Cellular Mechanisms Involved in the Pathogenesis of Airway Remodeling in Chronic Lung Disease

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Abstract

Background/Aims: Epithelial cells and lung fibroblasts play an important role in the development of chronic lung disease but the exact mechanisms responsible for this role have not been fully clarified. Our objective was to investigate the involvement of these cells in the inflammatory response associated to chronic lung disease.

Methods Human lung fibroblasts (MRC-5) and bronchial epithelial cells (NuLi-1) were challenged with pro-inflammatory (interleukin (IL)-1β, and hypoxia), and inhibitory (simvastatin) stimuli. Expression of markers of local inflammation (IL-8, monocyte chemoattractant protein-1, nuclear factor-κB), systemic inflammation (C-reactive protein, serum amyloid A) and proteases (matrix metalloproteinase's 9 and 12) were assessed by polymerase chain reaction and ELISA. Apoptosis/necrosis was also analyzed by flow cytometry.

Results Although both cell types upregulated their expression of local and systemic inflammation and protease activity markers when they were treated with IL-1β, MRC-5 showed a higher gene and protein expression compared to NuLi-1 and a different expression in MMP at 48 hours. Under hypoxic conditions, we observed a decrease in serum amyloid gene expression in MRC-5, which was further attenuated by simvastatin. Apoptosis was not altered by any stimulus.

Conclusions The inflammatory responses occurring in airway cells differ from one another. Fibroblasts seem to be the main cells involved in the inflammatory response associated with chronic lung disease, with a role roughly equivalent to that of protein imbalance. Apoptosis is not a predominant mechanism in these cells.

Introduction

Airway remodeling occurring in chronic lung disease is a multicellular response to mucosal injury which results in epithelial cell state changes, enhanced extracellular deposition and the expansion of profibrotic myofibroblast populations. Previous evidence suggests that obstructive lung diseases have certain triggers, inflammatory responses and features of remodeling in common [1] and that the inflammation which appears in the respiratory system precedes the onset of bronchial obstruction [2,3]. In this regard, recent research efforts have focused on the evaluation of early chronic lung disease [4,5].

One relevant question involves determining where the inflammatory process that triggers the bronchial obstruction begins. Of all the cell types involved, epithelial cells and lung fibroblasts seem to play a central role. Epithelial cells are a first barrier protecting the airway from exposure to the environment, and their alteration not only makes the airway more vulnerable, but also alters signaling pathways involved in inflammatory processes and repair [6]. Fibroblasts are believed to play an essential role in inflammatory responses within the respiratory system [7]. Collectively, inflammation can influence the epithelium and fibroblasts, leading to airway remodeling [8]. The mechanistic basis underlying COPD is complex and there

are different biological pathways involved, including imbalances in proteases and their inhibitors and apoptosis [9]. However, to date, no studies have assessed cell behavior under pro- and anti-inflammatory conditions as a starting point for research into chronic lung disease pathogenesis.

The purpose of the present study was to examine the roles of fibroblasts and epithelial cells in the main pathways involved in the development of chronic lung disease. By treating these cells with inflammatory cytokine, interleukin (IL)-1β, and hypoxia, we explored the potential involvement of these cells in the pathogenesis of chronic lung disease. In addition, we investigated the inhibitory effect of simvastatin on the response of these cells to both stimuli (ie inflammatory cytokine and hypoxia). The results of these experiments will help reveal the alterations in biological pathways in these cell types, which should lay the basis for future research to further our understanding of the workings of chronic lung disease.

Methods

Cell culture

MRC-5 (human lung fibroblast cell line) cells were obtained from the European Collection of Authenticated Cell Cultures (ECACC, Salisbury, UK) and NuLi-1 (human airway epithelial cell line) cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The MRC-5 cells were kept in Eagle´s Minimum Essential Medium (EMEM) (Gibco Technologies, Rockville, MD, USA), supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 U/mL) at 37 °C in a humidified atmosphere containing 5% CO_2. NuLi-1 cells were grown in serum-free Bronchial Epithelial Cell Growth Medium (BEGM, Lonza, and Walkersville, MD, USA). This medium consisted of Bronchial Epithelial Basal Medium (BEBM) and SingleQuot additives, with the exception of the gentamicin-amphotericin B. In addition, the medium was supplemented with 50 µg/mL G418 disulfate salt (Sigma GmbH, Germany). The cells were then cultured in collagen-coated cell culture flasks (Nunc).

Cell stimulation and inhibition

The cells were grown to 80% confluence, washed and incubated with serum-free medium for 24 hours, and then incubated with 10 ng/mL IL-1β (R&D Systems, Minneapolis, MN, USA) or hypoxia (1% O_2) for 24 and 48 h and/or 30 µM simvastatin sodium salt (Calbiochem, Darmstadt, Germany). The concentrations of IL-1β and simvastatin were chosen by preliminary concentration-response studies. The simvastatin treatment was started 45 min prior to cytokine treatment, as described previously [10]. We used untreated cells as controls for the IL-1β and simvastatin experiments and cells in normoxic conditions (5% O_2) for the hypoxia experiments. Altogether, there were 5 groups of experiments per cell type: IL-1β, simvastatin, simvastatin + IL-1β, hypoxia, and simvastatin + hypoxia. Each experiment was repeated six times.

Selection of biomarkers

We selected markers involved in the pathogenesis of chronic lung disease: IL-8, monocyte chemoattractant protein-1 (MCP-1), and nuclear factor-κB (NF-κB) as markers of local inflammation; C-

reactive protein (CRP) and serum amyloid A (SAA) as markers of systemic inflammation; and finally, matrix metalloproteinase (MMP) 9 and 12 as markers of proteases.

Gene expression

Gene expression was measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR). The total RNA was isolated from the cultured cells using the High Pure RNA isolation kit (Roche Mannheim, Germany). RNA was reverse transcribed using the iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). Each 25 µL amplification reaction mixture comprised 5 µL cDNA (10 ng/µL), 12.5 µL SYBR® Green PCR Master Mix (Stratagene, La Jolla, CA, USA), and 7.5 µL primers/H_2O. RT-qPCR was performed on an Applied Biosystems® 7900 Real-Time PCR System (Life Technologies, Grand Island, NY, USA) under the following cycling conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Expression of 18 s rRNA was used as an internal control for the normalization of target gene expression. For SAA, all SAA1, SAA2, and SAA4 genes were studied. The list of primers used to amplify the genes of interest appears in Table 1. Relative gene expression values were evaluated with the $2^{-\Delta\Delta Ct}$ method [11].

Table 1
Primers used for reverse transcription quantitative PCR

Gene	Forward primer	Reverse primer
18 s	5'-TGAAATATCCAGAACATCTTA-3'	5'-GCAAAATTTATTGTCCCATCAT-3'
CRP	5'-GTGTTTCCCAAAGAGTCGGATA-3'	5'-CCACGGGTCGAGGACAGTT-3'
SAA1	5'-ATCAGCGATGCCAGAGAGAAT-3'	5'-GTGATTGGGGTCTTTGCCA-3'
SAA2	5'-AGCCAATTACATCGGCTCAG-3'	5'-ATTTATTGGCAGCCTGATCG-3'
SAA4	5’-GTCCAACGAGAAAGCTGAGG-3’	5’-AGTGACCCTGTGTCCCTGTC-3’
MCP1	5´-CCCCAGTCACCTGCTGTTAT-3´	5´-TGGAATCCTGAACCCACTTC-3´
NF-κB	5´-CCTGGATGACTCTTGGGAAA-3´	5´-TCAGCCAGCTGTTTCATGGTC-3´
MMP9	5´-TTGACAGCGACAAGAAGTGG-3´	5´-GCCATTCACGTCGTCCTTAT-3´
MMP12	5´-ACACATTTCGCCTCTCTGCT-3´	5´-ACACATTTCGCCTCTCTGCT-3´
IL-8	5´-CAGGAATTGAATGGGTTTGC-3´	5´-AAACCAAGGCACAGTGGAAC-3´

18s: 18 s ribosome; CRP: C-reactive protein; SAA: Serum Amyloid A; MCP1: Monocyte chemoattractant protein 1; NF-κB: Nuclear factor kappa-B; MMP: Matrix Metalloproteinase; IL-8: Interleukin-8.

Protein expression

The media of the cell cultures were collected, centrifuged at 2750 *g* for 5 min, and then stored at − 80 °C until further measurements were taken. We measured the cytokine levels by ELISA (Quantikine®, R&D

Systems for IL-8, MCP-1, and MMP-9; Anogen, Mississauga, Ontario, Canada for SAA; and Abcam, Cambridge, UK for CRP), following the manufacturer's protocol.

Apoptosis and necrosis analysis

For apoptosis analysis, the cells were trypsinized and washed twice with phosphate-buffered saline (PBS). Two hundred thousand cells were stained with Annexin V-CF Blue and propidium iodide (PI) following the manufacturer's instructions (Immunostep, Salamanca, Spain). The percentage of annexin V-positive and/or PI-positive cells was analyzed using a Fortessa II flow cytometer (Becton Dickinson; Erembodegem, Belgium).

Statistics

Means and standard error for continuous variables and frequencies and percentages for categorical variables were estimated. The gene expression data were compared according to the treatment, using the U-Mann Whitney test. All the testing hypotheses were performed at a 5% level of significance (two-tailed), using the statistical analysis software, IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, USA).

Results

Stimulation with IL-1β

Local inflammation markers

Cells stimulated with IL-1β for both 24 h and 48 h significantly increased mRNA expression and protein levels of IL-8 and MCP-1 compared with those of untreated cells (Fig. 1). Interestingly, MRC-5 cells presented a higher gene expression of IL-8 compared to Nuli-1 ($p = 0.029$). On the other hand, MRC-5 cells presented a higher gene and protein expression for MCP-1. NF-κB presented no changes.

Systemic inflammation markers

The fibroblasts stimulated with IL-1β significantly increased SAA mRNA expression compared with that of the control cells (Fig. 2A, B, and C), 98-fold for SAA1, 200-fold for SAA2, and 3.6-fold for SAA4. The levels of CRP mRNA, as well as CRP and SAA protein, did not change in cells treated with IL-1β compared with untreated cells (data not shown). The epithelial cells stimulated with IL-1β significantly increased their CRP mRNA expression compared with unstimulated cells (Fig. 2E). The levels of SAA protein were significantly higher in cells stimulated with IL-1β for 24 h compared with unstimulated cells ($p = 0.029$) (Fig. 2F). Nevertheless, we found no significant differences in SAA1, SAA2, and SAA4 mRNA expression (Fig. 2D).

Metalloproteinases

MMP9 and MMP12 mRNA expressions increased significantly in fibroblasts and epithelial cells incubated with IL-1β for 24 and 48 h compared with the control cell values (Fig. 3). Interestingly, at 48 hours, MMP12 mRNA expression decreased in lung fibroblasts (Fig. 3B), and conversely, this expression increased in epithelial cells (Fig. 3D).

Apoptosis/necrosis

We found no significant differences between unstimulated cells and cells treated with IL-1β with respect to cell death.

Culture in hypoxic conditions

The cells cultured under hypoxic conditions did not alter their expression of local inflammation markers, metalloproteinase markers, or apoptosis/necrosis rates. However, the lung fibroblasts under hypoxic conditions decreased their SAA1 and SAA2 mRNA expression (3.7-fold, $p = 0.014$)

Inhibition with simvastatin

Simvastatin alone, in bronchial epithelial cell, did not downregulate gene expression or protein levels with any of the biomarkers, compared to the corresponding untreated cells (data not shown). Simvastatin decreased SAA1 and SAA2 mRNA in human lung fibroblasts (1.2- and 1.6-fold, respectively, versus untreated cell) under hypoxic conditions. Simvastatin did not show a decrease in the inflammatory parameters after stimulation with IL-1ß.

Discussion

The present study shows an overall assessment of the cellular responses of bronchial epithelial cells and lung fibroblasts after inflammatory stimuli associated with chronic lung disease. Our results show that 1) IL-1β stimulates different biological pathways involved in chronic lung disease (IL-8 and MCP-1), except apoptosis and specific inflammatory pathways (NF-Kb) biomarkers; 2) The involvement of IL-1ß stimulation in systemic inflammatory and proteinase imbalance biomarkers is higher in lung fibroblasts; 3) Under hypoxia conditions, there is a systemic inflammatory decrease in fibroblasts; 4) simvastatin maintains this downregulation in fibroblasts; 5) as a consequence, the two cell types show a different inflammatory response.

The evaluation of biological pathways that underlie cellular physiology represents a vital step in our understanding of the normal cellular mechanisms, which may be implicated in the initial pathophysiology of chronic lung diseases such as COPD. These results highlight the biological pathways initially stimulated which could potentially trigger the inflammatory response responsible for the eventual onset of chronic lung disease. It is important to bear in mind that, for this study, we used commercial cell lines, which are involved in the remodeling of lung damage, induced by inflammatory stimulus. Therefore,

although the pathways explored are the ones, which participate in the pathogenesis of chronic lung disease, we have actually studied them in “normal” cells in culture. Due to changes in these different pathways in patients who develop chronic lung disease [12], the cells in the respiratory system of patients with this disease may respond differently. For this reason, our results should now be replicated with primary cultures obtained from patients with chronic lung disease in order to clarify their involvement in the pathogenesis of the disease.

Fibroblasts, together with protein imbalance, seem to be the main factors involved in the inflammatory response in chronic lung disease. According to our results, both bronchial epithelial cells and lung fibroblasts participate, each in a different way, in the inflammatory process, which begins in the bronchial epithelium with the release of IL-8, MCP-1 in bronchial epithelial cells and lung fibroblasts and of CRP and SAA in bronchial epithelial cells. Subsequently, the activated macrophages release metalloproteinase 9 and 12, which contribute to the degradation of elastin in the lung parenchyma. However, this inflammatory process in lung fibroblasts could be attenuated by simvastatin. Interestingly, that biomarker associated with a disease-specific response (NF-κB) or with more advanced disease was not significantly changed.

The fact that IL-1β stimulation is not always associated with increased protein levels of pro-inflammatory mediators is of particular interest. Epigenetic post-transcriptional regulation of protein synthesis has been well documented in cell physiology and is directly related to chronic lung disease pathogenesis [13–15]. Osei et all. found that co-cultures of lung fibroblast with bronchial epithelial cells significantly increased the expression of miR-146-a-5p. They demonstrated that miR-146a-5p expression played an anti-inflammatory role, downstream of the IL-1 pathway, subsequently reducing IL-8 release from lung fibroblast [16]. This regulation mechanism may explain our findings of the release of IL-8 in lung fibroblast stimulated with IL-1β.

Systemic inflammation in chronic lung disease, defined as increased levels of inflammatory markers from different biological pathways [17], has become another target for chronic lung disease-related research and its origin is a challenge for researchers. Not only may proteins originating from the lung exert systemic effects, but there is also a lack of correlation between airway cytokine concentrations and those in the circulation [18], and investigators have been unable to find an association between the inflammatory load of induced sputum and plasma [19]. This may be due to hepatic hyperstimulation during chronic lung disease, such as COPD, and this lung-liver feedback should be explored further. Serum amyloid A (SAA), an acute phase protein which occurs in high levels in the blood during infection, was considered to be produced by hepatocytes and subsequently secreted into serum [20]. However, one published study has demonstrated that SAA mRNA [21] is normally expressed in the epithelial components of a variety of human organs, and tissues could be released locally in certain organ-specific diseases [22]. Moreover, SAA can also be produced by macrophages and other extrahepatic cells as well as in the lung [23,24]. Our previous findings show that this SAA expression was different between pulmonary cell types [25]. Our results show SAA mRNA expression but no protein expression in lung fibroblast. In contrast, we

observed a protein expression of SAA, but no SAA mRNA expression in bronchial epithelial cells. This may be due to differences in the posttranscriptional regulation depending on the cell type. The existence of several SAA receptors has been demonstrated, such as toll-like receptor (TLR) 2 and TLR4, although the structure of SAA differs from the ligands classically associated with these receptors. Furthermore, SAA has ability to activate TLR2 and TLR4, which is of particular interest, given that these TLRs play important roles in the inflammatory response, such as in the modulation of airway epithelial cell regeneration [26]. On the other hand, SAA preferentially activates the histone H3 demethylase Jmjd3, and as a result, SAA not only triggers transcription factor activation but also influences gene expression through epigenetic regulation [27,28]. Our findings could be explained by several mechanisms, such as the modulation of TLR activity or epigenetic mechanisms. Both of these may act differentially between lung fibroblast and bronchial epithelial cells, leading to increased TLR mRNA expression and a higher uptake of SAA protein, or an inhibition of SAA protein synthesis in lung fibroblast cells, respectively. In addition, our results could indicate an overlap in bronchial epithelial cells between SAA kinetics and the kinetic of the C-reactive protein, which would be in line with previous studies [29].

The interactions between epithelial cells, fibroblasts and the extracellular matrix of the airway wall are intimately involved in a number of functions within the lung [30]. MMP play a key role in chronic lung disease pathogenesis, as well as degrading matrix proteins, and its presence is required for normal matrix processing and lung repair [31]. In addition, MMP-12 plays a pivotal role in the inflammatory process that leads to lung injury [32]. MMP-12 also plays a role in inflammatory lung disease and tissue remodeling [33,34]. Hence, an increased expression of MMP-9 and MMP-12 may cause an unwanted degradation of lung tissue. According to our results, both cell types, bronchial epithelial and lung fibroblast, participate actively and differentially during the initial injury in chronic lung disease. Given the potential importance of MMP-12 in chronic lung disease, it is of interest to show that, after an inflammatory stimulus, its production and release occur earlier in fibroblasts than in the epithelial cells. Our findings suggest that the fibroblasts tend to act as sentinel cells in the event of injury, orchestrating the early phases by secreting MMP-12. In addition, fibroblasts could modulate the response of epithelial cells, which is in line with previous studies [35,36]. These differences between MMPs may be of major importance when considering the role of a given MMP and, particularly, in selecting an MMP to inhibit together with a synthetic inhibitor.

We explored the effect of hypoxia as a stimulus that would be representative of acute pulmonary diseases, in which hypoxemia would occur as a consequence of the severity of the disease. Although current COPD recommendations no longer consider respiratory failure as a component when measuring the severity of the disease [37], patients with COPD and secondary hypoxemia should be classed as particularly serious cases from a clinical perspective, and they have serious prognostic implications [38]. Hypoxia and inflammation are intimately linked [39]. Some studies have shown "*in vitro*" evidence that hypoxia may aggravate airway inflammation through an effect on immune cells such as macrophages [40] or monocytes [41], and our study suggests that inflammation before hypoxia does not worsen local

inflammation near lung fibroblasts and bronchial epithelial cells. We observed that exposure of normal lung fibroblasts, in culture, to hypoxia resulted in decreased SAA mRNA expression. Nevertheless, patients with high SAA had greater dyspnea and more frequent interstitial lung disease [42]. Therefore, our results suggest that lung fibroblasts can be considered a cellular model, used to analyze the underlying mechanisms in the pathogenesis of overlap syndrome, or to improve hypoxia-induced inflammation, which could be facilitated by high altitude acclimatization. This expression of SAA mRNA remains downregulated after treatment with simvastatin. However, the effect of the statins depends on the underlying stimulus, mechanisms, microenvironmental conditions [43] and cell type used in the experiment. The relationship between pulmonary disease and simvastatin is complex [44]. A recent systematic review identified articles evaluating the clinical efficacy of statin therapy in chronic lung disease. Although statin treatment was associated with improvements in exercise capacity, lung function and health status, the authors found no associations with inflammatory markers [45]. Multiple retrospective studies have shown that statins are beneficial in chronic lung disease because their anti-inflammatory effects include suppression of the upregulation of pro-inflammatory cytokines, chemokines, adhesion molecules and MMPs by inflammatory cells [46,47], and their potential role in respiratory disease has been hypothesized [48]. Interestingly, the impact of simvastatin on epithelial cell lines is less obvious, despite a few reports that simvastatin inhibits alveolar destruction [49] and affects alveolar recovery [50].

On the other hand, the role of increased apoptotic alveolar cells in the peripheral lung is relevant in severe stable COPD patients with pulmonary emphysema [51,52]. Our results did not show any differences in the rate of apoptosis and necrosis when we compared cells stimulated with and without IL-1β. The role of apoptosis in cell physiology is probably related to particular presentations of the disease, in which there is an increased destruction of lung parenchyma, such as in emphysema. The rate of apoptosis is higher in epithelial cells because, during the pathogenesis of chronic lung disease, there is destruction of the lung tissue and development of pulmonary emphysema, while fibroblasts increase their proliferation and ability to produce fibrotic tissue [53]. Therefore, our model is probably not ideal for studying emphysema.

In summary, the present study is an overall assessment of the cellular behavior of lung epithelial cells and lung fibroblasts after inflammatory stimuli associated with chronic lung disease. We have shown that the different biological pathways involved in chronic lung disease are increased after stimulation with IL-1β. These results highlight the initially stimulated biological pathways that could potentially trigger the inflammatory response responsible for the eventual onset of chronic lung disease. Future studies should replicate this approach with primary cell cultures obtained from patients with chronic lung disease in order to clarify their involvement in the pathogenesis of the disease at more advanced stages.

Abbreviations

COPD
chronic obstructive pulmonary disease; MRC-5:human lung fibroblast cell line; Nuli-1:bronchial epithelial cell line, IL-1β:interleukin 1beta; IL-8:interleukin 8; MCP-1:monocyte chemo attractant protein; NF-

κβ:nuclear factor kappa beta; CRP:C-reactive protein, SAA:serum amyloid A; MMP:matrix metalloproteinase, RT-qPCR:reverse transcription quantitative polymerase chain reaction and PI:propidium iodide.

Declarations

“Not applicable” in this section

Consent for publication

“Not applicable” in this section

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

EAO: Substantial contributions to conception and design, data acquisition, or data analysis and interpretation; drafting the article or critically revising it for important intellectual content and final approval of the version to be published. **CCA:** Substantial contributions to conception and design, data acquisition, or data analysis and interpretation and final approval of the version to be published.

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CLR: Substantial contributions to conception and design, data acquisition, or data analysis and interpretation and final approval of the version to be published.

ROC: Drafting the article or critically revising it for important intellectual content and final approval of the version to be published.

CMH: Drafting the article or critically revising it for important intellectual content and final approval of the version to be published.

JLLC: Substantial contributions to conception and design, data acquisition, or data analysis and interpretation; drafting the article or critically revising it for important intellectual content and final approval of the version to be published

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Figures

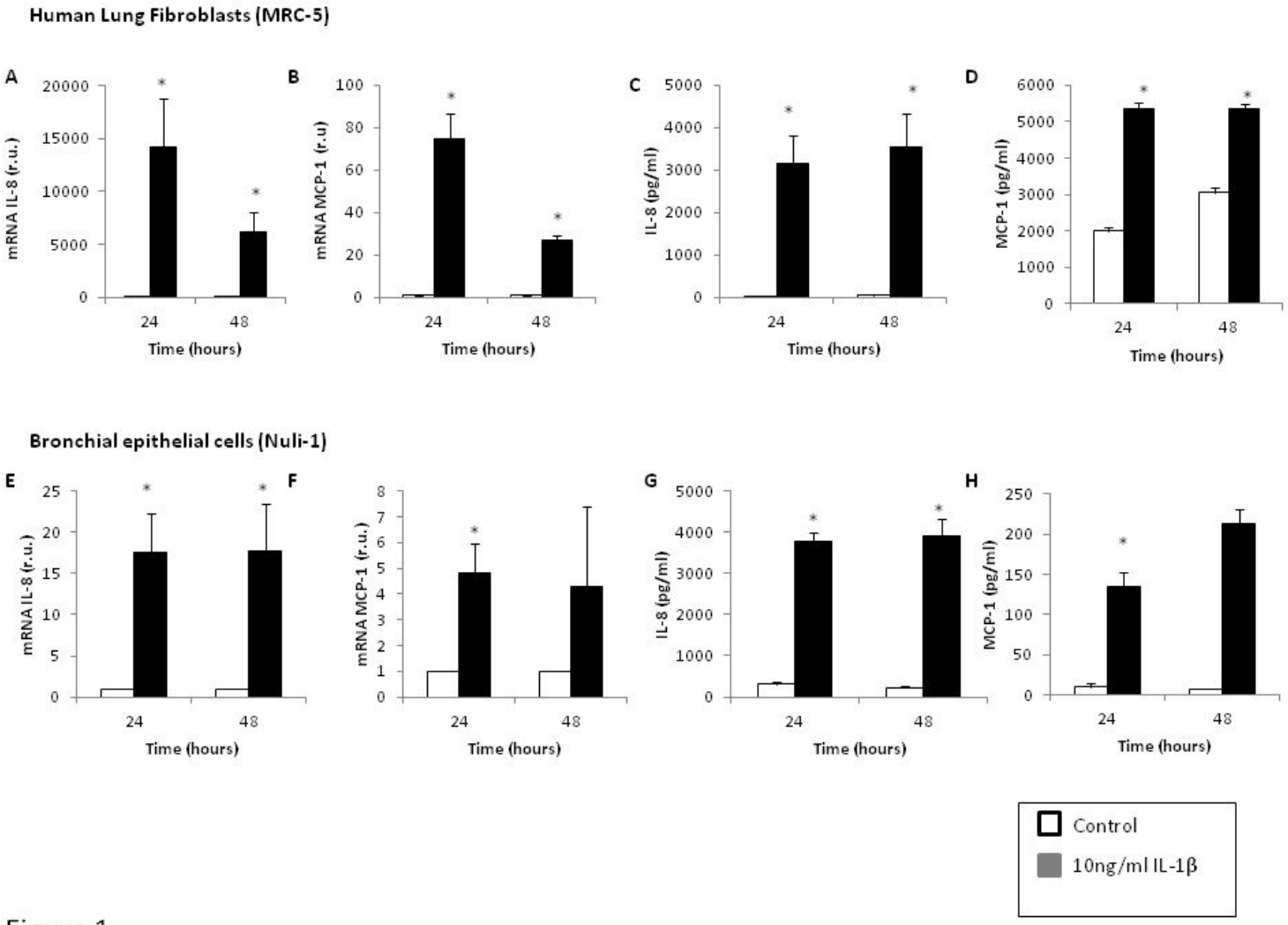

Figure 1

Figure 1

Effect of IL-1β on inflammatory mediator expression in fibroblasts and epithelial cells. mRNA expression of IL-8 (panel A), mRNA expression of MCP-1 (panel B), protein levels of IL-8 (panel C) and protein levels of MCP-1 (panel D) produced by human lung fibroblasts (MRC-5) without stimulation (control, white bars) and stimulated with IL-1β (10 ng/mL, black bars). mRNA expression of IL-8 (panel E), mRNA expression of MCP-1 (panel F), protein levels of IL-8 (panel G) and protein levels of MCP-1 (panel H) produced by human bronchial epithelial cells (NuLi-1) without stimulation (control, white bars) and stimulated with IL-1β (10 ng/mL, black bars). r.u= Relative units versus control; *p<0.05 versus control. Each bar of the figure is mean± SE n=6.

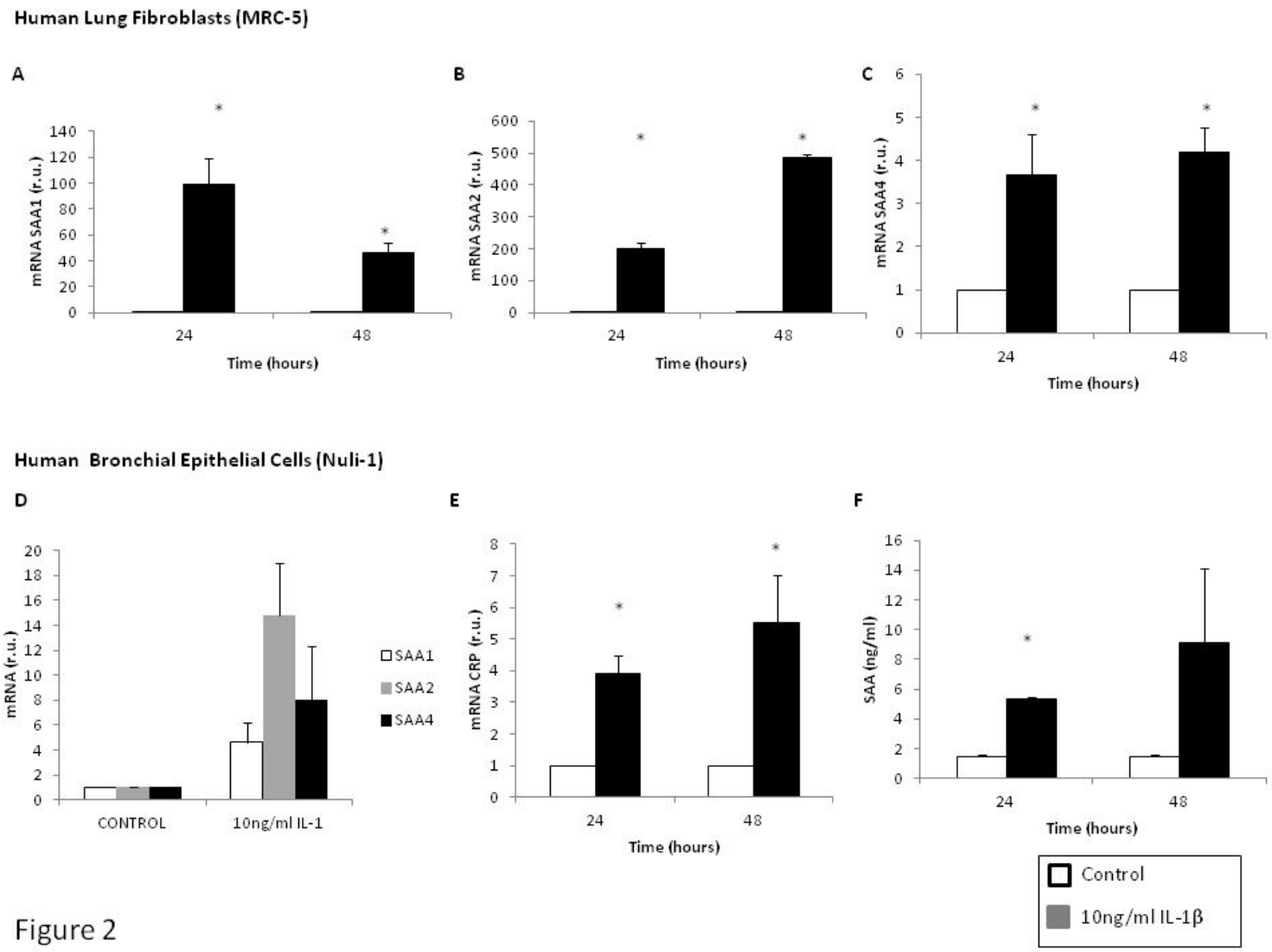

Figure 2

Figure 2

Effect of IL-1β on SAA and CRP expression in fibroblasts and epithelial cells. mRNA expression of SAA1 (panel A), SAA2 (panel B), and SAA4 (panel C) produced by human lung fibroblasts (MRC-5) without stimulation (white bars) and stimulated with IL-1β (10 ng/mL, black bars). mRNA expression of SAA (panel D), CRP (panel E) and SAA protein levels (panel D) produced by human bronchial epithelial cells (NuLi-1) without stimulation (white bars) and stimulated with IL-1β (10 ng/mL, black bars). r.u= Relative units versus control; *$p<0.05$ versus control. Each bar of the figure is mean± SE n=6.

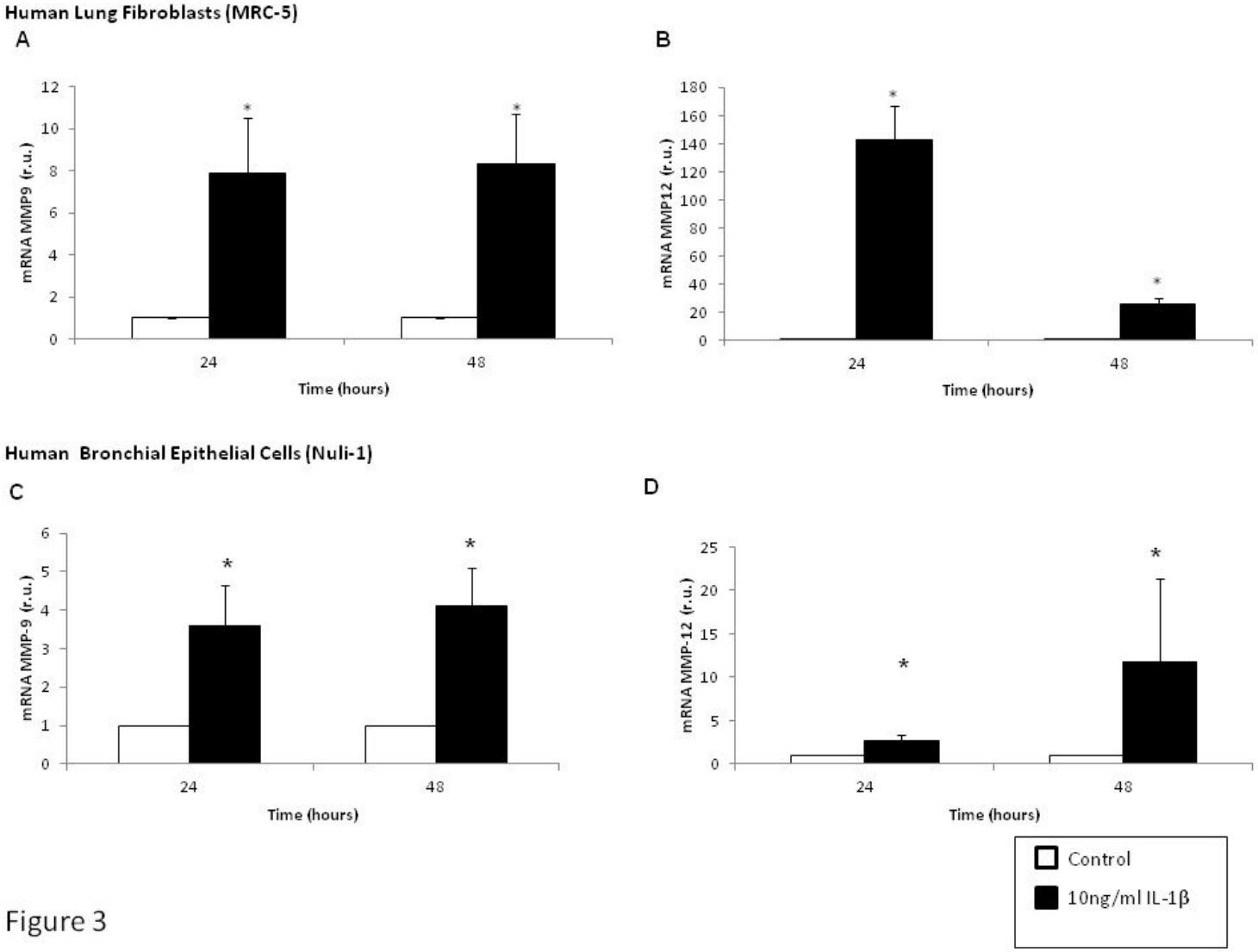

Figure 3

Figure 3

Upregulation of matrix metalloproteinase mRNA expression by IL-1β in fibroblasts and epithelial cells. Expression of MMP9 (panel A) and MMP12 (panel B) mRNA by human lung fibroblasts (MRC-5) without stimulation (white bars) and stimulated with IL-1β (10 ng/mL, black bars). Expression of MMP9 (panel C) and MMP12 (panel D) mRNA by human lung bronchial epithelial cells (NuLi-1) without stimulation (white bars) and stimulated with IL-