

Airway fibroblasts exhibit a synthetic phenotype in severe asthma

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Background: Platelet-derived growth factor (PDGF) may have a significant role in airway remodeling in asthma, because it is a powerful inducer of many airway fibroblast activities such as collagen synthesis.

Objective: To determine whether PDGF is a significant contributor to airway remodeling in patients with asthma by enhancing airway fibroblast procollagen I expression.

Methods: Six normal controls without asthma, 10 subjects with mild to moderate asthma, and 5 subjects with severe asthma underwent bronchoscopy with endobronchial biopsy. Biopsies were placed in Dulbecco modified Eagle medium and fibroblasts cultured in the presence and absence of PDGF isoforms -AA, -BB, and -AB (1, 5, 10, 100 ng/mL) and insulin-like growth factor 1 (100 ng/mL). Fibroblast procollagen I and PDGF receptors (PDGFRs) α and β expression were determined by ELISA.

Results: Platelet-derived growth factor BB significantly enhanced fibroblast procollagen I expression in patients with severe asthma compared with patients with mild/moderate asthma and normal controls. Furthermore, the baseline fibroblast expression of PDGFR- β was significantly greater in patients with severe asthma compared with the other groups. **Conclusion:** This pilot study suggests that airway fibroblasts from patients with severe asthma exhibit a synthetic phenotype, which may be driven by the overexpression of PDGFR- β . (*J Allergy Clin Immunol* 2005;115:534-40.)

Key words: PDGF, airway fibroblasts, asthma

Significant structural alterations of the bronchial wall (collectively termed *airway remodeling*) have been repeatedly demonstrated in many patients with asthma¹ and may lead to a state of irreversible airway obstruction.² Among these abnormalities, subepithelial basement membrane thickening has been described,^{3,4} which correlates with bronchial hyperresponsiveness.⁵ This morphologic change is believed to be caused by increased collagen (types I, III, and V) deposition by the airway fibroblasts.⁶

Abbreviations used

DMEM: Dulbecco modified Eagle medium
FVC: Forced vital capacity
IGF-1: Insulin-like growth factor 1
PDGF: Platelet-derived growth factor
PDGFR: Platelet-derived growth factor receptor

These fibroblasts, which include myofibroblasts, have also been shown to produce specific cytokines that may attract inflammatory cells to the airway, thus perpetuating inflammatory and fibrotic processes.⁷

Among the numerous growth factors that are active in airway inflammation and tissue repair, platelet-derived growth factor (PDGF) may have a prominent role in altering fibroblast activities linked to airway remodeling. PDGF is a powerful stimulator of fibroblast chemotaxis,^{8,9} proliferation,¹⁰⁻¹³ and collagen production.^{14,15} Furthermore, activated macrophages¹⁶ and eosinophils,¹⁷ 2 cell types that have often been linked to asthma,¹⁸ are capable of synthesizing and secreting PDGF. PDGF exists as a homodimer or heterodimer consisting of 2 polypeptide chains, denoted A and B, which can be dimerized via sulfhydryl bridges to form 3 bioactive isoforms (AA, BB, AB).¹⁹ The 3 isoforms bind differentially to cell surface PDGF- α and PDGF- β receptor subunits,²⁰ which differ in their biologic and functional activities.^{21,22} The α and β receptor subunits are dimerized and activated on binding of individual PDGF chains to the receptor subunits.²³ Binding studies have shown that the α -subunit of the receptor (PDGFR- α) can bind to either the PDGF-A chain or the PDGF-B chain, whereas the β -subunit (PDGFR- β) can bind only to the PDGF-B chain.²⁴ Although all of the PDGF isoforms and receptor types have been previously identified on human fibroblasts,²⁵ the expression and balance of the various components of this sophisticated system are virtually uncharacterized in the human lung. Consequently, it is unclear whether inflammatory and wound repair mechanisms within the context of asthma and airway remodeling might induce alterations in the PDGF system, which would perpetuate remodeling activities.

Given the prominent role of PDGF in inflammation and repair, we hypothesized that PDGF would be a significant contributor to fibroblast-induced remodeling in patients with asthma. More specifically, we hypothesized that fibroblasts derived from patients with asthma would

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exhibit a greater responsiveness to PDGF stimulation than fibroblasts from healthy controls, specifically with regard to procollagen production.

METHODS

Subjects

Five patients with severe asthma, 10 patients with mild asthma, and 6 normal, healthy individuals without asthma (nonasthmatic) were recruited from the general Denver community. Asthma severity was determined by using the American Thoracic Society guidelines,²⁶ and all subjects with asthma met diagnostic criteria for asthma,²⁷ exhibiting a methacholine PC₂₀ of less than 8 mg/mL, and were maintained on inhaled β_2 -agonists only. Exclusion criteria included use of cromolyn, nedocromil, inhaled or oral corticosteroids, leukotriene modifiers, and/or theophylline within 6 weeks before the study; an upper respiratory tract infection within 6 weeks before the study; immunotherapy within 3 months before the study; cigarette use; and significant nonasthma pulmonary disease or other medical illnesses. Informed consent was obtained from all subjects participating in the study, and all procedures were approved by the National Jewish Medical and Research Center Institutional Review Board and the Colorado State University Human Research Committee.

Protocol

Subjects underwent bronchoscopy with endobronchial biopsy as previously described.²⁸ The site of biopsy was randomized to either the right or left lower lobe, and 5 to 6 endobronchial biopsy specimens were taken from the tertiary carinae of the right or left lower lobes under direct visualization. Before bronchoscopy, spirometry was performed before and after the administration of 0.18 mg albuterol from a metered-dose inhaler and 0.4 mg atropine introduced intravenously. Lidocaine (4%) was used to anesthetize the upper airway, and lidocaine (1%) was applied to the laryngeal area, trachea, and orifice of the right lower or left lower lobe bronchi via the bronchoscope. Supplemental oxygen was administered throughout the procedure along with monitoring of heart rate and oxygen saturation. Subjects' vital signs were monitored in the laboratory after the procedure, and subjects were discharged once FEV₁ was 90% of the prebronchoscopy, postbronchodilator value.

Fibroblast culture

Fibroblasts were cultured from endobronchial biopsy tissue as previously described.²⁹ Briefly, biopsy specimens were rinsed and cut into several small pieces (approximately 5–6 per biopsy) and placed into wells (2 pieces/well) of a 24-well tissue culture plate containing Dulbecco modified Eagle medium (DMEM) supplemented with FBS (10%), streptomycin (100 μ g/mL), penicillin (10,000 U/mL), and gentamicin (100 μ g/mL). The tissue pieces were incubated at 37°C/5% CO₂ and cultured until cell growth established >50% confluency (approximately 8–20 days), with the media changed every 2 to 3 days. On reaching >50% confluency, the tissue pieces were removed and the cells were trypsinized, counted, tested for viability, stained for identification, and placed into 24-well plates for collagen experiments. In addition, cells were seeded in 15 $\times$ 60-mm culture dishes for subsequent cell passages (Becton Dickinson, Franklin Lakes, NJ). All cells were stained with monoclonal mouse antihuman antibodies against fibroblast antigen (Ab-1; Calbiochem, San Diego, Calif), vimentin (DAKO, Carpinteria, Calif) and α -smooth muscle actin (DAKO) to confirm fibroblast, myofibroblast, and mesenchymal cell specificity, respectively. First passage cells generated directly from the biopsy tissue were stained on cytospin slides, whereas second passage fibroblasts were grown

directly onto 18-mm coverslips (Fisher Scientific, Pittsburgh, Pa) placed in the culture dishes on repassage. Only cells from the first through fourth passages were used for experiments, given the potential concern for changes in cell function, including enhanced senescence, that may occur after several passages.³⁰

Quantification of procollagen I production

Fibroblast production of procollagen I was determined by using a sandwich ELISA. For this assay, cells were seeded at a density of 5×10^4 cells/well in 24-well culture plates containing 500 μ L DMEM supplemented with FBS (10%) and incubated for 24 hours to permit cell attachment. The cells were then rinsed with PBS, and fresh DMEM (without FBS) was added to the wells for an additional 24-hour incubation to establish quiescence. After this time, recombinant human PDGF isoforms AA, BB, and AB (1, 5, 10, and 20 ng/mL; R&D Systems, Minneapolis, Minn) or recombinant human insulin-like growth factor 1 (IGF-1; 100 ng/mL; R&D Systems), the latter as a positive control, and ascorbic acid (50 μ g/mL) were added to each well in triplicate and incubated for an additional 24 hours. After 24 hours, fibroblast culture supernatants were removed, diluted 1:100 in Enzyme Immunoassay buffer, and analyzed for procollagen type I by using a standard sandwich ELISA.

Briefly, microtiter plates (96-well; Nalge Nunc International, Rochester, NY) were coated (100 μ L into each well) with mouse antihuman procollagen I (Takara Assay kit PC-8-7 [Takara Bio Inc, Shiga, Japan] through Panvera) and diluted in PBS (pH 7.4) to a concentration of 1.0 μ g/mL. This medium was allowed to sit 16 to 24 hours at room temperature, at which time 225 μ L saturation buffer (0.1 mol/L potassium phosphate, 1.5 mmol/L sodium azide, 0.4 mol/L sodium chloride, 0.1 mmol/L EDTA, and 0.3% BSA) was added to each well. After blocking, ELISA plates were washed (10 mmol/L phosphate buffer containing 0.01% Tween), and samples (100 μ L) were added to wells in triplicate and incubated for 3 hours at 37°C. At this time, plates were again washed, and 100 μ L detection antibody (from Takara MK101 ELISA kit [Takara Bio Inc]) was added to each well. After an overnight incubation at 4°C, the plates were washed, and 3,3',5,5'-tetramethylbenzidine substrate was added. Plates developed for 10 to 30 minutes, and development was halted by the addition of 100 μ L 1 mol/L phosphoric acid. Plates were read at 450 nm, and standard curves were generated with a 4-parameter function on Delta Soft software (Elisa Tech, Aurora, Colo). The assay is sensitive over the range of 0.2 to 100 ng/mL.

Obtaining cell lysate

Whole fibroblast cell lysate samples were obtained in triplicate from fibroblast monolayer cultures (15 $\times$ 60-mm culture dishes). Briefly, the medium was removed, and the adherent cells were washed with PBS. The adherent fibroblasts were then gently detached from the tissue culture dish by using a cell scraper and 500 μ L PBS containing 50 mmol/L EDTA. A cell pellet was obtained from the cell solution by low speed centrifugation (4500 rpm in microfuge for 4.5 minutes at 4°C). After microcentrifugation, the supernatant was removed and discarded. Subsequently, 100 μ L ice-cold RIPA (Radio-Immunoprecipitation Assay) lysis buffer (Sigma-Aldrich, St Louis, Mo) (containing 1 $\times$ PBS, 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 10 mmol/L EDTA) with freshly added protease inhibitors (phenylmethylsulfonyl fluoride, 10 μ L/mL; sodium orthovanadate, 10 μ L/mL; and Sigma protease inhibitor cocktail for mammalian tissues, containing 104 mmol/L 4-(2-aminoethyl)benzenesulfonyl fluoride, 0.08 mmol/L aprotinin, 2.1 mmol/L leupeptin, 3.6 mmol/L bestatin, 1.5 mmol/L pepstatin A, 1.4 mmol/L E-64) was added to the cell pellet. The solution was gently mixed with a pipette and allowed to incubate on ice for 15 minutes to complete cell membrane disruption and was stored at -70°C . For analysis, the

TABLE I. Subject characteristics*

	Controls without asthma	Patients with mild/ moderate asthma	Patients with severe asthma	P value
Gender (M/F)	2/4	5/5	4/1	—
Age (y)	26.4 ± 3.7	34.6 ± 2.63	37 ± 3.7	.13
Medications	None	β ₂ -Agonists	β ₂ -Agonists	—
FEV ₁ (L)	3.67 ± 0.35	3.22 ± 0.25	2.6 ± 0.35	.12
FEV ₁ (% predicted)	102 ± 5.79	89.8 ± 4.10	64.7 ± 5.79	.0008

*Data are presented as means ± SEMs.

cell lysate samples were thawed gently on ice. The cell lysates were further homogenized by passing through a 21-gauge needle. The cell lysate was then spun in a microcentrifuge at 10,000g for 12 minutes at 4°C. After microcentrifugation, the supernatant fluid (total cell lysate) was transferred to a fresh centrifuge tube, and the resultant pellet was discarded. The cell lysates were kept on ice at all times.

Quantification of total cell protein

Total fibroblast protein in individual cell lysate samples was colorimetrically detected and quantified by using the bicinchoninic acid assay (Pierce, Rockford, Ill) according to the manufacturer's protocol. For this purpose, the cell lysates were diluted (1:10) and assayed in RIPA lysis buffer with freshly added protease inhibitors, as described. Plates were read at 570 nm, and standard curves were generated with linear fit on Delta Soft software. The assay is sensitive over the range of 15.6 to 2000 µg/mL.

Quantification of PDGFR-α and PDGFR-β

Baseline PDGFR protein expression was quantified in fibroblast cell lysates by using sandwich ELISAs specific to each of the receptors, α and β. Briefly, microtiter plates (96-well; NUNC, Roskilde, Denmark) were coated (100 µL into each well) with monoclonal mouse antihuman PDGFR-α or PDGFR-β antibodies (R&D Systems) diluted in PBS (pH 7.4) to a concentration of 2.0 µg/mL. This medium was allowed to sit 16 to 24 hours at room temperature, at which time the coating buffer was removed and 225 µL blocking buffer (0.1 mol/L potassium phosphate, 1.5 mmol/L sodium azide, 0.4 mol/L sodium chloride, 0.1 mmol/L EDTA, and 0.3% BSA) was added to each well. At the time of assay, ELISA plates were washed (PO₄/Tween buffer), and samples (100 µL) were added to wells in triplicate and incubated overnight at 4°C. After this time, plates were again washed, and 100 µL of a receptor-specific biotinylated polyclonal antibody conjugate was added to each well. The plates incubated for an additional 2 hours at room temperature on a shaker. The plates were then washed, and 100 µL avidin-horseradish peroxidase conjugate was added to each well. The plates incubated for 2 hours at room temperature on a shaker. Finally, the plates were washed, and TMB substrate was added. Plates developed for 5 to 10 minutes, and then development was halted by the addition of 100 µL 1 mol/L phosphoric acid. Plates were read at 450 nm, and standard curves were generated with 4-parameter function on Delta Soft software. The sensitivity of the PDGFR-α assay was 0.39 to 50 ng/mL, and that of the PDGFR-β assay was 0.039 to 5 ng/mL. PDGFR protein levels were normalized to the total cell protein present in a sample as obtained via the bicinchoninic acid assay, as described. Thus, PDGFR protein data were expressed as nanograms receptor protein/100 µg total cell protein.

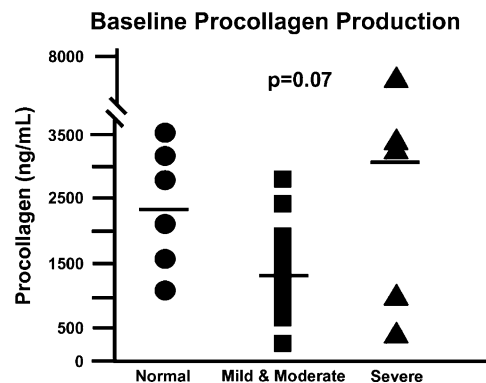


FIG 1. Baseline procollagen I expression from airway fibroblasts from the three study groups: normal controls (closed dots), mild/moderate asthma (closed squares), and severe asthma (closed triangles). $P < .07$ when 3 groups compared. Bars denote means.

Statistical analyses

The outcome variables were fibroblast procollagen I expression at baseline (negative or unstimulated control) and after exposure to PDGF-AA, PDGF-BB, PDGF-AB, and IGF-1 as well as baseline PDGFR expression (α and β). Data from multiple passages (first through fourth) within a subject were averaged to yield a single data point per condition. The procollagen I expression from the unstimulated, negative control and baseline receptor expression were compared between groups by using ANOVA as these data were normally distributed, as determined by the Shapiro-Wilk test.³¹ The comparisons of procollagen I expression after exposure to PDGF isoforms and IGF-1 were performed by using ANOVA after normalization of the response to the negative, unstimulated control. All tests were conducted at the 5% significance level. Finally, linear regression analyses were performed to evaluate the linear relationships between lung function and procollagen I expression for each subject group. Regression analyses were performed by using a mixed effects model for repeated measurements, with a random effect for subjects and a fixed effect for lung function parameters (FEV₁ % predicted and forced vital capacity [FVC] % predicted).

RESULTS

Subjects

Subject characteristics are shown in Table I.

Procollagen I expression

Immunostaining with vimentin, Ab-1, and α-smooth muscle actin confirmed the fibroblast phenotype, with 100% of the cells positive for vimentin, >95% positive for Ab-1 expression, and <5% positive for α-smooth muscle actin. The procollagen I expression from the unstimulated fibroblasts in each group is shown in Fig 1, and procollagen I expression after exposure to PDGF isoforms and IGF-1 as a percentage of negative, unstimulated control is shown in Fig 2. The comparison of procollagen I expression after exposure to PDGF isoforms and IGF-1 was performed by using ANOVA after normalization of the response to the negative, unstimulated control, because the difference between groups at baseline trended toward

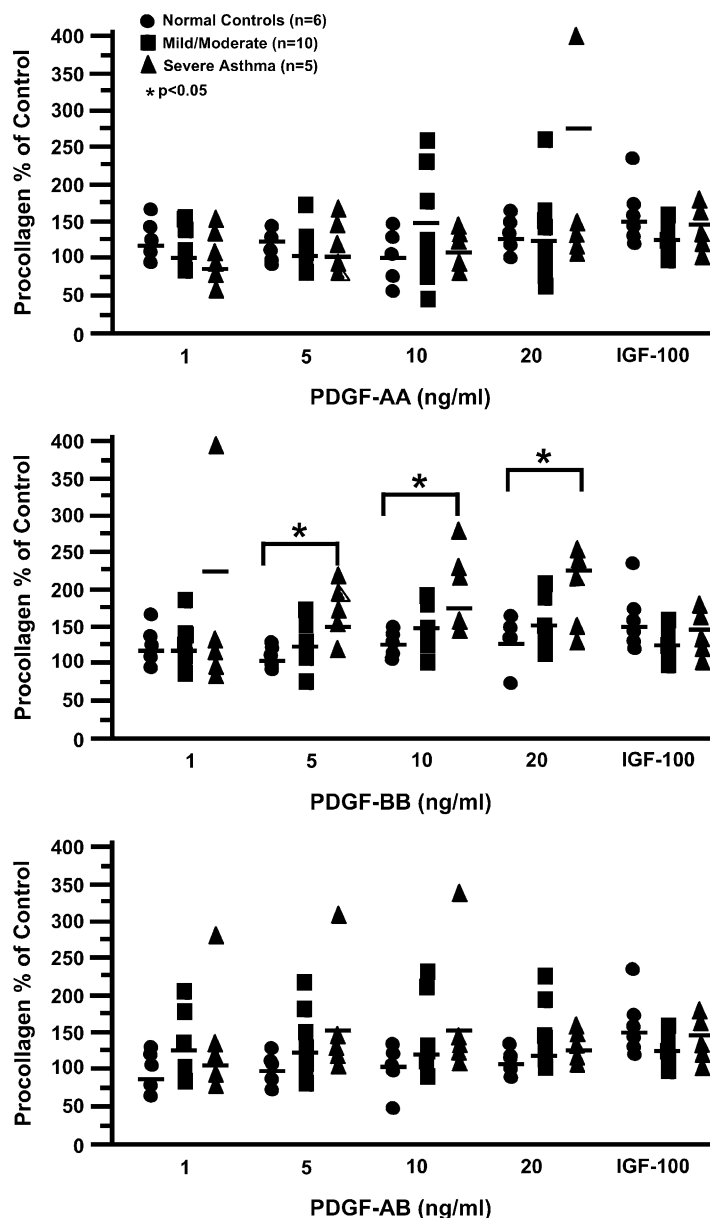


FIG 2. Fibroblast procollagen type I expression after exposure to PDGF isoforms AA (A), BB (B), and AB (C) and IGF-1 (A-C) in normal controls (closed dots), patients with mild/moderate asthma (closed squares), and severe asthma (closed triangles) expressed as percentage of negative, unstimulated control as means $\pm$ SEs. * $P < .05$.

significance at $P = .07$. Analyses indicated that there were no significant increases detected in procollagen expression with PDGF-AA or PDGF-AB stimulation at any of the concentrations, as shown in Fig 2, A and C, respectively. However, stimulation with PDGF-BB (5, 10, and 20 ng/mL) resulted in significantly greater procollagen I expression in the fibroblasts from patients with severe asthma compared with both patients with mild/moderate asthma and controls without asthma (5 ng/mL, $P = .04$; 10 ng/mL, $P = .05$; 20 ng/mL, $P = .03$; patients with severe asthma > patients with mild/moderate asthma and normal controls).

To explore further the significance of the PDGF-BB-induced procollagen I expression observed in the patients

with severe asthma, linear regression analyses were performed, and the relationship between procollagen I expression and lung function was determined for each group. Linear regression analyses revealed that the procollagen I expression in patients with severe asthma in response to PDGF-BB (all concentrations considered) was significantly and inversely related to both the FEV₁ % predicted (patients with severe vs mild/moderate asthma, $P = .001$; patients with severe asthma vs normal controls, $P = .024$) and the FVC % predicted (patients with severe vs mild/moderate asthma, $P = .010$; patients with severe asthma vs normal controls, $P = .041$). There were no significant relationships between the patients with mild/

moderate asthma and the normal controls with respect to these spirometric parameters (FEV₁ % predicted, $P = .503$; FVC % predicted, $P = .358$).

PDGFR expression

The baseline expression of PDGF receptors α and β is shown in Fig 3. Data were normalized to total cell protein and expressed as nanograms per 100 μ g total cell protein. Analyses revealed that there were no significant differences in baseline expression of PDGFR- α between the groups (patients with severe asthma, 7.6 ng/100 μ g protein; patients with mild/moderate asthma, 12.50 ng/100 μ g protein; normal controls, 11.33 ng/100 μ g protein; $P = .35$). However, there was a significantly greater baseline PDGFR- β expression in the group with severe asthma compared with the group with mild/moderate asthma and the normal control group (patients with severe asthma, 15.20 ng/100 μ g protein; patients with mild/moderate asthma, 13.30 ng/100 μ g protein; normal controls, 3.67 ng/100 μ g protein; $P = .0024$; patients with severe asthma > patients with mild/moderate asthma > normal controls).

DISCUSSION

Our data suggest that there are phenotypically distinct populations of airway fibroblasts between patients with asthma and normal, healthy individuals. In particular, the data presented here demonstrate that the fibroblasts of the group with severe asthma exhibited significantly increased procollagen I expression in response to PDGF-BB stimulation compared with the group with mild/moderate asthma and normal controls, which may be caused, at least in part, by increased PDGF- β receptor expression. In addition, the significant inverse relationship between procollagen I expression by airway fibroblasts after exposure to PDGF-BB and lung function in severe asthma suggests that this process may have physiologic consequences. It is of great interest that the mean fibroblast procollagen I expression of patients with severe asthma was 30% to 70% greater than that of both the patients with mild/moderate asthma and the normal controls with PDGF-BB stimulation (5, 10, 20 ng/mL). However, the patients with mild/moderate asthma also demonstrated increases in PDGFR- β expression, although less than the patients with severe asthma, without a corresponding increase in procollagen I expression. These data suggest that the increased expression of PDGF- β receptor only partly explains the mechanism of increased procollagen I expression in the patients with severe asthma.

The effects of PDGF on collagen expression by the airway fibroblasts have recently been shown to be regulated by the phosphatidylinositol 3 kinase (PI3K-Akt) pathway.³² The aberrant expression of this PDGF signaling pathway, which may be present in the patients with severe asthma, has been postulated as a mechanism that contributes to the progression of disease where fibrosis plays a role, including idiopathic pulmonary fibrosis,³³

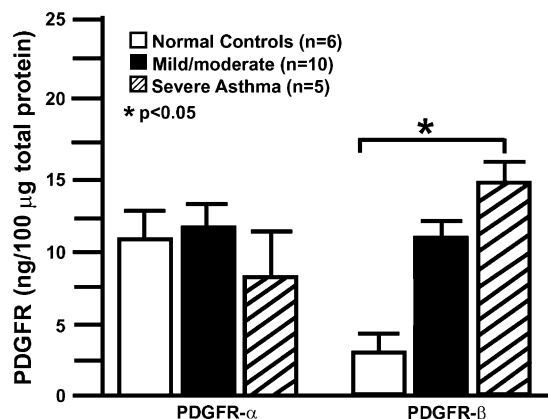


FIG 3. Fibroblast baseline PDGFR expression. Receptor protein levels are expressed in nanograms per 100 μ g total protein. Data from normal healthy control subjects are illustrated with open bars, patients with mild/moderate asthma with filled bars, and patients with severe asthma with shaded bars. * $P < .05$.

asbestosis,^{34,35} and airway remodeling in asthma.³⁰ We did not investigate this pathway here, but it certainly seems warranted, because it may provide an explanation for why PDGF results in different effects depending on asthma severity.

Our studies are in contrast with those of Dube et al.³⁶ Although these authors did report significant *in vitro* differences between the bronchial fibroblasts of individuals with and without asthma with respect to baseline DNA synthesis, cell senescence, and cell responsiveness to various mediators in their first investigation,³⁰ they described significant increases in fibroblast cell proliferation and procollagen expression in response to PDGF-BB only when endothelin 1 was simultaneously present in the cultures.³⁶ However, the details of the population with asthma chosen for their studies were not described. The population may not have included patients with severe asthma. Chanez et al.³⁷ have previously examined the expression levels of PDGF isoforms and PDGF receptor subunits in bronchial biopsies and bronchoalveolar lavage samples via immunohistochemistry and ELISA methods, respectively, and found no significant differences between patients with asthma and normal controls. The expression of PDGF and its receptors does not independently lend insight into either the functional reactivity of the mediators and their receptors or the potential effects on fibroblasts as an isolated cell type, as we report in our study. Although the significance of PDGF in the context of airway remodeling has not been extensively studied, our data are in agreement with those of several investigators who have described increased expression of PDGFR- β subunits in conjunction with other various conditions of inflammation and wound repair.³⁸⁻⁴¹ In addition, our data agree with those of Aubert et al.,¹⁶ who previously reported an enhanced expression of PDGFR- β in patients with asthma when analyzing total lung RNA samples in comparison with subjects with chronic obstructive pulmonary disease.

A limitation of our study and studies of this nature is the use of an *ex vivo* model as a representation of *in vivo* conditions, which arguably may not be a precise mimicry. It is feasible that fibroblasts existing within the milieu of the asthmatic airway, characterized by persistent inflammation and elevated cytokine levels, have undergone phenotypic alterations as a product of their environment that cause them to behave differently from those of healthy controls without asthma. However, it is unclear whether such phenotypic alterations persist once the cells have been removed from the inflammatory environment, such as with biopsy acquisition, or whether they are merely products of their environment, and once removed similarly undergo phenotypic alterations arising from the lack of inflammatory stimulus. Despite this limitation, we were able to detect differences between groups by using identical culture conditions and early passage cells. We think the ability to culture and perform experiments with cells isolated directly from well characterized subjects offers a perspective not attainable via cell lines and animal models.

To date, we have characterized the expression only of procollagen type I. Although type I collagen has been the collagen type most often described in the subepithelial fibrosis in asthmatic airways, and therefore has been thought to be the most important, we believe types III and V should also be described. We intend to conduct these analyses in the future.

Collectively, our data are in agreement with the physiological mechanism behind the currently accepted model of the PDGF receptor and isoform specificity proposed by Seifert et al.²⁴ In this model, the individual PDGFR subunits α and β differ in their specificity and binding affinity for the various PDGF isoforms, and the total and relative numbers of the α and β subunits present on a specific cell type determine the sensitivity of the cell to the different PDGF isoforms. Therefore, a population of cells exhibiting an enhanced expression of β but not α would differ comparatively from other cell populations in the greater numbers of functional $\beta\beta$ and $\alpha\beta$ receptor combinations. However, because $\beta\beta$ receptors can bind only to PDGF-BB and $\alpha\beta$ receptors can bind either with high affinity to PDGF-BB or with less affinity to PDGF-AB,²⁴ the overall cellular responsiveness in such a receptor expression scenario would result in an enhanced cellular responsiveness to PDGF-BB only. Thus, given that the biological activities of the PDGF isoforms on a target cell are ultimately dependent on the expression of receptor subtypes on that cell (ie, the spatial influence of receptor subunits coming together to dimerize), an enhanced expression of PDGFR- β subunit could influence the effectiveness of PDGF-BB stimulation.

In summary, our pilot study has shown that PDGF-BB augments airway fibroblast expression of procollagen I in patients with severe asthma compared with those having mild/moderate asthma or normal controls, and the expression inversely correlates with lung function. Furthermore, we have shown that the baseline expression of PDGFR- β is significantly greater in the fibroblasts derived from the

patients with severe asthma. We speculate that this overexpression of PDGFR- β may drive the expression of the synthetic fibroblast phenotype observed in our studies, having altered capabilities in collagen production. Immunohistochemical analyses that revealed a fibroblast population with $<5\%$ α -smooth muscle actin positivity rule out the expression of the myofibroblast phenotype as the source of enhanced collagen production observed in the fibroblasts of patients with asthma. The existence of a synthetic fibroblast phenotype in patients with asthma is of great interest and significance as a potential mechanism underlying the fibroblast-induced activities that are thought to contribute to airway remodeling in asthmatic airways.

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