last day of smoke exposure. Mice were treated with LY294002 or vehicle three times per week for the last 2 weeks of smoke exposure and throughout the infection. Phosphoinositide-3-kinase activation, antiviral responses, and infection were assessed at 7 days postinfection (dpi). (B) Phosphorylated Akt (pAkt) (S473) and IFN-β protein levels in lung homogenates. (C) Viral titers and (D) numbers of inflammatory cells in bronchoalveolar wash fluid (BALF). (E) Transpulmonary resistance and total lung capacity. Densitometry results were calculated as pAkt/Akt, HA, and RIG-I to glyceraldehyde phosphate dehydrogenase ratio and expressed as fold induction from media-treated control subjects. Data are mean ± SEM, n = 6–8, *P < 0.05 versus VIR, **P < 0.05 versus vehicle-treated Smk+VIR control subjects.

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Figure 6. Phosphoinositide-3-kinase (PI3K) p110α isoform levels are increased in chronic obstructive pulmonary disease (COPD) primary bronchoepithelial cells (pBECs), and its inhibition attenuates influenza virus entry and enhances antiviral responses independently of the mitogen-activated protein kinase pathway. (A) Healthy control subjects, patients with COPD, and healthy smoker pBECs were infected, and PI3K-p110α, p110β, and p110δ catalytic isoforms and -p85 regulatory subunit were measured 2 hours after infection. (B) pBECs were treated with siRNA to PI3K-p110α or Akt 24 hours before infection, and PI3K-p110α and Akt protein levels were measured 2 hours after infection. (C) pBECs were treated with PI-103 PI3K-p110α inhibitor (8 nM) 3 hours before infection, and phosphorylated Akt (pAkt) and influenza virus hemagglutinin (HA) protein were assessed at 2 hours and (D) IFN-β induction 24 hours after infection. For A, densitometry results were calculated as PI3K-p110, pAkt, or HA/glyceraldehyde phosphate dehydrogenase ratio and expressed as fold change from untreated control subjects, data are mean SEM, n = 3, *P < 0.05 versus healthy control subjects. For B, #P < 0.05 versus media-treated non–siRNA-treated control subjects, *P < 0.05 versus siRNA treatment. For C and D, *P < 0.05 versus non–PI-103-treated control subjects. siRNA = small interfering RNA.
Figure 7. Schematic representation of the role of the phosphoinositide-3-kinase (PI3K) signaling pathway in initial influenza virus entry and subsequent replication. Influenza virus entry is dependent on PI3K-p110α and pAkt. Once the virus enters the cells, the PI3K pathway is used in the replication process and also negatively regulates type I IFNs, leading to increased viral replication. Inhibition of PI3K-p110α suppresses viral entry, enhances antiviral responses, and attenuates infection.

- COPD
  - Influenza virus
  - PI3K inhibitor
  - P
  - Akt
  - p110α-specific inhibitor

- Influenza viral entry
- Type I IFNs
- Influenza viral replication

**COPD**

- **Influenza virus**
- **p110α**
- **PI3K inhibitor**
- **P**
- **Akt**
- **p110α-specific inhibitor**

**Influenza viral entry**

- **Type I IFNs**

**Influenza viral replication**

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Original Article

Targets for influenza in subjects with COPD and healthy subjects.

**COPD pBECs** and mice with experimental COPD supported increased influenza virus entry and replication. Infection of mice with experimental COPD exacerbated pulmonary inflammation and impaired lung function. These events were accompanied by decreased neutrophil influx and antiviral responses. These effects resulted from exaggerated PI3K activity and could be inhibited by global PI3K inhibition. PI3K-p110α was highly up-regulated in COPD pBECs and its specific inhibition reduced viral entry and increased antiviral response without having off-target effects. Furthermore, the RIG-I pathway was defective in COPD pBECs but exogenous IFN-β pretreatment partially restored infection-induced IFN-β responses.

Influenza viruses are thought to bind to SA residues for the initial attachment to BECs. Similar SA residue levels were detected on both COPD and healthy pBECs, and therefore did not explain the differences in susceptibility. Instead PI3K activity and levels of the p110α isoform were increased in COPD pBECs. Inhibition studies showed that these increases directly resulted in elevated viral entry and replication. This agrees with another study that demonstrated accumulation of influenza virus particles on the cell surface but the failure of virus particles to internalize when PI3K was inhibited (13). Influenza NS1 protein has been shown to activate the PI3K pathway (18); however, the elevated PI3K and viral entry in COPD pBECs is independent of influenza nonstructural protein 1. Viral entry was assessed after 2 hours and the nonstructural protein 1 only appears 6 hours after infection (18). This indicates that PI3K activity was enhanced before infection, and allowed for more viral binding and entry in COPD pBECs. PI3K also interacts with the MAPK pathway, particularly Jnk (18). Inhibitors used in this study suppressed PI3K activity without affecting the MAPK pathway, the inhibition of which did not affect viral entry.

The mouse model of cigarette smoke–induced experimental COPD is induced by similar exposures (concentration and volume) to those experienced by human smokers as we have previously described (21, 22). Increased infection in smoke-exposed mice was associated with reduced neutrophils and neutrophil-attracting cytokines TNF-α, MIP-1α, and KC. Neutrophils inhibit influenza infection (40, 41), and their depletion increases inflammation (42). Thus, the reduction in neutrophils observed in our study may facilitate infection and lead to more severe inflammation.

Infection of smoke-exposed mice also impaired lung function by increasing transpulmonary resistance and total lung capacity. Patients with COPD also have increased lung resistance that is associated with narrowing of the small airways (43, 44). Increased total lung capacity indicates hyperinflation and reduced gas trapping in damaged alveoli (45).

Postviral entry immune responses are important in limiting viral replication. We found increased infection in COPD pBECs and mice and also results from deficient antiviral responses including RIG-I, IFN-β, IFN-α, TNF-α, MIP-1α, and KC (46, 47). This is consistent with another study that demonstrated reduced antiviral responses to rhinovirus in subjects with COPD (48). Although RIG-I is the primary influenza PRR (13, 14), other PRRs, such as melanoma differentiation–associated protein 5, could also be impaired in COPD.

These defective responses were likely caused by increased activity of PI3K-p110α. The regulatory role of PI3K signaling in type I IFN responses remains unclear. PI3K is essential in interferon regulatory factor-7 nuclear translocation in plasmacytoid dendritic cells and IFN-β responses (49). However, in other studies inhibition of PI3K leads to increased IFN-β and reduced viral titers (19, 50), which is consistent with our observations. The precise underlying mechanisms of increased PI3K activation in COPD, and how elevated PI3K activity down-regulates antiviral responses, remain unclear. However, we do show that altered PI3K-p110α and Akt also occurs at the mRNA level because their transcripts were elevated in infected COPD pBECs.

We found that the increased susceptibility to infection was COPD-specific and not caused by cigarette smoke exposure. Acute smoke exposure inhibits RIG-I, IFN-β, and JAK-STAT activation after influenza or respiratory syncytial virus infection (46, 51). In our study COPD pBECs were obtained from patients who were all abstinent from smoking for more than 12 months. The dysfunctional PI3K signaling and increased influenza infection was not observed in pBECs from healthy smokers.

COPD is associated with epigenetic alterations. Immune cells from patients with COPD have reduced expression and activity of histone deacetylase 2, which is involved in the suppression of inflammatory gene expression and steroid resistance (52–54). Histone deacetylase 2 protein levels and activity are reduced in the lung tissue, alveolar macrophages, and bronchial biopsies of patients with COPD, which is associated with increased inflammation (52). Because oxidative stress activates the PI3K pathway (55), it is possible that chronic exposure to cigarette smoke or oxidative stress could progressively increase PI3K activation, influenza virus entry, inflammation, and resistance to steroid treatment through the reduction in histone deacetylase 2. The PI3K-p110β isoform is up-regulated in leukocytes from patients with COPD, and contributes to steroid insensitivity (55, 56). It is, however, unclear whether the PI3K-p110α isoform also contributes to steroid resistance, and if this occurs in pBECs from patients with COPD.

We also showed that RIG-I–mediated signaling could not be induced by Poly I:C and was defective in COPD pBECs. Exogenous IFN-β, however, partially...
restored antiviral responses, suggesting that IFN-β-mediated signaling is partially functional in COPD pBECs.

In summary, we demonstrate that the increased susceptibility to influenza in COPD involves increased influenza virus entry and deficient antiviral responses that allow greater replication, which amplies inflammation and impairs lung function. These effects involve exaggerated PI3K-p110α activity that promotes viral entry and reduces antiviral responses. Inhibition of PI3K, and the p110α subunit, reduced viral entry regardless of influenza strain and subtype, enhanced antiviral responses, and restored lung function. Targeting PI3K pathways and IFN-β may be novel therapeutic strategies for influenza in patients with COPD and the general population, particularly for novel highly pathogenic seasonal and pandemic influenza viruses.

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