ORIGINAL ARTICLE

Polymeric Immunoglobulin Receptor Down-regulation in Chronic Obstructive Pulmonary Disease

Persistence in the Cultured Epithelium and Role of Transforming Growth Factor-β

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Abstract

Rationale: The generation of protective secretory IgA relies on the epithelial polymeric immunoglobulin receptor (pIgR). pIgR expression is reduced in chronic obstructive pulmonary disease (COPD), but correlation to disease severity and underlying mechanisms remains unknown.

Objectives: To address the hypothesis that pIgR down-regulation in COPD concerns severe disease in relation to aberrant programming of the bronchial epithelium.

Methods: Surgical lung tissue and primary bronchial epithelium (cultured in air–liquid interface, ALI) obtained from a large series of patients (n = 116) were studied for pIgR expression and regulation.

Measurements and Main Results: pIgR immunostaining in the bronchial epithelium is decreased in severe COPD. In contrast, pIgR transcription was up-regulated in smokers with or without COPD. In ALI (vs. submerged) cultures, pIgR expression was strongly induced, whereas pIgR expression and IgA-transcytosis capacity were decreased in cultures from subjects with severe COPD as compared with control subjects. In addition, COPD cultures released more transforming growth factor-β_1 (TGF-β_1), reflecting increased epithelial TGF-β_1 immunostaining in COPD lung tissue. Finally, besides inducing epithelial dedifferentiation, exogenous TGF-β_1 dose-dependently inhibited pIgR production, whereas pIgR increased on blockade of TGF-β_1 activity during ALI differentiation.

Conclusions: pIgR down-regulation in COPD correlates with disease severity, and the bronchial epithelium reconstituted *in vitro* from these patients retains its aberrant imprinting for pIgR expression. This study also links pIgR down-regulation to TGF-β–driven reprogramming of the bronchial epithelium, which results in impaired lung IgA immunity in patients with COPD.

Keywords: chronic obstructive pulmonary disease; immunology; IgA

Chronic obstructive pulmonary disease (COPD) is the leading chronic respiratory disease, represents the fourth leading cause of medical consultancy and mortality, and, according to further increase in prevalence, is expected to represent the fifth leading cause of morbidity worldwide (1). It is mainly due to cigarette smoking, which induces in susceptible individuals

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At a Glance Commentary

Scientific Knowledge on the Subject: Down-regulation of polymeric immunoglobulin receptor (pIgR) has been reported in chronic obstructive pulmonary disease (COPD), potentially leading to decreased transport of IgA into airway secretions. Secretory IgA is believed to prevent adherence of pathogens and antigens to the surface epithelium, acting through so-called immune exclusion. Impaired IgA transport in COPD could therefore lead to defects in lung mucosal immunity and favor chronic inflammation. However, mechanisms of pIgR down-regulation, as well as correlation to disease severity, remained unknown.

What This Study Adds to the Field: This study of pIgR expression, in lung tissue and bronchoepithelial cell cultures obtained from patients with various stages of COPD, shows that pIgR protein down-regulation correlates with disease severity. In addition, pIgR down-regulation persists in the bronchial epithelium reconstituted *in vitro* from these patients and is associated with increased autocrine transforming growth factor (TGF)-β_1 release. Exogenous TGF-β_1 inhibits pIgR gene transcription while altering epithelial differentiation, whereas TGF-β_1 blockade during differentiation up-regulates pIgR expression. We suggest that pIgR down-regulation in severe COPD relates to the reprogramming of the airway epithelium by TGF-β.

neutrophilic inflammation of the airways and tissue remodeling. The latter includes changes of the bronchial epithelium, such as mucous (goblet cell) hyperplasia, squamous metaplasia, and subepithelial fibrosis (2).

IgA represents the most abundant immunoglobulin in mucosal secretions and participates in frontline defense mechanisms in the respiratory tract, along mucociliary clearance (3). It acts as an "innate" immunoprotein, scavenging particles and microbes before they reach and adhere to the epithelium, through so-called immune exclusion. Once adaptive immunity has been elicited through cognate dendritic T-cell interactions, production of IgA antibodies with high affinity to a specific antigen may occur. In addition, IgA may also regulate myeloid leukocytes by ligating specific FcαRI/CD89 receptors. To be able to exert its function within mucosal secretions, IgA produced by mucosal plasma cells (mainly as dimeric IgA) needs to be transported across the epithelium (3). This occurs through an active transcellular routing, owing to the polymeric immunoglobulin receptor (pIgR) expressed at the basolateral pole of epithelial cells. The dimeric IgA/pIgR complex is transcytosed across the epithelial cell up to the apical pole, where a proteolytic cleavage releases dimeric IgA bound to the main part of the extracellular domain of the pIgR, called secretory component (SC), to form secretory IgA (S-IgA).

Based on the protective roles of IgA in lung immunity, our first study (4) investigated pIgR/SC expression in lung explants from patients with very severe COPD and its relation to airway inflammation, showing that pIgR expression was strongly decreased as compared with control specimens from primary pulmonary hypertension and correlated to infiltration of submucosal glands by neutrophils. We could then demonstrate that neutrophil-derived serine proteinases (especially elastase and proteinase-3) could cleave pIgR/SC into nonfunctional fragments (5). However, a paradoxical up-regulation of pIgR expression was observed when human bronchial epithelial (Calu-3) cells were incubated with supernatants from activated neutrophils (5, 6) or with transforming growth factor-β (TGF-β) (6). More recently, it was also shown that this impaired pIgR expression was associated with morphological changes of the epithelium and could result in the formation of immune complexes in subepithelial tissues (7).

Previous studies have documented cellular and molecular regulatory mechanisms of pIgR expression and transcytosis; stimuli for pIgR up-regulation include IFN-γ and IL-4, as well as to a lower extent tumor necrosis factor (TNF)-α, IL-1β (8), and probably IL-17 (9). In contrast, mechanisms inhibiting pIgR expression remain unknown. The present study was designed to address the hypothesis that pIgR down-regulation in COPD is correlated to disease severity and to engagement of dedifferentiation pathways in the bronchial epithelium. We explored through an integrated approach of lung tissue and *in vitro* reconstituted primary bronchial epithelium from the same patients with various range of airflow limitation the regulatory mechanisms determining this epithelial defect. We hypothesized that pIgR down-regulation may relate to activation of the TGF-β pathway controlling epithelial differentiation. Some of the results of this study have been reported in the form of an abstract at the Munich Lung Conference 2013 (10).

Methods

Additional details are provided in the online data supplement.

Patients

One hundred sixteen patients were enrolled in this study, consisting of 55 (non)smoker control subjects and 61 patients with COPD, namely 18 with mild (global initiative for chronic obstructive lung disease [GOLD] stage I), 24 with moderate (GOLD stage II), and 19 with severe and very severe COPD (GOLD stages III and IV) undergoing lung resection surgery for a solitary lung tumor or for transplantation (for GOLD IV patients) (Table 1). They were recruited between 2007 and 2012. All patients underwent detailed clinical history and lung function testing, and patients with other lung diseases (such as asthma) were excluded from the study. All patients gave signed informed consent to the study protocol, which was approved by our local Ethical committee (Ref. #2007/19MARS/58).

Lung Tissue Sampling and Processing

Lung sections were obtained from the surgical specimens and processed for immunohistochemistry or for real-time quantitative polymerase chain reaction (RT-qPCR). One additional large sample was obtained for primary epithelial cell culture. According to optimal quality of samples, among the 116 enrolled patients (Table 1), expression analyses by RT-qPCR and immunohistochemistry were performed for 48 and 49 patients, respectively (*see* Table

Table 1. Patient Characteristics of the Study Population

	Nonsmokers	(Ex)-Smokers	Mild COPD	Moderate COPD	Severe COPD	All
Subjects, n	20	35	18	24	19	116
Sex, F/M	12/8	18/17	2/16	6/18	8/11	46/70
Smoking history, pack-years (never/former/current) (n = 107)	0*†‡§ (20/0/0)	28 ± 15§‖ (0/20/15)	47 ± 22‖ (0/8/10)	41 ± 20‖ (1/9/14)	50 ± 29*‖ (0/17/2)	31 ± 25 (21/54/41)
Inhaled corticosteroids, n	1	3	1	6	14	25
Inhaled corticosteroids, BDP equivalent, μg/d (n = 22)	568	1,603 ± 1,158	2,272	1,603 ± 978	2,219 ± 2,838	1,951 ± 2,247
Age, yr	65 ± 14	62 ± 14	66 ± 10	66 ± 10	61 ± 6	64 ± 12
BMI, kg/m^2 (n = 115)	26.3 ± 4.5	25.3 ± 6.7	25.8 ± 4.6	24.5 ± 4.2	23.5 ± 4.9	25.1 ± 5.3
FEV_1, % predicted (n = 113)	95 ± 16‡§	94 ± 21‡§	91 ± 14‡§	68 ± 8*†‖	29 ± 12*†‖	78 ± 29
FEV_1/VC ratio, % (n = 111)	77 ± 7†‡§	79 ± 8†‡§	64 ± 4*§‖	60 ± 9*‖	35 ± 11*†‖	65 ± 18
D_{LCO}, % predicted (n = 87)	77 ± 16§	74 ± 16‡§	63 ± 12	59 ± 16*	34 ± 19*‖	64 ± 20

Definition of abbreviations: BDP = beclomethasone dipropionate; BMI = body mass index; COPD = chronic obstructive pulmonary disease; D_{LCO} = diffusing capacity of carbon monoxide; F = female; M = male.
Data are mean ± SD unless otherwise specified. Demographic data, lung function tests, and smoking history as well as inhaled corticotherapy are stated for the patient groups, classified according to smoking history and the presence of airflow limitation. Patients with other lung diseases were excluded from the study. N is specified when data are missing.
*$P < 0.05$ versus (ex)-smokers.
†$P < 0.05$ versus mild COPD.
‡$P < 0.05$ versus moderate COPD.
§$P < 0.05$ versus severe COPD.
‖$P < 0.05$ versus nonsmokers.

E1 in the online supplement), and primary bronchoepithelial cultures were derived from 75 patients (Table E2).

Primary Cultures of Human Bronchial Epithelial Cells

A piece of large, cartilaginous bronchus away from the tumor site was selected to derive human bronchoepithelial cells (HBEC). Cultures were performed in air–liquid interface (ALI) for 2 weeks, to allow redifferentiation into a pseudostratified, mucociliary airway epithelium (11). ALI-HBEC were treated for 6 to 72 hours with TGF-β_1 (or IFN-γ as positive control) in the basolateral compartment, with cigarette smoke extract (CSE) at 2.5 or 5% for 3 or 6 days in the apical compartment, or exposed for 3 days to whole cigarette smoke (one exposure a day) using an exposure system, adapted from Beisswenger et al (12). For blocking experiments, anti-human TGF-β_1 antibody (Ab) or its control mouse IgG was added during the 2 first weeks of ALI. No significant cytotoxicity was observed (<10% decrease in 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay or <10% release of lactate dehydrogenase) in these conditions. For kinetics experiments, ALI cultures were performed for 1 to 5 weeks and compared with submerged cultures.

Immunoassays for pIgR and Epithelial/Mesenchymal Markers

Immunohistochemistry for pIgR, β-tubulin IV, MUC5AC, TGF-β, phospho-Smad2/3, and epithelial/mesenchymal markers. Serial paraffin lung sections were immunostained for pIgR/SC, TGF-β_1, and phospho-Smad 2/3. Paraffin sections of HBEC filters were stained for pIgR/SC, E-cadherin, high and low molecular weight cytokeratins, α-smooth muscle actin, vimentin, fibronectin, MUC5AC, and β-tubulin IV. Quantification of the staining intensity was performed with ImageJ software on tissue sections, stained during a same run and using the same threshold, which was adjusted using negative control and three test sections, as previously described (13). Results were expressed as the percentage of positive/stained area as well as the intensity of the staining (independent of the threshold) within the epithelium (Figures E1A–E1E). In addition, the subcellular apical-to-basal localization of the staining within the epithelium was evaluated.

Western blot for pIgR/SC. HBEC were analyzed for pIgR/SC expression by Western blot as previously described (5), and quantification was performed by using Quantity One software.

ELISA for SC, TGF-β, and fibronectin. SC immunoassay was performed as previously described (5). TGF-β_1 concentration was determined in both untreated (active TGF-β_1) and acidified supernatants (total TGF-β_1) by ELISA according to the manufacturer's instructions, as well as for TGF-β_2 and TGF-β_3. Fibronectin was detected by direct ELISA.

RT-qPCR Analysis for pIgR and TGF-β_1 mRNAs

Total RNA was isolated from lung tissue and was reverse-transcribed. Expression levels of pIgR and TGF-β_1 mRNA were quantified by RT-qPCR and normalized to the geometric mean of three housekeeping genes (RPS18, 18S-RNA, GAPDH), according to a previously described methodology (14). Transcript levels of these genes were stable in a series of lung RNA samples, with an average M value of 0.4413 identified by the geNorm algorithm.

Transcytosis Assay of Dimeric IgA

The transcytosis capacity of the epithelium was assayed in submerged and ALI cultures after incubation with dimeric IgA (added in the basolateral compartment) by quantifying S-IgA after 48 hours in the apical wash by sandwich ELISA. Monomeric IgA was used as control (undetectable S-IgA in the apical wash).

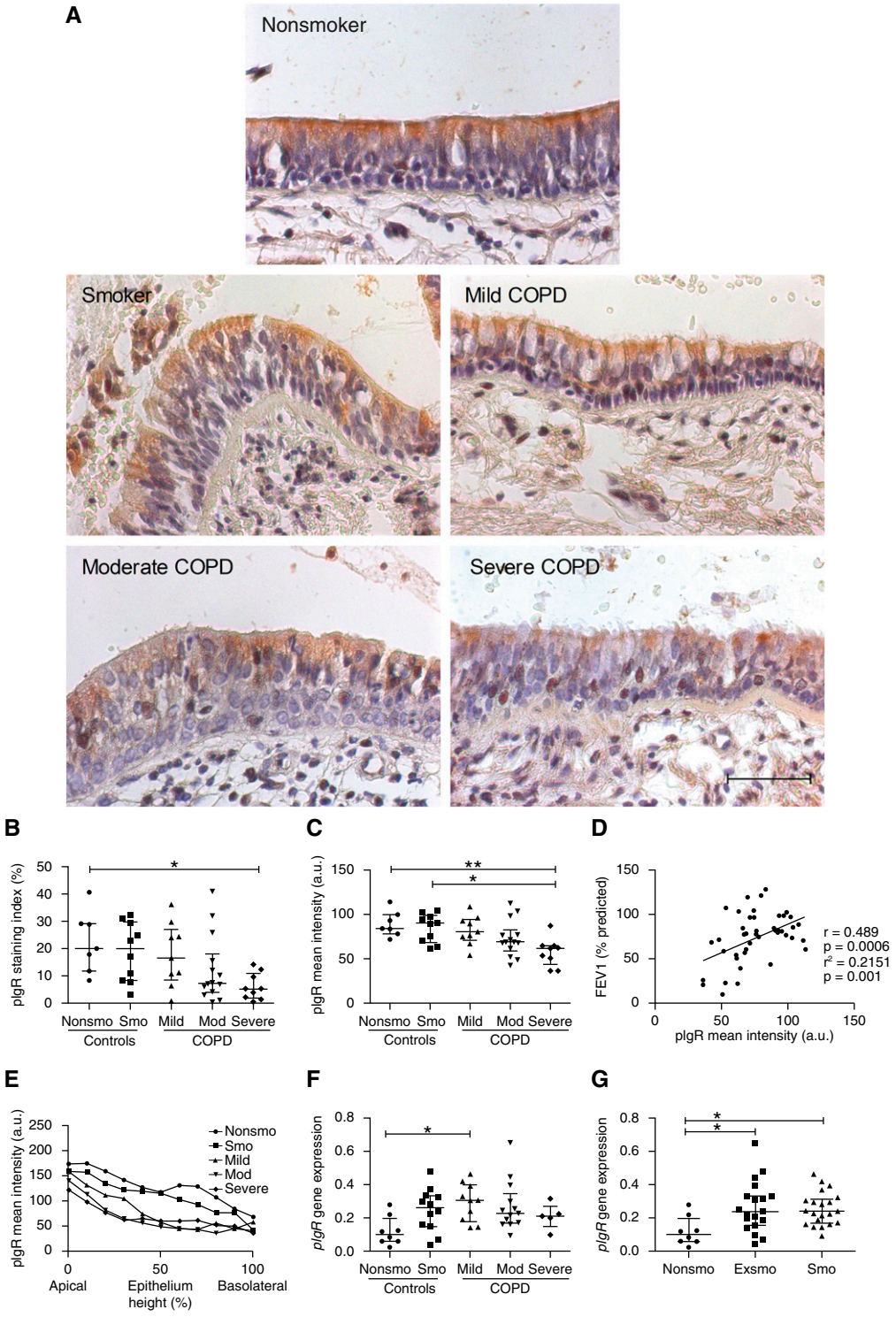

Figure 1. Polymeric immunoglobulin receptor (pIgR) protein content is decreased in large airways of patients with severe chronic obstructive pulmonary disease (COPD). (*A*) pIgR immunostaining is shown in lung tissue from a nonsmoker, a smoker, and patients with mild COPD, moderate COPD, or severe COPD. (*B*) Quantification of the percentage of positive area of pIgR staining in the epithelium using a computer-assisted method (n = 49). (*C*) Quantification of the intensity of pIgR staining in the epithelium (n = 49). (*D*) Correlation between the intensity of the pIgR staining and the FEV_1 (r = 0.489, P = 0.0006; linear regression: r^2 = 0.2151, P = 0.001). (*E*) Distribution of the pIgR staining from the apical to the basolateral side of the epithelium. (*F*, *G*) pIgR mRNA was assayed by real-time quantitative polymerase chain reaction in lung tissue from the same patients, and corrected for the geometric mean of the expression of the three housekeeping genes (n = 48). (*B*) *P = 0.035; (*C*) *P = 0.026, **P = 0.009; (*F*) *P = 0.02; (*G*) *P = 0.018 between nonsmokers and ex-smokers and *P = 0.014 between nonsmokers and smokers (Kruskall-Wallis test followed by Dunn *post hoc* test). Scale bar, 50 μm.

Statistical Analysis

Results were expressed as medians and interquartile ranges, unless otherwise stated. A P value less than 0.05 was considered as statistically significant.

Results

Decreased pIgR Expression in the Bronchial Epithelium from Severe COPD

We first evaluated pIgR immunostaining in our series of lung tissue from COPD and control patients. Expression of pIgR protein was decreased in large airways of patients with severe COPD, in terms of both percentage of stained area ($P = 0.035$) and staining intensity ($P = 0.009$), as compared with nonsmokers, whereas it was not significantly affected in milder stages (Figures 1A–1C). This decrease in pIgR expression was correlated to airflow limitation, as evidenced by reduced FEV_1 ($r = 0.489$, $P = 0.0006$; Figure 1D). The pIgR staining was preferentially localized at the apical pole of the epithelium, with an apical-to-basal gradient, both in control patients and patients with COPD (Figure 1E). Gene expression assessed by RT-qPCR in proximal airways from the same patients showed that pIgR transcription was increased in patients with mild COPD, as compared with nonsmoker control patients (Figure 1F). No pIgR mRNA up-regulation was observed in patients with moderate and severe COPD as compared with nonsmokers. The up-regulating effect of smoking was confirmed by analyzing groups irrespectively of COPD (Figure 1G), whereas the correlation between smoking history and pIgR expression was either very weak, at the mRNA level ($r = 0.318$, $P = 0.04$), or not significant, at the protein level ($r = -0.297$, $P = 0.053$).

These data indicated that decreased content of pIgR protein in the bronchial epithelium from COPD correlates with disease severity, whereas cigarette smoking and COPD disease process seem to exert divergent effects on active *pIgR* gene transcription.

Relation between pIgR/SC Production and Epithelial Cell Differentiation *In Vitro*

We next ascertained whether pIgR/SC expression by the bronchial epithelium reconstituted *in vitro* from proximal airway tissue of patients with COPD could recapitulate the *ex vivo* findings of pIgR down-regulation in this disease.

In the ALI culture model allowing epithelial differentiation (Figure 2A), the production of pIgR/SC was confirmed to be highly polarized to the apical compartment (apical-to-basal ratio ~ 15, $P = 0.0156$) (Figure 2B) and was dramatically increased on ALI culture ($\sim$50-fold, $P = 0.0156$) (Figure 2C). Accordingly, the capacity of HBEC to translocate dimeric IgA from the basolateral to the apical compartment (and generate S-IgA) strongly increased on ALI differentiation ($\sim$100-fold increase as compared with submerged cultures, $P = 0.016$; Figure 2D). This epithelial competence was acquired at 2 weeks and further increased up to 5 weeks of ALI culture both for the apical release of SC and the intracellular content of pIgR/SC (Figures 2E and 2F). Of note, pIgR expression was observed in both ciliated and goblet cells, whereas very weak staining was observed in undifferentiated basal cells (Figure E2).

Decreased pIgR/SC Expression in ALI-HBEC from Patients with COPD

We then compared the acquisition of pIgR/SC expression in ALI-HBEC from patients with COPD versus control patients. We observed that pIgR immunostaining was decreased in ALI-HBEC from patients with moderate and severe COPD as compared with ALI-HBEC from control nonsmokers and smokers, expressed as percent of stained area and mean staining intensity (Figures 3A–3C). In addition, pIgR expression in ALI-HBEC correlated with airflow obstruction in terms of FEV_1/VC ratio and FEV_1 (Figures 3D–3G). The capacity of ALI-HBEC to secrete SC and transcytose IgA was assessed (at 2 wk of ALI culture) in the different groups. SC release by ALI-HBEC was significantly reduced in cultures from severe COPD as compared with nonsmoker control subjects ($P = 0.02$) (Figure 3H), and the IgA-transcytosis capacity also tended to decrease without reaching statistical significance in severe COPD HBEC (Figure 3I). This indicated that down-regulation of pIgR expression observed in lung tissue from COPD persists in the bronchial epithelium reconstituted *in vitro* from such patients.

Effect of CSE on pIgR/SC Expression by ALI-HBEC

Because tissue data showed that pIgR transcription was increased in smokers, irrespectively of COPD, we evaluated whether incubation with CSE (soluble phase) or exposure to cigarette smoke (gaseous phase) could regulate pIgR/SC production in ALI-HBEC. In HBEC treated with CSE or exposed to CS, a decrease in pIgR/SC immunoreactive bands was observed (Figure E3), but this effect did not reach statistical significance after quantification and normalization to glyceraldehyde phosphate dehydrogenase level. In addition, no effect was observed at the mRNA level (Figure E3). These data suggested that the effect of cigarette smoking on epithelial pIgR is mainly indirect.

Up-regulation of Autocrine TGF-β_1 in the COPD Epithelium

We next explored mechanisms of pIgR down-regulation in COPD. Based on the findings linking pIgR expression to ALI-related differentiation of the bronchial epithelium, we wondered whether factors controlling cell differentiation and reactivated during ALI culture could regulate pIgR expression. TGF-β_1 was assessed and was readily detected in HBEC supernatants. TGF-β_1 (total form) was released in increased amounts by ALI-HBEC from patients with COPD ($P = 0.04$; Figure 4A), even though only a trend was observed for the active form (not shown). In these cultures, TGF-β_2 did not significantly differ between groups (Figure 4B), and TGF-β_3 was not detected (not shown).

In tissue, TGF-β_1 immunostaining within the epithelium appeared increased in large airways of patients with COPD expressed both in percentage of positive area and mean staining intensity (Figures 4C–4E), whereas quantification of TGF-β_1 mRNA by RT-qPCR in lung tissue did not show up-regulation in COPD (Figure 4F). In addition, phosphorylation of Smad 2/3 proteins—as functional readout of TGF-β activity—was increased in the bronchial epithelium from patients with severe COPD, as compared with nonsmokers (Figures 4G and 4H).

Down-regulation of pIgR Transcription in ALI-HBEC by TGF-β_1

The potential relationship between TGF-β_1 expression/activity and pIgR down-regulation was then assessed. First, incubating ALI-HBEC for 48 hours with TGF-β_1 decreased pIgR cellular immunoreactivity (Figure E4). In parallel,

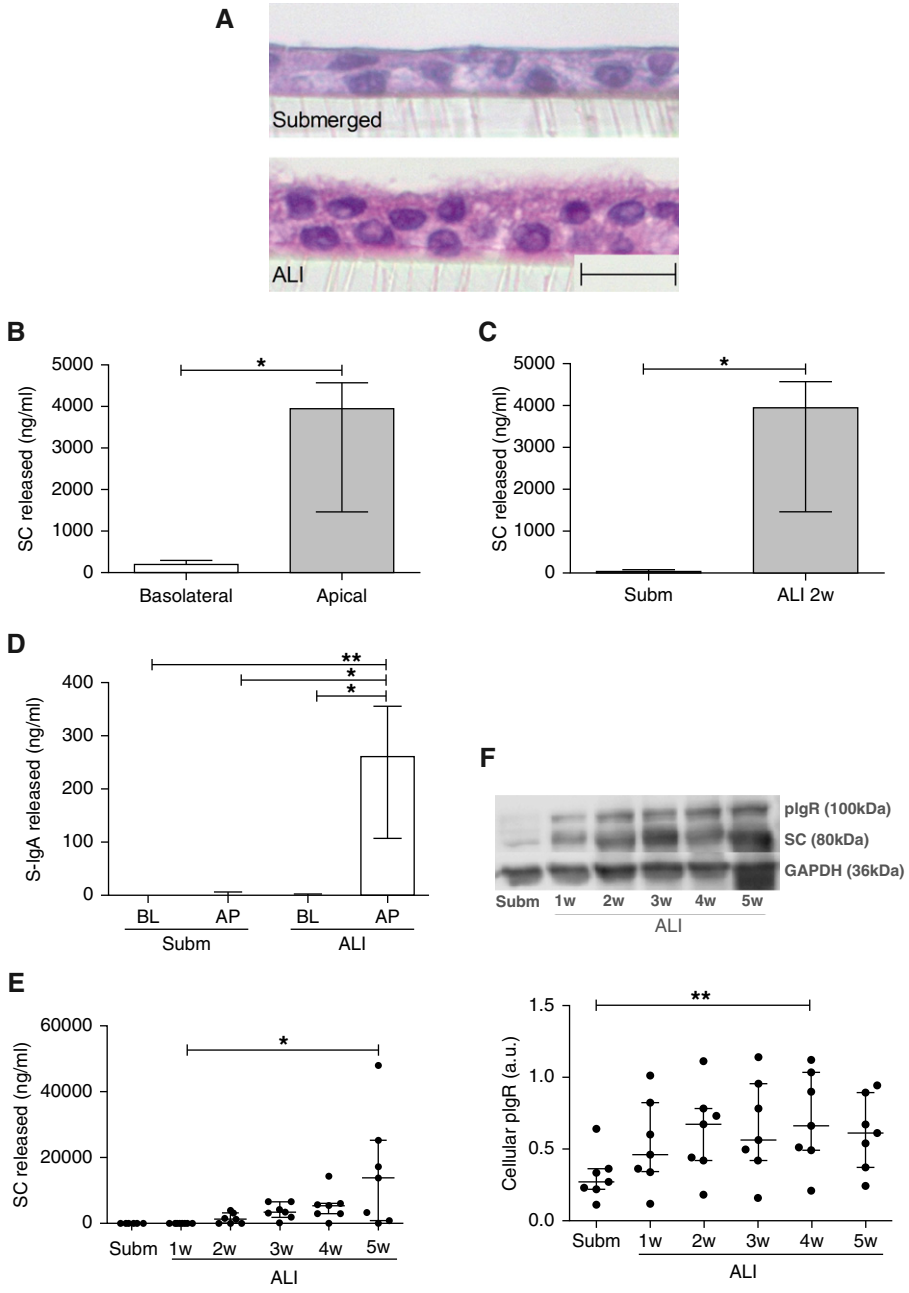

Figure 2. Polymeric immunoglobulin receptor (pIgR) expression is polarized and depends on air–liquid interface (ALI)-induced differentiation. (*A*) Microphotographs of human bronchoepithelial cells (HBEC) in submerged (*upper panel*) and ALI culture conditions (*lower panel*). (*B*) Secretory component (SC) production in the apical versus basolateral compartment in ALI-HBEC assessed by ELISA (n = 7). (*C*) SC released by HBEC in submerged versus ALI condition (n = 7). (*D*) Secretory IgA (S-IgA) released by HBEC in submerged versus ALI conditions, in apical and basolateral compartments (n = 8). (*E*) SC production in the apical compartment during 1 to 5 weeks of ALI culture (n = 7). (*F*) Immunoblot of pIgR expression during 1 to 5 weeks of ALI culture and quantification of intracellular content of pIgR protein during 5 weeks of ALI culture (n = 7). (*B*, *C*) **P* = 0.0156 (Wilcoxon rank test). (*D*) **P* = 0.04 between apical and basolateral supernatants in ALI conditions, **P* = 0.016 between apical supernatants in ALI versus submerged conditions, ***P* = 0.002 (Friedman test). (*E*) **P* = 0.032 (Friedman test). (*F*) ***P* = 0.009 (Friedman test). Scale bar, 20 μm.

in these conditions, a "dedifferentiation" of the bronchial epithelium was indicated by decreased expression of E-cadherin and cytokeratins and by induction of mesenchymal markers (vimentin, fibronectin) (Figure E4).

A dose–response decrease in apical SC release was confirmed in ALI-HBEC (from control patients) on TGF-β_1 treatment, in contrast to IFN-γ used as positive control (Figure 5A). This effect correlated with a dose-dependent decrease in pIgR mRNA (Figure 5B) and resulted in a reduced capacity to transcytose d-IgA and to generate S-IgA (Figure 5C).

To evaluate the relationship between TGF-β–driven pIgR down-regulation and epithelial dedifferentiation features, time-course experiments were performed. The kinetics of pIgR down-regulation on TGF-β_1 treatment was closely parallel to mesenchymal differentiation indicated by vimentin expression and fibronectin release, both affected from ∼ 24 hours of incubation (Figures 5D–5G). Finally, blocking TGF-β_1 in HBEC (from various donors) during ALI differentiation up-regulated pIgR expression (Figures 6A–6C). This effect of the blocking anti–TGF-β Ab on pIgR expression was dose dependent (maximal at 10 μg/ml), as shown for SC release, pIgR mRNA expression, and cellular pIgR content (Figures 6D–6G).

Discussion

Our study explored in lung tissue and bronchoepithelial cultures prospectively obtained from well-characterized patients with or without COPD and control subjects (e.g., for smoking) whether pIgR down-regulation in COPD relates to disease severity and, mechanistically, to epithelial dedifferentiation. The two major findings are first that pIgR down-regulation at the protein level correlates in COPD to airflow limitation and second that increased expression of TGF-β_1 by the COPD epithelium could recapitulate pIgR down-regulation, in addition to altering epithelial differentiation. Moreover, we also show that smoking *per se* stimulates pIgR transcription and, more importantly, that the abnormal reprogramming of the bronchial epithelium in COPD (including both up-regulated TGF-β_1, decreased pIgR, and IgA transcytosis) may persist *in vitro* after reconstitution on ALI. In the lung, pIgR down-regulation has been observed in COPD (4, 7) and in non-small cell lung cancer (15). Epithelial pIgR expression is also decreased in the upper airways from patients with chronic rhinosinusitis with nasal polyps and allergic rhinitis (16).

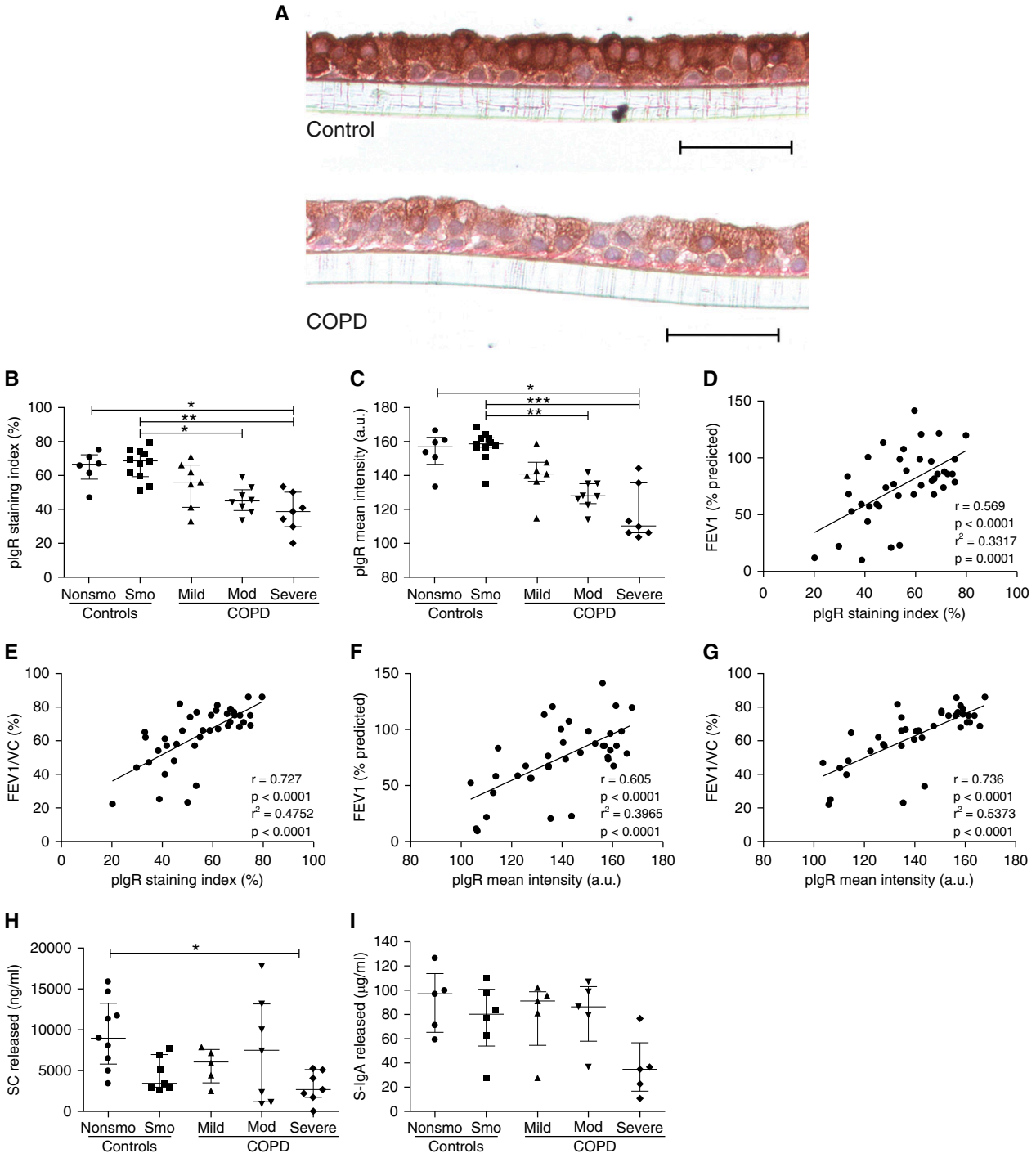

Figure 3. Polymeric immunoglobulin receptor (pIgR) expression and IgA transcytosis by the bronchial epithelium is decreased in human bronchoepithelial cells (HBEC) from patients with severe chronic obstructive pulmonary disease (COPD). (*A*) Immunostaining for pIgR in control HBEC from a nonsmoker patient (*upper panel*) and from a patient with severe COPD (*lower panel*). (*B*) Quantification of the percentage of positive area of pIgR staining in the epithelium using a computer-assisted method (n = 39). (*C*) Quantification of the intensity of pIgR staining in the epithelium using a computer-assisted method (n = 39). (*D*) Correlation between percentage of positive area of pIgR staining and FEV_1 ($r = 0.569$, $P < 0.0001$; linear regression: $r^2 = 0.3317$, $P = 0.0001$). (*E*) Correlation between percentage of positive area of pIgR staining and FEV_1/VC ratio ($r = 0.727$, $P < 0.0001$; linear regression: r^2=0.4752, $P < 0.0001$). (*F*) Correlation between pIgR staining intensity and FEV_1 ($r = 0.605$, $P < 0.0001$; linear regression: $r^2 = 0.3965$, $P < 0.0001$). (*G*) Correlation between pIgR staining intensity and FEV_1/VC ratio ($r = 0.736$, $P < 0.0001$; linear regression: $r^2 = 0.5373$, $P < 0.0001$). (*H*) Secretory component (SC) release in apical compartment in HBEC from control subjects and patients with COPD (n = 35). (*I*) Air–liquid interface (ALI) epithelium was assessed for its capacity to transcytose d-IgA and to generate secretory IgA (S-IgA) in the apical compartment (n = 26). (*B*) *$P = 0.01$ between smokers and moderate COPD, **$P = 0.001$, *$P = 0.021$ between nonsmokers and severe COPD; (*C*) **$P = 0.005$, ***$P < 0.0001$, *$P = 0.013$; (*H*) *$P = 0.021$ (Kruskall-Wallis test followed by Dunn *post hoc* test). Scale bar, 50 µm.

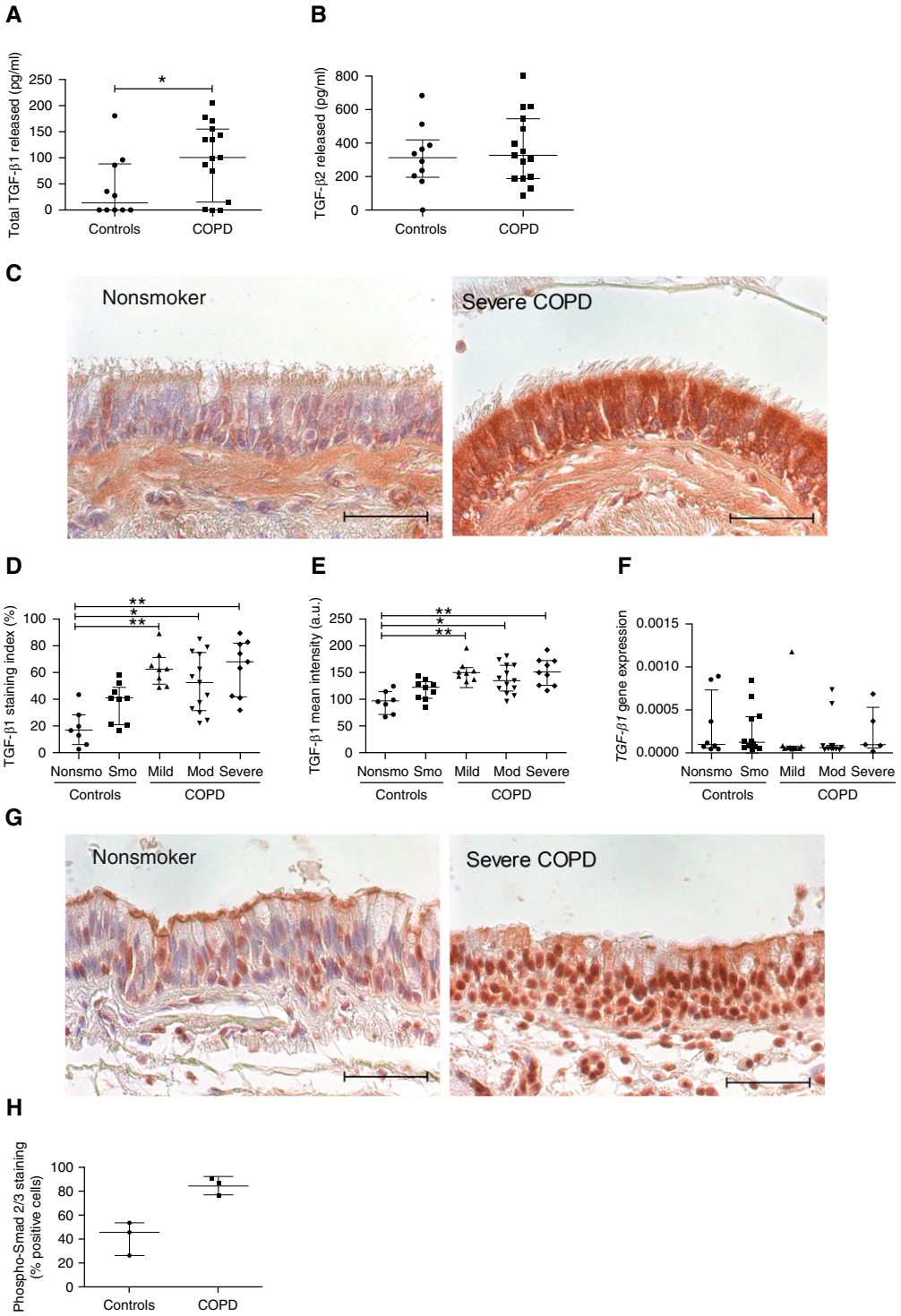

Figure 4. Up-regulation of transforming growth factor (TGF)-β_1 release by the chronic obstructive pulmonary disease (COPD) bronchial epithelium. (*A*) Constitutive total TGF-β_1 release by air–liquid interface (ALI)-human bronchoepithelial cells (HBEC) from control subjects and patients with COPD (n = 25). (*B*) TGF-β_2 release by ALI-HBEC from control subjects and patients with COPD (n = 25). (*C*) Representative immunohistochemistry (IHC) for TGF-β_1 in lung tissue from a nonsmoker and a patient with severe COPD. (*D*) Quantification of the percentage of positive area of TGF-β_1 staining in the epithelium (n = 46). (*E*) Quantification of the TGF-β_1 staining intensity in the epithelium (n = 46). (*F*) Expression of TGF-β_1 mRNA by real-time quantitative polymerase chain reaction in lung tissue from control subjects and patients with COPD, as corrected for the geometric mean of the three housekeeping gene expression (n = 48). (*G*) Representative immunohistochemistry for phospho-Smad 2/3 from nonsmokers and patients with severe COPD. (*H*) Quantification of the percentage of phospho-Smad 2/3–positive cells in the epithelium (n = 6). (*A*) *P = 0.04 (Mann-Whitney *U* test). (*D*) **P = 0.002 between nonsmokers and mild COPD, *P = 0.03, **P = 0.003 between nonsmokers and severe COPD (Kruskall-Wallis test followed by Dunn *post hoc* test). (*E*) **P = 0.002 between nonsmokers and mild COPD, *P = 0.04, **P = 0.003 between nonsmokers and severe COPD (Kruskall-Wallis test followed by Dunn *post hoc* test). Scale bar, 50 μm.

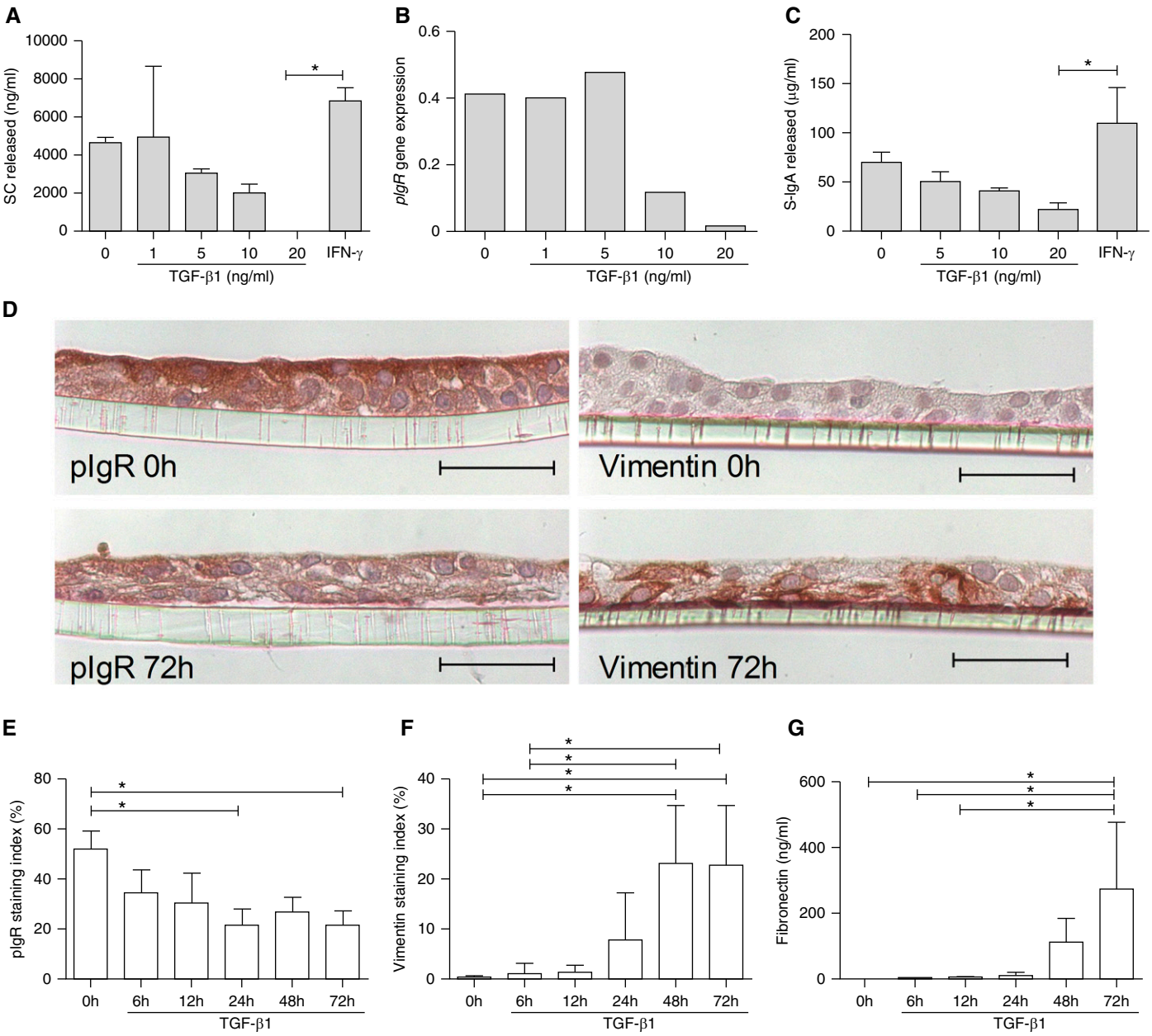

Figure 5. Down-regulation of polymeric immunoglobulin receptor (pIgR) expression and IgA transcytosis by TGF-β_1 (data are means ± SD). (*A*) Secretory component (SC) release was assessed in human bronchoepithelial cells (HBEC) treated by transforming growth factor (TGF)-β_1 (n = 3, with each condition in triplicates). (*B*) pIgR mRNA synthesis assessed by real-time polymerase chain reaction in HBEC treated by TGF-β_1 (n = 1). (*C*) secretory IgA (S-IgA) release was assessed in HBEC (from control patients) preincubated with increasing concentrations of TGF-β_1 or with 20 ng/ml of IFN-γ; (n = 3, with each condition in triplicates). (*D*) Kinetics of the effect of TGF-β_1 on pIgR and vimentin expression assessed by immunohistochemistry. Air–liquid interface (ALI)-HBEC were incubated with 10 ng/ml of TGF-β_1. (*E*) Quantification of the pIgR staining using computer-assisted method (n = 5). (*F*) Quantification of the vimentin staining (n = 5). (*G*) Release of fibronectin in basolateral medium (n = 5). (*A*) *P = 0.034. (*C*) *P = 0.018. (*E*) *P = 0.035 between 0 h and 24 h, *P = 0.02 between 0 h and 72 h. (*F*) *P = 0.02 between 0 h and 48 h, *P = 0.011 between 0 h and 72 h, *P = 0.046 between 6 h and 48 h, *P = 0.026 between 6 h and 72 h. (*G*) *P = 0.046 between 12 h and 72 h, *P = 0.026 between 6 h and 72 h, *P = 0.015 between 0 h and 72 h (Friedman test). Scale bar, 50 µm.

Although a correlation between pIgR immunostaining and airflow limitation has been observed in a narrow range of FEV_1 values, our previous study (4) could not properly address whether pIgR reduction was associated with disease severity, which is confirmed in the present study.

Importantly, pIgR expression is increased in smokers at the gene transcription level. These data are in line with a proteomic study reporting increased pIgR protein expression in sputum from smokers (17). However, we could not find increased immunoreactivity, at the protein level, within the bronchial epithelium from smokers. This discrepancy between protein and gene expression levels could relate to post-transcriptional mechanisms, such as degradation by neutrophil-derived proteinases (5), which could explain the absence of increased pIgR protein in

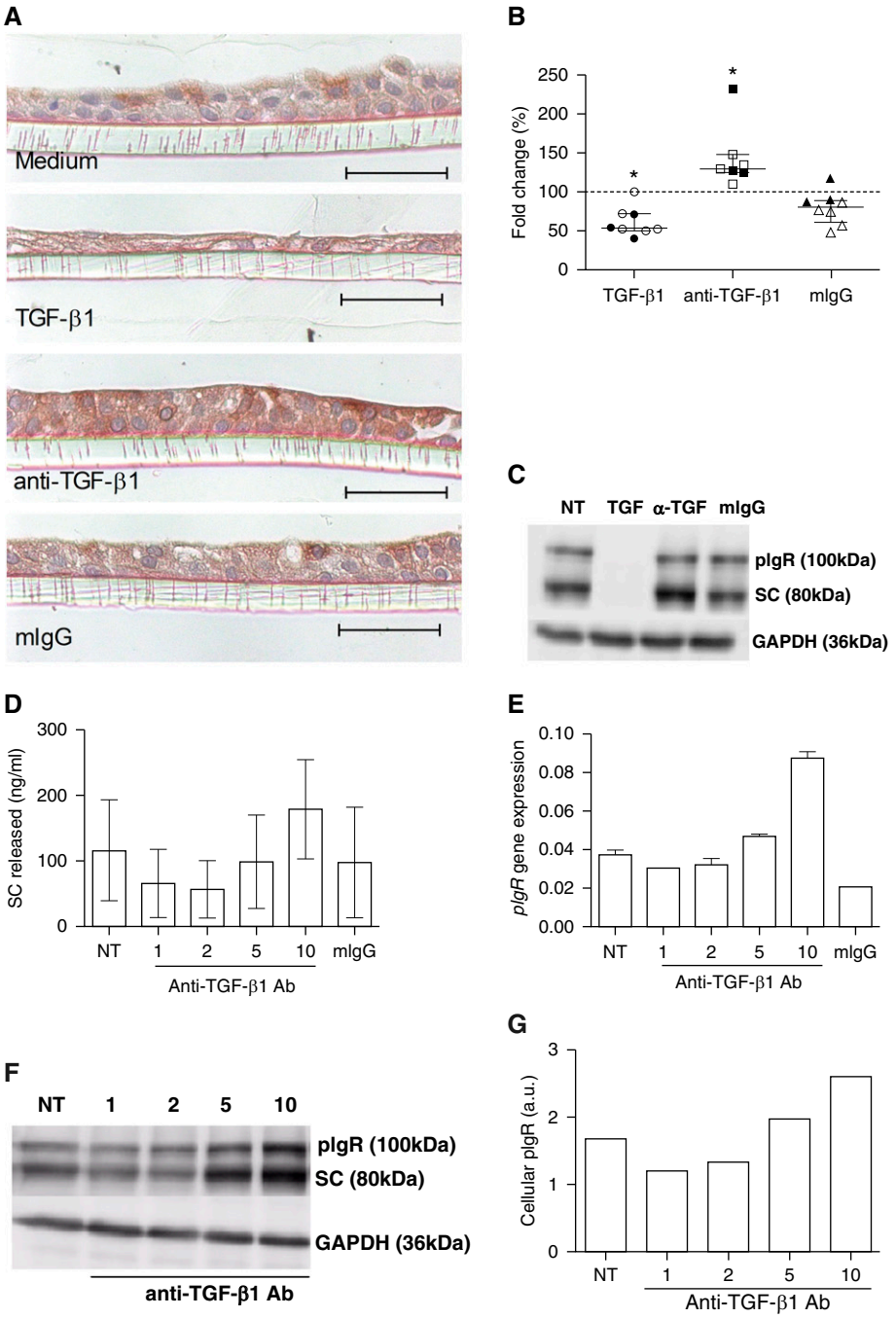

Figure 6. (*A*) Polymeric immunoglobulin receptor (pIgR) expression assessed by immunohistochemistry in blocking experiments using anti–transforming growth factor (TGF)-β_1 antibodies (Ab) (10 μg/ml) during the 2 weeks of air–liquid interface (ALI) differentiation, as compared with control mouse IgG (10 μg/ml) and to the effect of TGF-β_1 (10 ng/ml). (*B*) Quantification of pIgR staining using computer-assisted method (n = 8, including 5 control and 3 donors with chronic obstructive pulmonary disease [COPD] as *white* and *black dots*, respectively). (*C*) Cellular pIgR/secretory component (SC) content on TGF-β_1 blockade (one blot shown, representative of six experiments). (*D*) SC release was assessed in human bronchoepithelial cells (HBEC) incubated with increasing concentrations of anti–TGF-β_1 Ab (n = 2, with each condition in triplicates). (*E*) pIgR mRNA synthesis assessed by real-time polymerase chain reaction incubated with increasing concentrations of anti–TGF-β_1 Ab (n = 2, data are mean ± SD). (*F*) Cellular pIgR/SC content on TGF-β_1 blockade (one blot shown, representative of two experiments). (*G*) Quantification of the cellular content of pIgR protein on TGF-β_1 blockade. (*B*) **P* = 0.016 compared with medium (Wilcoxon rank test). Scale bar, 50 μm. NT = nontreated.

smokers and subjects with mild COPD despite up-regulated gene transcription. Alternatively, it could relate to the production of a modified pIgR/SC protein (e.g., via oxidative stress) with reduced immunoreactivity. In addition, *in vitro* we could not clearly observe a direct effect of cigarette smoke on pIgR expression; a trend for reduction was rather observed, in line

with a previous study reporting on pIgR/SC down-regulation in HBEC exposed to cigarette smoke for short time periods (18), but did not reach statistical significance. Overall, it could be suggested that cigarette smoke may have a dual effect on epithelial pIgR/SC, namely acute pIgR/SC down-regulation (18), and on the long-term (*in vivo*) stimulation of pIgR transcription, possibly via induction of inflammatory factors such as TNF-α or IFN-γ, which are up-regulated in COPD and able to promote inducible pIgR transcription (8). Importantly, this protective mechanism seems partly lost in the bronchial epithelium from patients with COPD, potentially as a result of TGF-β activation, which exerts an opposite action on pIgR transcription.

TGF-β represents a major signaling component of developmental, inflammatory, and remodelling pathways in the lung (19, 20). In lung tissue, our data are consistent with previous studies, which showed up-regulated TGF-β_1 synthesis within the small bronchial epithelium (21–24) as well as in submerged epithelial cultures from patients with COPD (23, 24). TGF-β_1 up-regulation in COPD was confirmed in gene microarray studies (25, 26). Here we show that up-regulated TGF-β_1 production by the COPD bronchial epithelium persists after *in vitro* reconstitution on ALI condition. This concerned specifically TGF-β_1, as TGF-β_2 was unchanged, and TGF-β_3 was not detected. Interestingly, increased TGF-β_1 release by COPD HBEC was associated with a decreased basal level of IgA transcytosis by HBEC from these patients. In addition, exogenous TGF-β_1 was able to dose-dependently reduce the capacity of the epithelium to transcytose polymeric IgA. This inhibitory effect of TGF-β on pIgR-mediated IgA transport was confirmed at the levels of pIgR/SC protein and mRNA. In contrast, in Calu-3 cells, we observed that TGF-β_1 up-regulated pIgR/SC production (6), suggesting differences in TGF-β signaling in this cell line as compared with primary cells. Moreover, we could also confirm (27) that ALI-HBEC undergo dedifferentiation on treatment by TGF-β_1, as indicated by decreased E-cadherin expression and induction of vimentin expression and fibronectin release. In contrast, Calu-3 cells seem to be resistant to epithelial to mesenchymal transition (28). In addition, similar kinetics were observed for TGF-β–driven pIgR down-regulation and induction of mesenchymal features, and blocking TGF-β activity during ALI differentiation could dose-dependently up-regulate epithelial pIgR expression. These findings indicate a link between up-regulated TGF-β activity and epithelial pIgR down-regulation, at least *in vitro*. Thus, this link between pIgR expression and TGF-β signaling should be integrated *in vivo* to several other pathways, which are activated in COPD at the level of the airway epithelium and which could either directly affect pIgR expression or cross-regulate TGF-β signaling (29).

The finding that the bronchial epithelium reconstituted *in vitro* on ALI condition may retain certain aberrant features observed in airway tissues from patients with COPD is of great interest. Previous studies reported such "memory" of aberrant functions of epithelial cells derived and cultured from lung tissue of patients with COPD or asthma, notably for antiviral immunity (30), oxidative response genes (31, 32), production of IL-8/CXCL8, mucus (33), or profibrotic factors (34). The latter study showed increased TGF-β_2, as well as vascular endothelial growth factor and periostin by ALI-HBEC from children with atopic asthma, further indicating that different chronic airway diseases are associated with activation of distinct remodeling pathways within the epithelium. As pIgR down-regulation is also observed in non-small cell lung cancer (15), it might be suggested that signaling pathways operating in COPD and lung cancer, which share some features at the level of epithelial changes, notably result in pIgR down-regulation. Importantly, TGF-β_1 expression is induced in mice very rapidly after smoke exposure, before inflammation is elicited, and becomes sustained on repeated exposure (35, 36). Our data support this concept by showing that the bronchial epithelium from smokers with COPD retains *in vitro* up-regulated TGF-β_1 expression. It remains to be elucidated whether changes in the expression of pIgR or TGF-β relate to genetic polymorphisms (37, 38) or to epigenetic reprogramming (such as DNA methylation) of the respiratory epithelium, in the context of ongoing inflammatory and oxidative reactions (39) in the COPD lung. A compartmentalized role for TGF-β has been reported in COPD (i.e., with gain-of-function in small airway disease and reduced signaling in emphysema) (36), but targeting TGF-β_1 has been shown to improve both airway and alveolar remodelling in cigarette smoke–induced experimental COPD (40).

Several lines of evidence suggest that decreased pIgR content in COPD may result in impaired mucosal defense against inhaled materials and pathogens. First, selective IgA deficiency has been previously associated with COPD (41). Second, several studies showed that IgA may limit the adhesion of pathogens to the respiratory epithelium (3) and may interfere with the assembly of respiratory viruses within the airway epithelium, potentially underlying the observation of the selective presence of EBV and CMV viruses in airways with reduced S-IgA content (7). Third, a higher colonization by *Haemophilus influenzae* has been associated in COPD with the production of IgA1 proteases (42) and with reduced sputum levels of pathogen-specific IgA antibodies (43), further supporting a role for IgA in COPD.

Altogether, our data indicate that cigarette smoking *per se* up-regulates pIgR transcription, probably through indirect mechanisms related to smoke-induced airway inflammation. In contrast, the bronchial epithelium from smokers with COPD displays down-regulated pIgR/SC, which correlates with disease severity. In addition, this defect persists *in vitro* on redifferentiation of the epithelium, as a result at least in part of increased TGF-β_1 activity. This epithelial reprogramming by TGF-β, which links defense and repair signaling pathways, could be involved in the disruption of IgA-mediated mucosal defense in the lung from patients with COPD. ■

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