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Transforming growth factor-β_1 is a potent inhibitor of secretory leukoprotease inhibitor expression in a bronchial epithelial cell line

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Transforming growth factor-β_1 is a potent inhibitor of secretory leukoprotease inhibitor expression in a bronchial epithelial cell line. F. Jaumann, A. Elssner, G. Mazur, S. Dobmann, C. Vogelmeier, for the Munich Lung Transplant Group. ©ERS Journals Ltd 2000.

ABSTRACT: Obliterative bronchiolitis (OB) is the major long-term complication following lung and heart-lung transplantation. In bronchoalveolar lavage fluid samples obtained from patients suffering from OB, a marked increase in the number of neutrophils and elevated expression of transforming growth factor (TGF)-β_1 had been found. The goal of the study was to evaluate whether TGF-β_1 is capable of interfering with the expression of the secretory leukoprotease inhibitor (SLPI), the dominating defence of the conducting airways against neutrophil elastase (NE).

The authors analysed the effects of TGF-β_1 on gene expression and protein release of SLPI by cultured human bronchial epithelial (BEAS-2B) cells. SLPI protein levels in the supernatants were quantified with a specific enzyme-linked immunosorbent assay; SLPI messenger ribonucleic acid (mRNA) levels were measured by reverse transcriptase polymerase chain reaction.

Incubation with TGF-β_1 induced a marked decrease in SLPI protein levels (1 ng·mL^{-1} TGF-β_1: stimulation index (SI; protein: relation to SLPI protein release of resting cells) = 0.56; 10 ng·mL^{-1} TGF-β_1: SI = 0.48; 50 ng·mL^{-1} TGF-β_1: SI = 0.37, $p<0.01$ each) and mRNA expression (1 ng·mL^{-1} TGF-β_1: SI (SI mRNA: relation to SLPI mRNA expression of resting cells) = 0.46; 10 ng·mL^{-1} TGF-β_1: SI= 0.31; 50 ng·mL^{-1} TGF-β_1: SI=0.18, $p<0.01$ each) in a dose dependent fashion. Simultaneous incubation of BEAS-2B cells with TGF-β_1 and NE also caused a significant reduction in SLPI synthesis (10 ng·mL^{-1} TGF-β_1 + 7.5 U·mL^{-1} NE: mRNA SI = 0.61, $p<0.05$; protein SI = 0.65, $p<0.05$; 50 ng·mL^{-1} TGF-β_1 + 7.5 U·mL^{-1} NE: mRNA SI = 0.52, $p<0.05$; protein SI = 0.58, $p<0.05$; 10 ng·mL^{-1} TGF-β_1: mRNA SI = 0.33, $p<0.01$; protein SI = 0.38, $p<0.01$).

In conclusion, the data suggest that the coincidence of neutrophilia and upregulation of transforming growth factor-β_1 in obliterative bronchiolitis may lead to uninhibited neutrophil elastase activity by downregulation of secretory leukoprotease inhibitor, with the consequence of ongoing injury to the epithelium.

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The transforming growth factor (TGF)-β family is composed of a group of structurally related multifunctional polypeptides that cause multiple effects on various cell types [1]. The TGF-βs are able to act as negative and positive regulators of cell growth and induce differentiation, transformation, tissue repair, fibrosis and inflammatory responses [2–7]. Apart from this pathophysiological role of TGF-β in several fibrotic lung diseases [4–8], recent reports emphasize that alveolar macrophages and bronchoalveolar lavage fluid (BALF) cells obtained from patients with obliterative bronchiolitis (OB) after lung transplantation show an increased expression of TGF-β compared to patients free from OB [9, 10]. OB is the most important complication in the long-term follow-up after lung and heart-lung transplantation, limiting life expectancy and quality of life [11]. While OB is considered to represent chronic graft rejection the exact cause is unknown.

Histopathologically, OB is characterized by inflammation in the early stages, followed by fibrosis of the lamina propria with the consequence of obliteration of the lumen [12, 13]. Another characteristic of OB is a significant neutrophilia detectable in BALF samples of affected patients [12–14]. Although the neutrophils that accumulate on the airway epithelial surface may aid in the clearance of micro-organisms, they have the potential to damage the epithelium. Therefore a causal relationship between airway neutrophilia and development of OB has been hypothesized [12]. Besides the release of reactive oxygen species, the potential harm of neutrophils is mainly mediated by neutrophil elastase (NE) [15, 16]. The proteolytic activity of NE is physiologically counterbalanced by several antiproteases. The major antiprotease in the conducting airways is secretory leukoprotease inhibitor (SLPI), a 12-kDa nonglycosylated serine antiprotease [17–19].

The aim of the study was to evaluate whether TGF-β_1 is capable of exerting regulatory effects on SLPI messenger ribonucleic acid (mRNA) expression and protein release in human bronchial epithelial cells, thereby potentially

affecting the defence of the epithelium against NE. The cell line used, BEAS-2B, is a simian vacuolating virus-40 transformed bronchial epithelial cell line established from healthy lung epithelium [20]. BEAS-2B shows and maintains typical epithelial cell morphology and function [21]. Therefore, BEAS-2B cells serve as an established model to investigate the pathogenesis of epithelial cell injury in airway diseases [22].

Materials and methods

Cell culture and stimulation

BEAS-2B cells (a gift from A. Gillissen, Bonn, Germany) were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Biochrom, Berlin, Germany) with 10% foetal calf serum (FCS; GIBCO, Eggenstein, Germany), streptomycin and penicillin. Cultures were grown in tissue culture flasks in a humidified gas environment with 95% air and 5% carbon dioxide at 37°C. After 80% confluence had been reached, the cells were seeded into six-well plates at a density of 1×10^5 cells·well^{-1} to grow again to 80% confluence. This was followed by stimulation with: 1) TGF-β_1 (R&D Systems, Minneapolis, MN, USA; 1–50 ng·mL^{-1}); 2) anti-TGF-β_1 neutralizing polyclonal antibody (R&D Systems; 800 ng·mL^{-1}); 3) NE (Elastin Products, Owensville, MO, USA; 1–10 U·mL^{-1}, 16,000U·mg protein^{-1}); and combinations of 1–3. The concentrations of TGF-β_1 were adapted to the concentrations found in the epithelial ling fluid (ELF) of the patients after lung transplantation (TGF-β_1 (ELISA) Kit; DRG, Marburg, Germany): mean±SEM: 3.9± 0.9 ng·mL ELF^{-1}; range: 0.0–21.5 ng·mL ELF^{-1} (n=33). The medium used was RPMI 1640 with 1% FCS; the incubation time 24 h. TGF-β_1 and anti-TGF-β_1 antibody had been coincubated for 1 h at 37°C before they were added to the cells. As a control, cells were incubated in fresh RPMI/FCS for the duration of the experiment. Following incubation, supernatants were obtained, centrifuged at 1,800×g and stored at -80°C. Cells were centrifuged at 289×g and also stored at -80°C.

Each condition was tested by a single incubation, and the experiment was performed on three separate occasions using cultures performed on separate days. The data are presented as the mean of three separate experiments.

Ribonucleic acid extraction

Frozen epithelial cells were lysed in ice cold TRIZOL™ reagent (GIBCO). Total ribonucleic acid (RNA) was extracted according to the methods recommended by the manufacturer and redissolved in water. Total RNA yield was calculated by measuring the absorbance (A) at 260 and 280 nm (assuming that A260 of 1 = 40 μg RNA). RNA purity was judged by determining the ratio of A260/A280. Only probes with a A260/A280 ratio from 1.6 to 2.0 were used for the following experiments.

First strand complementary deoxyribonucleic acid synthesis by reverse transcription

Firstly, 1.5 μg RNA in 12 μL ribonuclease (RNase)-free water and 1 μL of oligodeoxythymidine 12–18 (50 ng·μL^{-1}) were preheated to 70°C for 10 min and chilled on ice for 1 min. The RNA was reverse transcribed in RNase-free buffer containing 20 mM Tris-HCl pH 8.4; 50 mM KCl; 2.5mM $MgCl_2$; 500 μM deoxyribonucleoside triphosphate (dNTP) and 10 μM dithiothreitol (DTT). After 5 min preincubation at 42°C, 200 U Superscript II reverse transcriptase (GIB-CO) was added and reverse transcription (RT) for first strand complementary deoxyribonucleic acid (cDNA) synthesis was carried out for 50 min at 42°C. Reaction was terminated at 70°C for 10 min, followed by 1 min of chilling on ice. RNase H (GUBCO; 2 U) was added and followed by incubation for 20 min at 37°C in order to digest the mRNA strand of the formed mRNA/deoxyribonucleic acid (DNA) heteroduplex. The first strand cDNA was stored at -80°C.

Semiquantitative polymerase chain reaction

One microlite of cDNA was used per polymerase chain reaction (PCR). Primer sets used for the amplification of SLPI and reduced glyceraldehyde phosphate dehydrogenase (GAPDH) were: 1) GAPDH: forward: 5′-TGA AGG TCG GAG TCA ACG GAT TTG-GT-3′ reverse: 5′-CAT GTG GGC CAT GAG GTC CAC CAC-3′; size of PCR product: 900 base pairs (bp); 2) SLPI: forward: 5′-TGG AGG GCT CTG GAA AGT CCT TCA-3′; reverse: 5′-CTC CTC CAT ATG GCA GGA ATC AAG-3′; size of PCR product: 350 bp. Each 50-μL reaction mixture consisted of 5 μL 10×PCR buffer, 3 μL $MgCl_2$ (~1.5 mM), 1 μL 10 mM dNTP mix, 1 μL specific primer for GAPDH and SLPI (synthesized by MWG-Biotech, Ebersberg, Germany; ~10 μM), 0.5 μL *Taq* DNA polymerase (GIBCO; ~2 U) and 37.5 μL H_2O. The cycles (RoboCycler Gradient 40 with hot top; Stratagene, Heidelberg, Germany) used were: GAPDH: 94°C for 3 min / 94°C for 45 s / 60°C for 45 s / 72°C for 1 min for 22 cycles, followed by an extension step of 10 min at 72°C. The same cycle conditions were used for SLPI. The annealing temperature for SLPI was 68°C and PCR was run for 35 cycles. Products were electrophoresed on a 1% agarose gel and viewed on a 300-nm ultraviolet (UV) transilluminator (Cybertech, Berlin, Germany). Samples from RT reactions that did not contain reverse transcriptase were used as negative controls.

For quantification, PCR bands were stained with ethidium bromide (Sigma; Munich, Germany) and scanned by using a UV densitometer (Cybertech, Berlin, Germany). The SLPI signals were normalized in relation to their corresponding GAPDH signal from the same RNA, and expressed as an SLPI/GAPDH ratio. The data of the stimulation experiments are expressed as stimulation index (SI) in relation to SLPI mRNA expression of the resting cells. To confirm that correct PCR products had been amplified the gel bands were excised from the gel and sequenced (TopLab, Martinsried, Germany).

Secretory leukoprotease inhibitor enzyme-linked immunosorbent assay

A sandwich ELISA was established for the quantification of SLPI protein. A polyclonal goat anti-SLPI antibody (a gift from P. Birrer, University of Basle, Basle, Switzerland) was used as catcher. The second antibody was a polyclonal rabbit-anti-SLPI antibody (generated in laboratories at the University of Munich, Germany). An antirabbit/peroxidase (POD)-conjugate (Boehringer, Mannheim,

Germany) served as the third antibody. In the SLPI-ELISA recombinant SLPI (rSLPI; Synergen, Boulder, CO, USA) served as standard. After coating the plates at 37°C for 2 h with the first antibody in 50 mM $NaHCO_3$ (pH 9.6), the remaining protein binding sites were quenched (1 h, 37°C) with the blocking buffer (phosphate-buffered saline, 2% gelatin hydrolysate; Boehringer). Samples and standards (100 µL) were added to the wells and incubated overnight at 4°C. The second antibody was added (2 h, 37°C), followed by incubation with the POD-conjugated antibody (1 h, 37°C). Finally, 100 µL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate (Dako Corporation, Carpinteria, CA, USA) was added. The reaction was stopped with 3N H_2SO_4 after 15 min. The resulting absorbance was read at 450 nm in an automated ELISA reader (Eflab, Helsinki, Finland). All samples were run in duplicate. The lower limit of detection was 6 pg·mL^{-1} SLPI.

As with SLPI-mRNA, the protein levels are expressed as an SI: SLPI release by stimulated cells in relation to SLPI release by resting cells.

Statistics

Data are generally expressed as mean±SEM. For comparisons a paired t-test and correlation analysis according to Pearson were used. A p-value ≤0.05 was considered significant.

Results

Effects of transforming growth factor-β_1

All evaluated TGF-β_1 concentrations induced a reduction in SLPI transcript levels in a dose-dependent fashion: 1 ng·mL^{-1} TGF-β_1: SI=0.46; 10 ng·mL^{-1} TGF-β_1: SI=0.31; 50 ng·mL^{-1} TGF-β_1: SI=0.18. In parallel, the concentrations of SLPI protein in the supernatants diminished: 1 ng·mL^{-1} TGF-β_1: SI=0.56; 10 ng·mL^{-1} TGF-β_1: SI=0.48; 50 ng·mL^{-1} TGF-β_1: SI=0.37 (the data are presented as mean of three separate experiments, p<0.01 for each comparison to control; fig. 1). In contrast, following coincubation of TGF-β_1 with a neutralizing polyclonal antibody specific for TGF-β_1, the SLPI mRNA expression and protein release did not show any changes compared to control.

Effects of neutrophil elastase

Incubation of BEAS-2B cells with NE resulted in a dose-dependent increase in SLPI transcript levels: 1 U·mL^{-1} NE: SI=1.07; 2.5 U·mL^{-1} NE: SI=1.18; 5 U·mL^{-1} NE: SI=1.33; 7.5 U·mL^{-1} NE: SI=1.89; 10 U·mL^{-1} NE: SI= 2.42. This was paralleled by a similar rise of SLPI protein levels: 1 U·mL^{-1} NE: SI=1.10; 2.5 U·mL^{-1} NE: SI=1.20; 5 U·mL^{-1} NE: SI=1.46; 7.5 U·mL^{-1} NE: SI=2.59; 10 U·mL^{-1} NE: SI=3.12 (the data are presented as mean of three separate experiments, p<0.05 for experiments performed with ≥5 U NE compared with control; fig. 2).

Effects of coincubation with transforming growth factor-β_1 and neutrophil elastase

To determine whether the effects of TGF-β_1 on SLPI mRNA expression and protein release can be counterbalanced by NE, BEAS-2B cells were stimulated with increasing concentrations of TGF-β_1 (1–50 ng·mL^{-1}) in the presence of 7.5 U·mL^{-1} NE for 24 h. As a result, even in the presence of levels of NE shown above to be adequate to enhance SLPI synthesis, TGF-β_1 causes a reduction in SLPI mRNA expression and protein release. The extent of the inhibition was less than with TGF-β_1 alone, but nevertheless incubation with 1 ng·mL^{-1} TGF-β_1+7.5 U·mL^{-1} NE decreased SLPI mRNA expression and protein secretion by ~20%. By using higher doses of TGF-β_1, the effect was even more pronounced (the data are presented as the mean of three separate experiments: 1 ng·mL^{-1} TGF-β_1+7.5 U·mL^{-1} NE: mRNA SI=0.70, p=NS; protein SI=0.87, p=NS; 10 ng·mL^{-1} TGF-β_1+7.5 U·mL^{-1} NE: mRNA SI=0.61,

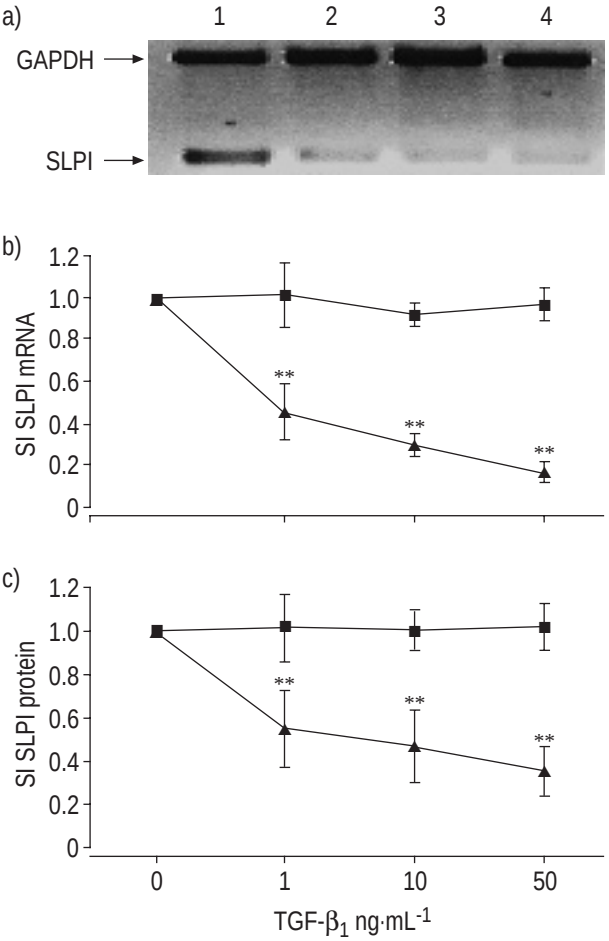

Fig. 1. – Secretory leukoprotease inhibitor (SLPI)/messenger ribonucleic acid (mRNA) transcript and protein levels following stimulation of cultured human bronchial epithelial (BEAS-2B) cells with various concentrations of transforming growth factor (TGF)-β_1. Cells were cultured with 1–50 ng·mL^{-1} TGF-β_1. SLPI mRNA expression was analysed by reverse transcription polymerase chain reaction. (a) Representative ethidium bromide-stained gel showing SLPI mRNA of BEAS-2B cells under resting conditions (lane 1) and following stimulation with 1 ng·mL^{-1} TGF-β_1 (lane 2), 10 ng·mL^{-1} TGF-β_1 (lane 3) and 50 ng·mL^{-1} TGF-β_1 (lane 4). Semiquantitative data were obtained by comparison to reduced glyceraldehyde-phosphate dehydrogenase (GAPDH) mRNA transcript levels (b). SLPI protein levels were quantified by a specific enzyme-linked immunosorbent assay (c). As a control, a neutralizing anti-TGF-β_1 antibody was added together with TGF-β_1. Data are expressed as the stimulation index (SI) in comparison to results obtained with resting cells. The data are presented as the mean±SEM of three separate experiments. ■: TGF-β_1 + anti-TGF-β_1; ▲: TGF-β_1.

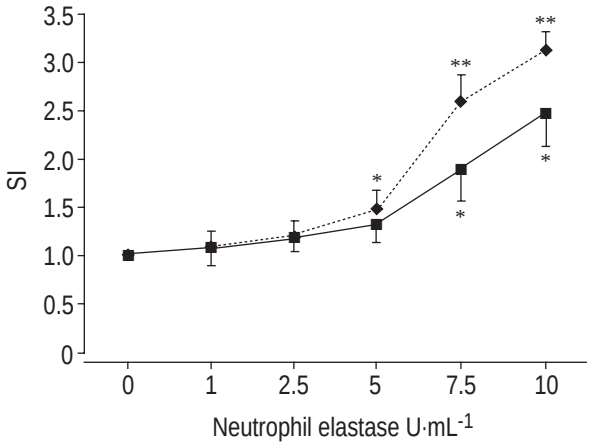

Fig. 2. – Secretory leukoprotease inhibitor (SLPI)/messenger ribonucleic acid (mRNA) transcript (■) and protein (◆) levels following stimulation of cultured human bronchial epithelial (BEAS-2B) cells with various concentrations of neutrophil elastase (NE). Cells were cultured with 1–10 U·mL^{-1} NE. The data are presented as the mean±SEM of three separate experiments. The SLPI protein release of 1×10^6 resting cells was 213±34 pg·mL^{-1}. *: p<0.05; **: p<0.01. SI: stimulation index.

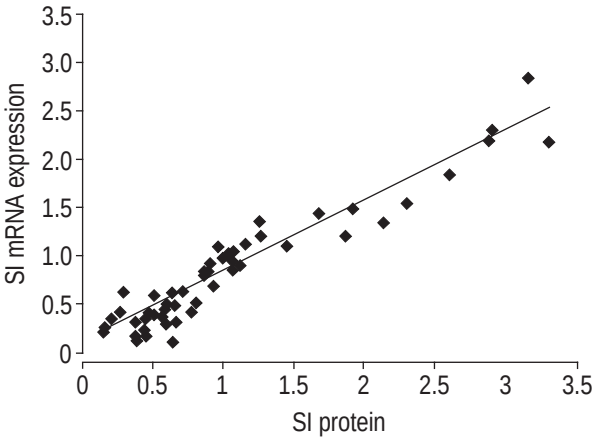

Fig. 4. – Comparison of secretory leukoprotease inhibitor protein release and messenger ribonucleic acid (mRNA) expression for all performed experiments. SI: stimulation index. r=0.91; p<0.001. Regression line is shown.

p<0.05; protein SI=0.65, p<0.05; 50 ng·mL^{-1} TGF-β_1+7.5 U·mL^{-1} NE: mRNA SI=0.52, p<0.05; protein SI=0.58, p<0.05; 10 ng·mL^{-1} TGF-β_1: mRNA SI=0.33, p<0.01; protein SI=0.38, p<0.01; fig. 3).

Comparing the SIs from SLPI mRNA and protein of all performed experiments revealed an excellent correlation (r=0.91; p<0.001; fig. 4).

Discussion

TGF-β_1 and NE are potentially involved in the pathogenesis of OB, the major long-term complication after lung and heart-lung transplantation. The goal of the study was to elucidate the effects of TGF-β_1, and NE on the synthesis and secretion of SLPI by human epithelial cells *in vitro*. The concentrations of TGF-β_1 used for stimulating BEAS-2B cells were in the same range as the levels found in the respiratory ELF of lung transplant recipients. It was found that TGF-β_1 reduces SLPI mRNA expression and protein release of the human bronchial epithelial cell line BEAS-2B. Simultaneous incubation with TGF-β_1 and NE also reduced SLPI expression and release, whereas NE alone had a stimulating effect.

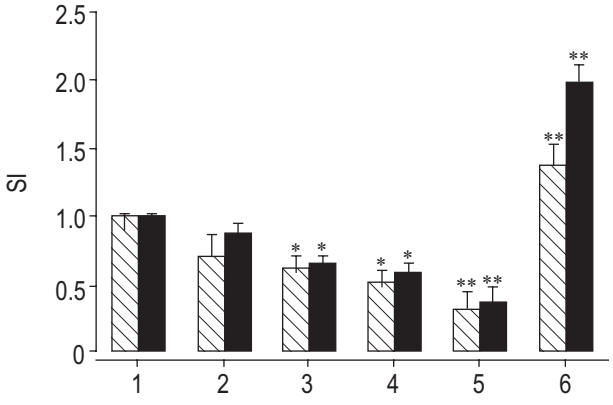

Fig. 3. – Secretory leukoprotease inhibitor (SLPI)/messenger ribonucleic acid (mRNA) transcript (▨) and protein (■) levels following stimulation of cultured human bronchial epithelial (BEAS-2B) cells with various concentrations of transforming growth factor (TGF)-β_1 in the presence of neutrophil elastase (NE). Cells were cultured with 1 ng·mL^{-1} TGF-β_1+7.5 U·mL^{-1} NE (lane 2); 10 ng·mL^{-1} TGF-β_1+7.5 U·mL^{-1} NE (lane 3); 50 ng·mL^{-1} TGF-β_1+7.5 U·mL^{-1} NE (lane 4); 10 ng·mL^{-1} TGF-β_1 (lane 5); and 7.5 U·mL^{-1} NE (lane 6). As a comparison, resting cells were used (lane 1). The data are presented as the mean±SEM of three separate experiments. The SLPI protein release of 1×10^6 resting cells was 201±31 pg·mL^{-1}. SI: stimulation index. *: p<0.05; **: p<0.01.

OB following lung or heart-lung transplantation is characterized by a marked increase in the number of neutrophils in BALF samples of affected patients (unpublished data) [12–14]. Although neutrophils contribute substantially to the host defence system of the lung, the massive neutrophil-dominated inflammation in OB may be associated with epithelial damage. This is suggested by other conditions with severe chronic neutrophilic inflammation like the lung involvement in cystic fibrosis, where a sharp increase in the number of neutrophils is associated with destruction of the bronchi [23]. Neutrophils may damage lung epithelium, by secretion of a variety of potent mediators, including reactive oxygen species and several proteases, in particular NE [24]. NE is a 29-kDa serine protease stored in the azurophilic granules of neutrophils, which following secretion is able to cleave elastin, several types of collagen, cell surface receptors and immunoglobulins, to alter composition and viscosity of sputum, and to damage endothelial and epithelial cells directly [25].

Physiologically, the human lung is protected against hazardous effects of NE by a shield of antiproteases. SLPI is the major antielastase of the conducting airways [18]. SLPI is a 12-kDa, nonglycosylated, disulphide-linked antiprotease secreted by cells of mucosal surfaces including the epithelium of the airways [17]. SLPI inhibits a variety of proteases, such as cathepsin G, trypsin, chymotrypsin, chymase, trypase, and NE. Based on enzyme kinetic studies, its major physiological function is probably the inhibition of NE [26].

In the lungs of patients with cystic fibrosis the protease defence is incapacitated, as evidenced by nearly complete degradation of SLPI and high concentrations of uncomplexed NE [27, 28]. As in cystic fibrosis, patients with OB

show elevated BALF levels of the neutrophil chemoattractant interleukin (IL)-8 [12, 29, 30]. IL-8 promotes NE release from neutrophils [31, 32]. Furthermore, NAKAMURA *et al*. [23] reported that NE can induce bronchial epithelial cells to synthesize and secrete IL-8.

With this as a background, it seems reasonable to assume that, as in cystic fibrosis, an insufficient anti-NE protective screen of the respiratory tract in OB would lead to ongoing injury of the epithelium of the airways. On the other hand, it has been shown that NE stimulates SLPI expression in a transformed human epithelial cell line (9HTEo-) and in primary human airway epithelial cells [33, 34].

MARCHAND *et al*. [35] found a decrease in SLPI protein release of primary human nasal surface epithelial cells after stimulation with NE. However, the concentrations of NE in these experiments were 2–200 times higher than the concentrations used in the current study. Also SALLENAVE *et al*. [36] reported an increase in SLPI protein and a decrease in SLPI mRNA after stimulation with NE in the alveolar epithelial cell line A549. They speculated about a specific cellular role of SLPI from their findings. However, using BEAS-2B cells, the current authors found an excellent correlation for the increase in both SLPI mRNA and protein. Thus the different cell systems might be responsible for the divergent results.

In contrast to cystic fibrosis, the initial inflammatory process in OB is followed by a fibroproliferative reaction leading to obliteration of the bronchiolar lumen. Thus, mechanisms involved in response to injury or repair are probably of equal importance for the pathogenesis of OB as the injury itself. Therefore, growth factors like the TGF-β family were addressed in studies on OB.

TGF-β_1 is known to stimulate production of fibronectin and collagen, and its overexpression in lung tissues has been associated with various fibrotic lung diseases [4–8]. Not surprisingly, it could be shown that the expression of TGF-β_1 by alveolar macrophages and BALF cells from lung transplant recipients suffering from OB is enhanced [9, 10].

To the authors′ knowledge, this study has shown for the first time that TGF-β_1 is capable of interfering directly with the anti-NE defence of the lung by reducing the expression and secretion of SLPI by bronchial epithelial cells. This effect was undoubtedly caused by TGF-β_1 itself as it was dose-dependent and could be blocked by the addition of a neutralizing anti-TGF-β_1 antibody. The down-regulation of SLPI could be a mere consequence of cell differentiation, as the used bronchial epithelial cell line may undergo squamous differentiation when exposed to TGF-β_1 [21, 37]. However, the concentrations of TGF-β_1 in these experiments were 10 times higher than the ones used in the current study and thus exceeded, by far, the physiological range. Furthermore, the incubation time was >1 week, while the cells were exposed to TGF-β_1 for just 24 h in the current study. Finally, using light microscopy no morphological changes of the cells were observed in the current study, in particular there was no transformation to "cobblestone" cells. For these reasons the current authors strongly believe that the down-regulation of rSLPI in this study is not caused by cell differentiation, but represents a direct effect of TGF-β_1.

On the epithelial surface of the OB-lung two factors potentially affecting SLPI levels "compete" with each other: one (NE) that may increase and another (TGF-β_1) that may decrease SLPI expression. In this context, it is important to evaluate the consequences of the presence of NE and TGF-β_1. Interestingly, when BEAS-2B cells were coincubated with NE+TGF-β_1, expression/secretion of SLPI was still significantly reduced, although suppression was somewhat attenuated in comparison to the experiments with TGF-β_1 alone. This observation indicates that the suppressing effect of TGF-β_1 overcomes the stimulatory effect of NE on SLPI synthesis. TGF-β_1, however, may not only interfere with the defence mechanisms of the epithelium of the lung by reducing SLPI levels. In a recent study ARSALANE *et al*. [38] demonstrated that TGF-β_1 is able to inhibit glutathione synthesis in the human alveolar epithelial cell line A549. Glutathione is the most important extracellular antioxidant of the human lung [39, 40]. Thus, TGF-β_1 may also impair defence against reactive oxygen species. SLPI and α_1-protease inhibitor carry methionine residues in their active centre [41]. Methionine can rather easily be oxidized, leading to a dramatic loss of the anti-NE activity of SLPI and α_1-protease inhibitor [26, 42]. Oxidative inactivation is the major mechanism by which neutrophils may overcome the protective shield against NE.

In summary, the present study demonstrates that *in vitro* exposure of the human bronchial epithelial cell line BEAS-2B to TGF-β_1 induces a decrease in SLPI mRNA and protein levels in the absence and presence of NE.

If further studies support these results, prophylaxis and/or therapy with aerosolized secretory leukoprotease inhibitor or α_1-protease inhibitor could be taken into consideration, *e.g.* for patients after lung and heart-lung transplantation.

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