Th1 cytokine-induced syndecan-4 shedding by airway smooth muscle cells is dependent on mitogen-activated protein kinases

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Tan X, Khalil N, Tesarik C, Vanapalli K, Yaputra V, Alkhouri H, Oliver BG, Armour CL, Hughes JM. Th1 cytokine-induced syndecan-4 shedding by airway smooth muscle cells is dependent on mitogen-activated protein kinases. Am J Physiol Lung Cell Mol Physiol 302: L700–L710, 2012. First published January 20, 2012; doi:10.1152/ajplung.00167.2011.—In asthma, airway smooth muscle (ASM) chemokine secretion can induce mast cell recruitment into the airways. The functions of the mast cell chemoattractant CXCL10, and other chemokines, are regulated by binding to heparan sulphates such as syndecan-4. This study is the first demonstration that airway smooth muscle cells (ASM) from people with and without asthma express and shed syndecan-4 under basal conditions. Syndecan-4 shedding was enhanced by stimulation for 24 h with the Th1 cytokines interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α), but not interferon-γ (IFNγ), nor the Th2 cytokines IL-4 and IL-13. ASM stimulation with IL-1β, TNF-α, and IFNγ (cytomix) induced the highest level of syndecan-4 shedding. Nonasthmatic and asthmatic ASM cell-associated syndecan-4 protein expression was also increased by TNF-α or cytomix at 4–8 h, with the highest levels detected in cytomix-stimulated asthmatic cells. Cell-associated syndecan-4 levels were decreased by 24 h, whereas shedding remained elevated at 24 h, consistent with newly synthesized syndecan-4 being shed. Inhibition of ASM matrix metalloproteinase-2 did not prevent syndecan-4 shedding, whereas inhibition of ERK MAPK activation reduced shedding from cytomix-stimulated ASM. Although ERK inhibition had no effect on syndecan-4 mRNA levels stimulated by cytomix, it did cause an increase in cell-associated syndecan-4 levels, consistent with the shedding being inhibited. In conclusion, ASM produce and shed syndecan-4 and although this is increased by the Th1 cytokines, the MAPK ERK only regulates shedding. ASM syndecan-4 production during Th1 inflammatory conditions may regulate chemokine activity and mast cell recruitment to the ASM in asthma.

IN ASThma, reModeling cHaNGes such as mucous hypersecretion and increased airway smooth muscle mass are widely accepted to be the consequence of chronic inflammation (1–3, 6), including mast cell degranulation (5, 8, 50). Infiltration of these cells into the airway interstitium and the increase in mast cell numbers in the smooth muscle are likely due to increased levels of CC and CXC chemokines (50, 57) such as CCL11 (eotaxin; Refs. 4, 32, 56) and CXCL10 (IP10; Ref. 4), respectively.

Chemokine functions such as their binding to cell surface receptors can be modulated by interactions with heparan sul-

Reagents. Recombinant human IFNγ (BD Biosciences), IL-1β, TNF-α, IL-4, and IL-13 (R&D Systems, Minneapolis, MN) were reconstituted and stored as recommended by the manufacturers and were all used at 10 ng/ml. The pharmacological inhibitors SP600125 (A.G. Scientific, San Diego, CA), GM6001, PD98059, SB203580
(Calbiochem, San Diego, CA), and actinomycin D (Sigma-Aldrich, Castlehill, Australia) were reconstituted in DMSO and stored at –20°C. Cycloheximide (Sigma-Aldrich) was stored at 4°C and reconstituted in water.

**ASMC culture.** Human ASMC were obtained from bronchial biopsies from nonasthmatic donors, donors with mild to moderate asthma, and resected lung tissue from donors undergoing surgery for lung cancer or from patients undergoing lung transplantation (refer to Table 1 for patient details). Samples were obtained with the donor’s informed consent and approval from Sydney South West Area Health Service, Australian Red Cross, and University of Sydney Human Ethics Committee.

ASMC were maintained in culture as previously described (24) in DMEM (Sigma-Aldrich) supplemented with 10% vol/vol FBS, 100 U/ml penicillin G, 100 μg/ml streptomycin sulphate, 25 μg/ml amphotericin B, 4 mM l-glutamine, and 20 mM HEPES (pH 7.4) and grown in humidified 5% CO2/air at 37°C. Cycloheximide (Sigma-Aldrich) was stored at 4°C and reconstituted in water.

**Patient details**

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FEV1, forced expiratory volume in 1 s; FVC, forearm vascular conductance; COPD, chronic obstructive pulmonary disease.

**Table 1.**
was reverse transcribed using RevertAid First Strand cDNA synthesis kit (Fermentas Life Sciences, Hanover, MD). The resulting cDNA (2.5 μg) was amplified by PCR using FAM-labeled SDC4 and VIC-labeled-18S rRNA TaqMan probes on an ABI Prism 7500 (Applied Biosystems) according to the following cycle parameters: 50°C for 2 min, 1 cycle; 95°C for 10 min and 40 cycles of 95°C for 15 s, 60°C for 1 min. Syndecan-4 mRNA levels were normalized against 18S rRNA levels.

Cell-associated syndecan-4 protein expression. ASMC were grown in six-well plates, and after treatment with cytokine(s) in the absence and presence of inhibitors for 0, 4, 8, and 24 h, whole cell lysates were prepared as previously described by Lator et al. (31). Each sample (25 μl) was subjected to SDS-PAGE and Western blotting. Syndecan-4 bands (molecular mass: ~30 kDa) were visualized using goat anti-human syndecan-4 polyclonal antibody (R&D Systems) diluted 1:1,000 and normalized to α-tubulin bands visualized using mouse anti-human α-tubulin monoclonal antibody (Santa Cruz Biotechnology) diluted 1:5,000. Band intensities were quantified by densitometric analysis using ImageJ version 1.43u software (Bethesda, MA). Syndecan-4 band densities were expressed relative to their respective α-tubulin band density.

Matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase release. ASMC were grown in six-well plates and after cytokine treatment for 4 and 24 h, the supernatants were collected and stored at −20°C. Samples (25 μl) and active- and pro-matrix metalloproteinase-2 (MMP-2)-positive controls were subjected to electrophoresis in 10% wt/vol polyacrylamide gels containing 2.5 mg/ml of gelatine (Sigma-Aldrich). Zymography was carried out by washing gels with 2.5% vol/vol Triton X-100 and then incubating them overnight at 37°C in enzyme activation buffer (50 mM Tris, 5 mM CaCl₂, 1 mM ZnCl₂, 0.5% vol/vol Triton X-100, and 0.01% wt/vol sodium azide pH 7.0). The gels were washed in distilled water and then stained (0.1% wt/vol Coomasie brilliant blue, 50% vol/vol methanol, and 10% vol/vol glacial acetic acid) for 1 h at room temperature. Gels were then destained for 4 h in solution containing 25% vol/vol methanol and 7% vol/vol glacial acetic acid. Pro- and active-MMP-2 bands were visualized using zymography by comparison with the pro- and active-MMP-2 control bands and quantified by densitometric analysis.

Pro- and active-MMP-2 levels were also quantified using a MMP-2 activity assay (Anaspec, Fremont, CA) that was run according to the supplied protocol.

Tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-2 levels in the ASMC supernatants were detected using ELISA kits (R&D Systems) according to the manufacturer’s protocols.

Data analysis. For all studies, statistical analyses were performed using Prism version 5.02 (GraphPad, San Diego, CA). Unless stated otherwise, all results were expressed as means ± SE for ASMC, and significance of results (P < 0.05) was determined by one-way repeated measures ANOVA with Bonferroni post hoc tests. For ELISA studies, shed syndecan-4 supernatant levels from duplicate treatments were averaged, adjusted for cell numbers, and expressed as picograms per 10⁵ cells. Significant syndecan-4 shedding in response to cytokine stimulation was determined by two-way Student’s t-test with Bonferroni post hoc test. In subsequent studies assessing the time dependence of cytokine-treatments, shed syndecan-4 levels were expressed as picograms per milliliters. Significance of results was determined by two-way repeated-measures ANOVA, one-way repeated-measures ANOVA, or Student’s t-test with Bonferroni post hoc tests. For real-time PCR studies, average results from all cell lines tested were expressed as fold change from unstimulated cells. Cell-associated syndecan-4 levels normalized to α-tubulin were expressed as fold change over 24 h unstimulated levels and the significance of results was tested by two-way repeated measures ANOVA. For gelatin zymography studies, results were expressed as a percentage of positive control MMP-2 levels per 10⁵ cells and significance of results was determined by two-way Student’s t-test with Bonferroni post hoc tests.

RESULTS

ASMC syndecan-4 shedding. Shed syndecan-4 was detected in the ASMC culture medium in the absence of any treatments. There was no difference in shed syndecan-4 levels between nonasthmatic (n = 9) and asthmatic (n = 7) ASMC (221 ± 84 vs. 212 ± 50 pg·ml⁻¹·10⁻⁵ cells, respectively) under basal conditions.

The individual Th1 cytokines IL-1β, TNF-α, but not IFNγ, as well as cytomix, increased syndecan-4 shedding. Stimulation with either IL-1β or TNF-α, but not IFNγ, for 24 h induced significant increases in shed syndecan-4 levels in both nonasthmatic (n = 7–9) and asthmatic (n = 7) ASMC (Fig. 1A; P < 0.05). Th1 cytokine-induced syndecan-4 shedding was not significantly different between nonasthmatic and asthmatic ASMC (Fig. 1, A–C). Cytomix induced the highest levels of syndecan-4 shedding (Fig. 1A; 847 ± 100 and 1,421 ± 462 pg·ml⁻¹·10⁻⁵ cells from nonasthmatic and asthmatic ASMC, respectively). Cytomix induced the highest rate of syndecan-4 shedding from ASMC in time-course studies (Fig. 1, B and C; n = 5).

In contrast, treatment of either nonasthmatic or asthmatic ASMC with the Th2 cytokines IL-4 and IL-13 individually or in combination did not mediate any increase in syndecan-4 shedding (data not shown).

ASMC syndecan-4 cell-associated protein expression. Under basal conditions, low levels of syndecan-4 expression were detected in ASMC. A significant increase in cell-associated syndecan-4 expression was observed with TNF-α and cytomix treatments for both nonasthmatic and asthmatic ASMC (Fig. 2, A–D; P < 0.05; n = 5). In both nonasthmatic and asthmatic ASMC, cytokine-induced syndecan-4 expression was maximal between 4–8 h, with expression levels diminishing at 24 h (Fig. 2, A and B). Similar to shed syndecan-4 levels, cytomix-treated ASMC produced the highest maximum and rate of syndecan-4 expression (Fig. 2, C and D). In asthmatic ASMC, cell-associated syndecan-4 expression was still significantly elevated at 24 h. In addition, there appeared to be a trend towards higher syndecan-4 expression in response to cytomix treatment in asthmatic (22.8 ± 4; n = 5; P < 0.001) compared with nonasthmatic (8.6 ± 5.2; n = 4; P < 0.05). In ASMC, however, due to high variability in syndecan-4 expression levels between different asthmatic ASMC lines, the difference did not achieve statistical significance.

ASMC MMP- and TIMP release. Nonasthmatic and asthmatic ASMC released MMP-2, TIMP-1, and TIMP-2. Both pro- and active-MMP-2, but not MMP-9 (data not shown), were detected in supernatants from ASMC (Fig. 3, A and B). Cytomix treatment of either nonasthmatic or asthmatic ASMC for 4 and 24 h did not mediate a further increase in either pro- or active-MMP-2 from unstimulated levels; however, in asthmatic untreated and cytomix-treated ASMC, active-MMP-2 levels significantly increased between 4 and 24 h (Fig. 3B).

TIMP-1 and TIMP-2 levels were high in the supernatants from nonasthmatic (52.7 ± 9.1 and 12.8 ± 2.3 ng/10⁵ cells respectively; n = 5) and asthmatic (56.9 ± 16 and 17.9 ± 3.3
ng/10^5 cells, respectively; n = 5) ASMC. Cytomix treatment did not alter TIMP-1 or TIMP-2 release (data not shown).

Although pretreatment with GM6001, a nonselective MMP inhibitor for MMP-1, -2, -3, -8, and -9, inhibited active MMP-2 in supernatants from cytomix-treated ASMC (Fig. 3C), it did not affect both cytomix-induced syndecan-4 shedding and cell-associated expression in ASMC (Fig. 3, D and E).

**Pathways leading to cytomix-induced syndecan-4 shedding.** The increase in syndecan-4 shedding 24 h after cytomix treatment was partially inhibited when nonasthmatic and asthmatic ASMC were pretreated with either the protein synthesis inhibitor cycloheximide or mRNA synthesis inhibitor actinomycin D (Fig. 4, A and B; P < 0.05; n = 5). The observed reduction in syndecan-4 shedding was preceded by complete inhibition of cytomix-stimulated increases in cell-associated syndecan-4 expression at 4 h (Fig. 4C; n = 3).

To investigate the role of MAPK in the regulation of syndecan-4 shedding, 30 μM PD98059, 10 μM SP600125, and 10 μM SB203580 were utilized as they inhibit cytokine-stimulated ERK, JNK, and p38 MAPK activation, respectively, in ASMC (17–18, 30–31). The ERK MAPK inhibitor PD98059 reduced cytomix-induced syndecan-4 shedding in both nonasthmatic and asthmatic ASMC (Fig. 5, A and B; P < 0.05 n = 5). In nonasthmatic ASMC, the JNK inhibitor SP600125 also significantly reduced cytomix-induced syndecan-4 shedding (Fig. 5A; P < 0.05; n = 5). A similar trend was observed in asthmatic ASMC; however, the difference did not achieve statistical significance (Fig. 5B; P = 0.06; n = 6). The p38 MAPK inhibitor SB203580 did not affect syndecan-4 shedding (Fig. 5, A and B; n = 5) but did reduce cytomix-induced p38 activation in the ASMC (Fig. 5C).

**Syndecan-4 production and MAPK inhibition.** Cytomix induced an increase in syndecan-4 mRNA levels (Fig. 6A; P < 0.05; n = 6). However, this effect was not modulated by the ERK and JNK MAPK inhibitors (Fig. 6A; n = 6). Cell-associated syndecan-4 protein levels in cytomix-stimulated ASMC were similar in the absence or presence of MAPK.
inhibitors at 4 h (Fig. 6B; n = 5). In contrast, at 24 h after cytomix stimulation, syndecan-4 protein expression was significantly higher in ASMC that were pretreated with the ERK inhibitor compared with vehicle-treated cells (Fig. 6B; P < 0.5; n = 7).

**DISCUSSION**

In asthma, there are increased numbers of activated eosinophils in the submucosa and mast cells in the smooth muscle bundles particularly, which may be partly due to the increased secretion of chemokines by the smooth muscle. As syndecan-4 can regulate chemokine functions, cell adhesion, and migration, we investigated syndecan-4 expression and its shedding by ASMC from people with and without asthma for the first time. Under basal conditions, the ASMC produced and shed syndecan-4. The proinflammatory Th1 cytokines IL-1β and TNF-α, but not IFNγ or the Th2 cytokines IL-4 and IL-13, induced increased syndecan-4 production and shedding. The shedding was regulated by MAPK. In addition, preliminary evidence that cell-associated syndecan-4 levels may remain elevated for longer on ASMC from people with asthma was found but further studies are required to establish whether or not this is so.

There are increasing reports of differences in signaling and transcription molecule expression in ASMC from people with asthma that underlie their reduced therapeutic responsiveness and altered proliferative and secretory responses (23–24, 37, 47–48, 51–52). In particular, in response to various cytokines, asthmatic compared with nonasthmatic ASMC production of IL-33 (43) and the chemokines CXCL10 (4), CXCL8 (21), and CCL15 (25) is increased, while production of other chemokines (CXCL9, CCL11, and CXCL12) is not (4, 43) and antioxidant production is reduced (39). Thus it was important to examine production and shedding of the proteoglycan syn-
decan-4 by asthmatic and nonasthmatic ASMC in response to the Th1 and Th2 cytokines.

Various stimuli have been shown to induce syndecan shedding such as mechanical strain (26, 33), insulin (46), thrombin (49), epidermal growth factor (49), and the phorbol ester PMA from a variety of cell types (15). Very recently the proinflammatory cytokines IFNγ and TNF-α were also shown to stimulate syndecan-1 and -4 shedding from lung epithelial cells (44). In this study, we showed that TNF-α, IL-1β or cytomix increased shed syndecan-4 levels, with the highest levels detected following treatment with cytomix.

In further studies to examine the kinetics of syndecan-4 shedding, we showed that cytomix stimulation induced syndecan-4 shedding to similar levels over 24 h in both nonasthmatic

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**Fig. 3. ASMC matrix metalloproteinase (MMP) expression and syndecan-4 shedding.** Serum-deprived nonasthmatic (black bars) and asthmatic (hatched bars) ASMC were treated with 10 ng/ml of IL-1β, TNF-α, and IFNγ combined (cytomix) for either 4 or 24 h. Pro (A) and active (B)-MMP-2 in ASMC supernatants were detected using gelatin zymography (n = 5). C: active-MMP-2 activity measured in ASMC supernatants harvested 24 h after cytomix treatment in the absence or presence of GM6001 from 2 nonasthmatic and 2 ASMC cell lines, D: shed syndecan-4 in ASMC supernatants collected after 24-h cytomix treatment in the absence or presence of the MMP inhibitor GM6001 were measured using ELISA. E: representative Western blots and grouped densitometric results from 3 nonasthmatic and 3 asthmatic primary ASMC cell lines for cell associated syndecan-4 expression in the presence or absence of GM6001. Results are expressed as the means ± SE fold change from unstimulated syndecan-4 levels normalized to α-tubulin. Statistical analyses were performed by Student’s t-test (A–D) or one-way repeated-measures ANOVA (E). *P < 0.05, compared with unstimulated.
and asthmatic ASMC. In contrast, the individual cytokines caused comparatively small increases in shedding. Shed syndecan-4 levels were still elevated at 24 h with cytomix; thus the elevated syndecan-4 shedding may occur for a longer time before returning to basal levels. The enhanced ability of cytomix stimulation to induce syndecan-4 shedding compared with the individual cytokines is likely to be dependent on TNF-α and IL-1β, as these two cytokines induced ASMC syndecan-4 shedding, whereas IFNγ appeared to have little effect by itself. However, the stimulatory effects of cytomix were larger than the sum of the individual effects of TNF-α and IL-1β, which is consistent with IFNγ contributing to syndecan-4 shedding in the presence of the other two cytokines.

Cytomix stimulation also induced increases in cell-associated syndecan-4 expression in both nonasthmatic and asthmatic ASMC. The observed decrease in cell-associated syndecan-4 levels after 8 h cytomix stimulation may be the result of shedding of the newly synthesized protein as shedding continued to increase for 8 h following cytomix treatment. These findings reflect those found for the effects of mechanical strain on vascular smooth muscle cells where syndecan-4 expression peaks by 4 h but drops below baseline, unstrained levels by 24 h (26).

Even though syndecan-4 shedding was similar in both nonasthmatic and asthmatic ASMC, there was a trend towards higher syndecan-4 expression in asthmatic ASMC compared with nonasthmatic ASMC. A limitation of our study is that ASMC cells could not be obtained from a larger number of healthy people and thus comparisons of syndecan-4 expression and release could only be made between asthmatic and nonasthmatic ASMC. However, the asthmatic ASMC syndecan-4 response to cytomix was highly variable and so the differences with nonasthmatic cells in cell-associated expression did not achieve statistical significance. In addition, this apparent increase in syndecan-4 expression in asthmatic ASMC over nonasthmatic ASMC was not observed following stimulation with the individual cytokines IL-1α, TNF-α, or IFNγ. Thus the airway smooth muscle in some people with asthma may express high levels of syndecan-4 under similar Th1 inflammatory conditions. However, asthmatic ASMC that express higher levels of syndecan-4 do not appear to shed more, as shedding was similar in nonasthmatic and asthmatic ASMC. Instead, asthmatic ASMC may be concentrating syndecan-4 protein on the cell. As syndecan-4 heparan sulfates bind various chemokines, increased cell surface syndecan-4 expression could result in the accumulation of chemokines in close proximity to the ASMC. In future studies, it would be of interest to examine the relationship between syndecan-4 cell surface expression and shedding and disease severity to clarify whether or not any changes in expression represent asthma pathophysiology or are part of a normal airway inflammatory response. ASMC from a larger number of people with asthma of differing severities, as well as healthy controls, would be needed to do this.

Syndecans are cleaved at the juxtamembrane domain of the extracellular domain by various enzymes, especially MMPs (7, 15). These include MMP-7 (34), MMP-9 (7), a disintegrin and metalloproteinase 17 (44), and the membrane-associated matrix metalloproteinase MT-MMP-1 (13). In this study pro- and active-MMP-2, but not MMP-9, were detected in the ASMC supernatants, but the pan MMP inhibitor GM6001 did not affect cytomix-induced ASMC syndecan-4 shedding and expression. As GM6001 does not inhibit all MMPs, further studies are required to determine whether other MMPs may be involved in syndecan-4 shedding.
The use of the pharmacological protein and mRNA synthesis inhibitors cycloheximide and actinomycin D, respectively, showed that the increase in syndecan-4 shedding and cell-associated expression by cytomix involves de novo protein and mRNA synthesis. These findings are consistent with the real-time PCR and Western blot studies where both syndecan-4 mRNA and cell-associated protein expression were elevated immediately after cytomix treatment. However, it is likely that shedding can occur in the absence of de novo protein synthesis as in most ASMC cell lines examined, neither cycloheximide, nor actinomycin D, completely abolished cytomix-induced shedding even though the cytomix-induced increase in cell-associated syndecan-4 expression was abolished by either cycloheximide or actinomycin D. Thus the Th1 cytokines are

Fig. 5. Effects of MAPK inhibitors on ASMC syndecan-4 shedding. Serum-deprived ASMC were treated with vehicle (0.1% vol/vol DMSO), PD98059 (PD; 30 μM), SB203580 (SB; 10 μM), or SP600125 (SP; 10 μM) for 45 min before and during stimulation with cytomix. Levels of syndecan-4 shed from nonasthmatic (A; ●) and asthmatic (B; ▪) ASMC after 24-h stimulation with cytomix were then measured using ELISA (mean values). Effects of PD (30 μM), SP (10 μM), and SB (10 μM) on ERK, JNK, and p38 MAPK phosphorylation (C) in the absence or presence of 30 min stimulation with cytomix in nonasthmatic ASMC (n = 4) was determined using Western blotting. Representative blots are shown on top, with each bar representing the means ± SE fold change from cytomix-mediated effects normalized to the respective total MAPK. Significance of effects mediated by MAPK inhibitors on syndecan-4 shedding were determined by Student’s t-test.
likely to activate the mechanisms that lead to new syndecan-4 production as well as syndecan-4 shedding.

Similar to this study, in vascular smooth muscle cells, mechanical strain-induced syndecan-4 shedding is also regulated by the MAPK signaling pathways (26). Hence, we used pharmacological inhibitors to explore the role of MAPK in regulating cytomix-induced syndecan-4 shedding. The effects of cytomix on syndecan-4 shedding were partially inhibited by the ERK and JNK MAPK inhibitors PD98059 and SP600125 but not by the p38 inhibitor SB203580. These results are in agreement with mechanical stress-induced syndecan-4 shedding in vascular smooth muscle cells (26). Interestingly, neither ERK nor JNK inhibition affected the cytomix-induced increase in ASMC syndecan-4 mRNA, which parallels the study on vascular smooth muscle cells that implicate MAPK in regulating syndecan-4 shedding rather than its production (26).

As ERK and JNK inhibitors reduced ASMC syndecan-4 shedding, we expected that these inhibitors would induce ASMC to retain their cell-associated syndecan-4 expression. Neither inhibitor affected cell-associated syndecan-4 levels in the first 4 h after cytomix addition. However, by 24-h postcytomix addition, syndecan-4 expression was higher in cells where ERK, but not JNK, was inhibited. These findings are further evidence that ERK regulates the activity of an as yet unidentified ASMC factor(s) that causes syndecan-4 shedding.

Despite evidence from our studies that JNK regulates syndecan-4 shedding by ASMC, its mechanism of action still remains to be elucidated. JNK inhibition reduced mechanical stress-induced syndecan-4 expression at early time points in vascular smooth muscle cells (26). However, in our ASMC studies, there was no evidence of this occurring, as the cytomix-induced syndecan-4 expression was similar in the absence or presence of the JNK and ERK inhibitors at 4 h. The shedding of syndecan from other cells is known to be regulated by multiple signaling pathways. For example, PKC activation, by either phorbol acetates (PMA) or stromal cell-derived factor-1 binding to syndecan-4, has been shown to induce syndecan-4 shedding from epithelial and HeLa cells (7).

This is the first study to validate the expression and shedding of syndecan-4 from human airway smooth muscle. In the lungs, syndecan-4 has been found expressed on interstitial fibroblasts. Recently, several studies (16, 20) have begun to characterize the role of syndecan-4 on lung fibroblasts. These studies found that the CXCL10-mediated inhibition of fibroblast migration is dependent on intact syndecan-4 containing heparan sulfate chains and this effect of CXCL10 was CXCR3-independent (20). In addition, the arrangement of α-smooth muscle actin, a myofibroblast marker in lung fibroblasts, is also dependent on the expression of full-length syndecan-4 containing heparan sulfate chains (16). Whether syndecan-4 has similar regulatory roles in ASMC still remains to be investigated. However, this is particularly fascinating because like syndecan-4, ASMC production of the mast cell chemoattractant CXCL10 is induced by Th1 cytokines. Furthermore, CXCL10 is often expressed in the airway smooth muscle in people with asthma and the mast cells in the smooth muscle are activated (8) and express the CXCL10 receptor CXCR3, whereas <50% of mast cells elsewhere in the airways express it (4). It is well accepted that activated mast cells induce and/or enhance airway smooth muscle contraction via release of mediators such as histamine and tryptase from their granules (22). Hence, increased expression of syndecan-4 during episodes of inflammation may indirectly contribute to airway hyperresponsiveness in people with asthma.

In addition to CXCL10, syndecan-4 binds and promotes the activation of TGF-β1 (12, 19). TGF-β1 is a profibrotic medi-
ator found elevated in bronchoalveolar lavage and lung tissue of people with asthma (40, 45, 54). TGF-β, has been implicated in subepithelial fibrosis in the airways in asthma due to its ability to stimulate increased extracellular matrix molecule production by ASM (23), fibroblasts (9), and epithelial cells (35). Under inflammatory conditions such as may occur during exacerbations of asthma, increased ASM syndecan-4 production may bind to and regulate TGF-β, which may lead to altered ASM matrix deposition and remodeling of the airway wall.

In summary, syndecan-4 was constitutively produced and shed by ASM and treatment with the Th1 cytokines IL-1β and TNF-α alone, or in combination with IFNy, increased production and shedding significantly. ASM syndecan-4 shedding was reduced by inhibitors of the MAPK ERK and JNK, but only ERK signaling appeared to lead directly to shedding. By accruing more syndecan-4 on their surface and also shedding it during Th1 cytokine-driven inflammation, asmatoc ASM may regulate inflammation and remodeling locally and thus alter the responsiveness of the airways in respiratory diseases such as asthma.

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


