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CHEST

Original Research

COPD

IL-27 Is Elevated in Patients With COPD and Patients With Pulmonary TB and Induces Human Bronchial Epithelial Cells to Produce CXCL10

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Background: **The role of IL-27 in the pathogenesis of airway inflammatory diseases remains elusive. We, therefore, have studied its concentrations in the sputum and plasma of patients with COPD and patients with pulmonary TB (PTB), and further investigated the mechanism-of-action effects of IL-27 on bronchial epithelial cells in vitro.**

Methods: **Human bronchial epithelial cells grown on air-liquid interface culture were activated by IL-27, alone, or in combination with other inflammatory cytokines in the presence or absence of various signaling molecule inhibitors. The expression of CXCL10 was detected by reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assay (ELISA). The underlying signaling pathways were studied by intracellular staining using flow cytometry, Western blot, ELISA, or siRNA.**

Results: **Significantly higher sputum and plasma concentrations of IL-27 were found in patients with COPD (n = 34; $P < .01$ and $P < .001$, respectively) or patients with PTB (n = 31; $P < .01$ and $P < .001$, respectively) than in healthy control subjects (n = 48). Sputum, but not plasma, IL-27 levels in patients with COPD correlated negatively with FEV_1 ($r = -0.51$, $P < .01$). Sputum, but not plasma, IL-27 in patients with PTB correlated positively with mycobacterial load in sputum ($r = 0.48$, $P < .05$). Further in vitro studies demonstrated that IL-27 could induce gene and protein expression of CXCL10 in bronchial epithelial cells, which was regulated by the activation of the phosphatidylinositol 3-OH kinase (PI3K)-Akt signaling pathway.**

Conclusions: **The production of IL-27 is related to the pathogenesis of COPD and PTB, and IL-27 induces the expression of CXCL10 in bronchial epithelial cells through the activation of the PI3K-Akt signaling pathway.** ***CHEST 2012; 141(1):121–130***

Abbreviations: ELISA = enzyme-linked immunosorbent assay; IFN = interferon; PBEC = primary human bronchial epithelial cell; PI3K = phosphatidylinositol 3-OH kinase; PTB = pulmonary TB; Th = T helper; TNF = tumor necrosis factor

Bronchial epithelial cells are actively involved in initiating, maintaining, and regulating both innate and adaptive immune responses in the airways.[1,2] Activated bronchial epithelial cells can produce a variety of inflammatory mediators that serve in a paracrine or autocrine manner to regulate airway inflammation.[3-5]

IL-27 is a pleiotropic cytokine consisting of EBI3 and p28 subunits.[6] It is an early product of activated antigen-presenting cells stimulated by toll-like receptor ligands or infectious agents.[7-9] IL-27 could drive the differentiation of the T helper (Th) cell type 1 (Th1) subset in the early stage of development,[6-8] and support germinal center function by enhancing IL-21 production and the function of follicular Th cells.[10] IL-27 is able to stimulate monocytes, mast cells, and keratinocytes to produce a variety of proinflammatory cytokines.[9,11] In addition, IL-27 exhibits antitumor activity by promoting effector responses of $CD8^+$ T cells and natural killer cells.[12,13] On the other hand, IL-27 plays an immunoregulatory role in suppressing the development of the Th1, Th2, and

Th17 cell subsets and driving the expansion of inducible regulatory T cells to produce antiinflammatory cytokine IL-10.[7,8,14]

Inflammatory cells, such as macrophages and dendritic cells, represent highly active cells that increase in the airways of patients with inflammatory diseases such as COPD and pulmonary TB (PTB).[15-17] Because activated macrophages and dendritic cells are the main sources of IL-27,[7] there might be an aberrant production of IL-27 in patients with COPD and PTB. However, its immunopathologic role in airway inflammation and its relationship to airway inflammatory diseases have not yet been elucidated. The aim of this study was to investigate IL-27 production in sputum and plasma obtained from patients with COPD and patients with PTB and to study how it activates human bronchial epithelial cells in airway inflammatory diseases.

Materials and Methods

Subjects

Patients and healthy smoking and nonsmoking control subjects were enrolled in this study. Patients with COPD were diagnosed using the criteria of the GOLD (Global Initiative for Chronic Obstructive Lung Disease). Patients with COPD who had an exacerbation during the 3 months prior to the visit were excluded. Patients with COPD who had used corticosteroids, theophylline, long-acting β_2-agonists, leukotriene antagonists, or antihistamines within the 3 months prior to the study were excluded, as were subjects who had histories of respiratory tract infection within the previous 2 months. The patients with PTB presented a clinical manifestation typical for *Mycobacterium tuberculosis*, which includes radiologic analysis, sputum smears, and culture positivity confirmed by bacteriologic examination. All patients with PTB were newly diagnosed, and no patient was treated with antiinflammatory drugs such as nonsteroidal antiinflammatory drugs, steroids, or others. The protocol described here was approved by the Clinical Research Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (approval No. 20080156), and informed consent was obtained from all participants according to the Declaration of Helsinki.

Sputum and Blood Sampling

Sputum was induced and taken according to the protocols described previously.[18] Ethylenediaminetetraacetic acid venous peripheral blood was also collected to obtain plasma. All sputum and plasma were stored at −80°C until analysis. The duration of time between sputum or plasma sampling and protein analysis of patient samples was ≤ 2 months.

Reagents

Recombinant human IL-1β, IL-4, IL-6, IL-12, IL-13, IL-17F, IL-23, IL-27, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α were purchased from R&D Systems. Inhibitor κB-α phosphorylation inhibitor BAY11-7082, extracellular signal-regulated kinase inhibitor U0126, c-Jun N-terminal kinase inhibitor SP600125, p38 mitogen-activated protein kinase inhibitor SB203580, phosphatidylinositol 3-OH kinase (PI3K) inhibitor LY294002, and Janus kinase inhibitor AG490 were purchased from Calbiochem Corp. PI3K p110γ inhibitor AS604850 and PI3K p110δ inhibitor IC87114 were purchased from Merck Biosciences. In all studies, the concentration of dimethyl sulfoxide was 0.1% (vol/vol).

Human Bronchial Epithelial Cell Culture

Primary human bronchial epithelial cells (PBECs) were purchased from ScienCell Research Laboratories and cultured in bronchial epithelial cell growth medium as described previously.[19] BEAS-2B cells were obtained from the American Type Culture Collection, and they were used instead of PBEC for efficient transfection.

Gene Expression of CXCL10

The sequences of PCR primers are described in Table 1. The level of CXCL10 gene expression was quantified by calculating the ratio of densitometric readings of the band intensity for CXCL10 and G3PDH from the same complementary DNA sample.

Enzyme-linked Immunosorbent Assay

The concentrations of IL-27, CXCL8, and IFN-γ were quantitated by enzyme-linked immunosorbent assay (ELISA) reagents from R&D Systems. CXCL10 levels in the supernatants and cell lysates were quantitated by a commercially available ELISA kit (Biosource), according to the manufacturer's instructions, and expressed as the amount recovered per 10^6 cells. The PI3K activity was also detected using an ELISA kit (Echelon Biosciences).

Analysis of Activated Intracellular Signaling Molecules

For flow cytometric analysis, permeabilized PBEC were stained with mouse antihuman phosphorylated Akt (BD Pharmingen) and phosphorylated PI3K (Cell Signaling Technology) or mouse IgG1 antibodies (BD Pharmingen), followed by fluorescein isothiocyanate-conjugated goat antimouse secondary antibodies. The expression of intracellular phosphorylated signaling molecules was then analyzed by flow cytometry (FACSCalibur, BD Biosciences). Western blot analysis of activated signaling molecules was performed as described previously.[9]

Knockdown of Gene Expression With siRNA

PI3K p110α, PI3K p110β, Akt, and control siRNA were purchased from Cell Signaling Technology. BEAS-2B cells

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Table 1—*Sequences of Primers and Predicted Sizes for Target Genes*

Chemokine/Primer	Predicted Size (Base Pairs)
CXCL10	
Forward, 5′-TGAATCAAACTGCGATTCTG-3′	275
Reverse, 5′-TTTCCTTGCTAACTGCTTTCAG-3′	
G3PDH	
Forward, 5′-ACCACAGTCCATGCCATCAC-3′	450
Reverse, 5′-TCCACCACCCTGTTGCTGTA-3′	

The amplification reaction was performed for 30 cycles, with denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s.

were transfected with 25 nM each of siRNA with 5 μL of Lipofectamine 2000 (Promega), and grown in F12/Dulbecco modified Eagle medium with 10% fetal bovine serum without antibiotics for 24 h. The cells and cell supernatants were then harvested after stimulation with IL-27, and subjected to Western blot or ELISA analysis.

Statistical Analysis

All data were expressed as mean ± SD. Differences between groups were assessed by Kruskal-Wallis test, Mann-Whitney *U* test, Student *t* test, or one-way analysis of variance with Bonferroni post hoc test. Non-parametric Spearman rank correlation coefficient was used to test correlations between two parameters. $P < .05$ was considered significantly different.

Results

Sputum and Plasma IL-27 in Patients With COPD and Patients With PTB

A total of 113 subjects (34 smokers with COPD, 31 nonsmokers with PTB, 26 healthy smokers, and 22 healthy nonsmokers) were recruited for this study, and the clinical characteristics of the subjects are summarized in Table 2. The concomitant medications for COPD were inhaled corticosteroids (21 patients), long-acting muscarinic antagonists (18 patients), and long-acting β_2-agonists (19 patients). No patients with COPD were receiving systemic corticosteroid therapy. The newly diagnosed patients with PTB were treated with a standard short-course anti-TB chemotherapy consisting of isoniazid, rifampin, pyrazinamide, and ethambutol.

Compared with healthy nonsmokers or smokers with normal lung function, sputum IL-27 was significantly elevated in patients with COPD ($P < .01$) and patients with PTB ($P < .01$) (Fig 1A). In addition, the levels of plasma IL-27 in patients with COPD and patients with PTB were significantly elevated compared with those of healthy nonsmokers ($P < .001$) and healthy smokers ($P < .001$) (Fig 1B). However, there was no significant correlation between sputum and plasma IL-27 levels in patients with COPD or those with PTB (data not shown).

Five patients with COPD and six patients with PTB were recruited after treatment for 8 weeks, and sputum concentrations of IL-27 showed reproducibility and the percentage change in IL-27 values was ≤ 10% in patients with COPD (Fig 1C). However, sputum IL-27 levels decreased in all six patients with PTB by week 8, and the percentage change in IL-27 levels was > 30% (Fig 1D). IL-27 levels in plasma showed kinetics similar to those observed in sputum (data not shown).

Association Between Sputum IL-27 and Lung Function in COPD and PTB

Sputum IL-27 concentrations in patients with COPD were negatively correlated with FEV_1 % predicted ($r = -0.51$, $P < .01$) (Fig 1E). However, IL-27 was not correlated with residual volume/total lung capacity ($r = 0.06$) (Fig 1F). No significant correlation

Table 2—*Patient Characteristics*

Characteristic	Nonsmokers (n = 22)	Healthy Smokers (n = 26)	COPD (n = 34)	PTB (n = 31)
Age, y	52 ± 11	53 ± 8	59 ± 8	51 ± 12
Sex, F (M)	6 (16)	7 (19)	10 (24)	9 (22)
Pack-y	0 ± 0	31 ± 18	36 ± 22	0 ± 0
Smoking status, current (former)	0	21 (5)	18 (16)	0
FEV_1, L	2.4 ± 0.7	2.5 ± 0.5	1.9 ± 0.8[a,b]	2.1 ± 0.9
FEV_1, % predicted	99.5 ± 12.9	101.5 ± 21.2	58.1 ± 11.5[c,d]	89.5 ± 22.9
FEV_1/FVC, %	78.4 ± 5.6	76.1 ± 6.9	56.1 ± 12.7[c,d]	71.2 ± 16.9
SaO_2, %	98.2 ± 1.5	98.5 ± 0.9	95.8 ± 1.5[e,f]	97.7 ± 0.8
Taking inhaled steroids	0	0	0	0

Data are presented as mean ± SD unless otherwise indicated. F = female; M = male; PTB = pulmonary TB; SaO_2 = oxygen saturation.
[a] $P < .01$ when compared with nonsmokers.
[b] $P < .01$ when compared with healthy smokers.
[c] $P < .001$ when compared with nonsmokers.
[d] $P < .001$ when compared with healthy smokers.
[e] $P < .05$ when compared with nonsmokers.
[f] $P < .05$ when compared with healthy smokers.

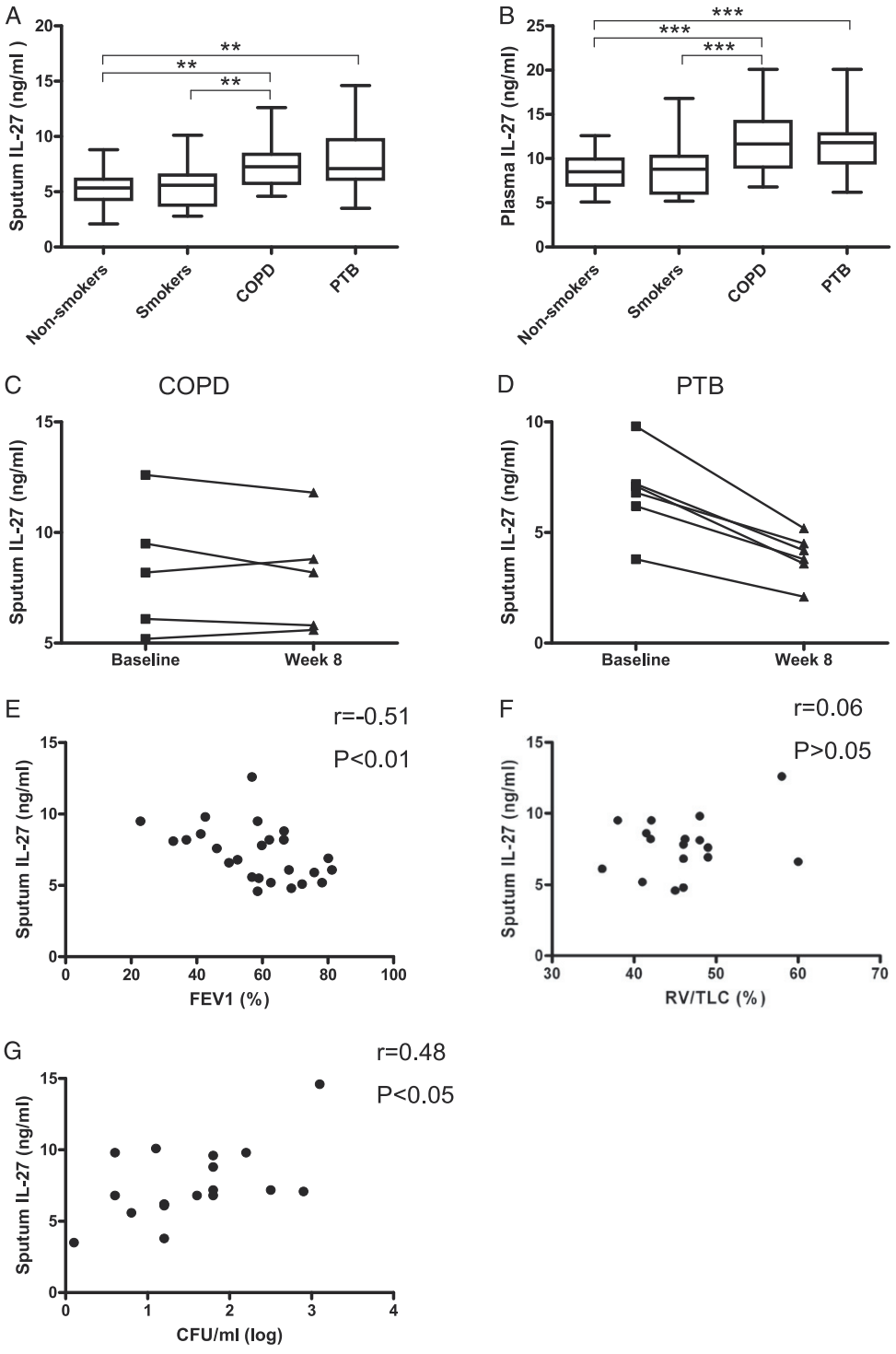

FIGURE 1. IL-27 concentrations in induced sputum and plasma were measured by dual antibody sandwich enzyme-linked immunosorbent assay. A standard curve was generated by using known amounts of recombinant human IL-27. Each sample was tested in duplicate and the concentrations were determined from the standard curve. ** $P < .01$, *** $P < .001$, when compared between groups denoted by horizontal lines. A, Induced sputum. B, Plasma. Patients with COPD and patients with PTB were recruited after 8 weeks of treatment to investigate the concentrations of IL-27. C, Five patients with COPD. D, Six patients with PTB. E, Correlation between sputum IL-27 and FEV_1 % predicted in patients with COPD. F, Correlation between sputum IL-27 and RV/TLC in patients with COPD. G, Correlation between sputum IL-27 and mycobacterial load (as assessed by log CFU) in induced sputum by quantitative mycobacterial culture. The nonparametric Spearman rank correlation test was used to test correlations between two parameters. CFU = colony forming unit; PTB = pulmonary TB; RV = residual volume; TLC = total lung capacity.

was detected between sputum IL-27 and sputum CXCL8 in patients with COPD ($r = 0.40$) (data not shown). In addition, plasma IL-27 was not correlated with any clinical indices, such as smoking status and lung function tests (data not shown). In patients with PTB, a positive correlation was observed between sputum IL-27 levels and mycobacterial load (as assessed by log CFU) in induced sputum ($r = 0.48$, $P < .05$) (Fig 1G). However, there was no detectable correlation between plasma IL-27 levels and mycobacterial load in induced sputum ($r = 0.25$) (data not shown). In addition, no significant correlation was detected between sputum IL-27 levels and sputum IFN-γ ($r = 0.32$) (data not shown).

Effects of IL-27 on CXCL10 Expression in PBEC: As shown in Figures 2A and 2B, IL-27 could induce CXCL10 gene expression in PBEC time dependently and dose dependently. In addition, the protein levels of CXCL10 in cell lysates were significantly increased, and peaked at the 12-h time point after stimulation with 10 and 100 ng/mL of IL-27 (Fig 2C), whereas the levels of CXCL10 in the supernatants peaked at 24 h and decreased at 48 h after stimulation (Fig 2D). However, IL-27 could not induce the expression of IL-6, TNF-α, or CXCL8 in PBEC (data not shown).

IL-27 Augments the Production of CXCL10 in Cytokine-Treated PBEC

As shown in Figure 3, stimulation of PBEC with each of the five cytokines IL-27, IFN-γ, IL-1β, TNF-α, and IL-17F induced the production of CXCL10. However, IL-4 and IL-13 and other IL-6/IL-12 family cytokines, including IL-6, IL-12, and IL-23, could not activate PBEC to express CXCL10. Furthermore, the combination of IL-27 and each of the four cytokines (IFN-γ, IL-1β, TNF-α, and IL-17F) significantly augmented CXCL10 production in comparison with that observed in PBEC activated by IL-27.

Effects of Signaling Molecule Inhibitors on IL-27-induced CXCL10 Production

The cytotoxicities of signaling molecule inhibitors on PBEC have been determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

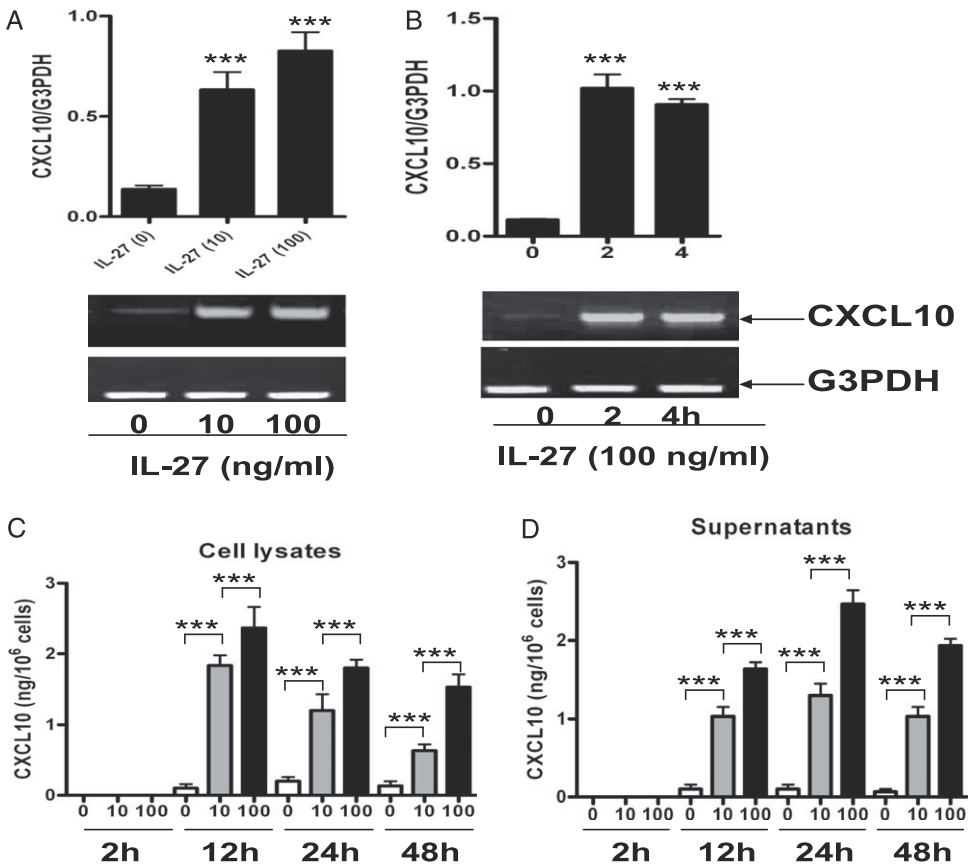

FIGURE 2. Gene and protein expression of CXCL10 in primary human bronchial epithelial cells (PBECs) grown on air-liquid interface culture. A, CXCL10 gene expression induced by IL-27 in PBECs. ***$P < .001$ vs medium control. B, Kinetic gene expression of CXCL10. ***$P < .001$ vs the level at the 0-h time point. C, CXCL10 protein levels in cell lysates of PBECs. D, CXCL10 protein levels in supernatants of PBEC. Results are expressed as the arithmetic mean ± SD from three independent experiments. ***$P < .001$ when compared between groups denoted by horizontal lines.

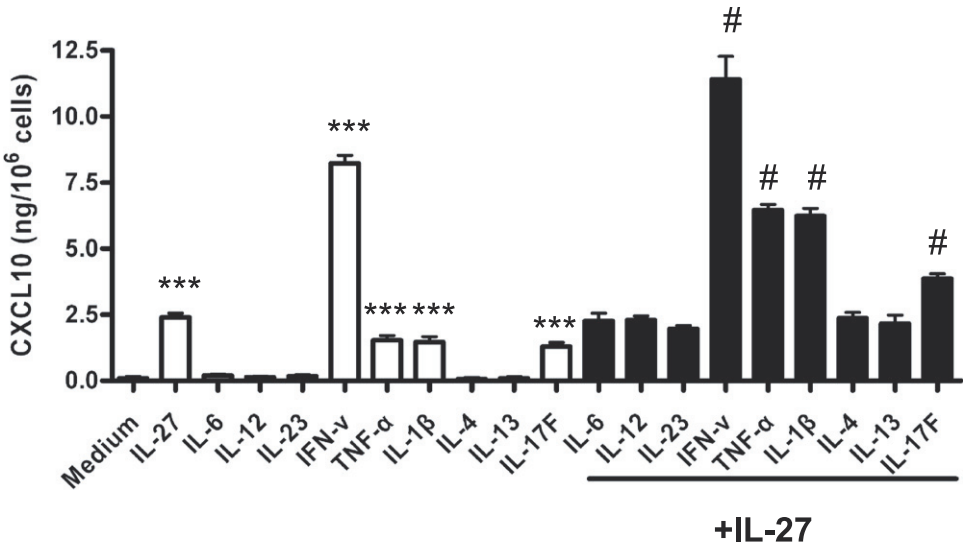

FIGURE 3. Effects of a Th1 cytokine (IFN-γ), Th17 cytokine (IL-17F), Th2 cytokines (IL-4 and IL-13), IL-6/IL-12 family cytokines (IL-6, IL-12, and IL-23), or other proinflammatory cytokines (IL-1β and TNF-α) on IL-27-induced CXCL10 production. PBECs were activated as indicated for 24 h, and CXCL10 protein levels in supernatants were determined by enzyme-linked immunosorbent assay. Results are expressed as the arithmetic mean ± SD from three independent experiments.***$P < .001$ vs medium control; #$P < .05$ vs IL-27-stimulated PBEC. IFN = interferon; Th = T helper; TNF = tumor necrosis factor. See Figure 2 legend for expansion of other abbreviation.

assay (data not shown), and the specificities of these inhibitors on their signaling pathways have been investigated largely by using different cell types, including PBECs.[19-21] Following previous studies and toxicity threshold values from the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, we selected the optimal concentrations of Janus kinase inhibitor AG490 (5 μM), NF-κB inhibitor BAY11-7082 (0.8 μM), PI3K inhibitor LY294002 (10 μM), extracellular signal-regulated kinase inhibitor U0126 (10 μM), p38 mitogen-activated protein kinase inhibitor SB203580 (20 μM), and c-Jun N-terminal kinase inhibitor SP600125 (5 μM) with significant inhibitory effects but without any cell toxicity. As shown in Figure 4A, LY294002 could significantly suppress IL-27-induced CXCL10 production in PBEC, whereas treatment with AG490, BAY11-7082, SB203580, SP600125, and U0126 did not affect CXCL10 release.

To avoid any nonspecific effects of this concentration of LY294002 (10 μM), lower concentrations of this compound were used. As shown in Figure 4B, LY294002 (1 μM) could also significantly downregulate IL-27-induced CXCL10 production. Even at the concentration of 0.5 μM of LY294002, IL-27-induced CXCL10 production was reduced, although it did not reach statistical significance. However, selective inhibition of PI3K p110γ by AS604850 or PI3K p110δ by IC87114 had no effect on IL-27-stimulated CXCL10 release (Fig 4B).

IL-27-Activated PI3K-Akt Pathway in PBEC

IL-27 could induce the phosphorylation of PI3K (Fig 5A) and Akt (Fig 5B) by intracellular fluorescence staining using flow cytometry. PI3K-Akt activation was highly reproducibly detectable in PBEC in response to IL-27 by means of ELISA and Western blot analyses (Fig 5C, 5D).

siRNA-PI3K p110α, siRNA-PI3K p110β, and siRNA-Akt Suppressed IL-27-Induced CXCL10 Expression

BEAS-2B cells were transfected with siRNA specific for PI3K p110α, PI3K p110β, and Akt, and Western blot analysis showed that the expression of PI3K p110α and PI3K p110β was completely inhibited by PI3K p110α- and PI3K p110β-specific siRNA, respectively (Fig 6A, 6B). In addition, the phosphorylation of Akt was significantly reduced by knockdown of PI3K p110α (by about 85%) and PI3K p110β (by about 70%) (Fig 6C).

As shown in Figures 6D and 6E, the induction of CXCL10 gene and protein expression by IL-27 was significantly inhibited in BEAS-2B cells transfected with siRNA for PI3K p110α, PI3K p110β, and Akt.

DISCUSSION

In this study, we demonstrated for the first time that sputum and plasma levels of IL-27 were significantly elevated in COPD and PTB patients compared with

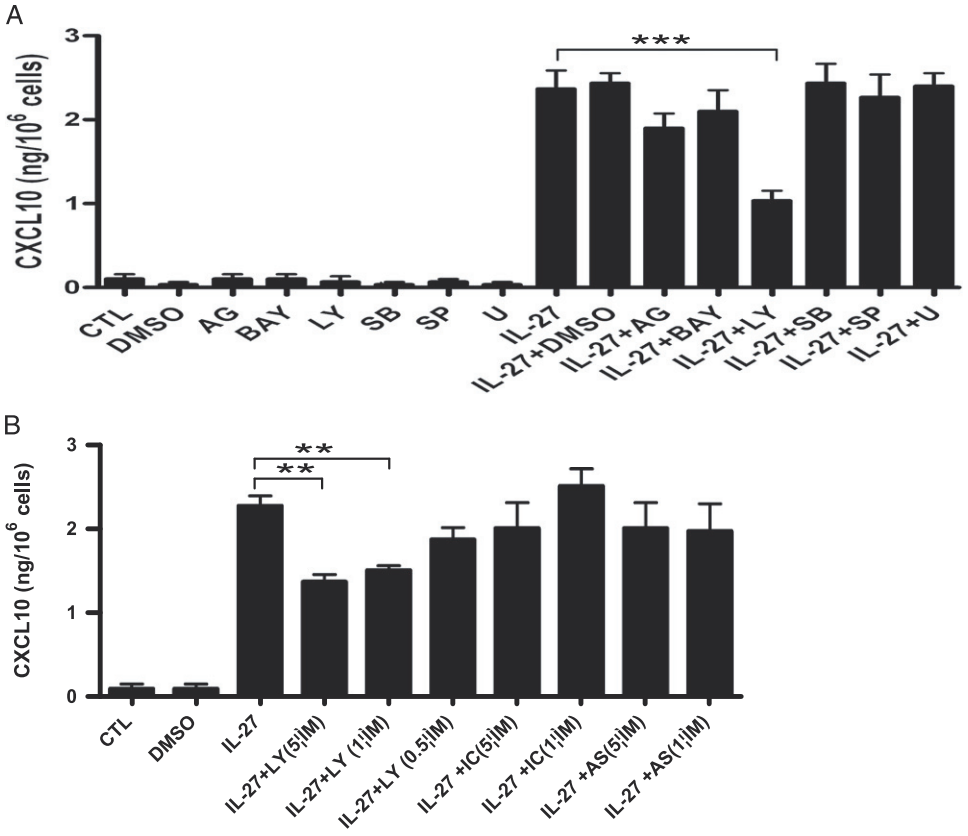

FIGURE 4. Effects of different signaling molecule inhibitors on IL-27-induced CXCL10 production in PBECs grown on air-liquid interface culture. A, The effects of AG, BAY, LY, SB, SP, or U on IL-27-induced CXCL10 release. B, Selective inhibition of pan-phosphatidylinositol 3-OH kinase (PI3K), PI3K p110γ, or PI3K p110δ activity on IL-27-induced CXCL10 release. PBECs were pretreated with AG (5 μM), BAY (0.8 μM), LY (0.5-10 μM), SB (20 μM), SP (5 μM), U (10 μM), AS (1-5 μM), or IC (1-5 μM) for 1 h, followed by incubation for a further 24 h with or without IL-27 (100 ng/mL) in the presence of inhibitors. The release of CXCL10 was determined by enzyme-linked immunosorbent assay. DMSO (0.1%) was used as the vehicle control. Results are expressed as the arithmetic mean ± SD from three independent experiments. $^{**}P < .01$, $^{***}P < .001$ when compared between groups denoted by horizontal lines. AG = AG490; AS = AS604850; BAY = BAY11-7082; CTL = medium control; DMSO = dimethyl sulfoxide; IC = IC87114; LY = LY294002; SB = SB203580; SP = SP600125; U = U0126. See Figure 2 legend for expansion of other abbreviation.

healthy control subjects. In addition, sputum, but not plasma, IL-27 concentrations in COPD showed a significant correlation with FEV_1, suggesting a possible link to disease severity. However, sputum IL-27 was not correlated with residual volume/total lung capacity, indicating that IL-27 may not be associated with alveolar wall destruction in COPD. In addition, sputum, but not plasma, IL-27 was correlated with mycobacterial load in sputum in patients with PTB, suggesting that IL-27 in sputum may reflect the intensity of TB infection. Additional COPD and PTB patients are currently being recruited to further evaluate the sputum and plasma conversion of IL-27 and the response to antiinflammation therapies.

Because bronchial epithelial cells are central participants in the pathogenesis of airway inflammatory diseases and we have recently found that IL-27 could enhance the expression of intercellular adhesion molecule 1 on the surface of bronchial epithelial cells,[19] we took bronchial epithelial cells as an in vitro cell model to study the potential role of IL-27 in the induction of cytokine and chemokine in bronchial epithelial cells. We found that IL-27 was able to induce the expression of CXCL10 but not IL-6, TNF-α, or CXCL8, which is consistent with previous reports that IL-27 could stimulate the production of CXCL10 in monocytes, keratinocytes, and fibroblast-like synoviocytes.[9,22,23] In fact, CXCL10 is a pivotal chemokine that contributes to both Th1- and Th2-type airway inflammation,[24,25] and a significantly increased production of CXCL10 has been seen in airway inflammatory diseases such as COPD, PTB, chronic bronchitis, and severe acute respiratory syndrome.[26-29] In this study, we found that IL-27 is a novel inducer of

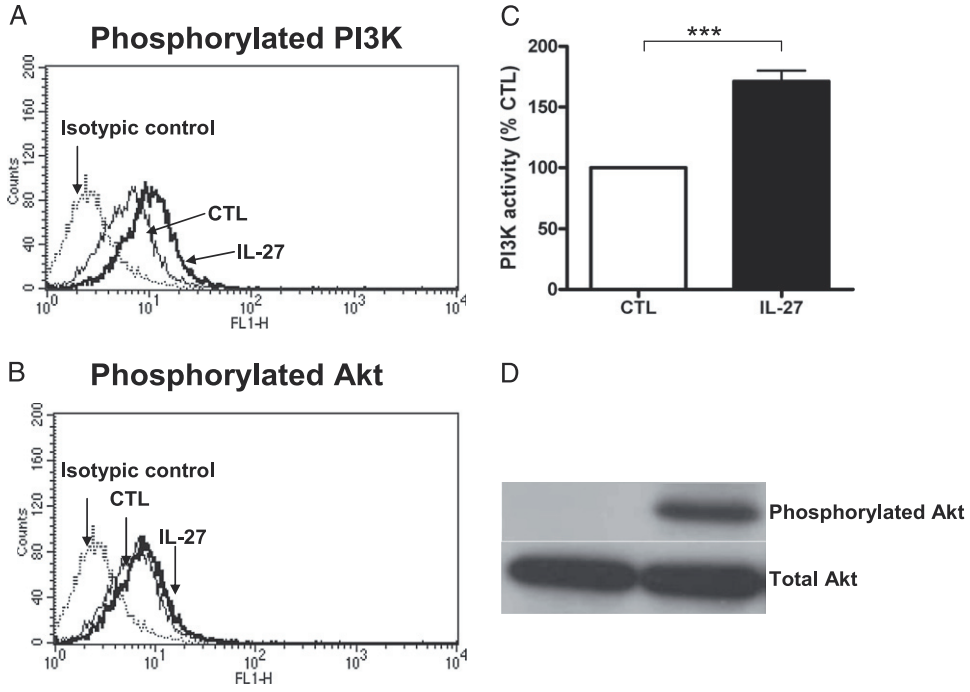

FIGURE 5. Effects of IL-27 on the activation of the PI3K-Akt signaling pathway in PBEC grown on air-liquid interface culture. PBEC were incubated with or without IL-27 (100 ng/mL) for 5 min. A, Intracellular expression of phosphorylated PI3K. B, Intracellular expression of phosphorylated Akt. The isotypic control represents the cell populations stained with antimouse IgG1 isotype control. C, Activation of PI3K was determined by enzyme-linked immunosorbent assay 15 min after stimulation with IL-27. ***$P < .001$ vs medium control. D, Phosphorylated Akt and total Akt levels were determined by Western blot analysis 15 min after stimulation with IL-27. The results are from three independent experiments. PI3K = phosphatidylinositol 3-OH kinase. See Figure 2 and 4 legends for expansion of abbreviations.

CXCL10 in bronchial epithelial cells, suggesting that the IL-27-CXCL10 axis may be particularly important in the development of airway inflammation.

Other cytokines, including IFN-γ, IL-17F, IL-1β, and TNF-α have also been described as being able to induce the production of CXCL10.[30,31] We, therefore, further investigated whether IL-27 could modulate the production of CXL10 in bronchial epithelial cells stimulated with either a Th1 cytokine (IFN-γ), Th17 cytokine (IL-17F), Th2 cytokines (IL-4 and IL-13), proinflammatory cytokines (IL-1β and TNF-α), or other IL-6/IL-12 family cytokines (IL-6, IL-12, and IL-23). We demonstrated that IL-27 could augment CXCL10 production from bronchial epithelial cells in combination with other inflammatory cytokines, including IFN-γ, IL-1β, TNF-α, and IL-17F. However, IL-4, IL-13, IL-6, IL-12, and IL-23 could not activate bronchial epithelial cells to produce CXCL10. This may be because the stimulation of bronchial epithelial cells by these cytokines could not provide an integrated response by activating CXCL10 gene transcription and protein translation via transcription factors, chromatin phosphorylation, and increasing nucleotide synthesis.

Regarding signaling pathways of IL-27, we demonstrated that IL-27-induced CXCL10 expression in bronchial epithelial cells is primarily regulated by the PI3K-Akt pathway. Furthermore, both PI3K p110α and PI3K p110β isoforms were important for IL-27-induced CXCL10 expression, whereas PI3K p110γ and PI3K p110δ had little effect. These results are reasonable because PI3K p110α and PI3K p110β are widely distributed, whereas PI3K p110γ and PI3K p110δ are enriched only in leukocytes.[32-34] However, inhibition of the PI3K-Akt pathway could partially suppress the expression of CXCL10 induced by IL-27 in bronchial epithelial cells, suggesting that other unidentified signaling pathways might contribute to CXCL10 expression.

Taken together, our results provide evidence that IL-27 might play an important role in airway inflammatory diseases including COPD and PTB. IL-27 was found to induce the expression of chemokine CXCL10 in bronchial epithelial cells via the activation of the PI3K-Akt signaling pathway. Elucidating the interactions between IL-27 and CXCL10 may be important in understanding and treating airway inflammatory diseases.

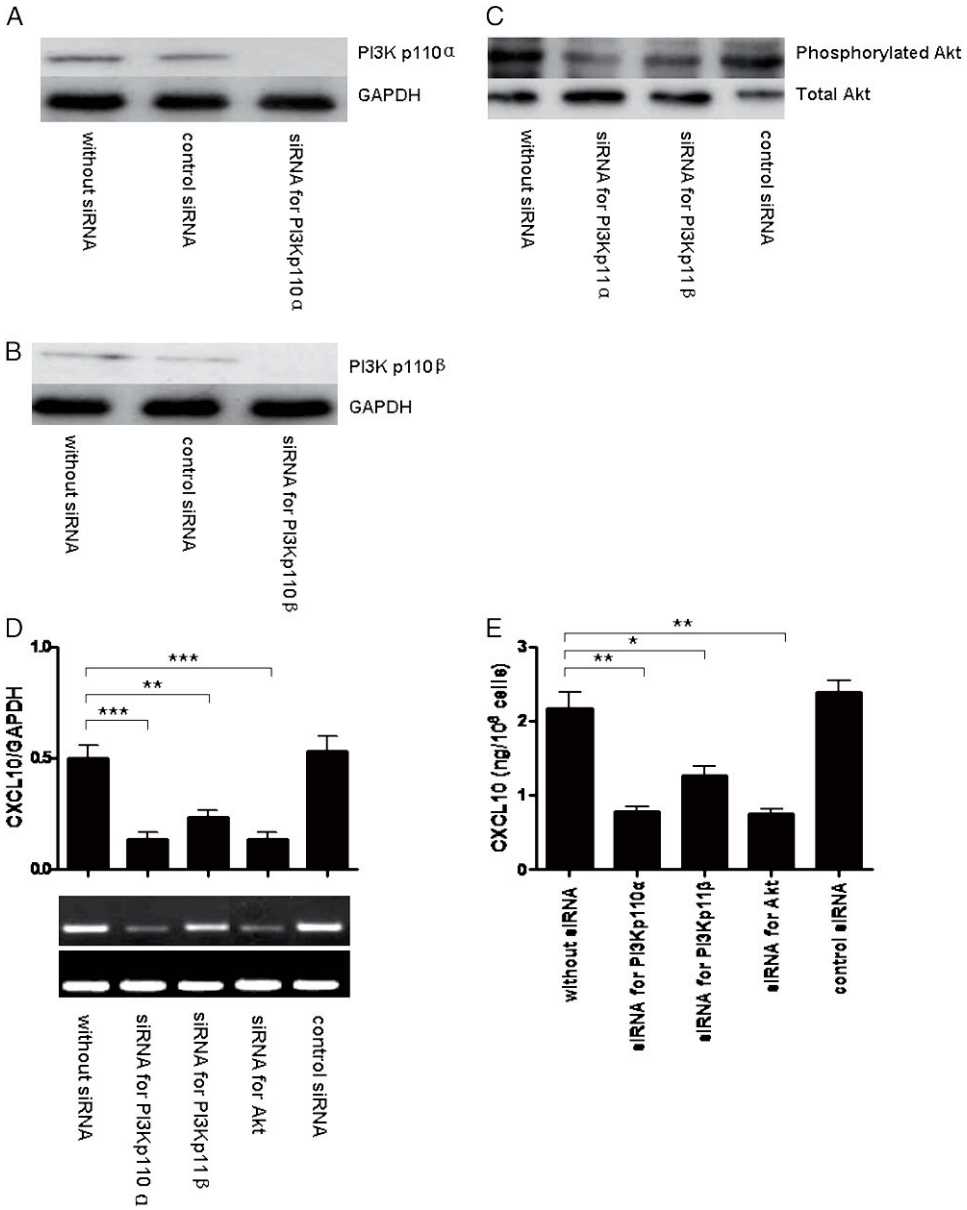

FIGURE 6. Effects of siRNA-PI3K p110α, siRNA-PI3K p110β, and siRNA-Akt on IL-27-induced CXCL10 expression. BEAS-2B cells were transfected with or without siRNA-PI3K p110α and siRNA-PI3K p110β, and their blocking effects were validated by Western blot. A, Blocking effects on the expression of PI3K p110α. B, Blocking effects on the expression of PI3K p110β. C, Blocking effects on the phosphorylation of Akt. BEAS-2B cells were transfected with siRNA-PI3K p110α, siRNA-PI3K p110β, and siRNA-Akt. The cells and cell supernatants were then harvested at 2 h and 24 h, respectively, after stimulation with 100 ng/mL of IL-27. D, Level of CXCL10 gene expression measured by reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). E, Level of protein production measured by RT-PCR and ELISA. Results are expressed as the arithmetic mean ± SD from three independent experiments. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ when compared between groups denoted by horizontal lines. GADPH = glyceraldehyde 3-phosphate dehydrogenase. See Figure 1 and 5 legends for expansion of other abbreviations.

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Dr Xu: contributed to the recruitment of subjects, the acquisition and interpretation of the data, and manuscript review.
Dr Huang: contributed to the recruitment of subjects, the acquisition and interpretation of the data, and manuscript review.
Dr Xiang: contributed to the recruitment of subjects, the acquisition and interpretation of the data, and manuscript review.

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