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Asthmatic bronchial fibroblasts demonstrate enhanced potential to differentiate into myofibroblasts in culture

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Background: Chronic inflammation and remodeling of the bronchial wall are basic hallmarks of asthma. It is known that mesenchymal cells in the lamina reticularis underlying the basement membrane of the thickened airway wall of asthmatics predominantly display the phenotype of myofibroblasts and express α-smooth muscle actin (α-SMA). Human bronchial fibroblasts (HBFs) transform *in vitro* into myofibroblasts under the influence of transforming growth factor (TGF-β). Differences in the reactivity of fibroblasts to TGF-β in cultures derived from healthy and asthmatic donors are elucidated here.

Material/Methods: Primary human bronchial fibroblasts (HBFs) were cultured from bronchial biopsies from non-asthmatic (n=7) and asthmatic (n=7) donors and treated with TGF-β_1 or TGF-β_2 to induce myofibroblast differentiation. Expression of α-smooth muscle actin (α-SMA) was assessed by immunocytochemistry and Western blotting. The cell size and shape parameters were measured by computer-aided methods.

Results: Regardless of whether TGF-β_1 or TGF-β_2 was used, asthmatic cells showed enhanced expression of the myofibroblast marker as confirmed by immunocytochemistry and immunoblotting. Analysis of the shape parameters of cells incubated in the presence of TGF-β_1 revealed that HBFs of asthmatics differ from those of non-asthmatics.

Conclusions: It is concluded that asthmatic HBFs cultured *in vitro* display some inherent features which facilitate their differentiation into myofibroblasts. These data indicate that increased reactivity of asthmatic fibroblasts to TGF-β may play a crucial role in asthma.

key words: **human bronchial fibroblasts • myofibroblasts • transforming growth factor beta • asthma**

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Background

Recent studies on bronchial asthma indicate that the chronic inflammation of the airways does not fully explain the pathophysiology of the disease. The structural changes in the bronchial wall are now considered to be the second fundamental factor in the development of asthma [1]. Airway remodeling is intensively studied in many laboratories as it seems to be resistant to currently available pharmacological treatment. This process is defined as a sequence of chronic structural changes leading to thickening of the airway wall, epithelial damage, subepithelial fibrosis, and increased deposition of extracellular matrix proteins [2,3].

It is generally accepted that fibroblasts in the asthmatic airways are stimulated not only to proliferate and produce collagens, but also to differentiate into myofibroblasts, the more contractile subset of fibroblasts which are involved in wound contraction and the development of fibrotic changes [4]. The process of myofibroblast differentiation has been studied in both animal and human models and was shown to be effectively induced by prolonged cell incubation with transforming growth factor-β (TGF-β) [5]. Damage to the epithelium, a characteristic feature of asthma, leads to increased release of TGF-β_1 and TGF-β_2 [6,7], the levels of which are also elevated in the bronchoalveolar lavage of asthmatics [8]. The aim of the present study was to evaluate whether TGF-β-stimulated fibroblasts in culture show an increased tendency to differentiate into myofibroblasts when derived from asthmatics.

Material and Methods

Isolation, culture, and characterization of human bronchial fibroblasts (HBFs)

Bronchial biopsies were obtained from the segmental bronchi during bronchoscopy using a fiberoptic bronchoscope (Olympus, Japan). Primary HBFs were isolated as described previously [9]. The cells were cultured in DMEM with 10% FCS at 37°C in a humidified atmosphere with 5% CO_2 and used between 5–8 passages. For some experiments, cells were incubated in serum-free DMEM supplemented with 0.1% bovine serum albumin (Sigma St. Louis, MO, USA) with or without human recombinant TGF-β_1 (source: CHO cells) or TGF-β_2 (source: BTI-Tn-5B1-4 (High-5) Insect Cells, PeproTech EC, London, UK).

Patient characteristics

The study was performed in two groups of subjects with a total of 14 individuals. The first group consisted of 7 non-asthmatics in whom diagnostic bronchoscopy ruled out any serious airway pathology, including asthma, fibrotic lung disease, sarcoidosis, and cancer (group NA: 2 males and 5 females; average age: 46 years; mean FEV_1: 112% predicted, range: 97–138%). The second group consisted of 7 patients diagnosed with moderate asthma (group AS: 2 males, 5 females; average age: 41 years; mean duration of asthma: 7 years; mean FEV_1: 89.7% of predicted, range: 72–118%). All patients were treated in the Department of Medicine of Jagiellonian University and were in stable clinical condition. The study was approved by the University's Ethics Committee (KBET/362/B/2003) and all the patients provided informed consent to participate.

Measurements of cell size and shape

A computer-based image analysis system was used to measure the size and shape of the bronchial fibroblasts from the asthmatics and healthy donors. The cells were rinsed with PBS, fixed with 3.7% formaldehyde, and stained with Coomasie brilliant blue R-250 (Merck, Darmstadt, Germany). Cell image analysis was performed using a basic system consisting of an inverted Olympus IMT-2 microscope equipped with a Hitachi KP-161 CCD camera, a personal computer, Photo Delux image grabber, and software for image analysis written by Mr. R. Tokarski [10,11]. The signal from the video camera was digitized. Each image of fibroblasts was subtracted from a background image to compensate for any unevenness in the illumination and camera response. The resulting image was then converted to a binary (black and white) image by selecting a threshold gray level above which all pixels were set to white and all others to black. This selection was performed under manual control to ensure the best correspondence between the original cells and the white areas of the binary image. The image was then compared with the original optical image and any selections not corresponding to single isolated cells were removed (Figure 1A,B).

To characterize the cell morphology quantitatively, the surface area of the cell projections (in μm^2) and the cell extension, dispersion, and elongation were measured (Figure 1C). The shape parameters were defined and calculated as described by Dunn and Brown (1986) [12]. The parameters were determined for at least 100 cells in each case. The mean and standard error for each parameter were calculated.

Staining of α-SMA

Cells growing on glass coverslips were washed with PBS, fixed in 3.7% paraformaldehyde for 5 min, and permeabilized in 0.1% Triton X-100 for 1 min. After 1 h in 0.3% H_2O_2, the coverslips were incubated for 24 hours at 4°C with a monoclonal antibody against human α-SMA (clone 1A4, Sigma, 1:400). For staining, the Strept ABC Complex/HRP Duet kit (Dako, Glostrup, Denmark) and the AEC Chromogen Kit (Sigma) were used according to the manufacturers' instructions. The level of myofibroblastic differentiation was measured by counting at least 200 cells.

Immunofluorescence

For the immunostaining of vimentin or α-SMA, cells growing on glass coverslips were washed with PBS, fixed in 3.7% paraformaldehyde for 15 min, and permeabilized in 0.1% Triton X-100 for 10 min at room temperature. Following fixation the slides were blocked with 3% bovine serum albumin for 10 min. Then the slides were incubated with mouse monoclonal antibody against vinculin (Sigma, 1:500) or mouse monoclonal antibody against human α-SMA (clone 1A4, Sigma, 1:400) for 1 hour at room temperature. The cells were washed five times with PBS and incubated with Alexa Fluor 488 gout anti-mouse IgG (clone, Sigma, 1:200) for 45 min at room temperature. Fluorescent images were taken with a Leitz Orthoplan microscope.

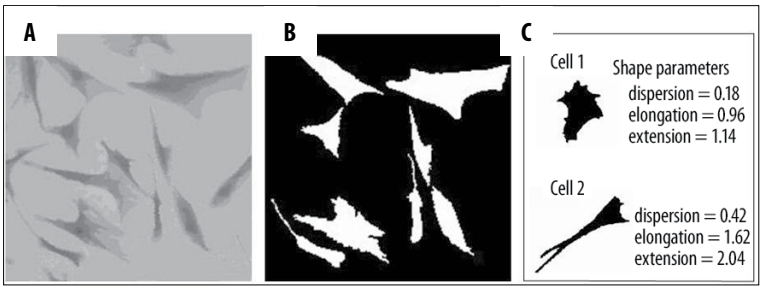


Figure 1. Representative digitized video image of HBFs (**A**) and an enhanced binary image of **A** after residual noise (**B**). In panel **B**, cells in contact and cells not fully in the frame have been removed. Two exemplary cells differing in shape and in dispersion, elongation, and extension are shown in panel **C**.

Preparation of cell extracts and immunoblotting

Cells were cultured in 6-cm-diameter Petri dishes until confluency. The cells were stimulated with TGF-β (10 ng/ml) for 48 hours. Fibroblasts were lysed with a lysis buffer (0.1 mol/l Tris-HC1, 15% glycerol, 2 mmol/l EDTA, 2% SDS, 10 mmol/l phenylmethytsulfonyl fluoride, and 10 μg/ml aprotinin, pH 7.4) and 10 μg of the total cellular protein was subjected to SDS-PAGE in 12% acrylamide gels under reducing conditions and transferred to a polyvinylidene difluoride membrane (Hybond-P, Amersham Pharmacia Biotech). After blocking in PBS-T (0.1% Tween 80 in PBS) containing 5% skimmed milk overnight at 4°C, the membrane was incubated for 1 hour with mouse monoclonal anti-α SMA actin (SIGMA) antibody (1:1000 in 5% skimmed milk in PBS-T) at 37°C. After washing three times, the membrane was incubated for 1.5 hours with anti-mouse IgG horseradish peroxidase-conjugated antibody (1:1000 in PBS-T containing 5% skimmed milk; Santa Cruz Biotechnology). The extensively washed membranes were incubated with chemiluminescent reagent (ECL-Western blotting analysis system, Amersham) for 60 seconds and exposed to Kodak X-Omat film. For quantitative measurements the films were digitalized and analyzed with an imaging system (ImageMaster VDS software, GeneTool, Syngene). The membranes were stained with Coomassie Brilliant Blue to check the uniformity of protein loading. The lowest value obtained for a band representing an NA patient was considered as 100%. The amount of protein was expressed relative to this lowest value. In some cases, α-SMA protein was not detected in the control samples and these wells were excluded from analysis.

Statistical analysis

The significance of differences in the proportion of cells expressing α-SMA between the cell groups (AS, NA) were determined by applying the nonparametric Mann-Whitney *U* test or the Wilcoxon matched-pairs rank test. Two methods were used to assess the statistical significance of changes in the cell projection parameters. In the case of shape parameters, the data sets were examined by one-way analysis of variance (ANOVA) with the Scheffè post hoc test. The GLM (general linear model) module of Statistica 6.0 was used for statistical calculations. The Kruskal-Wallis nonparametric variant of ANOVA was applied for comparisons of area and perimeter. The α correction according to Dunn-Sidak was applied in multiple comparisons.

RESULTS

After isolation, the cells were assessed microscopically to have fibroblast-like morphology. The levels of expression of α-smooth muscle actin (α-SMA), vimentin, and F-actin were also checked by means of the cytoimmunofluorescence staining or staining with TRITC-phalloidin. In all the primary cultures kept under normal conditions (DMEM+10%FCS), less than 0.5% of cells were very weakly stained for α-SMA and all cells expressed vimentin. In sparse culture they developed rather thin stress fibers and small focal adhesions. These results were considered proof that the isolated cells were not smooth muscle cells, but fibroblasts. This was the case in all the cell cultures we used. It is now believed that in cell culture on a rigid substrate, fibroblasts express a stable phenotype which can be called the proto-myofibroblastic phenotype [13].

Although the generation of myofibroblasts in pathological states (fibrosis, wound healing) *in vivo* is complex, the main inducers of this phenotype seems to be transforming growth factors β and the process can be reproduced *in vitro* by incubating the cell cultures in medium containing the active forms of TGF-β_1 or -β_2 for 1–3 days [14,15]. We cultured the HBFs in serum-free medium supplemented with the active form of human recombinant TGF-β_1 or TGF-β_2 at concentrations of 1, 5, 10, and 20 ng/ml for 48 hours. The *in vitro* differentiation of the HBFs to myofibroblasts was evaluated by an analysis of α-SMA protein expression and the incorporation into stress fibers using immunocytochemical methods. In contrast to control cultures incubated in DMEM with or without serum, the myofibroblasts in medium containing TGF-β were easily distinguishable because they displayed well-developed, thick, and α-SMA-positive stress fibers. The percentage of the TGF-β-induced. α-SMA-positive cells was 2–25% and varied from culture to culture. In individual cultures the effect was dose dependent up to 10 ng/ml, because of which a concentration of 10 ng/ml was chosen for further experiments. Our microscopic observations indicated that the incubation of HBFs in the medium containing TGF-β imposes an evident effect not only on α-SMA expression, but also on the cell size and shape (Figure 2). We did not observe significant differences between TGF-β_1- and β_2-treated HBF cultures.

To confirm these observations we measured the projected cell areas and cell shape parameters (extension, elongation, and dispersion) of fibroblasts (α-SMA-negative cells) and myofibroblasts (α-SMA-positive cells) from the same TGF-β_1-treated HBF culture. The parameters characterizing one representative cell culture are given in Figure 3. According to Dunn and Brown [12], extension is a measure of how much the shape differs from a circle. It has a value of zero if it is circular and increases without limit as it becomes less compact and is the sum of the elongation and dispersion. Elongation is a measure of how much the shape must be compressed along its long axis to min-

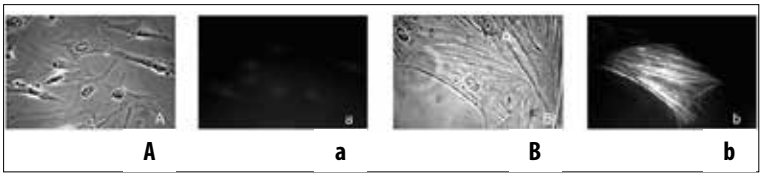


Figure 2. TGF-β_1-induced changes in the morphology of HBFs. HBFs (derived from asthmatics) were cultured in DMEM with 10% FCS for 24 hours, then with the same medium (**A,a**) or with DMEM supplemented with 0.1% BSA with 10 ng/ml TGF-β_1 (**B,b**) for 48 hours. The cells were fixed and stained for α-SMA. The cells were photographed with a Leitz Orthoplan microscope with phase contrast optics (**A,B**) and with epifluorescence mode (**a,b**). Bar = 20 µm

imize its extension. It changes from 0 for a circular shape and increases as the cell elongates. Elongation can never be less than 0 or greater than the extension. Dispersion is the minimum extension that can be attained by compressing the shape uniformly. The minimum dispersion of zero is achieved only if the shape is an ellipse and it can never have a value greater than that of the extension.

In all the cultures tested, the myofibroblasts population was characterized by a larger mean area (Figure 3A) and a smaller extension of the cell projection. The reduced extension value obtained for myofibroblasts is mainly due to the reduced elongation of the cell projections (Figure 3B–D).

The microscopic observations indicate that the incubation of HBFs in medium containing TGF-β_1 imposes an evident effect on the cell size and shape regardless of whether they are fully differentiated myofibroblasts (Figure 4). We sought to investigate whether this is more pronounced in the cell cultures derived from asthmatics. To answer this question the same quantitative analysis of the cell size and shape parameters was used. To get a statistically significant result we repeated the experiments (48-h incubation of cells without or with 10 ng/ml of TGF-β_1) using five cultures derived from healthy donors and five from asthmatics (Table 1, Figure 5). The cell cultures derived from various subjects differed significantly in their cell projection area (the range of the mean area was from 1915–5044 µm^2 in the NA group and 1704–5236 µm^2 in the AS group, Table 1A). However, the statistical analysis revealed that there was no difference in the areas of the cell projections of the HBFs from groups NA and AS, regardless of the cell culture conditions [serum-free medium with or without TGF-β_1 (Table 1) or DMEM with 10% FCS (data not shown)]. However, TGF-β_1 treatment caused a significant increase in the projected cell area in both groups of cultures (Table 1B, Figure 5A). Noteworthy results were obtained when the effect of TGF-β_1 on the extension of the HBF projections was studied. As shown in Table 1 and Figure 5B, the extension of the HBF projections was significantly reduced upon TGF-β_1 treatment in cell cultures derived from the subjects of both groups. In the case of this parameter there were significant differences between the NA and AS groups for both culture conditions (serum-free medium with and without TGF-β_1). Surprisingly, the extension seemed to be lower in the NA group (Table 1A).

To assess whether the potential of differentiation into myofibroblasts depends on the subject group (NA or AS), the percentage of cells expressing α-SMA was evaluated. In all the cultures tested in the serum-free condition, only a small proportion of cells (below 1%) showed a diffuse staining pattern of α-SMA that was not incorporated into stress fibers. In contrast, in the TGF-β_1-treated HBFs the myofibroblasts were easily distinguishable because they displayed well-developed, thick, and α-SMA-positive stress fibers. The percentage of α-SMA-positive cells was significantly higher in the AS group than in the NA (Figure 6A). The same result was obtained for cultures incubated for 48 hours in 10 ng/ml of TGF-β_2 (Figure 6B). The difference between the influence of TGF-β_1 and TGF-β_2 was not statistically significant (Wilcoxon signed-ranks test, $p>0.05$).

Although cytoimmunofluorescence identified a very low number of typical myofibroblasts in the TGF-β-untreated

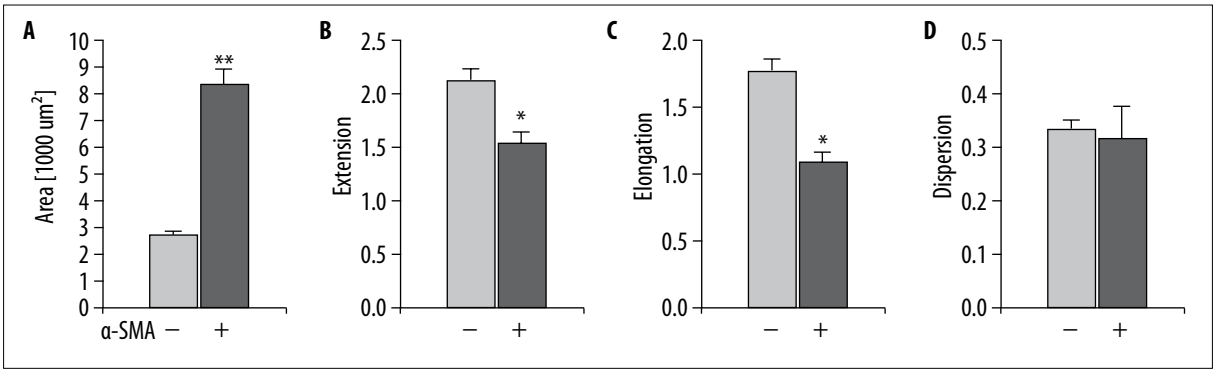


Figure 3. Comparison of α-SMA-negative fibroblasts and α-SMA-positive myofibroblasts. The cells were cultured in DMEM with 10% FCS for 24 hours and then with DMEM supplemented with 0.1% BSA with 10 ng/ml TGF-β_1 for 48 hours. The cells were fixed and stained for α-SMA. The area (**A**), extension (**B**), elongation (**C**), and dispersion (**D**) of the cell projections for α-SMA-negative and -positive cells derived from the NA and AS groups were determined. The presented values are the means ±*SE*. The parameters for at least 50 cells in each group were calculated. Significantly different populations in the same culture are indicated by *$p<0.05$ and **$p<0.01$. One representative result of four independent experiments is shown.

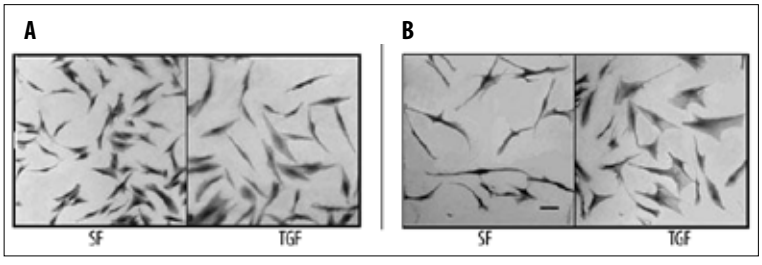


Figure 4. TGF-β_1-induced changes in the morphology of HBFs. The cells derived from the NA (**A**) and AS (**B**) groups were cultured in DMEM with 10% FCS for 24 hours and then with DMEM supplemented with 0.1% BSA with (TGF) or without (SF) 10 ng/ml TGF-β_1 for 48 hours. Cells were stained with Coomassie BB and photographed with an Olympus IMT inverted microscope. Bar = 20 µm.

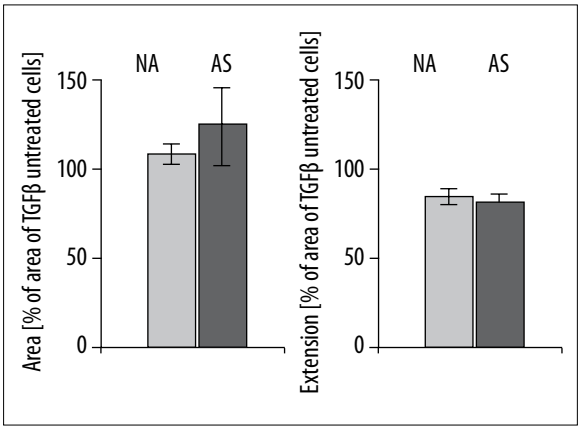


Figure 5. Size and shape of the HBF projections of the NA and AS groups. The cells were cultured in DMEM with 10% fetal calf serum for 24 hours and then with DMEM supplemented with 0.1% BSA with (TGF) or without (SF) 10 ng/ml TGF-β_1 for 48 hours. The area (A) and extension (B) of the cell projections for each experimental condition were determined and calculated as the percentage of the control value (SF). The results are the means of five separate experiments from both groups presented separately with the results of the statistical analysis in Table 1.

HBFs, α-SMA protein was detectable in whole-cell extracts of unstimulated cells by immunoblotting, predominantly in asthmatic cultures. In all the cultures tested, incubation in TGF-β_1 resulted in a pronounced increase in the expression of the marker protein (representative blots are shown in Figure 7A). When the amount of α-SMA protein was compared between the groups, a higher expression of the myofibroblast marker was evident in the HBFs from the asthmatics (Figure 7B,C).

Discussion

It is well known that transforming growth factor-β is one of the most important pro-fibrotic cytokines *in vivo* [15–17] and that it upregulates α-SMA expression in fibroblasts derived from different tissues and induces the differentiation of myofibroblasts *in vitro* [18,19]. Results using primary cells from human airways also support this view [6,20,21]. However, it is worth noting that in our experiments not all, but only a relatively small fraction of cells adopted a myofibroblastic phenotype upon TGF-β_1 or TGF-β_2 treatment (Figure 6). This observation suggests that HBFs represent heterogenic cell populations with subtypes characterized by slightly different features in which only some are somehow primed to differentiate. The heterogenicity of fibroblasts from asthmatics was confirmed by studies of Larsen et al. [21,22]. These authors compared primary cells derived from bronchoalveolar lavage fluid (BALF) and from bronchial biopsies and demonstrated that the first are more elongated, migrate faster, produce more α-SMA protein, and are characterized by a different profile of extracellular matrix protein production.

Our results indicate that the number of cells "primed" to differentiate into myofibroblasts was significantly higher in the populations of HBFs originating from asthmatic airways. To our knowledge this is the first report showing such differences between asthmatic and non-asthmatic fibroblasts. However, congruent results were obtained by Wicks et. al., who showed that several smooth muscle-related proteins and transcripts (calponin, heavy myosin chain, and desmin) are upregulated to a greater extent by TGF-β_2 in asthmatic HBFs than in healthy controls [23]. In contrast to our comparison of sparse, sub-confluent HBF cultures from asthmatics and non-asthmatics (Figures 6,7), they failed to show a difference in α-SMA expression. However, in very confluent cultures which Wicks et al. evidently used, the expression of α-SMA protein is generally very low (our results: data not shown) and the differentiation of myofibroblasts *in vitro* is strongly and inversely correlated with cell density [24].

It is believed that fibroblasts cultured *in vitro* are mechanically activated by contact with the rigid substrate and have a phenotype of proto-myofibroblasts, which represents an intermediate step between fibroblasts and myofibroblasts *in vivo* [13]. Our results indicate that the TGF-β-induced transition of bronchial fibroblasts into myofibroblasts is accompanied by striking cell shape changes (Figure 3) and this phenomenon was equally evident in both groups of HBF cultures. It was shown previously that TGF-β_1-treated fibroblasts with α-SMA-containing stress fibers are characterized by enhanced contractility and generate a higher traction force [25,26]. Moreover, Dugina et al. [27] showed that during myofibroblast differentiation a significant enlargement of focal adhesion (focal adhesion "supermaturation") takes place. These authors suggested that the process of fibroblast differentiation can affect cell adhesion and spread. Furthermore, our quantitative measurements of the size and shape parameters of fibroblasts derived from asthmatics and non-asthmatics revealed some statistically significant differences. In the HBF cultures examined in our experiments, TGF-β_1 treatment increased the area of the cell projections, possibly due to enhanced spreading of cells, regardless of whether the cells expressed α-SMA protein. The diminished extension of the cell projections was probably connected with the retraction of cell protrusions caused by enhanced cell traction force. Comparison of the HBF cultures of the NA and AS groups (Table 1) revealed that, statistically, the

Table 1. Size and shape of HBF projections of the NA and AS groups. (**A**) The cells were cultured in DMEM with 10% FCS for 24 hours and then with DMEM supplemented with 0.1% BSA with (TGF) or without (SF) 10 ng/ml TGF-β_1 for 48 hours. The area and extension of the cell projections for each experimental condition were determined. At least 100 cells were analyzed. Values are given as the mean ±*SE*. (**B**) In the case of the area of the cell projections, the statistical significance was determined by the nonparametric Kruskal-Wallis test. As multiple comparisons were performed, the confidence level was lowered according to the Dunn-Sidak correction to α=0.013. In the case of the extension parameter the statistical significance was determined by the Scheffè test. Significant *p* values are marked by (*).

A Patient's number	Area [μm²] ± SE		Extension ± SE	
	SF	TGF	SF	TGF
NA 1	2202±121	2583±103	3.39±0.9	2.79±0.8
NA 2	1915± 84	1927±102	2.76±0.7	2.36±0.7
NA 3	5044±243	5252±283	2.81±1.1	2.20±0.8
NA 4	4761±225	5332±281	3.09±1.0	2.85±1.0
NA 5	2720±144	3273±173	1.51±0.9	1.25±0.8
AS 1	1704± 99	2502±131	3.80±1.2	3.02±1.1
AS 2	1687±105	2306±132	3.88±0.9	3.04±0.9
AS 3	5236±269	5393±324	2.99±0.9	2.69±1.1
AS 4	5029±321	5141±322	3.27±1.1	2.57±0.9
AS 5	2637±233	5060±445	2.09±1.2	1.50±0.5

B	NA vs. AS		SF vs. TGF	
	SF	TGF	NA	AS
Area	0.0226	0.8445	0.0017*	0.0033*
Extension	$5{,}1\times10^{-8}$*	0,002*	$3{,}3\times10^{-9}$*	0,0001*

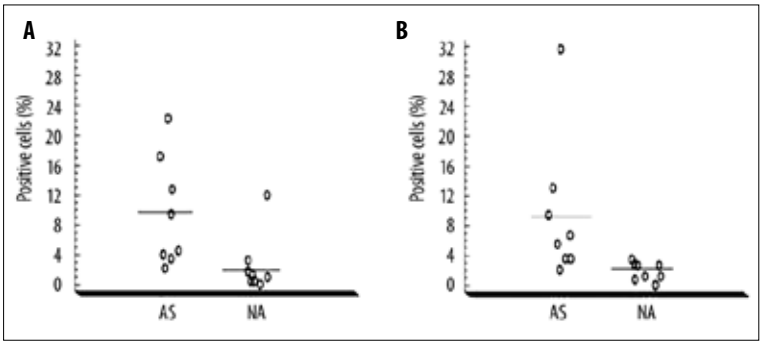
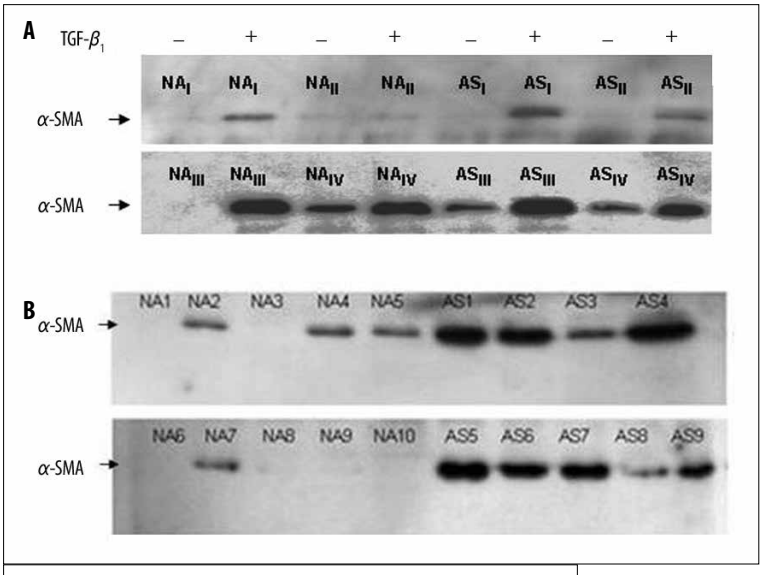


Figure 6. TGF-β-induced fibroblast to myofibroblast differentiation in the NA and AS groups. The HBFs were seeded on glass coverslips at a density of 7500 cells/cm² in DMEM with 10% FCS. After 24 hours the medium was changed to DMEM supplemented with 0.1% BSA with TGF-β_1 (**A**) or TGF-β_2 (**B**) at a concentration of 10 ng/ml for 48 hours. The cells were stained for α-SMA and the numbers of positive cells were counted. Each point represents the result obtained from a single culture. Bars represent the mean values. In the AS group the percentage of positive cells was significantly higher than in the NA group in both treatments (Mann-Whitney *U* test, $p<0.01$).

area was enhanced and the extension of the cell projections was reduced in the asthmatic fibroblasts.

Although the role of TGF-β in airway remodeling in asthma has been investigated in many laboratories, the mechanisms involved in the differentiation of phenotypic fibroblasts into myofibroblasts are only partially understood. It is now widely accepted that the expressions of both TGF-β_1 and TGF-β_2 increase in the asthmatic airways and that *in vitro* these cytokines induce the proliferation of fibroblasts and their differentiation into myofibroblasts and extracellular matrix protein synthesis [4,5,17]. Recently, Malavia et al. reported that in co-culture of human bronchial epithelial cells with normal human lung fibroblasts, prolonged cytokine exposure (e.g. to IL-13) and mechanical perturbations to the epithelium can cause the continuous release of TGF-β_2, which stimulates collagen production and secretion from fibroblasts [28]. Taken together, these observations suggest that further experiments are needed to explain whether the enhanced TGF-β-stimulated differentiation of asthmatic HBFs into myofibroblasts, accompanied by prominent cell shape changes, may be connected with increased secretion of some extracellular matrix proteins (e.g. collagen).



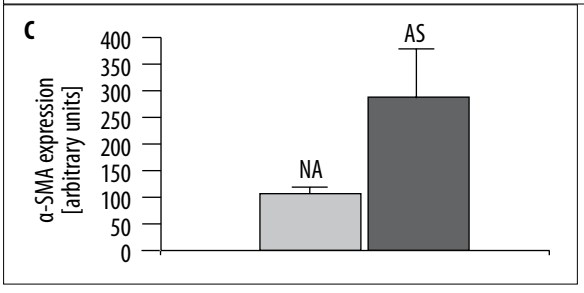


Figure 7. The expression of α-SMA in TGF-β_1-treated HBFs of the NA and AS groups. Protein extracts were subjected to SDS-PAGE electrophoresis and α-SMA protein was detected by immunoblotting. In immunoblot A, 10 μg of protein was used to visualize the small amount of α-SMA present in the TGF-β_1-untreated HBFs. In immunoblot B, 5 μg of protein extract per well was applied and the films were digitalized and analyzed by an imaging system (Genetools Syngen) to obtain semiquantitative data (C). The lowest value on each film obtained for a band representing an NA patient was considered as 100%. The amount of protein in other wells was expressed relative to this lowest value.

Conclusions

This study demonstrated that HBFs derived from asthmatics display some inherent features in culture which facilitate their differentiation into myofibroblasts. The exact molecular mechanism of the phenomenon reported here requires further studies and may be of considerable importance in explaining the bronchial remodeling in asthma.

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