

Original article

Mechanical strain increases cytokine and chemokine production in bronchial fibroblasts from asthmatic patients

Background: Mechanical strain and cytokine stimulation are two important mechanisms leading to airway remodeling in asthma. The effect of mechanical strain on cytokine secretion in airway fibroblasts is not known. The aim of this study was to determine whether bronchial and nasal fibroblasts differentially alter cytokine secretion in response to mechanical strain.

Methods: We measured secretion of the pro-fibrotic cytokine, interleukin-6 (IL-6), and the pro-inflammatory cytokines, IL-8 and monocyte chemotactic protein 1, before and after mechanical strain in bronchial fibroblasts obtained from asthmatic patients [asthmatic bronchial fibroblasts (BAF)] and normal volunteers [normal bronchial fibroblasts (BNF)], and in nasal fibroblasts (NF) obtained from nasal polyps. Cells were grown on flexible membranes and a mechanical strain of 30% amplitude, 1 Hz frequency was applied for 3, 6 and 24 h. Control cells were unstrained. IL-6, IL-8 and monocyte chemotactic protein 1 was measured after 24 h strain using enzyme-linked immunoassay; mRNA was measured by real time polymerase chain reaction. We also measured mRNA for versican, a matrix proteoglycan, known to be upregulated in the asthmatic airway wall.

Results: In unstrained conditions, no differences in cytokine secretion were observed. After 24 h strain, BAF secreted more IL-6 than BNF. Mechanical strain increased IL-8 mRNA in BAF, BNF and NF; and IL-6 and versican mRNA, in BAF, only.

Conclusions: Cytokine responses to mechanical strain varied in different airway fibroblast populations, and depended on the site of origin, and the underlying inflammatory state. Strain resulted in IL-6 upregulation and increased message for extracellular matrix protein in bronchial fibroblasts from asthmatic patients only, and may reflect these patients' propensity for airway remodeling.

**F. Le Bellego¹, H. Perera¹,
S. Plante², J. Chakir², Q. Hamid¹,
M. S. Ludwig¹**

¹Meakins-Christie Laboratories, McGill University Hospital Center, Montreal; ²Centre de Recherche, Hôpital Laval – Institut Universitaire de Cardiologie et de Pneumologie, Sainte-Foy, QC, Canada

Key words: airway remodeling; interleukin-6; interleukin-8; monocyte chemotactic protein 1; versican.

Mara S. Ludwig
Meakins Christie Laboratories
3626 St. Urbain St.
Montreal, QC
Canada H2X 2P2

Accepted for publication 4 May 2008

Asthma is a disease characterized by bronchoconstriction, airway inflammation, and airway remodeling. Airway inflammation involves the activation of various types of cells including inflammatory cells, such as eosinophils and T lymphocytes, and structural cells, such as airway epithelial cells, airway smooth muscle cells, and fibroblasts. These cells secrete different types of inflammatory mediators, including pro-inflammatory and pro-fibrotic cytokines and chemokines (1). While the role of pro-inflammatory mediators in contributing to asthma pathogenesis has been relatively well investigated, less attention has been directed at the potential importance of pro-fibrotic mediators, such as interleukin-6 (IL-6), in contributing to airway remodeling (2, 3).

Structural changes in the asthmatic airway wall include increased deposition of various extracellular matrix (ECM) components, such as collagen, elastin, glycoproteins and proteoglycans (PG) (4–7). Fibroblasts are the

putative cell responsible for matrix deposition (8). There is information available in the literature documenting the ability of fibroblasts to secrete chemokines and cytokines (9, 10), thereby suggesting the potential for an autocrine mechanism, wherein the release of cytokines by fibroblasts results in increased matrix secretion.

A further stimulus to change in the asthmatic airway wall is thought to be excessive mechanical strain. It has been hypothesized that airway constriction and heterogeneous distribution of ventilation results in increased mechanical strain or stress on the structural cells of the asthmatic airway wall (11). Mechanical strain has been shown to affect the production of ECM components, upregulating type I collagen in pulmonary fibroblasts, and type III and IV collagen in co-cultures of bronchial epithelial cells and lung fibroblasts (12, 13). We have shown in bronchial fibroblasts obtained from asthmatic subjects, that mRNA and protein for the large, aggregating PG, versican, is

increased in response to mechanical strain, in comparison to cells obtained from normal volunteers (14, 15). Mechanical strain also results in upregulation of inflammatory cytokines, such as IL-8 and monocyte chemotactic protein 1 (MCP-1), in airway epithelial and airway and vascular smooth muscle cells (16–19). However, there is no information currently available on whether mechanical strain induces cytokine production in bronchial fibroblasts. If excessive mechanical strain of bronchial fibroblasts results in increased secretion of cytokines, especially pro-fibrotic cytokines, then the fibroblast response to mechanical strain could lead to increased inflammation and/or signal to upregulate matrix deposition.

To investigate this question, we studied the effect of excessive mechanical strain on pro-inflammatory and pro-fibrotic cytokine and chemokine secretion in different types of airway fibroblasts. We questioned whether underlying inflammation would be necessary for the response and therefore, compared the response of bronchial fibroblasts from asthmatic patients to that of fibroblasts from normal volunteers. We also questioned whether there are phenotypic differences in cytokine secretion in different airway fibroblast populations, both in terms of basal secretion and in terms of response to mechanical stimulation, and so examined fibroblasts of nasal origin. We measured changes in IL-6, IL-8, and MCP-1, and assessed changes in versican mRNA as a marker of matrix upregulation.

Methods

Bronchial fibroblasts

Primary fibroblasts were isolated from bronchial biopsies of seven asthmatic patients and eight healthy volunteers (Table 1). All patients gave written informed consent, as approved by the Laval Hospital Ethics Committee. Asthmatic patients had mild disease, as characterized by the use of β agonist only. None had ever used inhaled or systemic corticosteroids. All asthmatic patients were nonsmokers, and atopic, confirmed with a positive skin reaction to at least one common allergen. Patients had PC_{20} methacholine ranging from 0 to 4.21 mg/ml and FEV_1 within the normal range. All normal subjects were nonatopic, nonsmokers and had PC_{20} methacholine greater than 16 mg/ml. Additional details on selection and evaluation of subjects, bronchoscopy and bronchial biopsy procedures, biopsy processing, identification and characterization of bronchial fibroblasts have been described in previous publications (20–22). Isolated fibroblasts were characterized by immunofluorescence and flow cytometry using a mouse anti-vimentin antibody, and a mouse anti-human fibroblast antigen Ab-1 antibody (Calbiochem, San Diego, CA, USA) that shows no cross-reactivity with epithelial cells, endothelial cells, smooth muscle cells, or other cell types. This identification confirmed the purity of bronchial fibroblast cell culture. Cells were used at fourth or fifth passage.

Nasal fibroblasts

Primary fibroblasts were isolated from biopsies of nasal polyps of three healthy, nonatopic volunteers. All patients gave written informed consent, as approved by the Ethics Committee at the

Table 1. Subject characteristics

Group	Sex	Age	PC_{20}	FEV_1 (%)	Atopy	Medication
Asthmatic	F	20	4.21	3.51 (105)	Yes	β -agonist only
Asthmatic	M	20	2	4.22 (98)	Yes	β -agonist only
Asthmatic	M	18	0.66	4.29 (94)	Yes	β -agonist only
Asthmatic	M	26	3.11	4.00 (76.7)	Yes	β -agonist only
Asthmatic	F	24	0.72	2.56 (84)	Yes	β -agonist only
Asthmatic	F	26	1.1	3.52 (101)	Yes	β -agonist only
Asthmatic	F	21	0.3	2.64 (85)	Yes	β -agonist only
Mean		22.1 $\pm$ 1.1	1.7 $\pm$ 0.5	3.5 $\pm$ 0.3		
Normal	M	28	> 256	4.76 (91)	No	No
Normal	F	22	> 128	3.52 (108)	No	No
Normal	M	22	> 128	4.58 (111)	No	No
Normal	F	25	> 128	3.30 (102)	No	No
Normal	M	32	25.5	4.01 (122)	No	No
Normal	F	26	96	2.93 (95)	No	No
Normal	M	24	41.76	3.28 (80)	No	No
Normal	M	23	> 128	5.06 (103)	No	No
Mean		25.3 $\pm$ 1.2		3.9 $\pm$ 0.3		

PC_{20} , provocative concentration of methacholine to cause a fall in FEV_1 of 20%; FEV_1 , forced expiratory volume in 1 s; % predicted is shown in parentheses; M, male; F, female.

McGill University Hospital Centre. All subjects were nonsmokers. Cells were used at fourth passage.

Cell culture

Fibroblasts were cultured in Dubelcco's modified Eagle's medium (DMEM; Gibco-BRL-Invitrogen, Burlington, ON, Canada) supplemented with 10% heat-inactivated fetal calf serum (FCS) (HyClone, Logan, UT, USA), 100 U/ml penicillin G, 100 μ g/ml streptomycin and 250 ng/ml amphotericin B (all from Gibco-BRL-Invitrogen) at 37°C in the presence of 5% CO_2 . Sub-culturing was carried out using trypsin (0.25%) (Gibco-BRL-Invitrogen).

Mechanical stimulation of cultured fibroblasts

Cells were seeded on type I collagen-coated six-well BioFlex silastic-bottom culture plates (Flexcell International Corp., McKeesport, PA, USA) at a concentration of 1.5×10^5 cells/well. Cells were grown in the BioFlex plates until $\sim 90\%$ confluence. Cells were serum-starved by replacing the medium with DMEM without FCS, with antibiotics and antimycotics for 24 h. Plates were then transferred to the baseplate of the cell stretching device (FX-3000 Flexercell strain unit; Flexcell International, McKeesport, PA, USA) and placed in a 37°C, 5% CO_2 incubator. Application of a negative pressure caused a downward deformation of the flexible silastic membrane to which the cells were attached. A biaxial strain of 30% amplitude at a frequency of 1 Hz was applied for 3, 6 and 24 h. Strain of this amplitude corresponds approximately to inspiratory capacity (16). Control cells were cultured in BioFlex plates but not submitted to cell stretch. Cell layer and supernatants were harvested at various time points, and processed. Cell viability was assessed by Trypan blue exclusion.

Human cytokine array

A human cytokine-array kit (RayBio human cytokine antibody array III and 3.1 map) was purchased from RayBiotech (Norcross, GA, USA). Briefly, the membranes were blocked with a blocking

buffer, and then 1 ml of supernatant from each sample was added and incubated at 4°C overnight (samples were run in duplicate). The membranes were washed, and 1 ml of primary biotin-conjugated antibody was added and incubated at room temperature for 2 h. The membranes were incubated with 2 ml of horseradish peroxidase-conjugated streptavidin at room temperature for 30 min. The membranes were developed by using enhanced chemiluminescence detection. Densitometric analysis was performed using image analyzer software, the FluorChemtm FC 800 system (Alpha Innotech, San Leandro, CA, USA), which measures the sum of all the pixel values after background correction.

IL-6, IL-8 and MCP-1 enzyme-linked immunoassay

Cytokine release in cell supernatants was assessed by sandwich enzyme-linked immunoassay (ELISA). After 24 h of strain, fibroblast supernatants were collected and stored frozen at -80°C until quantification by using commercially available ELISA kits specific for human cytokine (RayBiotech). The detection limits were 1 pg/ml for IL-8, 6 pg/ml for IL-6, and 5 pg/ml for MCP-1.

RNA extraction and cDNA synthesis

Total cellular RNA was isolated using RNeasy mini kit extraction columns (Qiagen Inc., Mississauga, ON, Canada) and reverse-transcribed. For each sample, 1 µg of total RNA was incubated at 65°C for 5 min with 25 ng/µl oligodT and 0.5 mM dNTP and then at 42°C for 50 min with the following mix: 10 mM DTT, First-Strand Buffer, 10 U RNase inhibitor and 200 U SuperScriptTM II Reverse Transcriptase (Invitrogen).

Real-time polymerase chain reaction

Quantification of the mRNA message coding for IL-6, IL-8, MCP-1, and versican, was performed by real-time polymerase chain reaction (PCR) using the Roche LightCycler PCR (Roche Diagnostics, Laval, QC, Canada). S9 was measured as the housekeeping gene. Primers were designed to span an intron (sequences are shown in Table 2). Versican primers were purchased from Qiagen Inc. Real-time PCR reactions were performed in a volume of 20 µl containing 1 µl of cDNA, 5 µM of each primer and 10 µl of QuantiTectTM SYBR[®]

Table 2. Sequences and predicted size of primers used for real-time PCR

Cytokine/primer	Predicted size of amplicon (base pairs)
IL-6	
FP 5'-GGTACATCCTCGACGGCATC-3'	
RP 5'-GCCTCTTTGCTGCTTTCACAC-3'	79
IL-8	
FP 5'-GCAGAGGGTTGTGAGAAG-3'	
RP 5'-TCTTGATTGCATCTGGCAAC-3'	169
MCP-1	
FP 5'-GATCTCAGTGCAGAGGCTCG-3'	
RP 5'-TGCTTGCTCAGGTGGTCCAT-3'	153
S9	
FP 5'-TGCTGACGCTTGATGAGAAG-3'	
RP 5'-CGCAGAGAGAAGTCGATGTG-3'	307
Versican	
Hs_CSPG2_1_SG QuantiTect Primer Assay (Qiagen)	97

Green PCR containing DNA polymerase, dNTP mix, buffer, MgCl₂ and fluorescent dyes (Qiagen Inc.). Quantification of endogenous ribosomal protein S9 was performed as a control to correct for variations in cDNA content among samples. The expression of S9 remained stable under our experimental conditions. Results were analyzed with the LightCycler software version 3.5.3, and the melting curve was used to check for the specificity of the amplification products. Quantification of the PCR products was performed by using a standard curve obtained by simultaneously amplifying serial dilutions of the amplicon. Values obtained for each cytokine were normalized to S9 in the same sample.

Statistical analysis

The data were analyzed using GraphPad software. Data are reported as mean ± standard error. Kruskal-Wallis (nonparametric ANOVA) with Mann-Whitney tests (nonparametric unpaired *t*-tests) for multiple comparisons, were used to analyze differences in proteins measured by ELISA. Kruskal-Wallis with Dunn's tests (standard nonparametric multiple comparison tests) for multiple comparisons, were used to analyze differences in mRNA. Results were considered statistically significant at *P* levels < 0.05.

Results

Screening of cytokine and chemokine secretion by cytokine array

Normal bronchial fibroblasts (BNF), asthmatic bronchial fibroblasts (BAF) and nasal fibroblasts (NF) were cultured on collagen type 1 coated plates and submitted to excessive mechanical strain for 24 h. Control samples were unstrained. Supernatants were collected and analyzed using cytokine arrays. Measurements were carried out in duplicate. Positive signals of varying intensity were obtained for IL-6, IL-8, and MCP-1 in all fibroblasts (Fig. 1). Other cytokines involved in remodeling, such as TGFβ1, EGF and RANTES, were not up-regulated in response to mechanical strain; their signals at baseline, were, at best, modest.

Cytokine and chemokine secretion measured by ELISA

We focused on those cytokines whose signal was most evident on the cytokine array. Measurements were made on supernatants collected after application of excessive mechanical strain for 24 h. Control samples were unstrained. Under nonstrained conditions, there were no significant differences in cytokine secretion among the different fibroblast populations (Fig. 2A-C, left hand panels). After 24 h strain, BAF secreted significantly more IL-6 than BNF (*P* < 0.05) (Fig. 2A-C, right hand panels).

IL-6 mRNA expression is increased in BAF in response to mechanical strain

Expression of IL-6 mRNA was measured in samples at baseline, and after 3, 6 and 24 h of mechanical strain (Fig. 3). Control samples were unstrained. In BAF, mRNA expression for IL-6 was increased after 3 h of

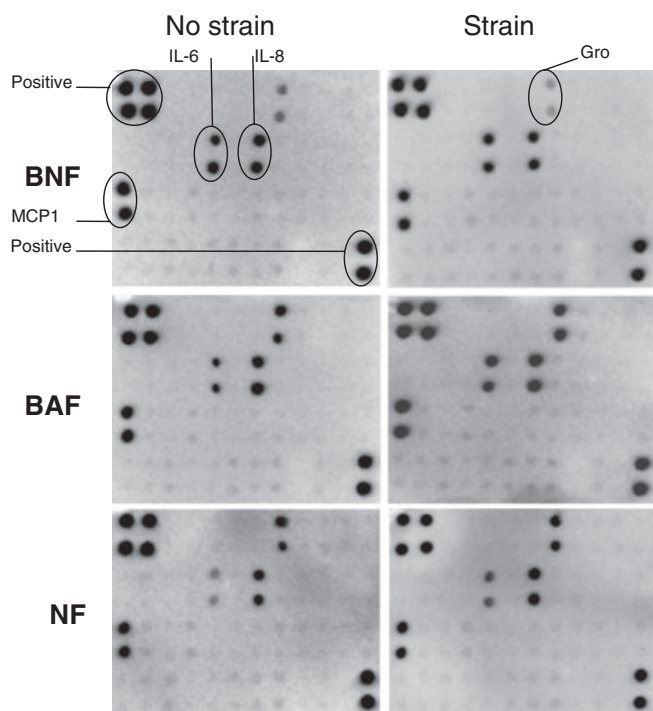


Figure 1. Screening of cytokine and chemokine secretion in supernatant using cytokine arrays. After 24 h of culture, with or without strain, supernatants were collected and analyzed. Array membranes showed well-defined signals for IL-6, IL-8, MCP-1 and Gro, under baseline conditions (no strain) and after 24 h of strain. Cytokine arrays were performed in duplicate. BNF, normal bronchial fibroblasts; BAF, asthmatic bronchial fibroblast; NF, nasal fibroblasts.

mechanical strain ($P < 0.05$). In BNF, IL-6 mRNA was decreased at the 6 and 24 h timepoints relative to the 3 h timepoint ($P < 0.05$). In NF, mechanical strain had no effect on IL-6 mRNA. There were no changes in IL-6 mRNA expression over the 24 h time period in cultured fibroblasts without strain (control conditions), excepting a decrease in IL-6 mRNA at 24 vs 6 h in BAF, a finding which reflected a decrease well below the baseline value.

IL-8 mRNA expression is increased in response to mechanical strain

In all three fibroblast populations, IL-8 mRNA expression was increased after 3 h of mechanical strain (Fig. 4). In BAF, IL-8 mRNA expression was still high at the 6 and 24 h timepoints. There was a significant increase in IL-8 mRNA expression in unstrained BAF at the 3 h timepoint (control conditions).

MCP-1 mRNA expression in response to mechanical strain

MCP-1 mRNA expression was significantly increased in BNF at the 3 h timepoint, as compared to the 24 h timepoint (Fig. 5). There were no significant changes in

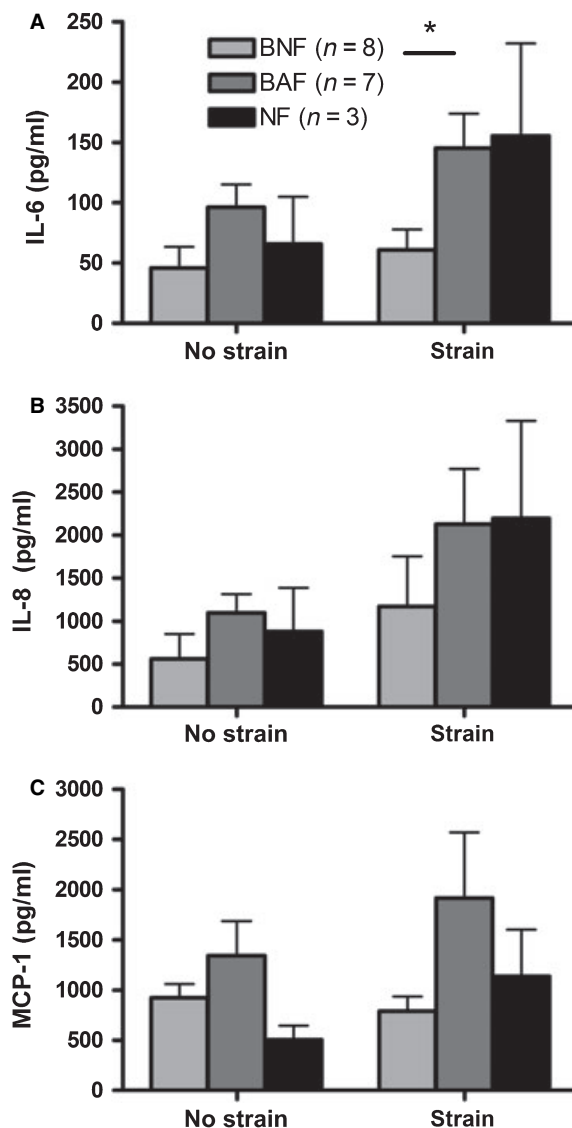


Figure 2. Cytokine and chemokine secretion in supernatant measured by ELISA. Cells were cultured with or without strain for 24 h, supernatant collected and analyzed. (A) IL-6 secretion; (B) IL-8 secretion; (C) MCP-1 secretion. *n*, represents the number of patients. BNF, normal bronchial fibroblasts; BAF, asthmatic bronchial fibroblast; NF, nasal fibroblasts. * $P < 0.05$.

MCP-1 mRNA expression over the 24 h time period in cultured fibroblasts without strain (control conditions).

Versican mRNA expression is increased in BAF in response to mechanical strain

Versican mRNA expression was significantly increased in BAF after 6 h of mechanical strain ($P < 0.05$ vs the 3 h timepoint) (Fig. 6). The relatively elevated level persisted at 24 h. There were no significant changes in versican mRNA expression over the 24 h time period in cultured fibroblasts without strain (control conditions).

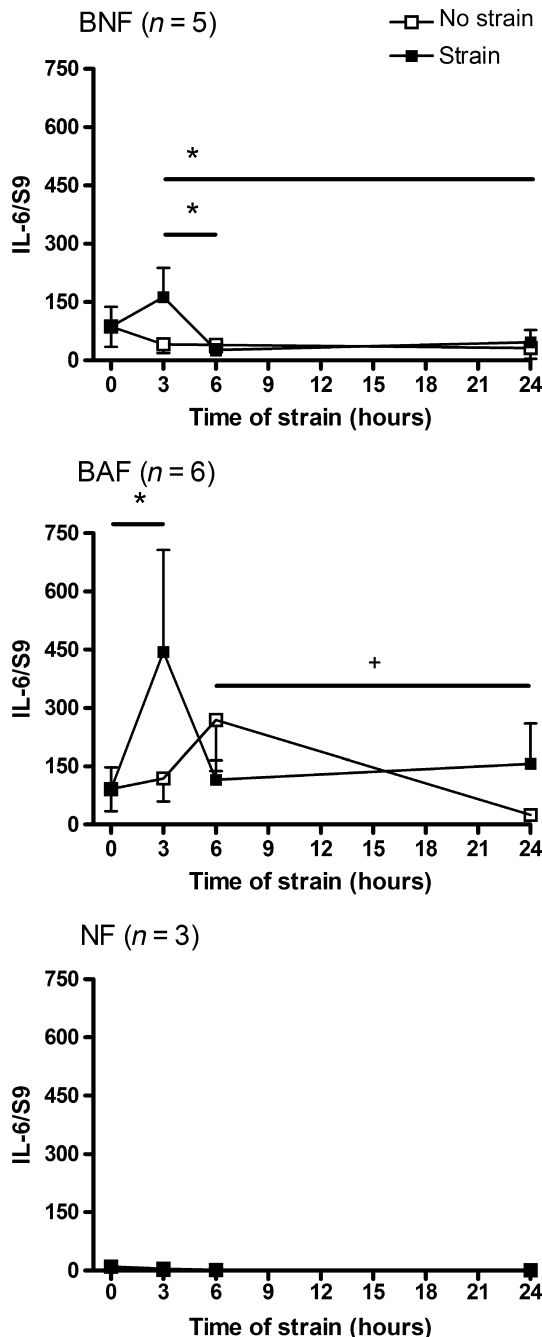


Figure 3. IL-6 mRNA expression. Cells were cultured with or without strain for different durations (3, 6 and 24 h). mRNA was extracted and quantified by real time PCR. BNF, normal bronchial fibroblasts; BAF, asthmatic bronchial fibroblast; NF, nasal fibroblasts. *n*, represents the number of patients. **P* < 0.05 for strained cells; + *P* < 0.05 for unstrained cells.

Discussion

The present study shows that excessive mechanical strain leads to differences in cytokine and chemokine message and secretion in various types of airway fibroblasts. IL-8 mRNA was increased in response to mechanical strain in

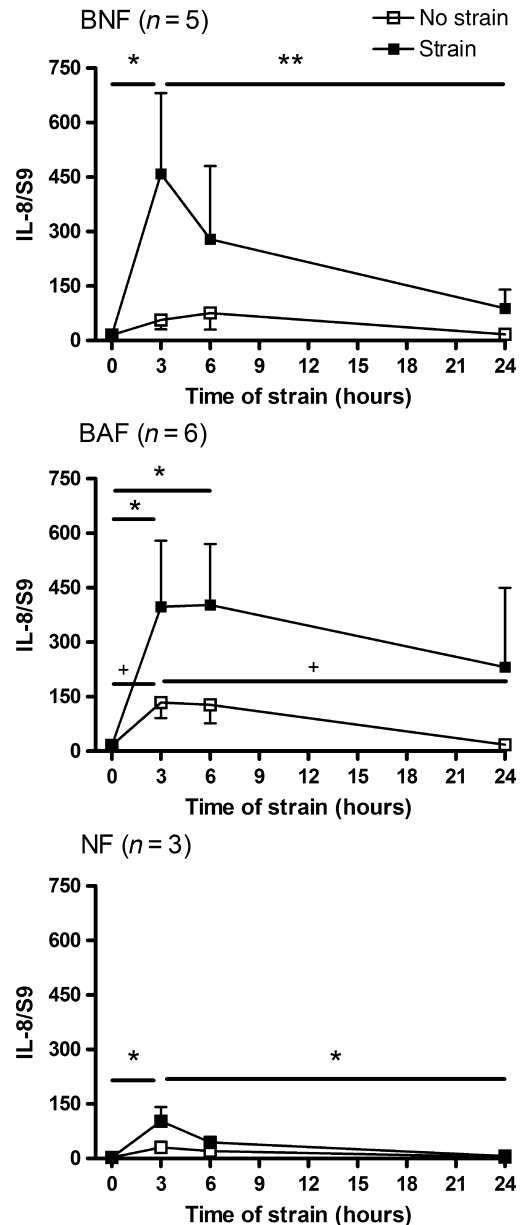


Figure 4. IL-8 mRNA expression. Cells were cultured with or without strain and for different durations (3, 6 and 24 h). mRNA was extracted and quantified by real time PCR. BNF, normal bronchial fibroblasts; BAF, asthmatic bronchial fibroblast; NF, nasal fibroblasts. *n*, represents the number of patients. **P* < 0.05; ***P* < 0.01 for strained cells, + *P* < 0.05 for unstrained cells.

all fibroblasts studied. On the other hand, IL-6 message was increased in bronchial fibroblasts from asthmatic patients only. Further, bronchial fibroblasts from asthmatic patients secreted more IL-6 after excessive mechanical strain than did bronchial fibroblasts from normal volunteers. Along with the upregulation of the pro-modeling cytokine, IL-6, message for the ECM proteoglycan, versican, was increased in response to excessive mechanical strain in these same cells.

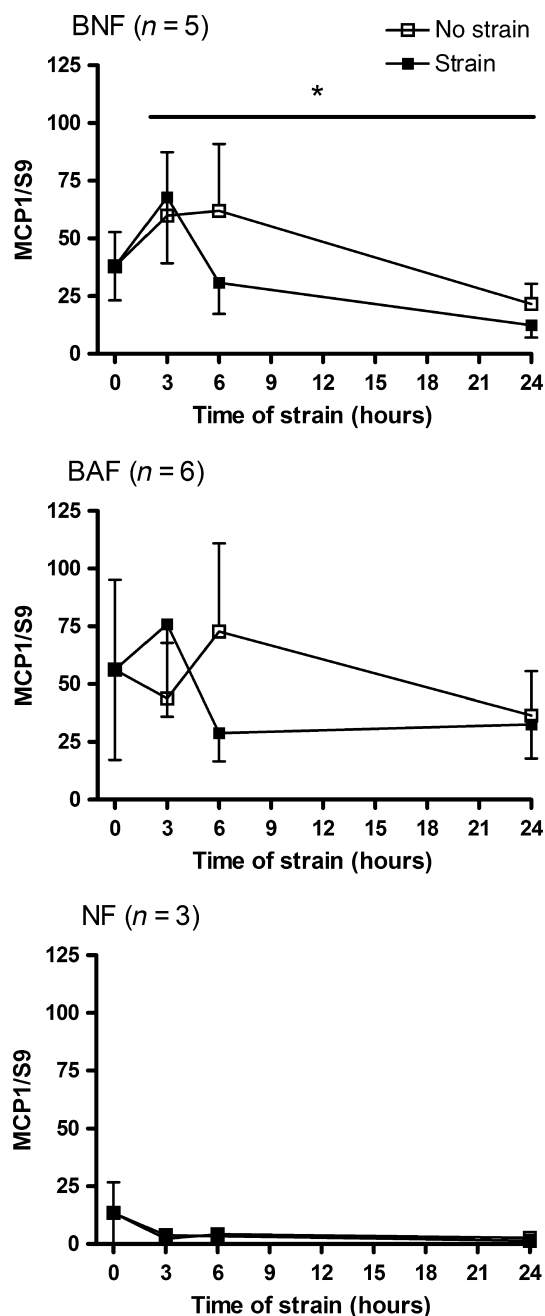


Figure 5. MCP-1 mRNA expression. Cells were cultured with or without strain for different durations (3, 6 and 24 h). mRNA was extracted and quantified by real time PCR. BNF, normal bronchial fibroblasts; BAF, asthmatic bronchial fibroblast; NF, nasal fibroblasts. *n*, represents the number of patients. **P* < 0.05, for strained cells.

Fibroblast populations from different parts of the airway tree have been shown to demonstrate different cytokine profiles. Kotaru et al. (23) reported that fibroblasts isolated from the proximal airways behave differently than fibroblasts isolated from the distal lung, in terms of cytokine and collagen secretion, and response to

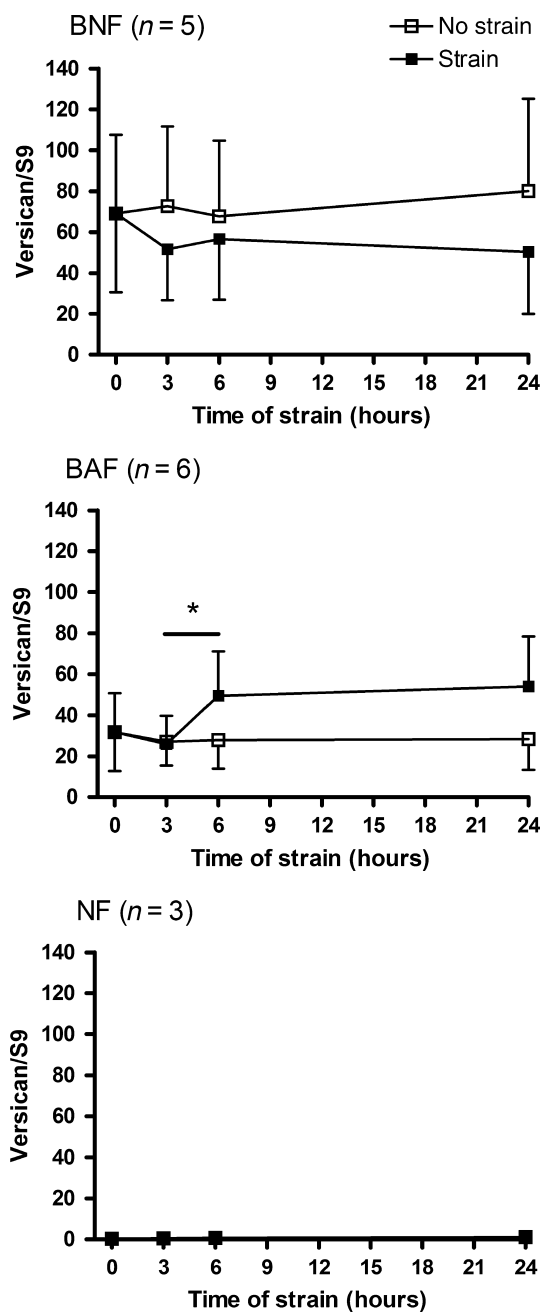


Figure 6. Versican mRNA expression. Cells were cultured with or without strain for different durations (3, 6 and 24 h). mRNA was extracted and quantified by real time PCR. BNF, normal bronchial fibroblasts; BAF, asthmatic bronchial fibroblast; NF, nasal fibroblasts. *n*, represents the number of patients. **P* < 0.05, for strained cells.

cytokine stimulation. In the current experiment, no differences in basal cytokine secretion were evident; however, the cytokine response to mechanical strain varied amongst the different fibroblast populations. The ability of lung cells to respond to mechanical stimulation with upregulation of cytokines has been previously

demonstrated in bronchial and alveolar epithelial cells (16, 18, 24), but not in bronchial fibroblasts. To our knowledge, no previous data are available on the effects of mechanical strain on nasal fibroblasts.

The mechanism by which fibroblasts upregulate cytokine secretion in response to mechanical strain is not known, but one putative pathway is via stimulation of MAP kinase phosphorylation. A number of studies have now shown that mechanical strain causes activation of MAP kinase signaling in various types of fibroblasts (lung, chick embryo, cardiac) including a study we have published in these same populations of fibroblasts (15, 25, 26). Furthermore, in that study, we showed that bronchial fibroblasts obtained from asthmatic patients had a different profile of MAP kinase phosphorylation in response to mechanical strain, than fibroblasts obtained from normal volunteers. In airway smooth muscle cells, mechanical strain has been shown to cause increases in IL-8 through activation of ERK 1/2 and p38 kinases (19). It seems plausible that these signaling pathways are involved in the response we document in the current experiment. Another interesting question is whether mechanical strain influences fibroblast differentiation. Along these lines, Choe et al. (27) have shown that mechanical strain of lung fibroblasts resulted in myofibroblast differentiation (as assessed by number of α smooth muscle actin positive cells present in a tissue engineered airway wall model containing epithelial cells and human fetal lung fibroblasts). Whether fibroblasts from asthmatic patients would be more sensitive to this effect is not known.

Airway fibroblasts from asthmatic and control subjects responded differently to mechanical strain, in terms of upregulation of cytokine message and secretion. IL-8 message was increased in response to excessive mechanical strain in all three fibroblast populations, BNF, BAF and NF. Vlahakis et al. (16) showed a significant increase in IL-8 in alveolar epithelial cells submitted to a 30% stretch amplitude, while Oudin and Pugin (18) showed an IL-8 response in bronchial epithelial cells submitted to a 20% stretch. In our study, it is interesting to note that the upregulation of IL-8 mRNA was most sustained in the asthmatic cells. Further, BAF showed enhanced IL-8 message even in those cells not submitted to the strain stimulus, simply with time in culture. After 24 h of mechanical strain, IL-6 protein was significantly greater in BAF as compared to BNF. IL-6 message was increased after 3 h of mechanical strain in BAF, only. These differences may reflect differences in the underlying inflammatory milieu and subsequent alterations in phenotype. Cytokines are up-regulated in the inflammatory state characteristic of asthmatic disease. IL-8 is an important member of the chemokine family, mediates the activation and migration of neutrophils and is involved in the initiation and amplification of inflammation (28). IL-6 contributes to the inflammatory process, but may also play an important role as a pro-

remodeling cytokine. Kuhn et al. (29) reported that IL-6 transgenic mice showed significant airway remodeling and sub-epithelial collagen accumulation. Other investigators have reported that IL-6 or IL-6 like cytokines have relevance to asthmatic airway remodeling (2, 30). Our observation that mechanical strain up-regulates IL-6 in the BAF only, may reflect the tendency for these cells to contribute to the remodeling characteristic of this disease.

We investigated the effect of mechanical strain on versican message as a marker of extra-cellular matrix. Versican is a large aggregating proteoglycan, which deposition is increased early in the fibrotic process (31). It forms a provisional matrix upon which subsequent collagen and other fibrotic proteins are laid down (32). We have shown in recently published experiments, investigating similar populations of bronchial fibroblasts obtained from asthmatic and control subjects, that versican protein and message is upregulated in response to excessive mechanical strain (14, 15). In the current experiment, we corroborate our previous findings.

The observation that mechanical strain differentially up-regulates pro-remodeling cytokines and extra-cellular matrix proteins in airway fibroblasts from asthmatic patients has particular relevance for asthma. Asthma is characterized by remodeling of the airway wall which contributes to abnormal airway physiology, including airway narrowing and airway hyper-responsiveness. In addition, asthmatic airways are subject to excessive mechanical stimulation as a consequence of heterogeneous airway narrowing and ventilation distribution (11). In the current study, excessive mechanical strain resulted in increased secretion of the pro-remodeling cytokine, IL-6, and the extra-cellular matrix protein, versican. One can postulate an autocrine mechanism whereby mechanical strain causes increased secretion of IL-6 which leads to enhanced matrix deposition. Alternately, the strain itself may result in increased ECM secretion, independent of the cytokine effect. Perhaps these two effects function synergistically.

In conclusion, we show that bronchial fibroblasts from asthmatic patients respond in a unique manner to excessive mechanical stimulation, with increased secretion of pro-inflammatory and pro-remodeling cytokines, coincident with, or perhaps causal to, the up-regulation of matrix components. This phenotype, specific to the BAF, likely contributes to the airway remodeling characteristic of this disease.

Acknowledgments

This work was supported by the J T Costello Memorial Research Fund, Canadian Institutes of Health Research, the McGill University Health Center Research Institute (FLB) and Fond de Recherche en Santé du Québec (FLB).

References

- Barnes PJ, Chung KF, Page CP. Inflammatory mediators and asthma. *Pharmacol Rev* 1988;**40**:49–84.
- O'Hara KA, Kedda MA, Thompson PJ, Knight DA. Oncostatin M: an interleukin-6-like cytokine relevant to airway remodelling and the pathogenesis of asthma. *Clin Exp Allergy* 2003;**33**: 1026–1032.
- Knight DA, Ernst M, Anderson GP, Moodley YP, Mutsaers SE. The role of gp130/IL-6 cytokines in the development of pulmonary fibrosis: critical determinants of disease susceptibility and progression? *Pharmacol Ther* 2003;**99**:327–338.
- Chu HW, Halliday JL, Martin RJ, Leung DY, Szefer SJ, Wenzel SE. Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. *Am J Respir Crit Care Med* 1998;**158**:1936–1944.
- Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig MS. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. *Am J Respir Crit Care Med* 1999;**160**:725–729.
- Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001;**164**:S28–S38.
- Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; **1**:520–524.
- Gizycki MJ, Adelroth E, Rogers AV, O'Byrne PM, Jeffery PK. Myofibroblast involvement in the allergen-induced late response in mild atopic asthma. *Am J Respir Cell Mol Biol* 1997;**16**: 664–673.
- Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001;**108**:430–438.
- Teran LM, Mochizuki M, Bartels J, Valencia EL, Nakajima T, Hirai K et al. Th1- and Th2-type cytokines regulate the expression and production of eotaxin and RANTES by human lung fibroblasts. *Am J Respir Cell Mol Biol* 1999;**20**:777–786.
- Tschumperlin DJ, Drazen JM. Mechanical stimuli to airway remodeling. *Am J Respir Crit Care Med* 2001; **164**:S90–S94.
- Breen EC. Mechanical strain increases type I collagen expression in pulmonary fibroblasts in vitro. *J Appl Physiol* 2000;**88**:203–209.
- Swartz MA, Tschumperlin DJ, Kamm RD, Drazen JM. Mechanical stress is communicated between different cell types to elicit matrix remodeling. *Proc Natl Acad Sci USA* 2001;**98**:6180–6185.
- Ludwig MS, Ftouhi-Paquin N, Huang W, Page N, Chakir J, Hamid Q. Mechanical strain enhances proteoglycan message in fibroblasts from asthmatic subjects. *Clin Exp Allergy* 2004; **34**:926–930.
- Le Bellego F, Plante S, Chakir J, Hamid Q, Ludwig MS. Differences in MAP kinase phosphorylation in response to mechanical strain in asthmatic fibroblasts. *Respir Res* 2006;**7**:68.
- Vlahakis NE, Schroeder MA, Limper AH, Hubmayr RD. Stretch induces cytokine release by alveolar epithelial cells in vitro. *Am J Physiol* 1999; **277**:L167–L173.
- Jiang MJ, Yu YJ, Chen YL, Lee YM, Hung LS. Cyclic strain stimulates monocyte chemotactic protein-1 mRNA expression in smooth muscle cells. *J Cell Biochem* 1999;**76**:303–310.
- Oudin S, Pugin J. Role of MAP kinase activation in interleukin-8 production by human BEAS-2B bronchial epithelial cells submitted to cyclic stretch. *Am J Respir Cell Mol Biol* 2002;**27**: 107–114.
- Kumar A, Knox AJ, Boriek AM. CCAAT/enhancer-binding protein and activator protein-1 transcription factors regulate the expression of interleukin-8 through the mitogen-activated protein kinase pathways in response to mechanical stretch of human airway smooth muscle cells. *J Biol Chem* 2003;**278**:18868–18876.
- Dube J, Chakir J, Laviolette M, Saint MS, Boutet M, Desrochers C et al. In vitro procollagen synthesis and proliferative phenotype of bronchial fibroblasts from normal and asthmatic subjects. *Lab Invest* 1998;**78**: 297–307.
- Goulet F, Boulet LP, Chakir J, Tremblay N, Dube J, Laviolette M et al. Morphologic and functional properties of bronchial cells isolated from normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 1996;**15**:312–318.
- Laliberte R, Rouabhia M, Bosse M, Chakir J. Decreased capacity of asthmatic bronchial fibroblasts to degrade collagen. *Matrix Biol* 2001;**19**: 743–753.
- Kotaru C, Schoonover KJ, Trudeau JB, Huynh ML, Zhou X, Hu H et al. Regional fibroblast heterogeneity in the lung: implications for remodeling 2. *Am J Respir Crit Care Med* 2006;**173**: 1208–1215.
- Jafari B, Ouyang B, Li LF, Hales CA, Quinn DA. Intracellular glutathione in stretch-induced cytokine release from alveolar type-2 like cells 1. *Respirology* 2004;**9**:43–53.
- Chiquet M, Sarasa-Renedo A, Tunc-Civelek V. Induction of tenascin-C by cyclic tensile strain versus growth factors: distinct contributions by Rho/ROCK and MAPK signaling pathways. *Biochim Biophys Acta* 2004;**1693**: 193–204.
- MacKenna DA, Dolfi F, Vuori K, Ruoslahti E. Extracellular signal-regulated kinase and c-Jun NH2-terminal kinase activation by mechanical stretch is integrin-dependent and matrix-specific in rat cardiac fibroblasts. *J Clin Invest* 1998;**101**:301–310.
- Choe MM, Sporn PH, Swartz MA. Extracellular matrix remodeling by dynamic strain in a three-dimensional tissue-engineered human airway wall model. *Am J Respir Cell Mol Biol* 2006;**35**:306–313.
- Pease JE, Sabroe I. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. *Am J Respir Med* 2002; **119**:25.
- Kuhn C, III, Homer RJ, Zhu Z, Ward N, Flavell RA, Geba GP et al. Airway hyperresponsiveness and airway obstruction in transgenic mice. Morphologic correlates in mice overexpressing interleukin (IL-11 and IL-6 in the lung. *Am J Respir Cell Mol Biol* 2000;**22**:289–295.
- Ammit AJ, Moir LM, Oliver BG, Hughes JM, Alkhouri H, Ge Q et al. Effect of IL-6 trans-signaling on the pro-remodeling phenotype of airway smooth muscle 1. *Am J Physiol Lung Cell Mol Physiol* 2007;**292**:L199–L206.
- Iozzo RV. Matrix proteoglycans: from molecular design to cellular function. *Annu Rev Biochem* 1998;**67**: 609–652.
- Bensadoun ES, Burke AK, Hogg JC, Roberts CR. Proteoglycan deposition in pulmonary fibrosis. *Am J Respir Crit Care Med* 1996;**154**: 1819–1828.