Effects of decorin and biglycan on human airway smooth muscle cell proliferation and apoptosis

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D’Antoni ML, Torregiani C, Ferraro P, Michoud M-C, Mazer B, Martin JG, Ludwig MS. Effects of decorin and biglycan on human airway smooth muscle cell proliferation and apoptosis. Am J Physiol Lung Cell Mol Physiol 294: L764–L771, 2008. First published February 2, 2008; doi:10.1152/ajplung.00436.2007.—Proteoglycans (PG) are altered in the asthmatic airway wall. Because PGs are known to affect cell proliferation and apoptosis, we hypothesized that alterations in PG might influence the airway smooth muscle (ASM) hyperplasia observed in the asthmatic airway. Human ASM cells were seeded on plastic or plates coated with decorin (Dcn), biglycan (Bgn), or collagen type I (Col I) (1, 3, and 10 μg/ml). Cells were stimulated with platelet-derived growth factor (PDGF), and cell number was assessed at 0, 48, and 96 h. Cell proliferation was measured by bromodeoxyuridine (BrdU) incorporation and apoptosis number was assessed at 0, 48, and 96 h. Cell proliferation was induced by PDGF, and cell number was assessed at 0, 48, and 96 h. Cell proliferation was measured by bromodeoxyuridine (BrdU) incorporation and apoptosis number was assessed at 0, 48, and 96 h.

Asthma is an inflammatory disease of the airways characterized by reversible airway obstruction, airway hyperresponsive-ness, and airway remodeling. Changes in extracellular matrix (ECM) protein deposition and increases in airway smooth muscle (ASM) mass are two components of airway remodeling that contribute to the pathophysiology of asthma (17). ECM can influence ASM cell function. Seeding ASM cells on different ECM matrices affects the rate of cell proliferation and contractile protein expression and function (3, 12). ECM produced by asthmatic ASM cells has been shown to differentially affect proliferation of nonasthmatic ASM (18). Migration and cell survival have also been shown to be influenced by the type of ECM upon which ASM cells were cultured (6, 22).

Alterations in ECM proteins are a characteristic feature of asthmatic airway remodeling. Modifications in ECM include increases in collagen I, III, and V, changes in glycoproteins, such as fibronectin and tenasin, and changes in deposition of various proteoglycans (PG), such as versican, biglycan, and decorin (4, 13, 17, 20, 24). Biglycan and decorin are members of the small leucine-rich PG (SLRP) family (14). Decorin has one chondroitin/dermatan sulfate side chain, whereas biglycan has two (14). Decorin is involved in collagen fibril formation, since it binds to several collagen subtypes, including types I, III, and V (1, 36, 39). Decorin has also recently been shown to protect collagen fibers from collagenase degradation because of the manner in which it coats the fibril (7). Decorin binds transforming growth factor (TGF)-β and regulates the effects of this cytokine by modulating its bioavailability (11, 45). Biglycan interacts with many types of collagen, including types I, III, and VI (5, 35, 42); this SLRP also binds TGF-β (11); however, its functional effects are generally less well described.

Although there is little information available on the effects of decorin on lung cell proliferation, decorin has been shown to decrease cell proliferation in both cancer and vascular systems via its interaction with the epidermal growth factor receptor (16, 30). It has been recently reported that biglycan enhances vascular smooth muscle cell migration and proliferation (38). Decorin has been shown to be both anti- and pro-apoptotic, depending on the cell type investigated. Whereas decorin protects against apoptosis in endothelial cells (34), renal tubular epithelial cells (33), and macrophages (44), this PG induces programmed cell death in squamous cell carcinoma lines (40). Biglycan has been shown to decrease apoptosis in mesangial cells (32).

In the human and rat lung, biglycan is mainly located in the inner area of the airway wall, more specifically, in the subepithelial region and within the smooth muscle bundles (13, 24). Decorin has been shown to be deposited in the reticular basement membrane and, more prominently, in the adventitial layer of the airway wall (20, 25, 27). Biglycan deposition is generally increased in both human asthma and in animal models of asthmatic disease (4, 13, 20, 24, 25). Changes in decorin are more variable. Decorin has been shown to be increased in the airway wall in rodent models of asthma (25, 28); however, deposition has been reported as unchanged or decreased in the airway wall of human asthmatics (4, 20, 24, 27).

ASM mass is increased in the asthmatic airway wall. Both proliferation and apoptosis have been shown to be involved in...
ASM remodeling in an animal model of asthma (26). We hypothesized that PGs, such as decorin and biglycan, may influence ASM mass through effects on proliferation and apoptosis. To investigate this question, human airway smooth muscle cells were cultured on decorin, biglycan, and collagen type I matrices, and the effects on cell number, proliferation, and apoptosis were determined.

METHODS

Materials. The following reagents were purchased from Sigma (Oakville, Ontario, Canada): decorin, biglycan, collagen type I, transferrin, insulin, BSA (fraction v), and ascorbic acid. The following reagents were obtained from Gibco-BRL-Invitrogen (Burlington, Ont., Canada): DMEM, F-12 nutrient mixture (HAM), FBS, penicillin, streptomycin sulfate, amphotericin B, trypsin-EDTA solution, and Hank's balanced salt solution (HBSS). Platelet-derived growth factor (PDGF) was purchased from R&D Systems (Minneapolis, MN). Flow cytometry reagents were purchased as a fluorescein isothiocyanate (FITC) bromodeoxyuridine (BrdU) Flow Kit and as an Annexin V-FITC Apoptosis Detection Kit I from BD Pharmingen (San Diego, CA). Camptothecin (CAM) was purchased from Axxora (San Diego, CA).

Human airway smooth muscle cell isolation and culture. Cultures of human airway smooth muscle cells (HASMCs) were prepared from surgical and lung transplant specimens as previously described (8). Segments of lobar or main bronchus measuring 5 × 2 mm were incubated in HBSS containing collagenase type IV (640 U), soybean trypsin inhibitor (10 mg), and elastase type IV (100 U). The digested tissue was then filtered through a 125-µm Nytex mesh, and the resulting cell suspension was centrifuged. The pellet was reconstituted in culture medium containing 5% FBS, insulin, human fibroblast growth factor, gentamicin sulfate, amphotericin B, and human epidermal growth factor (Cambrex Bio Science Walkersville, Walkersville, MD) and plated in a 25-cm² culture flask.

HASMCs were then cultured in medium containing 1:1 DMEM (supplemented with glucose, L-glutamine, 110 mg/m LCMD F-12 nutrient mixture (supplemented with l-glutamine) containing 10% FBS, 0.4 µg/ml insulin, 5 µg/ml transferrin, 100 µM ascorbic acid, 0.1% BSA, and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B). The medium was changed every 48 h. Positive immunohistochemical staining for smooth muscle-specific α-actin and positive identification of myosin light chain kinase and calponin by Western blot analysis ensured that cells were ASM cells. This project was approved by the local Institutional Review Board.

Coating of culture wells with ECM. Purified decorin and biglycan (both from bovine articular cartilage) and collagen type I (rat tail) were diluted in PBS to concentrations of 1, 3, and 10 µg/ml, and these solutions were used to coat six-well plates. The plates were then incubated at 37°C for a minimum of 8 h to allow adsorption of the ECM proteins. The plates were washed one time with PBS to remove excess, unbound ECM proteins. Plates were incubated with PBS + 0.1% BSA at room temperature for 30 min to prevent any non-specific protein binding.

Cell counting. HASMCs were harvested during passages 2 to 6 by washing two times with PBS and detaching them with 0.25% trypsin-0.04% EDTA solution. The trypsin was neutralized with the addition of 0.5 (vol/vol) of DMEM-F-12 10% FBS medium, and then the cells were resuspended in DMEM-F-12 containing 0.5% FBS. The cells were subsequently plated in six-well plates at a density of 6,250 cells/cm² and left undisturbed for 48 h. After this 48-h growth-arrest period, cells were exposed to 10 ng/ml of the mitogen PDGF-BB diluted in 0.5% FBS DMEM-F-12. The medium was changed every 48 h thereafter during the course of the experiment. Cell number was assessed at 0, 48, and 96 h using a standard hemocytometer and trypsin blue exclusion (Fig. 1).

Measurement of proliferation and apoptosis using flow cytometry. Proliferation experiments were carried out as per the BD Pharmingen FITC BrdU Flow Kit. Briefly, cells were cultured and grown as described above. After PDGF stimulation (24 h), 10 µM BrdU was added to the cell medium. After a 24-h incubation period with BrdU, cells were fixed, permeabilized, and stained for BrdU using a FITC-conjugated mouse anti-BrdU monoclonal antibody. Samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA) and CellQuest Pro software (BD Biosciences). Negative control samples containing no BrdU were used to establish levels of background autofluorescence and to define the BrdU positive gate (Fig. 1).

Apoptosis was measured in experiments using the BD Pharmingen Annexin V-FITC Apoptosis Detection Kit I. Briefly, cells in 0.5% FBS DMEM-F-12 medium, cultured and grown as described above, were seeded on decorin (1, 3, and 10 µg/ml) or plastic. Post-PDGF stimulation (48 h), supernatant and trypsinized cells were pooled and incubated with Annexin V-FITC and propidium iodide (PI) and analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA) and CellQuest Pro software (BD Biosciences). As a positive control, CAM (100 µM) was added to cells cultured on plastic (Fig. 1).

Addition of exogenous decorin. To measure the effect of adding decorin to the medium on cell number, a 96-well plate system was used. Cells were plated at a cell density of 6,250 cells/cm² either on decorin-coated wells (1, 3, and 10 µg/ml) or on plastic. Post-PDGF stimulation (48 h), supernatant and trypsinized cells were pooled and incubated with Annexin V-FITC and propidium iodide (PI) and analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA) and CellQuest Pro software (BD Biosciences). As a positive control, CAM (100 µM) was added to cells cultured on plastic (Fig. 1).

Coating of plates with decorin and collagen in combination. To study the effects of culturing cells on plates coated with both decorin and collagen, cells suspended in 0.5% FBS DMEM-F-12 were plated at a cell density of 6,250 cells/cm² in 96-well plates coated with either decorin (1, 3, and 10 µg/ml), collagen (1, 3, and 10 µg/ml), or a mixture of both (1, 3, and 10 µg/ml). We also examined whether the order of coating affected the outcome, i.e., whether plates were first coated with decorin, and then collagen, or vice versa. Finally, we varied the relative concentrations of collagen and decorin (from 0.3 to 0.5).
300 μg/ml) to determine threshold effects of the matrix interactions. After 48 h, the cells were stimulated with PDGF (10 ng/ml). After PDGF stimulation (48 and 96 h), cell number was assessed by crystal violet staining, as described above.

**Digestion of glycosaminoglycan (GAG) side chains with chondroitin ABC.** To determine the influence of GAG side chains on cell number, HASMC were seeded on two distinct decorin coatings. One was the usual decorin coating, and the other consisted of the protein core alone. Decorin solutions (1, 3, and 10 μg/ml) were allowed to passively adsorb at 37°C for a minimum of 8 h, and then unbound decorin was aspirated. To digest GAG side chains, 0.05 units chondroitinase ABC in a reaction buffer containing 50 mM Tris, 60 mM sodium acetate, pH 8, and 0.02% BSA was added to the decorin coating. Reaction buffer alone was added to the nondigested, control samples. The samples were then incubated at 37°C for 4 h. The plates were washed one time with PBS and then incubated at room temperature for 30 min with PBS + 0.1% BSA. After being washed two times with PBS, cells suspended in 0.5% FBS DMEM-F-12 were seeded at a cell density of 6,250 cells/cm². After 48 h, the cells were stimulated with PDGF (10 ng/ml), and, 48 and 96 h later, cell number was assessed by crystal violet staining.

**Statistical analysis.** The data were analyzed using GraphPad software and reported as means ± SE. ANOVA with Dunnett's multiple-comparison test was used to analyze differences within groups. Differences were considered statistically significant at P < 0.05.

**RESULTS**

**Effect of culturing HASMC on various ECM.** HASMC were seeded on six-well plates coated with decorin, biglycan, or collagen type I (1, 3, and 10 μg/ml), and cell number was assessed 0, 48, and 96 h post-PDGF stimulation. Results are shown in Fig. 2. A significant decrease in cell number was observed at 0, 48, and 96 h when cells were seeded on decorin at 10 μg/ml compared with cells seeded on plastic and 0.5% FBS (P < 0.01) (Fig. 2A). There was a significant decrease in cell number at 0 h when cells were seeded on biglycan (10 μg/ml) compared with cells seeded on plastic and 0.5% FBS (P < 0.05). This difference in cell number did not persist at 48 and 96 h (Fig. 2B). There was an increase in cell number in cells seeded on collagen I (Fig. 2C). This increase was significant with 10 μg/ml collagen I at 48 h (P < 0.01) and with 3 μg/ml and 10 μg/ml collagen I at 96 h (P < 0.05 and 0.01, respectively) compared with control cells seeded on plastic and 0.5% FBS. In all experiments, cells stimulated with 10% FBS showed the greatest increases in cell number.

**Effect of decorin and biglycan on HASMC proliferation.** Cell proliferation was assessed at 48 h post-PDGF by measuring BrdU incorporation with flow cytometry. BrdU incorporation for each group of cells was calculated as a percentage of the control sample (plastic, 0.5% FBS). Cells grown on decorin matrix (10 μg/ml) demonstrated significantly decreased BrdU incorporation compared with the control samples (P < 0.05) (Fig. 3B, top). No significant differences in BrdU incorporation were observed in cells seeded on biglycan (Fig. 3B, bottom) or collagen (data not shown) compared with the control.

**Effect of decorin and biglycan on HASMC apoptosis.** We examined the effect of decorin and biglycan on HASMC apoptosis. Growth on decorin (10 μg/ml) resulted in a significant increase in the percentage of cells staining positive for annexin V compared with the control samples (plastic, 0.5% FBS) (47.3 ± 16.9 vs. 10.15 ± 1.6% at 48 h, P < 0.05) (Fig. 4). In cells seeded on decorin (10 μg/ml), the annexin V positive, PI negative cell population was also significantly increased relative to control (plastic, 0.5% FBS) (Fig. 4B, top). CAM and decorin caused similar levels of increase in both annexin V and PI staining (Fig. 4B, top). There were no significant increases in annexin V or PI staining in cells seeded on biglycan (Fig. 4B, bottom).

**Effect of decorin coating vs. addition of exogenous decorin to the medium.** Cells were either seeded on a decorin coating or on plastic with decorin subsequently added to the culture
Fig. 3. Bromodeoxyuridine (BrdU) incorporation measured using flow cytometry for cells seeded on decorin, biglycan (1, 3, and 10 μg/ml), and plastic. BrdU incorporation was measured 48 h post-PDGF stimulation. A: flow cytometry plots of side scatter (SSC) vs. BrdU fluorescence for 4 individual samples: plastic, 0.5% FBS no BrdU (top left); plastic and 0.5% FBS (top right); biglycan (10 μg/ml) (bottom left) and decorin (10 μg/ml) (bottom right). B: quantification of BrdU plots for decorin (top) and biglycan (bottom). BrdU incorporation is represented as %BrdU incorporation relative to the control sample (plastic and 0.5% FBS). Percentages represent means ± SE of duplicate values from 5 independent experiments. *P < 0.05 vs. plastic and 0.5% FBS.

Fig. 4. Annexin V and propidium iodide (PI) staining measured using flow cytometry 48 h after PDGF stimulation on decorin, biglycan (1, 3, and 10 μg/ml), or plastic and in cells exposed to camptothecin (CAM; 100 μM). A: flow cytometry plots of annexin V vs. PI fluorescence for cells seeded on plastic and 0.5% FBS (top left); CAM (top right); biglycan (10 μg/ml) (bottom left) and decorin (10 μg/ml) (bottom right). B: quantification of plots for decorin (top) and biglycan (bottom). Bars represent the total amount of Annexin V staining: PI negative staining is shown in the filled portion of the bar; PI positive staining is shown in the open portion of the bar. Percentages represent means ± SE of duplicate values from 4 independent experiments. *P < 0.05 and **P < 0.01 for total annexin V staining vs. plastic and 0.5% FBS; *P < 0.05 and **P < 0.01 for PI positive and negative subpopulations vs. their respective controls (plastic and 0.5% FBS).
medium. Cell number was assessed at 48 h by crystal violet staining. Cells seeded on decorin coating (10 μg/ml) showed a decrease in absorbance at 550 nm, which corresponded to a significant decrease in cell number compared with the control (plastic, 0.5% FBS) \((P < 0.01)\) (Fig. 5A). When decorin was added to the medium (1–30 μg/ml), no significant decrease in cell number was observed.

**Effect of decorin and collagen I in combination.** Cells were seeded on decorin alone, collagen I alone, or a matrix containing a mixture of both decorin and collagen I (Fig. 5B). Cell number was assessed by crystal violet staining at 48 h. When collagen I and decorin were combined as a matrix coating, the decrement in cell number due to decorin was abrogated, and the cells had similar absorbance values to the cells seeded on collagen alone. We also investigated whether the order in which the decorin and collagen were adsorbed to the surface of the well affected cell proliferation. There was no difference in the effect of matrix on cell number as a consequence of the order of matrix coating, i.e., decorin or collagen I adsorbed first or second (data not shown). Varying the relative concentrations of decorin to collagen and collagen to decorin from 0.03:1 to 30:1 showed that a modest amount of collagen (relative concentration of collagen-decorin of 1:10) reversed the decorin-induced decrease in cell number, whereas only at the highest relative concentration of decorin (30:1) was the promitogenic effect of collagen mitigated (data not shown).

**Decorin effect on cell proliferation is mediated through the GAG side chain.** Cells were seeded either on intact decorin, to which reaction buffer was added, or on adsorbed decorin digested with chondroitinase ABC. Cells were stimulated with PDGF, and cell number was assessed by crystal violet staining at 48 h (Fig. 5C). Cells seeded on intact decorin coating (1, 3, and 10 μg/ml) showed a decrease in absorbance at 550 nm, which corresponded to a significant decrease in cell number at 48 h compared with the control (plastic, 0.5% FBS) \((P < 0.01)\). Cells seeded on the chondroitinase-digested decorin (1 and 3 μg/ml) had absorbance values that were slightly higher (increased cell number) than the control \((P < 0.01)\).

**DISCUSSION**

The results of this study demonstrate that culturing cells on different ECM matrices can variably affect HASMC number. Culture on the SLRP decorin resulted in a persistent decrease in cell number via its effects on both proliferation and apoptosis. These effects were specific to decorin, since culture on biglycan, a related SLRP, resulted in an initial decline in ASM cell number that recovered over time. Neither cell proliferation nor apoptosis, measured at the 48-h time point was affected. Conversely, culture on a collagen-coated matrix enhanced cell number relative to control.

There is relatively little information available in the literature on the effects of PG on ASM cell growth. Decorin has been shown to have an antiproliferative effect on cancer cells of epithelial origin (15, 31); on the other hand, biglycan enhanced vascular smooth muscle proliferation (38). Versican, the large aggregating PG, can either enhance or decrease vascular smooth muscle proliferation, depending on the specific versican isoform (37). Heparin, the glycosaminoglycan side chain bound to basement membrane PG, has...
been shown to have antiproliferative effects on human and canine ASM (9, 19).

The effect that collagen exerted on cell number in the current experiment is consistent with data reported in the literature. Hirst and colleagues (12) showed that human ASM proliferation was enhanced when cells were cultured on collagen and fibronectin matrices but decreased with growth on a laminin matrix. Bonacci et al. (2) showed a similar effect of collagen on bovine ASM cell number.

BrdU incorporation measured at 48 h post-PDGF stimulation was significantly decreased in cells grown on decorin matrices. Although BrdU incorporation measures DNA synthesis, not proliferation per se, the significant decrease in this marker implicates a decrease in proliferation in contributing to the decrement in cell number. However, the decrease in BrdU incorporation was relatively modest and unlikely to account for the magnitude of change in cell number. Cells grown on biglycan showed no decrease in BrdU incorporation relative to control. Surprisingly, there was no increase in BrdU incorporation in cells grown on collagen matrix, despite a significant increase in cell number in this preparation. These differences in the relative magnitude of the signal may relate to the time point at which BrdU incorporation was measured. Preliminary time course data showed that the amount of BrdU incorporation varied, depending upon the time point sampled (from 24 to 48 h post-PDGF stimulation). BrdU incorporation at the 48-h time point in the cells grown on collagen may reflect cell confluency in samples in which proliferation was enhanced. Alternately, the increase in cell number for cells grown on collagen matrix may have reflected the antiapoptotic effects of collagen on ASM cells, as reported by Freyer et al. (6). Because our primary interest was the potential antiproliferative effects of small PGs, we chose a time point that better reflected the activity of these ECM proteins. These findings did not preclude the possibility that enhanced apoptosis could also play a role in the sustained decrease in cell number seen with growth on decorin. To further investigate this question, we measured the effect of growth on PG matrices on HASMC apoptosis.

Decorin caused a significant increase in HASMC apoptosis. At 48 h post-PDGF stimulation, both annexin V and PI staining was increased on cells grown on decorin at concentrations of 3 and 10 μg/ml. We believe this represented “true apoptosis” rather than a combination of apoptosis and necrosis, since annexin V positive, PI negative staining, an index of early apoptosis, was also significantly increased compared with the plastic and 0.5% FBS control. The level of apoptosis was similar in degree to that induced by CAM (which promotes apoptosis by inhibiting topoisomerase I activity) (10). Hence, the effect of decorin on HASMC number reflected a combination of decreased proliferation and increased apoptosis. Although cell number was significantly decreased at time 0 when cells were seeded on biglycan (10 μg/ml) compared with the plastic control, by 48 h, cell growth had normalized relative to control conditions. Hence, growth on biglycan did not cause a sustained decrement in cell number, as reflected by the normal rates of proliferation and apoptosis in these samples.

To further examine the mechanisms by which decorin exerted its effect on HASMC, cells were exposed to decorin added exogenously to the culture system. A decrease in cell number was observed only when cells were seeded on decorin matrix, not when decorin was added exogenously to the medium (in concentrations up to 30 μg/ml). These results suggest that the effect of decorin was not due to the sequestration of PDGF by decorin protein (21). When cells were plated on a combination of decorin and collagen I, the decrease in cell number was abrogated. Hence, the presence of collagen seemed to override the effect of decorin alone. Removing GAG side chains with chondroitinase ABC digestion similarly reversed the decrease in cell number associated with the intact molecule. In fact, there was a significant, albeit slight, increase in cell number in cells exposed to chondroitinase digestion. This modest increase is probably explained by the small degree of variability in the measurements and is unlikely to be of physiological significance. However, we cannot exclude the possibility that the addition of buffer medium augmented cell proliferation in some fashion.

These last data implicate the GAG side chain as critical to the antiproliferative and/or proapoptotic effect. Chondroitin/dermatan sulfate are the GAG side chains present on decorin. Some information is available in the literature on the effect of GAGs on cell proliferation. Several investigators have reported that heparin and heparin-like molecules can reduce proliferation of HASMCs (9, 19). In his experiments, Kanabar et al. (19) showed that chondroitin sulfate A and B had less marked effects despite concentrations much greater (up to 10 mg/ml) than those used in the current experiment. Studies in fibroblasts have shown that exogenously added chondroitin sulfate (CS) A, B, and C had no effect on fibroblast growth in culture, whereas dermatan sulfate suppressed proliferation (29, 41). Johnson et al. (18) showed that ECM produced by asthmatic ASMs, which contained increased perlecan (a heparin sulfate PG) and decreased chondroitin sulfate, enhanced ASM proliferation. These data are consistent with those of the current experiment, since digestion of CS led to normalization of cell number. On the other hand, culture on biglycan, which also has CS side chains, did not result in persistent decreases in cell number. This observation suggests that it is not simply the presence of a CS side chain that leads to the proapoptotic, antiproliferative effect. Differences in glycosylation pattern or the specific subtype of CS side chain may be important.

Another issue is the ability of the GAG side chain to influence cell adhesion. Hirst and colleagues (12) have shown that cell attachment is affected by the matrix upon which cells are plated, at least early in the process. Whereas attachment of ASM cells varied initially, depending on whether collagen, laminin, or fibronectin was the matrix of interest, by 6 h after seeding, attachment was not different. San Antonio and colleagues (29) reported that addition of exogenous heparin to media inhibited the ability of fibroblasts to attach to collagen matrices. A similar effect of decorin on attachment of fibroblasts has also been reported (43). Although we observed some differences in initial attachment and spreading of HASMC, depending on the matrix employed, these differences resolved with time in culture; perhaps the initial decrement in cell number in cells grown on biglycan matrix reflected initial differences in cell attachment.

The ability of decorin to reduce ASM cell number may have important implications for asthmatic disease. Airway remodeling is a characteristic feature of asthma; PG contribute significantly to this process (4, 13, 20, 23, 24). We have reported data showing that decorin is deposited both internal
and external to the smooth muscle layer. The antiproliferative or proapoptotic effect of decorin could serve to limit the growth of ASM beyond its usual compartment. Pepe et al. (23) examined bronchial biopsy specimens from patients with severe asthma and showed that a characteristic feature of remodeling in these airways was encroachment of ASM in the subepithelial layer. Normal deposition of decorin could influence this process. It is interesting to consider the data recently published by Matsushita et al. (20) showing that decorin was decreased in the airway wall of fatal asthmatics. The loss of the modulating effect of decorin may have contributed to the excess ASM mass typical in the airways of these patients.

A further consideration is how well the in vitro cell culture conditions reflect the in vivo situation in the airway wall. The relative concentrations of these different ECM molecules may vary significantly in the microenvironment surrounding the ASM cell. We can draw some information from the data that show that a relatively modest amount of collagen can override the decorin effect and that a high concentration of decorin is required to abrogate the promitogenic collagen effect. Nonetheless, there may well be large fluctuations in relative concentrations of these molecules in the regions surrounding an individual ASM cell, depending on the cell types involved in matrix deposition and the degree to which matrix deposition is upregulated as a part of the pathological process. Furthermore, other mitogens and/or cytokines are likely to be important in influencing these effects and in stimulating the cell surface receptors involved in promitorigenic or proapoptotic processes. Such limitations are present in cell culture experiments generally and serve as a cautionary note when extrapolating findings from in vitro experiments to the in vivo setting.

In conclusion, we have shown that the PG decorin decreases ASM proliferation and increases ASM apoptosis. These effects were specific to decorin, since they were not demonstrated by biglycan, another member of the SLRP family. The GAG side chain, rather than the core protein, seemed to be important in modulating this effect. The ability of decorin to limit ASM growth could have important implications for airway remodeling in asthma.

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