

Transforming growth factor β_1 increases fibronectin deposition through integrin receptor $\alpha_5\beta_1$ on human airway smooth muscle

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Background: Integrin receptors signal to and from the extracellular matrix. Altered expression of the integrin receptors, such as the fibronectin receptor $\alpha_5\beta_1$, might be implicated in extracellular matrix accumulation in airway remodeling in asthma.

Objective: We examined the effect of TGF- β stimulation on integrin $\alpha_5\beta_1$ expression and the role of $\alpha_5\beta_1$ in fibronectin deposition and proliferation.

Methods: Integrin subunit α_5 and β_1 expression in airway smooth muscle (ASM) from subjects with and without asthma was examined by means of PCR and flow cytometry. The effect of blocking $\alpha_5\beta_1$ receptor on ASM proliferation to FBS was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay. Cells were stimulated with TGF- β in the presence or absence of extracellular signal-regulated kinase, phosphoinositide-3 kinase, or p38 inhibitors and antibodies to the α_5 and β_1 subunits. The effect of blocking $\alpha_5\beta_1$ receptor on fibronectin deposition was assessed by means of immunocytochemistry.

Results: Proliferation of ASM cells from asthmatic and nonasthmatic subjects was inhibited by blocking the fibronectin receptor subunit $\alpha_5\beta_1$. TGF- β -induced $\alpha_5\beta_1$ was extracellular signal-regulated kinase dependent but not phosphoinositide-3 kinase or p38 dependent. Blockade of the $\alpha_5\beta_1$ receptor inhibited TGF- β -induced fibronectin matrix deposition.

Conclusion: Through its increased expression by the profibrotic stimulus TGF- β , integrin $\alpha_5\beta_1$ might be important in regulating fibronectin deposition. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Human airway smooth muscle, integrins, transforming growth factor β , proliferation, fibronectin, fibrosis, extracellular matrix

Airway remodeling is a characteristic feature of persistent asthma and includes increased airway smooth muscle (ASM)

Abbreviations used

ASM: Airway smooth muscle
CT: Cycle threshold
DMEM: Dulbecco modified Eagle's medium
ECM: Extracellular matrix
ERK: Extracellular signal-regulated kinase
fb-mAb: Function-blocking mAb
ITS: Insulin-transferrin-selenium
PI3K: Phosphoinositide-3 kinase

mass and altered extracellular matrix (ECM) deposition. Although the mechanisms leading to airway remodeling are not well understood, profibrotic stimuli, such as TGF- β , can induce the deposition of structural proteins and hence contribute to the remodeling process.

TGF- β , a 25-kd protein, is secreted as an inactive protein that is activated by the release of the noncovalently bound latency-associated peptide. Levels of TGF- β are increased in the airways of asthmatic subjects and correlate with basement membrane thickness, suggesting a role for TGF- β in airway remodeling.^{1,2} Furthermore, TGF- β has been implicated in the deposition of ECM proteins in several fibrotic diseases, and we have recently reported that TGF- β induces fibronectin and collagen I deposition from human ASM cells.³

Cell-ECM interactions play a critical role in many physiologic processes, including proliferation, matrix deposition, migration, and secretion. Fibronectin, an ECM protein known to be increased in the airways of asthmatic subjects, modulates cellular function.^{4,5} Altered ECM deposition from asthmatic ASM cells in culture can also modulate cellular responses, supporting an important role for cell-ECM interactions.⁶ These cell-ECM interactions are mediated, at least in part, through integrin receptors present on the cell surface.

Integrins are heterodimeric transmembrane glycoproteins consisting of an α and β subunit. Currently, 18 α and 8 β subunits have been identified and exist in a variety of combinations.⁷ The β_1 family of integrins are involved in a variety of human ASM responses including survival, proliferation, and cytokine secretion.^{5,8-10} Each integrin confers ligand specificity, and $\alpha_5\beta_1$ is the major receptor for fibronectin binding at distinct Arg-Gly-Asp RGD sites. TGF- β can induce expression of integrin receptors on various cell types, including mesangial cells, hepatic stellate cells, and fibroblasts.¹¹⁻¹³

Integrins not only receive signals from the ECM but also participate in ECM deposition. Fibronectin is secreted as a soluble protein and is assembled into an insoluble matrix outside

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of the cell. $\alpha_5\beta_1$ is important in matrix assembly,¹¹ and levels of $\alpha_5\beta_1$ correlate with the degree of fibronectin accumulation in other disease states.¹⁴ For example, overexpression of $\alpha_5\beta_1$ in CHO cells results in increased fibronectin deposition.¹⁵ Whether increased levels of $\alpha_5\beta_1$ expression can be induced by TGF- β in ASM cells and whether this modulates fibronectin accumulation is not known.

In this study we show that the proliferation of asthmatic ASM cells is inhibited by function-blocking antibodies to $\alpha_5\beta_1$. We demonstrate that TGF- β increases $\alpha_5\beta_1$ expression through the extracellular signal-regulated kinase (ERK) signaling pathway in human ASM cells from asthmatic and nonasthmatic subjects. Furthermore, we demonstrate that the fibronectin receptor plays a critical role in fibronectin assembly.

METHODS

Cell culture

Primary human ASM cells were obtained from lung specimens in accordance with the human ethics committees of the University of Sydney and the South West Sydney Area Health Service. ASM cells from nonasthmatic subjects (56.6 ± 2.5 years) were isolated from airways of subjects undergoing lung resection for either lung transplantation or carcinoma. ASM cells from asthmatic subjects (32.7 ± 2.9 years) were obtained from deep endobronchial biopsies or lung transplantation. Subject details are shown in Table E1 in the Online Repository (available at www.jacionline.org). ASM bundles were dissected free from surrounding tissue and grown as explants, as previously described.¹⁶ ASM characterization was confirmed by means of light microscopy and immunohistochemistry for smooth muscle α -actin and calponin expression. ASM cell cultures took 2 to 3 months to establish distinguishing them from fibroblasts, which establish after 3 weeks. In addition, ASM cells in culture reached confluence after 2 weeks, as opposed to 1 week for fibroblasts, indicating that our cultures were pure for muscle. ASM cells were used between passages 4 and 8. For all experiments, ASM cells were seeded at a density of 1×10^4 cells/cm².

Role of integrin receptor $\alpha_5\beta_1$ in proliferation of ASM cells

TGF- β at 1 ng/mL failed to induce proliferation (see Fig E1 in the Online Repository at www.jacionline.org). Thus the decision was taken to use FBS as a mitogenic stimulus to assess the role of integrins in proliferation. ASM cells were seeded in 96-well tissue-culture plates in Dulbecco modified Eagle's medium (DMEM) containing 0.5% FBS for 8 hours. Cells were then equilibrated in 0.1% FBS for 24 hours. Cells were preincubated with integrin function-blocking mAbs (fb-mAbs; 10 μ g/mL) for 30 minutes before stimulation with 5% FBS. Mouse IgG was used as a control. Proliferation was assessed by means of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] assay on day 6, as previously described by Hirst et al.¹⁷ Absorbance was measured at a test wavelength of 570 nm and a reference wavelength of 655 nm.

Real-time PCR

Human ASM cells were seeded in 6-well plates for 24 hours in DMEM containing 5% FBS. Medium was changed to DMEM containing 0.1% insulin-transferrin-selenium (ITS) for 24 hours before stimulation of the cells with DMEM containing 0.1% ITS and TGF- β (1 ng/mL). For inhibitor or fb-mAb experiments, cells were preincubated with LY294002 (3 μ mol/L), wortmannin (100 nmol/L), U0126 (5 μ mol/L), SB203580 (10 μ mol/L), SB202474 (10 μ mol/L), or fb-mAbs α_5 or β_1 (10 μ g/mL) for 30 minutes before TGF- β stimulation. Total RNA was extracted from the cells at various time points up to 24 hours (Macherey-Nagel, Easton, Pa). Real-time PCR was performed for analysis of the integrin subunits α_5 and β_1 (Assays on demand, Applied Biosystems, Calif) and for fibronectin and 18s rRNA for each subject. The cycle thresholds (CTs) for the gene of interest and 18s rRNA were

determined by using 7000 system sequence detection software (Version 1.2.3; Applied Biosystems). Data were normalized to 18s rRNA. Results from triplicate wells from each treatment were averaged, and data were calculated as fold expression relative to control.

Flow cytometric analysis of cell-surface integrins

Human ASM cells were analyzed for cell-surface expression of integrin subunits α_5 and β_1 . ASM cells were seeded in 6-well plates in DMEM containing 1% antibiotics and 5% FBS for 24 hours, equilibrated in 0.1% ITS for 24 hours, and stimulated with TGF- β (1 ng/mL) for up to 48 hours. ASM cells were then washed twice in PBS and harvested with phenol-red free trypsin-EDTA (0.02% wt/vol) for 3 minutes. Cells were incubated with PBS containing 5% BSA for 5 minutes and then washed in 0.1% BSA-PBS. Cells were resuspended in PBS containing 1% BSA for 30 minutes on ice. After centrifugation (2500 rpm), cells were incubated with the specific anti-integrin mAbs (Chemicon, Temecula, Calif) or isotype control (2 μ g/mL) for 30 minutes on ice. After washing in 0.1% BSA-PBS, cells were incubated with an R-phycoerythrin-conjugated anti-mouse antibody in the dark on ice for 30 minutes. Cells were then washed twice in PBS and fixed in 4% paraformaldehyde for 20 minutes. Cells were analyzed on a FACSCalibur Sort flow cytometer (Becton Dickinson, North Ryde, NSW, Australia). Typically, 10,000 events were acquired in the gated region of the forward-scatter/side-scatter plots to examine ASM cell-surface expression of integrin subunits. Data files were generated with CellQuest Pro software (Becton Dickinson, North Ryde, NSW, Australia) and analyzed with Flowjo software (Treestar, Inc, Ashland, Ore). Isotype staining controls were used as a guide to set regions to determine the percentage of positive events. Only 1% or less of events in these controls was allowed in the positive region. The geometric mean is expressed as a fold increase in fluorescence intensity above the geometric mean determined for the relevant control for each treatment.

Immunofluorescence

ASM cells were seeded in chamber slides (Labtek, Nalge Nunc International, NY) in DMEM containing 5% FBS for 24 hours, equilibrated in DMEM containing 0.1% ITS for 24 hours, and stimulated in the presence or absence of fb-mAbs to α_5 and β_1 (10 μ g/mL) with TGF- β (1 ng/mL) for 48 hours. Cells were fixed in 4% paraformaldehyde solution for 15 minutes at room temperature and incubated with PBS containing 0.1% BSA for 30 minutes and then incubated with rabbit anti-fibronectin antibody (1:500; Sigma-Aldrich, St Louis, Mo) for 90 minutes at room temperature. They were washed with PBS containing 0.1% BSA and incubated with goat anti-rabbit tetramethylrhodamine isothiocyanate (Sigma-Aldrich)-conjugated secondary antibody for 30 minutes at room temperature in the dark. Cells were counterstained with Hoechst before imaging with a microscope (BX51; Olympus, Center Valley, Pa) and commercially available imaging software (IM1000; Leica Microsystems, Wetzlar, Germany). Images were quantitated by scoring on a scale of 0 to 3 (0, no staining; 3, very strong staining) by 6 independent observers.

Statistical analysis

Data are expressed as means $\pm$ SEM obtained from cells cultured from *n* subjects. Data were analyzed by using the Student paired *t* test or 1-way ANOVA with Bonferroni post-hoc testing where appropriate. A *P* value of less than or equal to .05 was considered significant.

RESULTS

Fibronectin receptor is involved in ASM cell proliferation

ASM cells in culture from asthmatic and nonasthmatic subjects express the integrin subunits α_5 and β_1 . Proliferation of asthmatic and nonasthmatic ASM cells was significantly attenuated after incubation with a β_1 fb-mAb (asthmatic, $53.0\% \pm 12.13\%$ reduction vs 5% FBS, *P* < .05, *n* = 9; nonasthmatic, $45.9\% \pm 3.74\%$

reduction, $P < .05$, $n = 14$). Blocking with an α_5 fb-mAb in combination with β_1 further attenuated proliferation of asthmatic and nonasthmatic ASM cells (asthmatic, $69.8\% \pm 12.6\%$ reduction vs 5% FBS, $P < .05$, $n = 9$; nonasthmatic, $69.1\% \pm 7.17\%$ reduction, $P < .05$, $n = 14$). Treatment with isotype-matched control antibodies had no significant effect on proliferation (Fig 1). This is the first study to report this in asthmatic ASM cells and confirms previous findings by Nguyen et al⁹ in nonasthmatic ASM cells.

TGF- β increases fibronectin levels

We confirm, in an independent set of experiments, our previous finding that TGF- β induces fibronectin mRNA.³ TGF- β stimulation of asthmatic and nonasthmatic ASM cells for 24 hours induced fibronectin mRNA (asthmatic, 2.73 ± 0.66 -fold vs 0.1% ITS, $P < .05$, $n = 7$; nonasthmatic, 2.15 ± 0.29 -fold, $P < .05$, $n = 8$; data not shown).

TGF- β increases fibronectin receptor expression

We examined the cell-surface expression of the integrin subunits α_5 and β_1 on human ASM cells. Integrin subunits α_5 and β_1 were universally expressed on asthmatic and nonasthmatic ASM cells, with greater than 90% of cells expressing these subunits ($96.7\% \pm 2.3\%$ of nonasthmatic cells and $99.7\% \pm 0.1\%$ of asthmatic cells express α_5 , see Fig E2 in the Online Repository at www.jacionline.org; $92.3\% \pm 3.3\%$ of nonasthmatic cells and $99.5\% \pm 0.3\%$ of asthmatic cells express β_1). The percentage of cells expressing these subunits was unaffected by TGF- β stimulation ($P > .05$). However, the level of expression of α_5 and β_1 was increased after TGF- β stimulation for 48 hours on nonasthmatic cells ($P < .05$, $n = 6-7$). An increase in α_5 cell-surface protein expression on asthmatic ASM cells was also observed ($P = .05$, $n = 4$); however, TGF- β stimulation did not increase β_1 expression on asthmatic ASM cells. Control samples showed no significant isotype-control antibody binding to human ASM cells (Fig 2).

To determine whether the observed increase in cell-surface expression was associated with changes at the gene level, we examined mRNA expression of the subunits at various time points relative to the housekeeping control 18s rRNA. Expression of the α_5 subunit mRNA was increased by TGF- β at 24 hours in both asthmatic (Δ CT: 11.85 ± 0.4 in unstimulated cells and 10.54 ± 0.34 in TGF- β -stimulated cells; 2.63 ± 0.38 -fold, $P < .05$, $n = 5$) and nonasthmatic (Δ CT: 11.47 ± 0.3 in unstimulated cells and 10.43 ± 0.4 in TGF- β -stimulated cells; 2.25 ± 0.3 -fold, $P < .05$, $n = 9$; Fig 3) ASM cells. Expression of β_1 mRNA was increased in nonasthmatic ASM cells (Δ CT: 9.79 ± 0.5 in unstimulated cells and 9.19 ± 0.5 in TGF- β -stimulated cells; 1.65 ± 0.24 -fold, $P < .05$, $n = 6$). Although there was an apparent increase in β_1 mRNA in asthmatic cells caused by TGF- β , this did not reach statistical significance (Δ CT: 9.63 ± 0.5 in unstimulated cells and 9.06 ± 0.2 in TGF- β -stimulated cells; 1.60 ± 0.31 -fold, $P > .05$, $n = 5$; Fig 3, B).

ERK pathway, but not p38 or phosphoinositide-3 kinase pathway, is involved in TGF- β -induced $\alpha_5\beta_1$ expression

Chemical inhibitors of the ERK, phosphoinositide-3 kinase (PI3K), and p38 pathways were used to examine the signaling pathways involved in TGF- β -induced α_5 expression. The ERK inhibitor U0126 decreased TGF- β -induced α_5 mRNA expression in both asthmatic (0.47 ± 0.07 -fold reduction; $P < .05$, $n = 5$) and

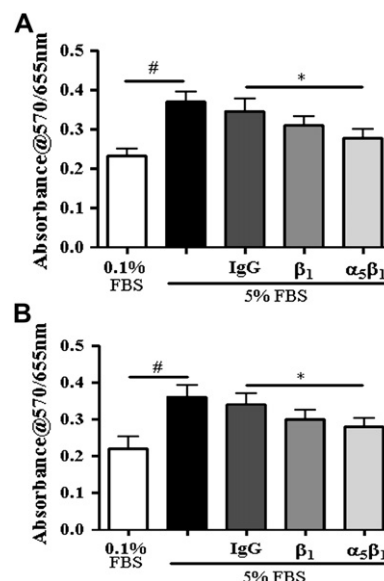


FIG 1. Integrin-dependent FBS-induced proliferation of nonasthmatic (A; $n = 14$) and asthmatic (B; $n = 9$) ASM cells. Proliferation was assessed after culture for 6 days in the presence or absence of fb-mAbs to β_1 either alone or in combination with anti- α_5 . Data are means $\pm$ SEMs of triplicate values from individual experiments. * $P < .05$ compared with IgG control. # $P < .05$ compared with 0.1% FBS.

nonasthmatic (0.4 ± 0.11 -fold reduction; $P < .05$, $n = 5$) ASM cells. PI3K inhibitors LY294002 and wortmannin had no effect on TGF- β -induced α_5 expression in nonasthmatic ($P > .05$, $n = 6$) or asthmatic ($P > .05$, $n = 6$) ASM cells (Fig 4). Inhibition of p38 (inhibitor SB203580) also had no effect on TGF- β -induced α_5 expression (data not shown).

Blocking $\alpha_5\beta_1$ reduces fibronectin accumulation

Fibronectin matrix deposition was visualized by means of immunofluorescence. TGF- β stimulation increased fibronectin matrix deposition in asthmatic (ITS score: 1.67 ± 0.10 ; TGF- β score: 2.5 ± 0.25 ; $P < .05$; $n = 3$) and nonasthmatic (ITS score: 1.36 ± 0.49 ; TGF- β score: 2.29 ± 0.44 ; $n = 4$) ASM cells. However, the presence of blocking antibodies to the α_5 and β_1 subunits together reduced fibronectin deposition in asthmatic ($\alpha_5\beta_1$ score: 1.0 ± 0.17 ; $P < .05$; $n = 3$) and nonasthmatic ($\alpha_5\beta_1$ score: 0.79 ± 0.28) ASM cells (Fig 5). Blocking the α_5 and β_1 subunits did not appear to reduce cell numbers, as visualized by Hoescht staining of cell nuclei.

DISCUSSION

In the present study we have shown that TGF- β induces expression of the major fibronectin receptor $\alpha_5\beta_1$ through an ERK-dependent pathway and that $\alpha_5\beta_1$ plays a role in both fibronectin matrix assembly and proliferation of asthmatic and nonasthmatic ASM cells.

TGF- β was selected as the stimulus with which to assess $\alpha_5\beta_1$ expression on ASM cells because it is a potent inducer of ECM proteins, including fibronectin,³ and is considered to play a significant role in fibrosis. Furthermore, levels of TGF- β are increased in the airways of subjects with asthma.¹ TGF- β significantly induced expression of fibronectin and its receptor, $\alpha_5\beta_1$, at both the mRNA and protein levels, suggesting that enhancement is regulated at the level of transcription, although other mechanisms, such as

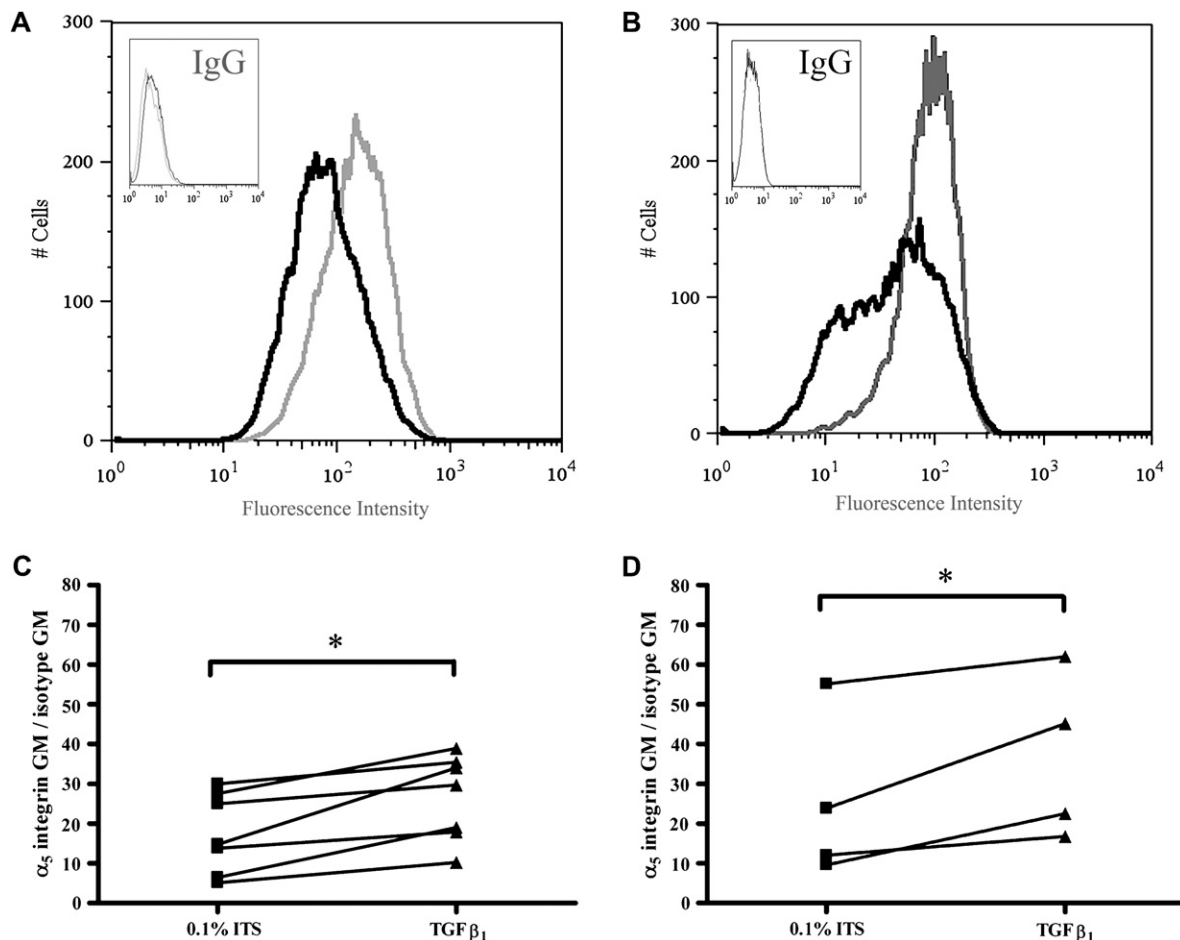


FIG 2. Cell-surface expression of α_5 on ASM cells from nonasthmatic (A and C) and asthmatic (B and D) subjects. Representative flow cytometric histograms of nonasthmatic (Fig 2, A) and asthmatic (Fig 2, B) ASM cells showing α_5 expression after stimulation in the presence (gray line) or absence (black line) of TGF- β (1 ng/mL) for 48 hours are shown. Inset panels show isotype controls. * $P \leq .05$ compared with 0.1% ITS. GM, Geometric mean.

receptor recycling, could also contribute. TGF- β has been reported to modulate integrin expression in many cell types, including epithelial cells, fibroblasts, mesangial cells, and hepatic cells^{11-13,18}; however, this is the first study to show TGF- β -induced α_5 expression on asthmatic ASM cells. Although we did not observe differences between asthmatic and nonasthmatic ASM cells in α_5 mRNA or protein expression after stimulation with TGF- β , the fact that TGF- β levels are significantly greater in the asthmatic airway than the nonasthmatic airway would indicate a greater potential for increased α_5 expression in asthma.

TGF- β mediates its responses by interacting with its cell-surface receptors to form a complex of type I and type II receptors. The classical signaling cascade after interaction of TGF- β with its receptors is through the Smad pathway, although TGF- β is also known to signal through Smad-independent pathways (ERK, PI3K, and p38).^{3,19,20} We have previously shown that ERK, PI3K, and p38 play a role in TGF- β -induced fibronectin expression,³ and therefore we were interested to determine whether these pathways are also involved in TGF- β -induced integrin expression. The results of the present study indicate a role for the ERK signaling pathway in TGF- β -induced α_5 expression because the mitogen-activated protein kinase inhibitor U0126 partially inhibited α_5 mRNA. Similarly, a role for ERK in modulating integrin

expression has been reported in other studies. For example, in osteoblasts upregulation of the β_1 integrin subunit by bone morphogenetic protein-4, a member of the TGF- β superfamily, is decreased by inhibition of the ERK pathway.²¹ ERK has also been implicated in integrin-fibronectin signaling,²² and in myoblasts increased $\alpha_5\beta_1$ promotes ERK activation.²³ Whether the reduction in α_5 mRNA is a direct response to ERK inhibition or whether it is a secondary response to reduced fibronectin deposition was not examined, although it is a possibility. In addition, ERK has been implicated in COX-2-induced α_5 expression in human lung carcinoma cells.²⁴ We know that TGF- β also induces COX-2 expression in human ASM cells,²⁵ although whether COX-2 plays a role in α_5 expression was not determined. Inhibition of the PI3K pathway had no effect on integrin expression, although we have previously reported it to play a role in TGF- β -induced fibronectin in asthmatic, but not nonasthmatic, ASM cells.³ The previous study suggested intrinsic differences between asthmatic and nonasthmatic matrix regulation. Whether different integrin receptors, such as α_4 , which binds the CS-1 domain of an alternatively spliced fibronectin, are regulated by different signaling pathways has not been determined.

Inhibition of the signaling pathways did not completely abrogate α_5 mRNA induction, suggesting that other signaling

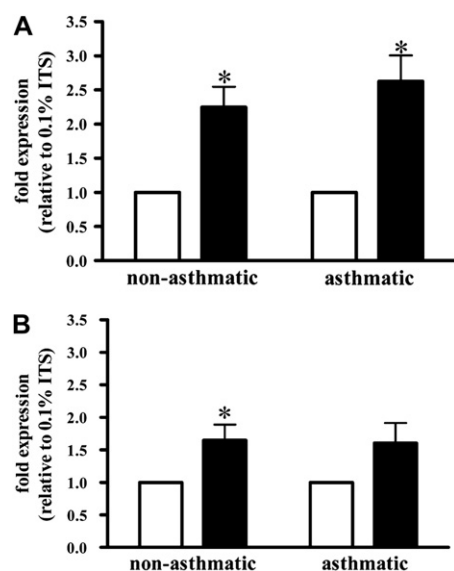


FIG 3. Production of α_5 (A) and β_1 (B) mRNA by nonasthmatic (n = 6-9) and asthmatic (n = 5) ASM cells in response to TGF- β (1 ng/mL; black bars) at 24 hours. Results are expressed as a fold increase above the time-matched control (0.1% ITS; white bars). * $P \leq .05$ compared with control.

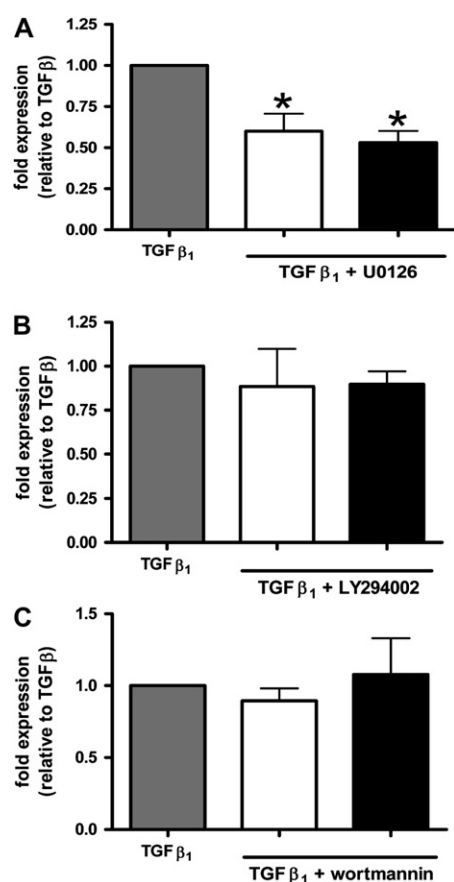


FIG 4. Production of α_5 mRNA by nonasthmatic (white bars) and asthmatic (black bars) ASM cells after stimulation with TGF- β (1 ng/mL) in the presence or absence of the ERK inhibitor U0126 (A) and the PI3K inhibitors LY294002 (B) or wortmannin (C) at 24 hours. Results are expressed relative to TGF- β -induced expression (gray bars). * $P < .05$ compared with TGF- β .

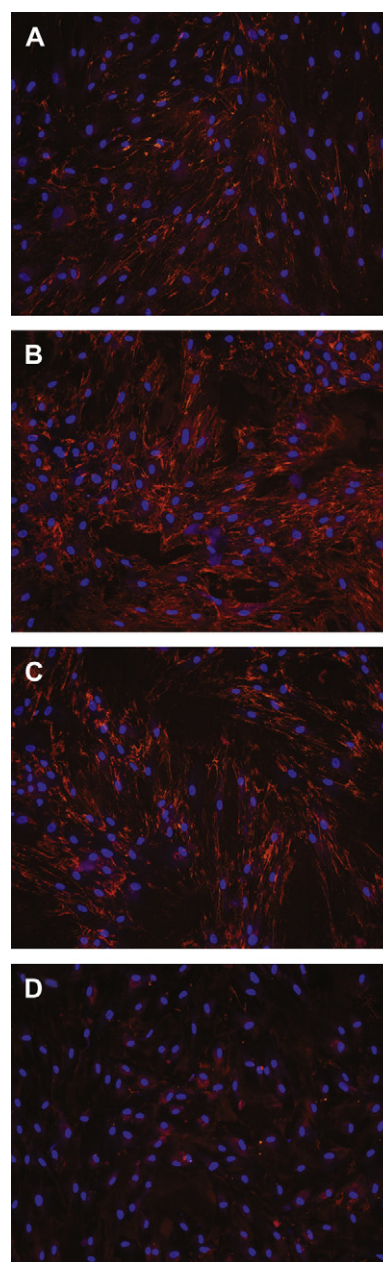


FIG 5. The effect of $\alpha_5\beta_1$ function-blocking antibodies on fibronectin deposition. Fibronectin matrix visualized by means of immunofluorescence (red staining) in untreated (0.1% ITS) cells (A), TGF- β -stimulated cells (B), TGF- β -stimulated cells exposed to isotype control antibodies (C) and TGF- β -stimulated cells exposed to α_5 and β_1 fb-mAbs (D) is shown. A representative experiment from 1 nonasthmatic subject is shown.

pathways not examined in this study might also play a role. The signaling molecules protein kinase C δ , phosphatidylcholine-specific phospholipase C, and c-Jun N-terminal kinase have been implicated in TGF- β -induced fibronectin.^{20,26} Whether these pathways also play a role in integrin α_5 expression in human ASM cells remains to be determined.

Integrins provide an important link between the intracellular signaling processes and the extracellular environment. Expression of fibronectin and its receptor are coregulated,²⁷ and the fibronectin receptor must bind to fibronectin to organize it into an insoluble matrix. Therefore any change in the level of $\alpha_5\beta_1$

expression could potentially alter fibronectin accumulation. Increased expression of $\alpha_5\beta_1$ has been associated with disease states,^{11,28} and it is possible that increased expression of $\alpha_5\beta_1$ on human ASM cells might directly contribute to the increased fibronectin deposition associated with asthma. We report in this study that $\alpha_5\beta_1$ is required for fibronectin matrix assembly because blocking the integrin receptor decreased fibronectin matrix expression. This is in agreement with a study by Pickering et al,²⁸ who determined that fibronectin assembly in the rat injured carotid artery requires $\alpha_5\beta_1$ expression. Although Freyer et al⁸ reported that the $\alpha_5\beta_1$ receptor was necessary for cell survival, the decrease in fibronectin assembly could not be accounted for by a loss of cells, hence further indicating a role for $\alpha_5\beta_1$ in fibrosis.

Increased ECM assembly might contribute to the structural remodeling associated with asthma and might enhance mitogen-induced smooth muscle proliferation. Nguyen et al⁹ showed that in nonasthmatic ASM cells $\alpha_5\beta_1$ plays a role in fibronectin-enhanced proliferation. Here we demonstrate for the first time in asthmatic ASM cells a role for $\alpha_5\beta_1$ in FBS-induced proliferation. Therefore the results of this and other studies suggest a role for $\alpha_5\beta_1$ in airway remodeling. It is feasible that altered integrin-ECM accumulation might contribute to the increased bronchial hyperresponsiveness associated with asthma by changing the stiffness of the airways or the ability of the airways to contract and relax or increasing airway wall thickness.²⁹⁻³¹

In summary, the data presented indicate a significant role for the integrin receptor $\alpha_5\beta_1$ in TGF- β -induced fibronectin deposition. Specific inhibition of the integrin receptor might provide a therapeutic target for the control of the increased matrix assembly and increased smooth muscle bulk associated with airway remodeling and asthma.

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Clinical implications: These findings provide a mechanism for the increase in TGF- β -induced fibronectin and therefore provide a possible target to reduce the remodeling process that occurs in asthma.

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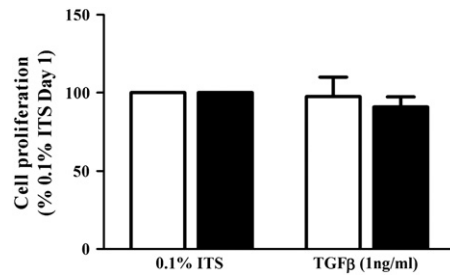


FIG E1. The effect of TGF- β (1 ng/mL) stimulation on the proliferation of ASM cells from nonasthmatic ($n = 6$; *white bars*) and asthmatic ($n = 5$; *black bars*) subjects. Proliferation was assessed after stimulation for 24 hours in the presence or absence of TGF- β (1 ng/mL). Results are expressed as a percentage of the time-matched control (0.1% ITS).

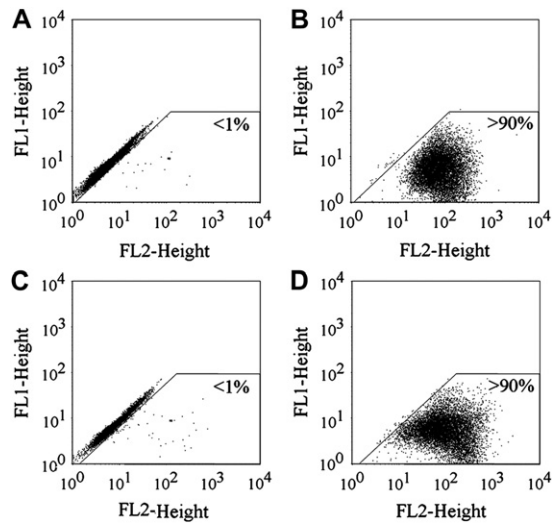


FIG E2. Representative flow cytometric plots showing the number of integrin α_5 -positive ASM cells from nonasthmatic (**B**) and asthmatic (**D**) subjects compared with an isotype-matched IgG antibody (nonasthmatic [**A**] and asthmatic [**C**]).

TABLE E1. Patient information

Patient no.	Sex	Age (y)	Procedure	Disease	Nonasthmatic/asthmatic	Smoking history	Treatment
1	F	83	Resection	Carcinoma	Nonasthmatic	NA	NA
2	M	60	Resection	Carcinoma	Nonasthmatic	Yes	NA
3	F	56	Explanted lungs	Emphysema	Nonasthmatic	Exsmoker	NA
4	F	52	Resection	Carcinoma	Nonasthmatic	No	NA
5	F	60	Resection	Carcinoma	Nonasthmatic	Exsmoker	NA
6	M	66	Resection	COPD	Nonasthmatic	Yes	NA
7	M	61	Resection	Carcinoma	Nonasthmatic	NA	NA
8	F	38	Resection	Carcinoma	Nonasthmatic	NA	NA
9	M	55	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
10	M	20	Bronchoscopy	No disease	Nonasthmatic	No	N
11	F	52	Resection	Carcinoma	Nonasthmatic	NA	NA
12	F	46	Resection	Carcinoma	Nonasthmatic	NA	NA
13	F	60	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
14	M	31	Bronchoscopy	No disease	Nonasthmatic	No	N
15	M	62	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
16	M	79	Resection	Carcinoma	Nonasthmatic	NA	NA
17	M	75	Resection	Carcinoma	Nonasthmatic	NA	NA
18	F	61	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
19	M	58	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
20	M	59	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
21	M	53	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
22	F	59	Resection	Right middle lobe syndrome	Nonasthmatic	NA	NA
23	F	47	Explanted lungs	Emphysema	Nonasthmatic	Exsmoker	NA
24	M	53	Resection	Colon carcinoma solitary metastasis to lung	Nonasthmatic	NA	NA
25	M	78	Resection	Carcinoma	Nonasthmatic	NA	NA
26	F	73	Resection	Carcinoma	Nonasthmatic	NA	N
27	M	42	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
28	F	67	Resection	Carcinoma	Nonasthmatic	NA	NA
29	F	58	Explanted lungs	Emphysema	Nonasthmatic	NA	NA
30	M	77	Resection	Carcinoma	Nonasthmatic	NA	NA
31	M	76	Resection	Emphysema	Nonasthmatic	Exsmoker	NA
32	F	22	Bronchoscopy	No disease	Nonasthmatic	NA	NA
33	F	68	Resection	Carcinoma	Nonasthmatic	NA	NA
34	M	58	Resection	Carcinoma	Nonasthmatic	NA	NA
35	M	44	Explanted lungs	Pulmonary fibrosis	Nonasthmatic	NA	NA
36	F	44	Explanted lungs	Primary pulmonary hypertension	Nonasthmatic	NA	NA
37	M	62	Resection	Carcinoma	Nonasthmatic	NA	NA
38	NA	NA	Explanted lungs	Donor/trauma	Nonasthmatic	NA	NA
39	M	69	Bronchoscopy	Cough	Nonasthmatic	NA	NA
40	M	16	Explanted lungs	Donor	Nonasthmatic	NA	N
41	F	63	Resection	Carcinoma	Nonasthmatic	NA	A
42	NA	NA	Explanted lungs	Donor	Nonasthmatic	NA	NA
43	M	NA	Explanted lungs	Donor	Nonasthmatic	Yes	NA
44	M	33	Bronchoscopy	Asthma	Asthmatic	NA	NA
45	M	20	Bronchoscopy	Asthma	Asthmatic	No	B, C
46	M	27	Bronchoscopy	Asthma	Asthmatic	No	B, C
47	M	23	Bronchoscopy	Asthma	Asthmatic	No	B
48	M	22	Bronchoscopy	Asthma	Asthmatic	No	B
49	M	25	Bronchoscopy	Asthma	Asthmatic	No	B
50	F	19	Bronchoscopy	Asthma	Asthmatic	No	B, C
51	F	43	Bronchoscopy	Asthma	Asthmatic	NA	B, C
52	F	49	Bronchoscopy	Asthma	Asthmatic	NA	B, C
53	M	45	Bronchoscopy	Asthma	Asthmatic	NA	NA
54	M	38	Bronchoscopy	Asthma	Asthmatic	NA	NA
55	M	42	Bronchoscopy	Asthma	Asthmatic	NA	NA
56	M	40	Bronchoscopy	Asthma	Asthmatic	No	NA
57	F	34	Bronchoscopy	Asthma	Asthmatic	NA	B, C
58	M	21	Bronchoscopy	Asthma	Asthmatic	NA	NA
59	M	22	Bronchoscopy	Asthma	Asthmatic	NA	NA

(Continued)

TABLE E1. (Continued)

Patient no.	Sex	Age (y)	Procedure	Disease	Nonasthmatic/asthmatic	Smoking history	Treatment
60	M	22	Bronchoscopy	Asthma	Asthmatic	No	B
61	M	26	Bronchoscopy	Asthma	Asthmatic	No	B
62	M	25	Bronchoscopy	Asthma	Asthmatic	NA	NA
63	M	30	Bronchoscopy	Asthma	Asthmatic	NA	NA
64	M	20	Bronchoscopy	Asthma	Asthmatic	No	B, C
65	M	78	Bronchoscopy	Bronchiectasis/asthma	Asthmatic	No	B, C
66	M	15	Explant lungs	Asthma	Asthmatic	NA	B, C
67	M	58	Bronchoscopy	Asthma	Asthmatic	No	B, C
68	M	40	Bronchoscopy	Asthma	Asthmatic	NA	B, C

F, Female; NA, not available; M, male; COPD, chronic obstructive pulmonary disease; N, no treatment; A, anticholinergics; B, bronchodilators; C, corticosteroids.