A role for PI3K δ in the impairment of glucocorticoid responsiveness in COPD

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Abstract

Background: Glucocorticoid function is markedly impaired in the lungs of patients with chronic obstructive pulmonary disease. This reduction in glucocorticoid sensitivity may be due to an oxidant-mediated elevation in phosphoinositol 3-kinase δ signaling.

Objective: To determine the role of phosphoinositol 3-kinase δ in the reduced glucocorticoid responsiveness in chronic obstructive pulmonary disease.

Methods: Peripheral lung was obtained from 24 patients with chronic obstructive pulmonary disease, 20 age-matched smokers with normal lung function and 13 non-smokers. Peripheral blood monocytes were isolated from 9 chronic obstructive pulmonary disease patients and 7 age-matched smokers with normal lung function and from healthy volunteers.

Results: The expression of phosphoinositol 3-kinase δ and Akt phosphorylation were elevated in macrophages from patients with chronic obstructive pulmonary disease patients as compared to control groups of age-matched smokers and non-smokers. *In vitro*, oxidative stress induced phosphorylation of Akt in monocytes and macrophages which was abolished in by selective inhibition of phosphoinositol 3-kinase δ , but not phosphoinositol 3-kinase γ . Dexamethasone was less effective at repressing lipopolysaccharide-induced granulocyte-macrophage colony stimulating factor and CXC motif chemokine 8 release in blood monocytes from chronic obstructive pulmonary disease patients as compared to age-matched smokers. This reduced sensitivity was reversed by inhibition of phosphoinositol 3-kinase δ but not phosphoinositol 3-kinase γ .

Conclusion: Phosphoinositol 3-kinase δ expression and signaling is increased in the lungs of chronic obstructive pulmonary disease patients. Selective inhibition of phosphoinositol 3-kinase δ may restore glucocorticoid function in chronic obstructive pulmonary disease patients and may therefore present a potential therapeutic target.

Key Messages:

- Phosphoinositol 3-kinase δ expression and signaling is elevated in macrophages from the lungs of patients with chronic obstructive pulmonary disease.
- Oxidative stress selectively signals through the phosphoinositol 3-kinase δ isoform.
- Selective inhibition of phosphoinositol 3-kinase δ restores glucocorticoid sensitivity in monocytes from patients with chronic obstructive pulmonary disease.

Capsule Summary:

This study shows that selective inhibition of phosphoinositol 3-kinase δ may be a potential therapeutic strategy to restore glucocorticoid responsiveness in patients with chronic obstructive pulmonary disease.

Key words: phosphoinositol 3-kinase, oxidative stress, Akt, macrophage, glucocorticoid insensitivity, chronic obstructive disease

Abbreviations: PI3K: phosphoinositol 3-kinase; GC: glucocorticoid; COPD: chronic obstructive pulmonary disease; GM-CSF: granulocyte and macrophage colony stimulating factor; CXCL-8: CXC motif chemokine 8.

Introduction

Glucocorticoids fail to adequately suppress chronic inflammation in several diseases including chronic obstructive pulmonary disease (COPD) and severe asthma [1-3]. The precise molecular mechanism(s) of this reduction in glucocorticoid function remain unclear but is 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 in the lungs of COPD patients [4;5].

Oxidative stress impairs the activity of the glucocorticoid receptor (GR) co-repressor histone deacetylase-2 (HDAC-2) which consequently reduces the ability of glucocorticoids to mediate transrepression of pro-inflammatory genes [6-11]. HDAC-2 expression and activity is also reduced in COPD and is therefore likely to be important in the development of the reduced glucocorticoid responsiveness seen in 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 phosphatidyl 3-kinase (PI3K) δ signaling, as demonstrated in transgenic mice expressing a kinase dead PI3K δ [8]. The PI3K δ isoform may 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 (RTKs) and G-protein coupled receptors (GPCRs) and serve to initiate intracellular signaling cascades by generation of the lipid secondary messenger phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P₃). PtdIns(3,4,5)P₃ serves as a docking site for the pleckstrin homology (PH)- domain of proteins such as the serine-threonine kinase

Akt [14]. In addition to growth factors, chemokines and cytokines, PI3Ks may also be regulated by oxidative stress [15].

The class I PI3K isoforms PI3K γ and δ are predominantly expressed in leucocytes and play a central role in inflammatory cell function including respiratory burst and migration as well as in high affinity IgE receptor (Fc ϵ RI) and chemokine receptor signaling [16-20]. Consequently, a number of studies have implicated both PI3K γ and δ 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 COPD patients. We demonstrate that there is an increase in the expression of the PI3K δ isoform in macrophages in the peripheral lungs of COPD patients compared to those from healthy smokers and non-smokers. Because we have previously shown that an oxidant mediated reduction of glucocorticoid function is prevented by inhibition of PI3K δ function in mice [8], we examined the role of PI3K δ underlying the glucocorticoid insensitivity of blood monocytes from COPD patients using a selective PI3K δ inhibitor. We showed that selective inhibition of PI3K δ but not PI3K γ mediated a reversal of glucocorticoid insensitivity, indicating a potential role for PI3K δ in glucocorticoid insensitivity in 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 COPD patients, 20 smokers and 13 non-smokers (Table 1). 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 prior to surgery. Peripheral venous blood was collected from 9 COPD patients and 7 smokers with normal lung function (Table 1). Pulmonary function tests were performed as previously described [24]. COPD was defined according to international guidelines as the presence of post-bronchodilator FEV₁/FVC ratio<70% [25]. Peripheral venous blood was also collected for functional *in vitro* studies from healthy volunteers. The study was approved by the local ethics committee of the University Hospital of Ferrara and all patients gave 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 non-consecutive fields (x40). Macrophages were identified by morphological staining. Normal nonspecific IgG from the animals in which the primary antibodies were raised in was used for negative controls (Santa Cruz Biotechnology, Santa Cruz, CA, USA). All staining and cell counting was performed in a blinded manner. The number of positively-stained cells was expressed as a percentage of the total cells counted. Antibodies used were: anti-PI3K δ antibody (Santa Cruz Biotechnology); anti-p-Akt antibody (Epitomics, Burlingame, CA, USA).

Monocyte Isolation & Treatments.

Peripheral blood mononuclear cells (PBMCs) were isolated from whole venous blood by Histopaque (Sigma, Dorset, UK) according the manufacturer's instructions. Monocytes were isolated by selective adhesion as previously described [26]. Cell culture and all treatments were performed using RPMI 1640 GlutaMAX phenol red free media (Invitrogen, Paisley, UK) with 1% foetal calf serum (FCS). Monocytes were treated with or without inhibitors for 45 minutes and then stimulated with or without either LPS (3ng/ml) or hydrogen peroxide

(H₂O₂; 100µM) for 1 hour. Reagents used: LPS (Sigma), dexamethasone (Sigma), LY294002 (Merck Biosciences, Nottingham, UK), IC87114 (Caltag Medsystems Ltd, Buckingham, UK) and AS604850 (Merck Biosciences).

Alveolar Macrophage Isolation & Treatment.

Bronchoalveolar lavage (BAL) was performed on patients with normal lung function as previously described [27]. Macrophage purity was ($94.8 \pm 5.3\%$) as assessed by QuickDiff (Harleco, Gibbstown, NJ, USA) staining of cytopins. Macrophages were collected from the BAL by 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 & Immunoblotting.

Proteins were extracted using a RIPA Buffer (50mM Tris HCl pH 8.0, 150mM NaCl, 0.25% Deoxycholate, 2mM EDTA, 0.1% SDS, 0.5% NP40, phosphatase inhibitors, protease inhibitors). Protein quantification was assessed by BCA assay (Perbio, Northumberland, UK). Immunoblotting was performed as previously described [9]. All blots were stripped and re-probed for loading controls as previously described using Chemicon stripping buffer as per manufacturer's instructions (Millipore, Watford, Herts, UK). *Antibodies:* Akt, p-Akt, (Cell Signaling Technology, Herts, UK); PI3Kδ (Abcam, Cambridge, UK).

ELISA.

Monocytes were treated with or without inhibitors for 45 minutes, with or without dexamethasone for 10 minutes then with or without LPS (3ng/ml) for 16 hours. CXCL-8 and

GM-CSF ELISAs were performed using DuoSet kits (R&D Systems) according to manufacturer's instructions.

Statistical Analysis.

Data was analysed by one-way ANOVA to determine statistically significant variance between the groups for each endpoint assessed. Statistical significance between groups was then calculated by the non-parametric Mann-Whitney t-test using GraphPad Prism software. Data is expressed as mean $\pm$ SEM. Differences were considered significant if $p < 0.05$.

Results

PI3K δ expression and phosphorylation of Akt (ser473) are elevated in macrophages in the lungs of COPD patients. Lung macrophages from COPD patients are less responsive to glucocorticoid suppression of pro-inflammatory 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 PI3K δ , but not of PI3K γ , signaling [8]. An elevation of PI3K δ activation may therefore represent a mechanism of glucocorticoid insensitivity in COPD. PI3K δ staining in macrophages in the peripheral lungs of COPD patients was increased compared to smokers with normal lung function ($p=0.0032$) and non smokers ($p=0.0007$) (Figure 1). However, the expression of PI3K δ in macrophages from the peripheral lungs of smokers with normal lung function was not increased compared to those from non-smokers with normal lung function ($p=0.105$).

Phosphorylation of Akt at serine 473 (p-Akt-ser473), a marker of Akt activation, was elevated in peripheral lung macrophages in COPD compared to smokers with normal lung function ($p=0.0033$) and non-smokers ($p=>0.0001$) (Figure 2). P-Akt-ser473 staining in

peripheral lung macrophages was also elevated in smokers with normal lung function compared to non-smokers (p=0.0022).

Oxidative stress induced phosphorylation of Akt (ser473) in a PI3K δ -dependent manner in blood monocytes and BAL macrophages. PI3K δ and γ are activated from distinct receptor types (receptor tyrosine kinases and G-protein coupled receptors respectively). LPS (3ng/ml)-induced phosphorylation of Akt has been shown to be dependent on PI3K δ , but not on PI3K γ signaling in monocytes [28;29]. Akt phosphorylation is also induced by oxidative stress; however the role of PI3K and thereafter the individual isoform(s) remain unknown. Treatment of blood monocytes isolated from healthy volunteers with H₂O₂ (100-200 μ M) induced a concentration- and time-dependent increase of Akt (ser473) phosphorylation (Figures 3A and 3B). Both LPS and oxidant-induced phosphorylation of Akt was inhibited by the selective PI3K δ inhibitor IC87114 (1 μ M) and by the pan-PI3K inhibitor LY294002 (10 μ M) but not by the selective PI3K γ inhibitor AS604850 (1 μ M) (Figures 3C and 3D). Similarly, both LPS- and oxidant-induced Akt phosphorylation was reduced by both IC87114 and LY294002 but not by AS604850 in BAL macrophages (Figures 3E and 3F).

Selective inhibition of PI3K δ but not PI3K γ restores glucocorticoid responsiveness in COPD blood monocytes. Glucocorticoid repression of pro-inflammatory mediator release from lung macrophages is impaired in COPD patients [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. Monocytes isolated from smokers (Table 1) were used as a control group which have been exposed in a similar fashion to the main etiological factor in the development of COPD (cigarette smoke) but have normal lung function and do not show a similar relative reduction in GC responsiveness as compared to COPD patients.

Compared to smokers, dexamethasone (10nM to 1μM) was less effective at suppressing both GM-CSF and CXCL-8 release (Figure 4). There was no significant change in the IC₅₀ of dexamethasone in COPD patients as compared to smokers.

Selective inhibition of both PI3Kδ (1μM IC87114) and PI3Kγ (1μM AS604850) alone had no impact on basal or LPS-stimulated GM-CSF or IL-8 release (Figure 4A and 4D). Inhibition of PI3Kγ had no impact on the impaired ability of dexamethasone to inhibit LPS-stimulated IL-8 and GM-CSF release in monocytes from COPD patients (Figure 4C and 4F). However, selective inhibition PI3Kδ restored the ability of dexamethasone (10nM-1μM) to suppress the LPS-induced release of GM-CSF and CXCL-8 to levels close to those observed in the control smokers (Figure 4B and 4E). Neither addition of IC78114 nor AS604850 resulted in any effect on the IC₅₀ of dexamethasone.

Discussion

We demonstrated that PI3Kδ expression and Akt phosphorylation in peripheral lung macrophages from COPD patients are increased compared to those from healthy smokers or normal subjects. Furthermore, selective inhibition of PI3Kδ, but not PI3Kγ, restored glucocorticoid-mediated repression of pro-inflammatory mediator release from monocytes isolated from COPD patients 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 to PI3Kγ knock-out and to wild type when exposed to cigarette smoke [8]. Therefore, activation of PI3Kδ and Akt phosphorylation may contribute to the impairment of glucocorticoid responsiveness seen in 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 mechanismss of this impairment of glucocorticoid function remains unclear but is likely to involve an oxidant-mediated alteration in function and/or signaling of several kinases and GR co-repressors [2;3;30]. However, although the PI3K γ and δ 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 PI3K δ and PI3K γ play key roles in the function of both the innate and adaptive immune responses; however, the impact 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 PI3K δ but not PI3K γ indicting that an oxidant-mediated induction of PI3K signaling is mediated primarily through PI3K δ . In mice, genetic abolition of PI3K δ but not PI3K γ signaling protects against an oxidant-mediated reduction in glucocorticoid function [8]. The expression of GR α 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 GR α transactivation, suggesting that glucocorticoid-mediated GR α activation is still functional. Furthermore, glucocorticoid sensitivity is

protected in mice that are devoid of PI3K δ signaling without any increase in the expression of GR α . [8].

The role of PI3K δ in the relative reduction of glucocorticoid responsiveness in patients with COPD is unknown. Here the 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 μ mol/L, selective inhibition of both PI3K δ and PI3K γ had no significant effect on LPS-induced proinflammatory mediator release. However, consistent with the in vitro and in vivo models, selective inhibition of PI3K δ but not PI3K γ 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 PI3K δ 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 may 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 COPD patients showed an increase in the expression of PI3K δ in macrophages compared to control groups of age matched smokers with normal lung function and non-smokers. PI3K δ 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 elevated in macrophages in the peripheral lungs of COPD patients compared to age matched smokers with normal lung function and age-matched non-smokers. This indicates that the observed elevation in the expression of PI3K δ in the macrophages from COPD patients translates into an increase in PI3K δ 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 COPD patients is likely to facilitate an enhanced and chronic activation of the PI3K δ /Akt pathway. Lipid peroxidation, which is able to generate a self-perpetuating cycle of reactive oxygen species, is also elevated in COPD and is likely to play an important role in the chronic up regulation of these and many other pathways [4;33-35].

Interestingly, age-matched healthy smokers also displayed increased PI3K δ 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 PI3K δ -mediated Akt signaling, this might be related to an increased exposure to oxidants through cigarette smoke. Regulated activation of the PI3K δ /Akt signaling 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 PI3K δ /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 PI3K δ /Akt pathway but might also result in the increase of PI3K δ expression. This in turn might allow for a hyperactivation of the PI3K δ 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 GR α function and therefore glucocorticoid-mediated immunosuppression [6-9;11]. HDAC-2 expression and activity is reduced in COPD and correlates with disease severity [13]. Hyperphosphorylation of HDAC-2 impairs its function, and we have shown that PI3K δ signaling is, in part, responsible for the hyperphosphorylation and inactivation of HDAC-2 and consequent reduction in glucocorticoid responsiveness [8;36]. The significant elevation in PI3K δ /Akt signaling seen in COPD is therefore likely to reflect the enhancement of the oxidant burden and overwhelmed extra- and intracellular anti-oxidant defences in the lungs of COPD patients [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 PI3K δ /Akt signaling in lung macrophages from COPD patients might therefore contribute to the reduced activity and expression of HDAC-2 resulting in a reduction in the responsiveness to GCs. 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 PI3K δ 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 PI3K δ 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 PI3K δ /Akt signaling pathway, which would allow the development of novel anti-inflammatory, glucocorticoid-restorative, or both therapeutic strategies across these diseases. Selective inhibition of PI3K δ 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.

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466 Table 1. Characteristics of subjects.

Group	Age	Sex	Smoking Status	Pack Years	FEV1 (litres)	FEV1 (% pred)	FEV1/FVC %	GOLD Stage	Chronic Bronchitis	Emphysema (NETT Score)
Peripheral Lung Section Study										
COPD	68.9±6.9	20M, 4F	10 Cur, 14 For	38.7±15.1	2.1±0.5	*74.7±16.7	57.2±9.5	<u>1</u> = 9 <u>2</u> = 12 <u>3</u> = 3	11 Yes, 13 No	13 Score 0 6 Score 1 3 Score 2 2 Score 3
Smoker	70.4±6.7	19M, 1F	9 Cur, 11 For	49±31.5	2.5±0.7	92.3±14.4	75.5±4.5	N/A	9 Yes, 11 No	11 Score 0 3 Score 1 4 Score 2 2 Score 3
NS	67.8±8.1	1M, 12F	NA	NA	2.1±0.5	101.5±22.6	76.4±3.5	NA	None	None
Glucocorticoid Functional Study										
COPD	76.1±3.1	8M, 1F	2 Cur, 7 For	49.9±29.7	1.4±0.5	*52.7±x13.4	52.7±7.6	<u>1</u> = 0 <u>2</u> = 5 <u>3</u> = 4	?????	??????
Smoker	58±6.5	6M, 1F	5 Cur, 2 For	33.9±x9.9	3.0±0.6	101.4±x11.4	78.6±5.0	NA	?????	??????

467 Data is depicted as Mean ± SD. FEV1/FVC ratio is post bronchodilator for subjects with

468 COPD but not smokers or non-smokers. *Abbreviations: GOLD = Global initiative for*469 *chronic obstructive lung disease (GOLD) guideline classification of COPD patients; pred =*470 *predicted; NS = non-smoker; M = male; F = female; Cur = current; For = former; FEV1 =*471 *forced expiratory volume in 1 second; FVC = forced vital capacity; BD: bronchodilator.*

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Figure legends

Figure 1. Expression of PI3K δ in macrophages in the peripheral lungs of non-smokers, smokers and COPD patients. Representative pictures (x40) of PI3K δ staining in peripheral lung sections from (A) non-smokers, (B) smokers and (C) COPD patients. (D) Percentage of macrophages positively stained for PI3K δ in the peripheral lung sections from non-smokers (n=11), smokers (n=19) and COPD patients (n=17). Histograms represent the mean $\pm$ SEM. *Abbreviations; NS: non-smoker.*

Figure 2. Expression of p-Akt in macrophages in the peripheral lung of non-smokers, smokers and COPD patients. Representative pictures (x40) of p-Akt (ser473) staining in peripheral lung sections from (A) non-smokers, (B) smokers and (C) COPD patients. (D) Graphical depiction of the percentage of macrophages positively stained for p-Akt (ser473) in the peripheral lung sections from non-smokers (n=11), smokers (n=19) and COPD patients (n=17). Histograms data represent the mean $\pm$ SEM. *Abbreviations; NS: non-smoker.*

Figure 3. Impact of oxidative stress on Akt phosphorylation: Role of PI3K δ and γ isoforms. H₂O₂-induced phosphorylation of Akt (ser374) in a (A) concentration and (B) time dependant manner. Immunoblots of the impact of selective inhibition of PI3K δ , PI3K γ and pan-PI3K activity on either (C, E) LPS or (D, F) H₂O₂-induced phosphorylation of Akt (ser374) in (C, D) blood monocytes isolated from the venous blood of healthy volunteers and (E, F) alveolar macrophages isolated from the BAL of volunteers with normal lung function. Images are representative of n=3-6. *P<0.05; ** P<0.01. *Abbreviations; conc: concentration; h: hour.*

Figure 4. Selective inhibition of PI3K δ restores glucocorticoid responsiveness in monocytes from COPD patients. (A+D) impact of IC87114 and AS604850 on LPS-induced GM-CSF

release and CXCL-8 release. Functional repression of LPS stimulated GM-CSF (*B+C*) and IL-8 (*E+F*) release by dexamethasone in COPD patients (squares; ■, n=9) compared to smokers with normal lung function (circles; ●, n=7). Impact of IC87114 (*B+E*) and AS604850 (*C+F*) on dexamethasone-mediated repression of LPS-induced GM-CSF and CXCL-8 release in COPD patients (diamonds; ♦) close to that seen in smokers with normal lung function. **P*<0.05. *Abbreviations; M: Molar.*

Figure 1

Figure 2

Figure 3

Figure 4