A role for phosphoinositol 3–kinase δ in the impairment of glucocorticoid responsiveness in patients with chronic obstructive pulmonary disease

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Background: Glucocorticoid function is markedly impaired in the lungs of patients with chronic obstructive pulmonary disease (COPD). This reduction in glucocorticoid sensitivity might be due to an oxidant-mediated increase in phosphoinositol 3–kinase (PI3K) δ signaling.

Objective: We sought to determine the role of PI3Kδ in the reduced glucocorticoid responsiveness in patients with COPD.

Methods: Peripheral lung tissue was obtained from 24 patients with COPD, 20 age-matched smokers with normal lung function, and 13 nonsmokers. Peripheral blood monocytes were isolated from 9 patients with COPD and 7 age-matched smokers with normal lung function and from healthy volunteers.

Results: The expressions of PI3Kδ and Akt phosphorylation were increased in macrophages from patients with COPD compared with those from control groups of age-matched smokers and nonsmokers. *In vitro* oxidative stress induced phosphorylation of Akt in monocytes and macrophages, which was abolished by means of selective inhibition of PI3Kδ but not PI3Kγ. Dexamethasone was less effective at repressing LPS-induced GM-CSF and CXC motif chemokine 8 release in blood monocytes from patients with COPD compared with age-matched smokers. This reduced sensitivity was reversed by inhibition of PI3Kδ but not PI3Kγ.

Conclusion: PI3Kδ expression and signaling is increased in the lungs of patients with COPD. Selective inhibition of PI3Kδ might restore glucocorticoid function in patients with COPD and might therefore present a potential therapeutic target. (J Allergy Clin Immunol 2010;125:1146-53.)

Key words: *Phosphoinositol 3–kinase, oxidative stress, Akt, macrophage, glucocorticoid insensitivity, chronic obstructive disease*

Glucocorticoids do not adequately suppress chronic inflammation in several diseases, including chronic obstructive pulmonary disease (COPD) and severe asthma.[1-3] The precise molecular mechanism or mechanisms of this reduction in glucocorticoid function remain unclear but are likely to involve oxidative stress. Oxidants, particularly those derived from both exogenous sources, such as cigarette smoke, and endogenous sources, including inflammatory cell respiratory burst, are a prominent component of the chronic inflammation seen in the lungs of patients with COPD.[4,5]

Oxidative stress impairs the activity of the glucocorticoid receptor (GR) corepressor histone deacetylase 2 (HDAC-2), which consequently reduces the ability of glucocorticoids to mediate transrepression of proinflammatory genes.[6-11] HDAC-2 expression and activity is also reduced in patients with COPD and is therefore likely to be important in the development of the reduced glucocorticoid responsiveness seen in patients with this disease.[12,13] Cigarette smoke exposure reduces HDAC activity and impairs glucocorticoid function in mice, which can be prevented by the abolition of phospholipid kinase phosphoinositol 3–kinase (PI3K) δ signaling, as demonstrated in transgenic mice expressing a kinase-dead PI3Kδ.[8] The PI3Kδ isoform might therefore play a role in the development of reduced glucocorticoid function under conditions of oxidative stress.

The PI3K family is involved in a plethora of cellular functions, including cell growth, motility, proliferation, and survival.[14] PI3Ks are activated by cell-surface receptors, such as receptor tyrosine kinases and G protein–coupled receptors, and serve to initiate intracellular signaling cascades by means of generation of the lipid secondary messenger phosphatidylinositol-3,4,5-triphosphate. Phosphatidylinositol-3,4,5-triphosphate serves as a docking site for the pleckstrin homology domain of proteins, such as the serine-threonine kinase Akt.[14] In addition to activation by growth factors, chemokines, and cytokines, PI3Ks might also be regulated by oxidative stress.[15]

The class I PI3K isoforms PI3Kγ and PI3Kδ are predominantly expressed in leukocytes and play a central role in inflammatory cell function, including respiratory burst and migration, as well as

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Abbreviations used
BAL: Bronchoalveolar lavage
COPD: Chronic obstructive pulmonary disease
CXCL-8: CXC motif chemokine 8
GR: Glucocorticoid receptor
HDAC: Histone deacetylase
PI3K: Phosphoinositol 3–kinase

in high-affinity IgE receptor (FcεRI) and chemokine receptor signaling.[16-20] Consequently, a number of studies have implicated both PI3Kγ and PI3Kδ as playing central roles in both innate and adaptive inflammatory responses, and therefore these have become attractive therapeutic targets.[21-23]

We examined the expression and functional role of PI3K in lung tissue and monocytes obtained from patients with COPD. We demonstrate that there is an increase in the expression of the PI3Kδ isoform in macrophages in the peripheral lungs of patients with COPD compared with that seen in healthy smokers and nonsmokers. Because we have previously shown that an oxidant-mediated reduction of glucocorticoid function is prevented by inhibition of PI3Kδ function in mice,[8] we examined the role of PI3Kδ underlying the glucocorticoid insensitivity of blood monocytes from patients with COPD by using a selective PI3Kδ inhibitor. We showed that selective inhibition of PI3Kδ but not PI3Kγ mediated a reversal of glucocorticoid insensitivity, indicating a potential role for PI3Kδ in glucocorticoid insensitivity in patients with COPD.

METHODS

Human study subjects

All subjects were recruited from the Section of Respiratory Medicine of the University Hospital of Ferrara, Italy. Peripheral lung tissue was collected from 24 patients with COPD, 20 smokers, and 13 nonsmokers (Table I). All subjects were undergoing elective surgery for lung cancer, and COPD was diagnosed retrospectively; these subjects were not taking bronchodilator, theophylline, antibiotic, antioxidant, and/or glucocorticoid therapy in the last month before surgery. Peripheral venous blood was collected from 9 patients with COPD and 7 smokers with normal lung function (Table II). Pulmonary function tests were performed as previously described.[24] COPD was defined according to international guidelines as the presence of a postbronchodilator FEV_1/forced vital capacity ratio of less than 70%.[25] Peripheral venous blood from healthy volunteers was also collected for *in vitro* studies. The study was approved by the local ethics committee of the University Hospital of Ferrara, and all patients provided written informed consent.

Lung tissue processing and immunohistochemistry

Lung tissue processing and immunohistochemistry were performed as previously described.[24] The total numbers of macrophages were counted in 20 nonconsecutive fields (magnification ×40). Macrophages were identified by means of morphologic staining. Normal nonspecific IgG from the animals in which the primary antibodies were raised was used for negative controls (Santa Cruz Biotechnology, Santa Cruz, Calif). All staining and cell counting was performed in a blind manner. The number of positively stained cells was expressed as a percentage of the total cells counted. The antibodies used were as follows: anti-PI3Kδ antibody (Santa Cruz Biotechnology) and anti–p-Akt antibody (Epitomics, Burlingame, Calif).

Monocyte isolation and treatments

PBMCs were isolated from whole venous blood by using Histopaque (Sigma, Dorset, United Kingdom), according to the manufacturer's instructions. Monocytes were isolated by means of selective adhesion, as previously described.[26] Cell culture and all treatments were performed with RPMI 1640 GlutaMAX phenol red free media (Invitrogen, Paisley, United Kingdom) with 1% FCS. Monocytes were treated with or without inhibitors for 45 minutes and then stimulated with or without either LPS (3 ng/mL) or hydrogen peroxide (H_2O_2; 100 μmol/L) for 1 hour. Reagents used were as follows: LPS (Sigma), dexamethasone (Sigma), LY294002 (Merck Biosciences, Nottingham, United Kingdom), IC87114 (Caltag Medsystems Ltd, Buckingham, United Kingdom), and AS604850 (Merck Biosciences).

Alveolar macrophage isolation and treatment

Bronchoalveolar lavage (BAL) was performed on patients with normal lung function, as previously described.[27] Macrophage purity was 94.8% ± 5.3%, as assessed with QuickDiff (Harleco, Gibbstown, NJ) staining of cytospin preparations. Macrophages were collected from the BAL fluid by means of selective adhesion in 1640 GlutaMAX phenol red free RPMI media (Invitrogen) with 10% FCS. Treatments were performed in RPMI media supplemented with 1% FCS. Cell culture and treatments were performed as for monocytes described above.

Protein extraction and immunoblotting

Proteins were extracted with an RIPA Buffer (50 mmol/L Tris HCl [pH 8.0], 150 mmol/L NaCl, 0.25% deoxycholate, 2 mmol/L EDTA, 0.1% SDS, 0.5% NP40, phosphatase inhibitors, and protease inhibitors). Protein quantification was assessed by using the BCA assay (Perbio, Northumberland, United Kingdom). Immunoblotting was performed as previously described.[9] All blots were stripped and reprobed for loading controls, as previously described, with Chemicon stripping buffer per the manufacturer's instructions (Millipore, Watford, Herts, United Kingdom). Antibodies used were as follows: Akt, p-Akt, (Cell Signaling Technology, Herts, United Kingdom), and PI3Kδ (Abcam, Cambridge, United Kingdom).

ELISA

Monocytes were treated with or without inhibitors for 45 minutes, with or without dexamethasone for 10 minutes, and then with or without LPS (3 ng/mL) for 16 hours. CXC motif chemokine 8 (CXCL-8) and GM-CSF ELISAs were performed by using DuoSet kits (R&D Systems, Minneapolis, Minn), according to the manufacturer's instructions.

Statistical analysis

Data were analyzed by means of 1-way ANOVA to determine statistically significant variance between the groups for each end point assessed. Statistical significance between groups was then calculated by using the nonparametric Mann-Whitney *t* test with GraphPad Prism software (GraphPad Software, Inc, La Jolla, Calif). Data are expressed as means ± SEMs. Differences were considered significant at a P value of less than .05.

RESULTS

PI3Kδ expression and phosphorylation of Akt (ser473) are increased in macrophages in the lungs of patients with COPD

Lung macrophages from patients with COPD are less responsive to glucocorticoid suppression of proinflammatory genes.[12] Mice exposed to oxidative stress, such as cigarette smoke, are also relatively glucocorticoid insensitive, an effect that is prevented by the selective abolition of PI3Kδ, but not PI3Kγ, signaling.[8] An increase in PI3Kδ activation might therefore represent a mechanism of glucocorticoid insensitivity in patients with COPD. PI3Kδ staining in macrophages in the peripheral lungs of patients with COPD was increased compared with that seen in smokers

TABLE I. Characteristics of subjects for peripheral lung sections

Group	Age (y)	Sex	Smoking status	Pack-years	FEV_1 (L)	FEV_1 (% predicted)	FEV_1/FVC ratio (%)	GOLD stage	Chronic bronchitis	Emphysema (NETT score)
COPD	68.9 ± 6.9	20 M, 4 F	10 current, 14 former	38.7 ± 15.1	2.1 ± 0.5	74.7 ± 16.7	57.2 ± 9.5	stage 1 = 9 stage 2 = 12 stage 3 = 3	11 yes, 13 no	score 0 = 13 score 1 = 6 score 2 = 3 score 3 = 2
Smoker	70.4 ± 6.7	19 M, 1F	9 current, 11 former	49 ± 31.5	2.5 ± 0.7	92.3 ± 14.4	75.5 ± 4.5	NA	9 yes, 11 no	score 0 = 11 score 1 = 3 score 2 = 4 score 3 = 2
Nonsmoker	67.8 ± 8.1	1 M, 12 F	NA	NA	2.1 ± 0.5	101.5 ± 22.6	76.4 ± 3.5	NA	None	None

Data are presented as means ± SDs. The FEV_1/FVC ratio is after bronchodilator for subjects with COPD but not smokers or nonsmokers.
GOLD, Global Initiative for Chronic Obstructive Lung Disease guideline classification of patients with COPD; *F*, female; *FVC*, forced vital capacity; *M*, male; *NA*, not applicable; *NETT*, National Emphysema Treatment Trial.

TABLE II. Characteristics of subjects from the glucocorticoid functional study

Group	Age (y)	Sex	Smoking status	Pack-years	FEV_1 (L)	FEV_1 (% predicted)	FEV_1/FVC ratio (%)	GOLD stage
COPD	76.1 ± 3.1	8 M, 1 F	2 current, 7 former	49.9 ± 29.7	1.4 ± 0.5	52.7 ± 13.4	52.7 ± 7.6	stage 1 = 0 stage 2 = 5 stage 3 = 4
Smoker	58 ± 6.5	6 M, 1 F	5 current, 2 former	33.9 ± 9.9	3.0 ± 0.6	101.4 ± x11.4	78.6 ± 5.0	NA

Data are depicted as means ± SDs. The FEV_1/FVC ratio is after bronchodilator for patients with COPD but not smokers or nonsmokers.
GOLD, Global Initiative for Chronic Obstructive Lung Disease guideline classification of patients with COPD; *F*, female; *FVC*, forced vital capacity; *M*, male; *NA*, not applicable.

with normal lung function ($P = .0032$) and nonsmokers ($P = .0007$, Fig 1). However, the expression of PI3Kδ in macrophages from the peripheral lungs of smokers with normal lung function was not increased compared with that seen in those from nonsmokers with normal lung function ($P = .105$).

Phosphorylation of Akt at serine 473 (p-Akt-ser473), a marker of Akt activation, was increased in peripheral lung macrophages in patients with COPD compared with that seen in smokers with normal lung function ($P = .0033$) and nonsmokers ($P < .0001$, Fig 2). p-Akt-ser473 staining in peripheral lung macrophages was also increased in smokers with normal lung function compared with that seen in nonsmokers ($P = .0022$).

Oxidative stress induced phosphorylation of Akt (ser473) in a PI3Kδ-dependent manner in blood monocytes and BAL macrophages

PI3Kδ and PI3Kγ are activated from distinct receptor types (receptor tyrosine kinases and G protein–coupled receptors, respectively). LPS (3 ng/mL)–induced phosphorylation of Akt has been shown to be dependent on PI3Kδ but not PI3Kγ signaling in monocytes.[28,29] Akt phosphorylation is also induced by oxidative stress; however, the role of PI3K and thereafter the individual isoform or isoforms remain unknown. Treatment of blood monocytes isolated from healthy volunteers with H_2O_2 (100-200 μmol/L) induced a concentration- and time-dependent increase of Akt (ser473) phosphorylation (Fig 3, *A* and *B*). Both LPS- and oxidant-induced phosphorylation of Akt was inhibited by the selective PI3Kδ inhibitor IC87114 (1 μmol/L) and by the pan-PI3K inhibitor LY294002 (10 μmol/L) but not by the selective PI3Kγ inhibitor AS604850 (1 μmol/L; Fig 3, *C* and *D*). Similarly, both LPS- and oxidant-induced Akt phosphorylation was reduced by both IC87114 and LY294002 but not by AS604850 in BAL macrophages (Fig 3, *E* and *F*).

Selective inhibition of PI3Kδ but not PI3Kγ restores glucocorticoid responsiveness in blood monocytes from patients with COPD

Glucocorticoid repression of proinflammatory mediator release from lung macrophages is impaired in patients with COPD.[12] Here we measured the degree of suppression of LPS-induced release of GM-CSF and CXCL-8 by dexamethasone in monocytes from patients with COPD. As a control group, monocytes isolated from smokers (Table II) who have been exposed in a similar fashion to cigarette smoke, the main causative factor in the development of COPD, but have normal lung function and do not show a similar relative reduction in glucocorticoid responsiveness as seen in patients with COPD were used. Compared with smokers, dexamethasone (10 nmol/L to 1 μmol/L) was less effective at suppressing both GM-CSF and CXCL-8 release from COPD patient monocytes (Fig 4). There was no significant change in the inhibitory concentration of 50% of dexamethasone in patients with COPD compared with that seen in smokers.

Selective inhibition of both PI3Kδ (1 μmol/L IC87114) and PI3Kγ (1 μmol/L AS604850) alone had no effect on basal or LPS-stimulated GM-CSF or IL-8 release (Fig 4, *A* and *D*). Inhibition of PI3Kγ had no effect on the impaired ability of dexamethasone to inhibit LPS-stimulated IL-8 and GM-CSF release in monocytes from patients with COPD (Fig 4, *C* and *F*). However, selective inhibition PI3Kδ restored the ability of dexamethasone (10 nmol/L to 1 μmol/L) to suppress the LPS-induced release of

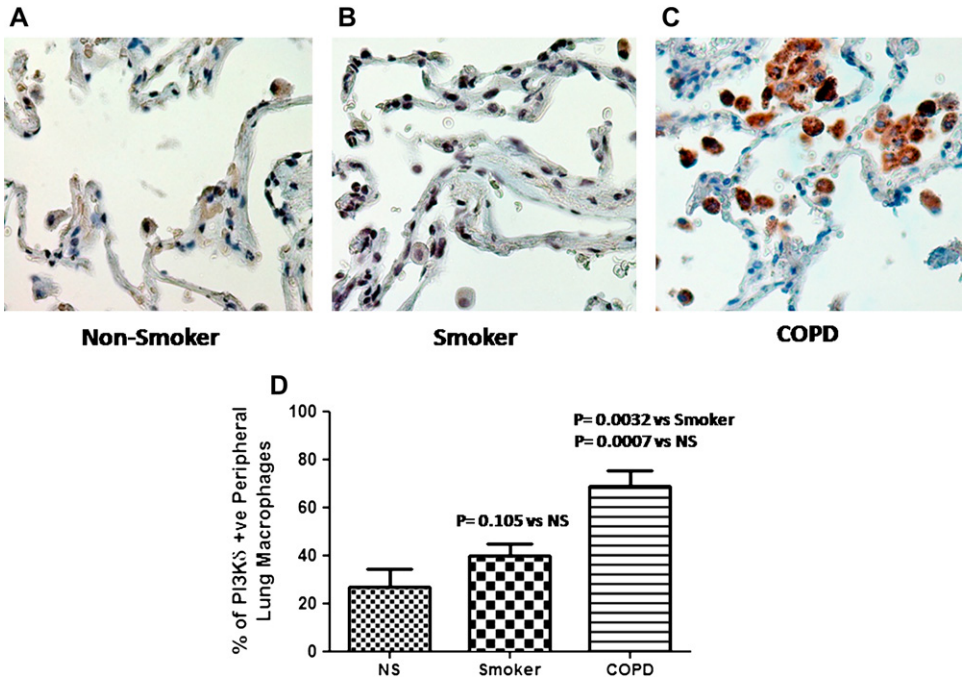

FIG 1. Expression of PI3Kδ in macrophages in the peripheral lungs of nonsmokers, smokers, and patients with COPD. Representative images (magnification ×40) of PI3Kδ staining in peripheral lung sections from nonsmokers **(A)**, smokers **(B)** and patients with COPD **(C)** are shown. **D**, Percentage of macrophages positively stained for PI3Kδ in the peripheral lung sections from nonsmokers (n = 11), smokers (n = 19), and patients with COPD (n = 17). Histograms represent means ± SEMs. *NS*, Nonsmoker.

GM-CSF and CXCL-8 to levels close to those observed in the control smokers (Fig 4, *B* and *E*). Addition of neither IC78114 nor AS604850 resulted in any effect on the inhibitory concentration of 50% of dexamethasone.

DISCUSSION

We demonstrated that PI3Kδ expression and Akt phosphorylation in peripheral lung macrophages from patients with COPD are increased compared with those from healthy smokers or control subjects. Furthermore, selective inhibition of PI3Kδ but not PI3Kγ restored glucocorticoid-mediated repression of proinflammatory mediator release from monocytes isolated from patients with COPD to levels seen in control subjects. These data are consistent with our recent demonstration that transgenic mice lacking an active PI3Kδ kinase isoform have functional glucocorticoid-mediated immunosuppression compared with that seen in PI3Kγ knock-out and wild-type mice when exposed to cigarette smoke.[8] Therefore activation of PI3Kδ and Akt phosphorylation might contribute to the impairment of glucocorticoid responsiveness seen in patients with COPD.

The failure of glucocorticoids to suppress the chronic inflammatory response in diseases such as COPD and severe asthma represents a growing unmet medical need and a major disease management problem. The exact mechanism or mechanisms of this impairment of glucocorticoid function remain unclear but are likely to involve an oxidant-mediated alteration in function, signaling, or both of several kinases and GR corepressors.[2,3,30] However, although the PI3Kγ and PI3Kδ isoforms are attractive targets for both innate and adaptive immunity, their potential role in the pathogenesis of COPD remains unclear.

Oxidants and, consequently, the redox status of the cell are key regulators of physiological cellular function and pathophysiological changes in disease.[4,30] The increase of the oxidant burden in the lungs of patients with COPD is an important component of chronic inflammation and might play an important role in the relative impairment of glucocorticoid function. Oxidants can alter PI3K signaling either indirectly though inactivation/activation of a receptor by altering phosphatase and tensin homolog (PTEN)-activity or directly by deactivating protein phosphatases.[15] Both PI3Kδ and PI3Kγ play key roles in the function of both the innate and adaptive immune responses; however, the effect of oxidants on the function of the individual PI3K isoforms remains unknown. Here oxidative stress induced a time- and concentration-dependent induction of Akt phosphorylation in peripheral blood monocytes. This oxidant-mediated induction of Akt phosphorylation was abolished in both monocytes and lung macrophages by selective inhibition of PI3Kδ but not PI3Kγ, indicating that an oxidant-mediated induction of PI3K signaling is mediated primarily through PI3Kδ. In mice genetic abolition of PI3Kδ but not PI3Kγ signaling protects against an oxidant-mediated reduction in glucocorticoid function.[8] The expression of GRα is also reduced in both the lungs of patients with COPD and in cigarette smoke–exposed mice.[8] However, this is unlikely to have a major role in the reduction of the glucocorticoid's anti-inflammatory actions because glucocorticoids still confer unwanted side effects in patients with COPD, which are likely mediated through GRα transactivation, suggesting that glucocorticoid-mediated GRα activation is still functional. Furthermore, glucocorticoid sensitivity is protected in mice that are devoid of PI3Kδ signaling without any increase in the expression of GRα.[8]

The role of PI3Kδ in the relative reduction of glucocorticoid responsiveness in patients with COPD is unknown. Here the

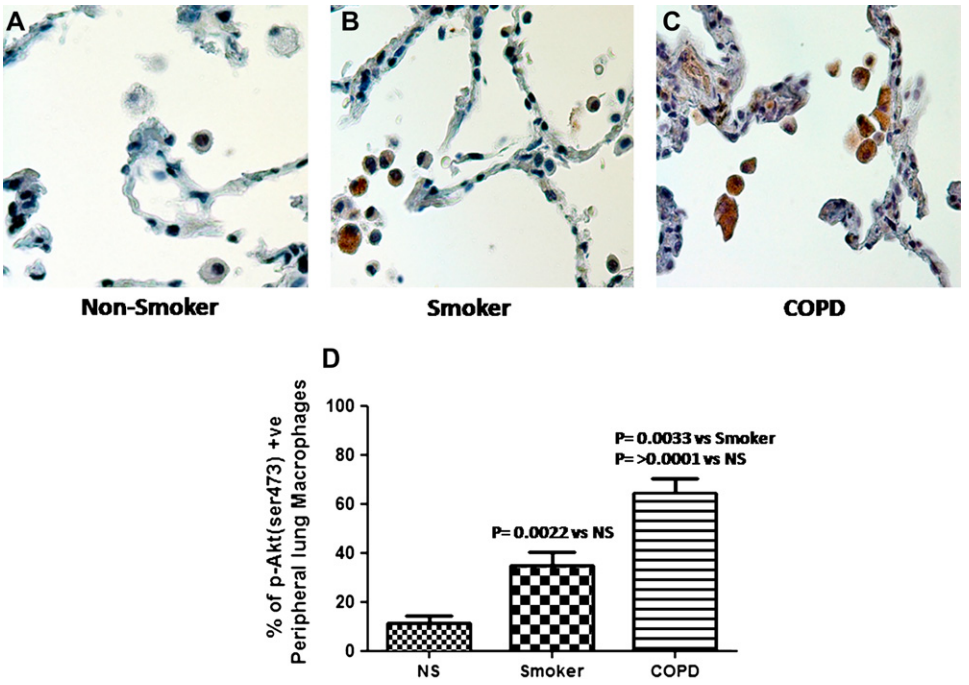

FIG 2. Expression of p-Akt in macrophages in the peripheral lungs of nonsmokers, smokers, and patients with COPD. Representative images (magnification ×40) of p-Akt (ser473) staining in peripheral lung sections from nonsmokers **(A)**, smokers **(B)**, and patients with COPD **(C)** are shown. **D**, Graphic depiction of the percentage of macrophages positively stained for p-Akt (ser473) in the peripheral lung sections from nonsmokers (n = 11), smokers (n = 19), and patients with COPD (n = 17). Histograms represent means ± SEMs. *NS*, Nonsmoker.

ability of dexamethasone to suppress proinflammatory mediator release was reduced in monocytes isolated from the peripheral blood of patients with COPD compared with that seen in age-matched smokers with normal lung function. At concentrations of less than 10 μmol/L, selective inhibition of both PI3Kδ and PI3Kγ had no significant effect on LPS-induced proinflammatory mediator release. However, consistent with the *in vitro* and *in vivo* models, selective inhibition of PI3Kδ but not PI3Kγ restored the ability of dexamethasone to repress proinflammatory mediator release to levels close to or comparable with the repression seen in age-matched smokers with normal lung function. The relative reduction in glucocorticoid responsiveness seen here in monocytes is also seen in alveolar macrophages from patients with COPD and can be restored by treatment with theophylline, a known inhibitor of PI3K. Indeed, both alveolar macrophages and monocytic cell lines are susceptible to oxidant-mediated glucocorticoid insensitivity, which is reversed by theophylline, suggesting a common mechanism.[10,12] Therefore an increase in PI3Kδ signaling might represent part of the mechanism by which glucocorticoid function is impaired in patients with COPD. Interestingly, an increase in PI3K/Akt signaling associated with oxidative phosphorylation has also been implicated in the development of glucocorticoid resistance in patients with acute lymphoblastic leukemia.[31] This might represent a mechanistic commonality of oxidant-driven glucocorticoid insensitivity between diseases.

Although blood monocytes represent a readily available source of primary inflammatory cells that also display apparent disease characteristics, it is also important to look in the lung at the site of disease. Alveolar macrophages are major inflammatory cells in COPD, playing an important role in both the chronic inflammation and tissue destruction.[1,32] Immunohistochemical staining of peripheral lung tissue from patients with COPD showed an increase in the expression of PI3Kδ in macrophages compared with that seen in control groups of age-matched smokers with normal lung function and nonsmokers. PI3Kδ is the main PI3K isoform responsible for phosphorylation of Akt in monocytes and macrophages, as both demonstrated here in primary human cells and also in primary murine cells.[29] Akt phosphorylation was also increased in macrophages in the peripheral lungs of patients with COPD compared with that seen in age-matched smokers with normal lung function and age-matched nonsmokers. This indicates that the observed increase in the expression of PI3Kδ in macrophages from patients with COPD translates into an increase in PI3Kδ signaling. Although Akt activation is transient when it is mediated by a single acute insult *in vitro*, the heightened, chronic inflammatory response and oxidant burden in patients with COPD are likely to facilitate an enhanced and chronic activation of the PI3Kδ/Akt pathway. Lipid peroxidation, which is able to generate a self-perpetuating cycle of reactive oxygen species, is also increased in patients with COPD and is likely to play an important role in the chronic upregulation of these and many other pathways.[4,33-35]

Interestingly, age-matched healthy smokers also displayed increased PI3Kδ signaling/Akt phosphorylation compared with that seen in nonsmokers, yet these age-matched healthy smokers do not show a relative reduction in responsiveness to glucocorticoids comparable with that which is seen in patients with COPD. Because oxidants can induce PI3Kδ-mediated Akt signaling, this might be related to an increased exposure to oxidants through cigarette smoke. Regulated activation of the PI3Kδ/Akt signaling

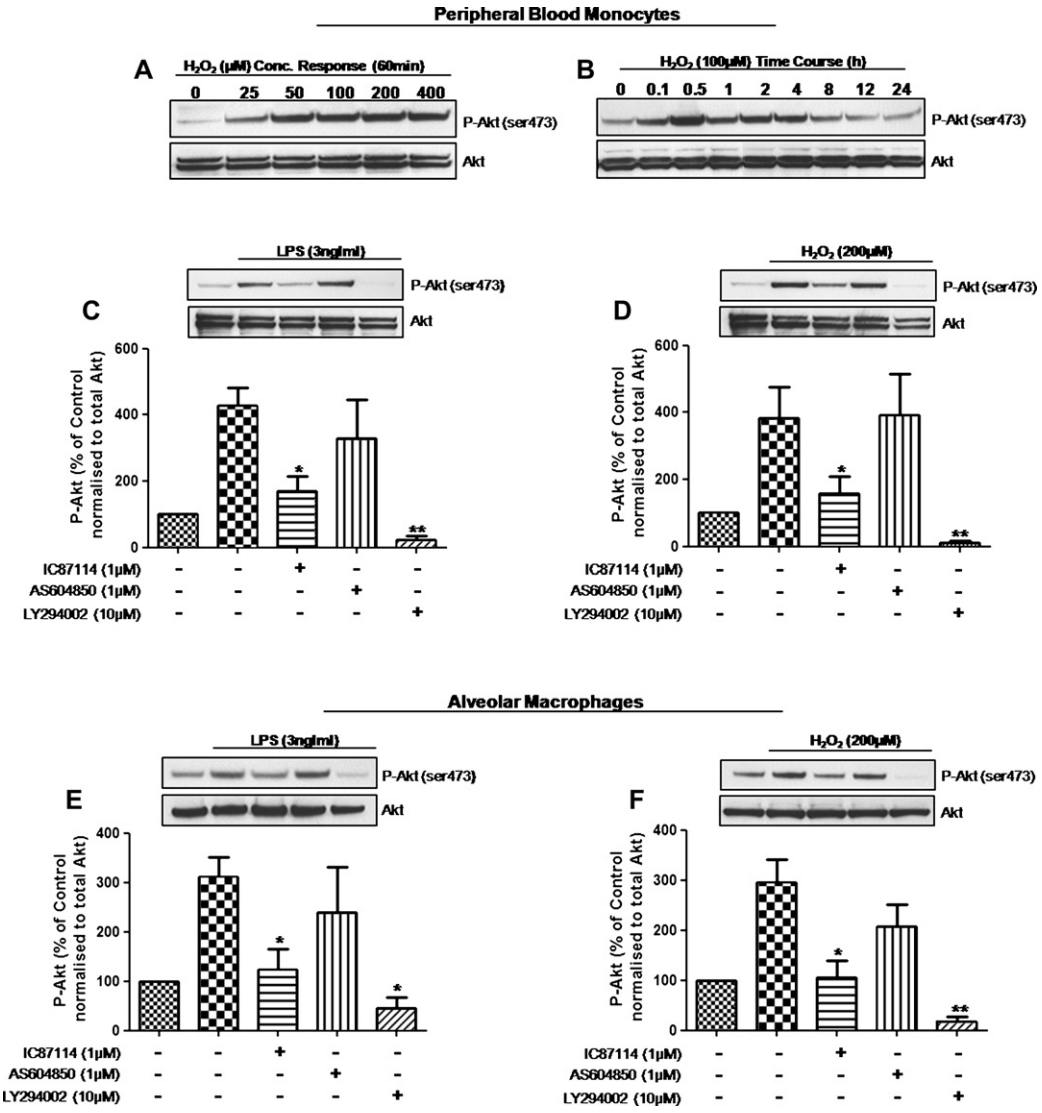

FIG 3. Effect of oxidative stress on Akt phosphorylation: role of PI3Kδ and PI3Kγ isoforms. H_2O_2-induced phosphorylation of Akt (ser374) in a concentration-dependent **(A)** and time-dependent **(B)** manner is shown. Immunoblots of the effect of selective inhibition of PI3Kδ, PI3Kγ, and pan-PI3K activity on either LPS-induced (**C** and **E**) or H_2O_2-induced (**D** and **F**) phosphorylation of Akt (ser374) in blood monocytes isolated from the venous blood of healthy volunteers (Fig 3, *C* and *D*) and alveolar macrophages isolated from the BAL fluid of volunteers with normal lung function (Fig 3, *E* and *F*) are also shown. Images are representative of 3 to 6. $*P < .05$ and $**P < .01$. *conc*, Concentration.

pathway is integral in the orchestration of leukocyte functions for both the innate and adaptive immune responses.[14,18-20] Many of these responses are sensitive to glucocorticoids, and therefore regulated activation of the PI3Kδ/Akt pathway is unlikely to impair glucocorticoid function. However, the chronic and increased nature of the oxidant burden and inflammatory response in the lungs of patients with COPD might not only activate the PI3Kδ/Akt pathway but might also result in the increase of PI3Kδ expression. This in turn might allow for a hyperactivation of the PI3Kδ pathway beyond that seen in smokers with normal lung function. Cigarette smoke reduces glucocorticoid responsiveness through covalent modification and inactivation of HDAC-2 activity, which is fundamental for GRα function and therefore glucocorticoid-mediated immunosuppression.[6-9,11] HDAC-2 expression and activity is reduced in patients with COPD and correlates with disease severity.[13] Hyperphosphorylation of HDAC-2 impairs its function, and we have shown that PI3Kδ signaling is, in part, responsible for the hyperphosphorylation and inactivation of HDAC-2 and consequent reduction in glucocorticoid responsiveness.[8,36] The significant increase in PI3Kδ/Akt signaling seen in patients with COPD is therefore likely to reflect the enhancement of the oxidant burden and overwhelmed extracellular and intracellular antioxidant defenses in the lungs of patients with COPD.[4,5] Macrophages isolated from the lungs of patients with COPD display a relative unresponsiveness to glucocorticoid immunosuppression, which might account, at least in part, for the failure of glucocorticoids to significantly repress the chronic inflammatory response in the lungs of patients with COPD.[12] This chronic enhancement of PI3Kδ/Akt signaling in lung macrophages from patients with COPD might therefore contribute to the

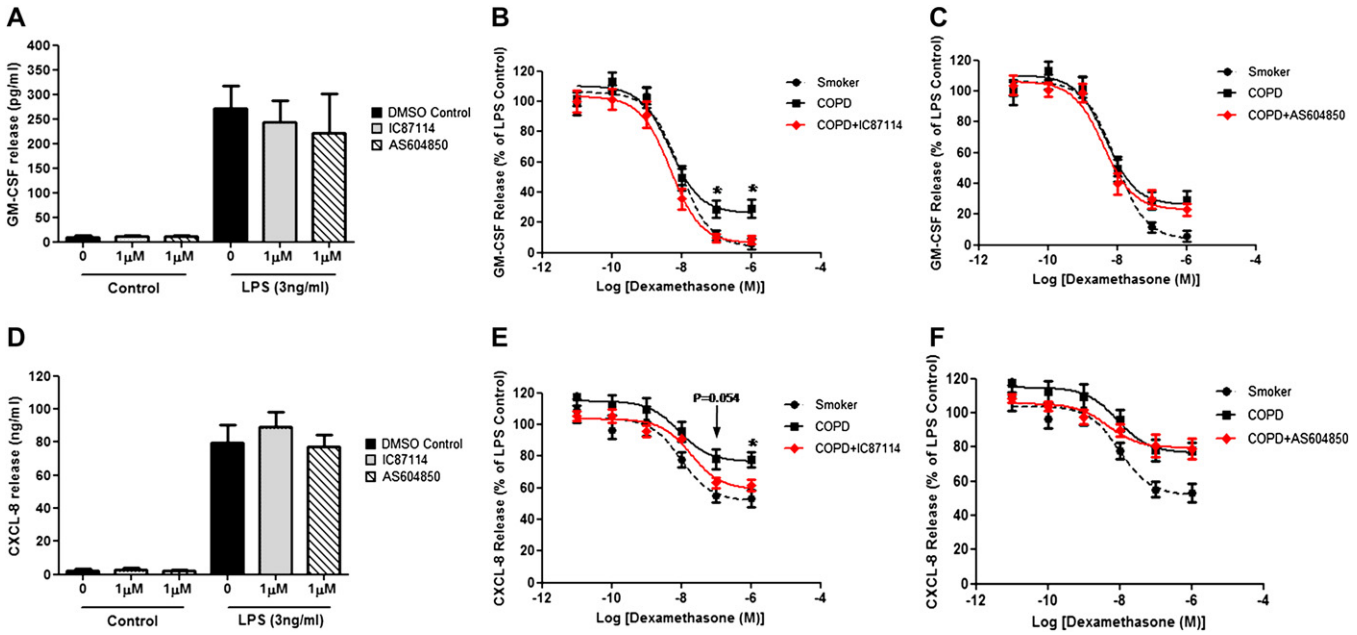

FIG 4. Selective inhibition of PI3Kδ restores glucocorticoid responsiveness in monocytes from patients with COPD. **A** and **D**, Effect of IC87114 and AS604850 on LPS-induced GM-CSF release and CXCL-8 release. Functional repression of LPS stimulated GM-CSF (**B** and **C**) and IL-8 (**E** and **F**) release by dexamethasone in patients with COPD (*squares*, n = 9) compared with that seen in smokers with normal lung function (*circles*, n = 7). Effect of IC87114 (Fig 4, *B* and *E*) and AS604850 (Fig 4, *C* and *F*) on dexamethasone-mediated repression of LPS-induced GM-CSF and CXCL-8 release in patients with COPD *(diamonds)* close to that seen in smokers with normal lung function. **P* < .05.

reduced activity and expression of HDAC-2, resulting in a reduction in the responsiveness to glucocorticoids. However, because of the complexity of the disease, it is likely that a number of pathways are involved. Therefore a global analysis of kinase expression and signaling relating to oxidant-mediated alterations and significant differences between patients with COPD and control smokers might be required to ascertain the potential pathways involved.

Taken together, these data show that PI3Kδ expression and signaling are increased in lung macrophages from patients with COPD, which might be responsible, in part, for the impairment of glucocorticoid function in this disease. The increased oxidant burden in the lungs of patients with COPD is also likely to play a role in this increase of PI3Kδ signaling and consequently thereafter the reduction in glucocorticoid function. An increased oxidant burden is also associated with a number of other relatively glucocorticoid-insensitive diseases, including severe asthma and cystic fibrosis. Similarly to COPD, these diseases lack effective alternative anti-inflammatory therapies. Further investigation into the possible mechanism or mechanisms of relative glucocorticoid unresponsiveness in these diseases might reveal common mechanisms, such as a chronic hyperactivation of the PI3Kδ/Akt signaling pathway, which would allow the development of novel anti-inflammatory, glucocorticoid-restorative, or both therapeutic strategies across these diseases. Selective inhibition of PI3Kδ might therefore provide a therapeutic strategy for the restoration of glucocorticoid function in patients with COPD, as indicated here, and perhaps in other relatively glucocorticoid-unresponsive conditions, such as severe asthma and cystic fibrosis, in which oxidative stress is a prominent component.

Key messages

- **PI3Kδ expression and signaling are increased in macrophages from the lungs of patients with COPD.**
- **Oxidative stress selectively signals though the PI3Kδ isoform.**
- **Selective inhibition of PI3Kδ restores glucocorticoid sensitivity in monocytes from patients with COPD.**

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