Use of a three-dimensional cell culture model to study airway smooth muscle-mast cell interactions in airway remodeling

Claudia C. Ceresa, Alan J. Knox, and Simon R. Johnson

Divisions of Therapeutics and Molecular Medicine and Respiratory Medicine, University of Nottingham, Nottingham, United Kingdom

Submitted 20 August 2008; accepted in final form 30 March 2009

Ceresa CC, Knox AJ, Johnson SR. Use of a three-dimensional cell culture model to study airway smooth muscle-mast cell interactions in airway remodeling. Am J Physiol Lung Cell Mol Physiol 296: L1059–L1066, 2009. First published April 3, 2009; doi:10.1152/ajplung.90445.2008.—Increased airway smooth muscle (ASM) mass and infiltration by mast cells are key features of airway remodeling in asthma. We describe a model to investigate the relationship between ASM, the extracellular matrix, mast cells, and airway remodeling. ASM cells were cultured in a three-dimensional (3-D) collagen I gel (3-D culture) alone or with mast cells. Immunocytochemistry and Western blotting of ASM in 3-D cultures revealed a spindle-shaped morphology and significantly lower α-smooth muscle actin and vimentin expression than in ASM cultured in monolayers on collagen type I or plastic (2-D culture). In 3-D cultures, basal ASM proliferation, examined by Ki67 immunocytochemistry, was reduced to 33 ± 7% (P < 0.05) of that in 2-D cultures. The presence of mast cells in cocultures increased ASM proliferation by 1.8-fold (P < 0.05). Gelatin zymography revealed more active matrix metalloproteinase (MMP)-2 in 3-D than in 2-D culture supernatants over 7 days. Functional MMP activity was examined by gel contraction. The spontaneous gel contraction over 7 days was significantly inhibited by the MMP inhibitor ilomastat. Mast cell coculture enhanced ASM gel contraction by 22 ± 16% (not significant). Our model shows that ASM has different morphology, with lower contractile protein expression and basal proliferation in 3-D culture. Compared with standard techniques, ASM synthetic function, as shown by MMP production and activity, is sustained over longer periods. The presence of mast cells in the 3-D model enhanced ASM proliferation and MMP production. Airway remodeling in asthma may be more accurately modeled by our system than by standard culture systems.

asthma; extracellular matrix; matrix metalloproteinase; proliferation

Airway remodeling describes a series of structural changes that occur as a complication of chronic inflammatory diseases of the airways. Airway remodeling in asthma is categorized by airway smooth muscle (ASM) hyperplasia, an increase in the number of subepithelial myofibroblasts, a change in the extent and nature of airway extracellular matrix (ECM), mast cell infiltration of ASM, increased vascularity, epithelial damage, metaplasia, and mucus gland hyperplasia (2). In asthma, remodeling starts in infancy, is poorly responsive to asthma therapies, and leads to an accelerated loss of lung function. Airway remodeling therefore results in increased asthma symptoms, as well as increased use of medication and health care resources (2, 24).

The use of cultured primary ASM cells has provided a large amount of information on the role of ASM and has shown ASM to be a key player in airway remodeling (31). In remodeling, ASM cells are increased in number and produce a large array of pro- and anti-inflammatory molecules, both of which orchestrate airway inflammation and contribute to tissue remodeling (20, 23). The behavior of the ASM cell in remodeling with respect to proliferation, migration, and synthetic function is determined by a range of stimuli, including growth factors, cytokines, chemokines, proteases, and ECM proteins, which can be affected by airway inflammation (5, 16, 30, 40). In the airway, the ECM is complex: including collagen I, III, IV, and V (33), laminin, nidogen, and the proteoglycans fibronectin and tenascin, which are components of all basement membranes (27). We and others have demonstrated that ECM and other components of the asthmatic airway can alter ASM function to promote remodeling, with ECM proteins affecting ASM proliferation (22), migration (16), apoptosis (11), β-agonist signaling (10), and matrix metalloproteinase (MMP) activity (16). Recently, the presence of mast cells in ASM bundles has been identified as a specific feature of airway remodeling in asthma (3, 4), and studies suggest that mast cells may affect ASM differentiation (38) and, in turn, that ASM can promote mast cell survival and activation (19). In vivo, the presence of mast cells has been associated with bronchial hyperresponsiveness (34), although exactly how these observations are related to airway remodeling has not been extensively investigated.

In vivo, ASM cells exist in a three-dimensional (3-D) environment, with ECM and cell-cell contacts surrounding the cell, rather than on one surface, as is the case in commonly used two-dimensional (2-D) culture systems. Because of this complex arrangement of ASM cells in the airway, study of ASM cell morphology in vivo is difficult and is reflected by a very small number of published studies. In acutely dissociated ASM cells from canine airways, different phenotypes are present; the majority of these are elongate cells, which strongly express contractile proteins (14). In human airways, the majority of ASM cells are spindle shaped, with elongated nuclei; however, when analyzed by immunohistochemistry, levels of contractile protein expression varied independent of this spindle-shaped morphology (14). A small number of in vivo studies have confirmed that the ASM cell number is increased in human asthma (39) and have started to examine how airway inflammation can lead to changes in the expression of contractile proteins and contractile force. In sensitized rat airways, allergen challenge is associated with increased force but, interestingly, reduced contractile protein expression (29). Similarly, reduced contractile protein expression is associated with increased contractile force in bovine tracheal muscle strips after incubation with ECM proteins, including collagen I (6). These apparently paradoxical observations may be due to cytoskeletal reorganization in the ASM cells (26).
Therefore, the influence of these cell-cell and cell-matrix interactions on airway remodeling is difficult to study in vivo and with standard cell culture systems. A recent workshop sponsored by the National Heart, Lung, and Blood Institute highlighted the need to develop methods with which to associate "in vitro observations to in vivo disease state with regard to ASM function" (32). To more closely model the behavior of ASM cells in the airway environment and incorporate the effect of ECM and mast cell interactions on ASM biology, we have adapted a 3-D collagen gel culture system to study ASM phenotype, proliferation, and synthetic function and how this may be altered in airway remodeling. Our culture system has revealed differences in ASM phenotype and function in the 3-D environment, suggesting that this technique may be a useful adjunct with which to study airway remodeling.

**METHODS**

**Cell culture.** Primary human ASM were derived from bronchial smooth muscle obtained from patients undergoing surgical resection for carcinoma. ASM were dissected free from epithelium, digested with collagenase, and subcultured in DMEM containing 10% FCS and 2 mM glutamine, as previously described (15). The resulting ASM cells were characterized by light microscopy and the presence of α-smooth muscle actin (α-SMA) by immunocytochemistry. Cells from three donors were used between passages 2–6. The study was approved by the Nottingham Local Ethical Research Committee, and all patients gave informed consent. HMC-1 cells, a human mast cell line derived from a patient with mast cell leukemia (9), were a gift from Peter Bradding (University of Leicester). HMC-1 cells were cultured in Iscove’s modified Dulbecco’s medium, as previously described (7). HMC-1 cells were activated by incubation with 5 ng/ml PMA and the calcium ionophore A23187 (25 ng/ml) for 12 h, as described elsewhere (3). After activation, mast cells were washed twice with phosphate-buffered saline to avoid any possible direct effect of PMA/calcium ionophore on the ASM cells in cocultures.

To make 3-D ASM cultures, we suspended ASM cells $(1 \times 10^5$/ml) in a solution of 0.75 mg/ml collagen I in DMEM. The solution was transferred into the wells of a 24-well tissue culture plate in 0.5-ml aliquots, and the gels were allowed to polymerize by incubation at 37°C (28). This process resulted in a 17-mm-diameter, 5-mm-thick gel plug containing $5 \times 10^4$ ASM cells. Two control culture conditions were used: 1) standard tissue culture plastic, where an equal number of ASM cells were seeded directly in a 24-well tissue culture dish (plastic), and 2) identical collagen gels that were cast in 24-well plates without ASM cells. When set, an equal number of ASM cells were seeded on the gel as a monolayer (2-D). All experiments were completed in serum-free conditions unless otherwise stated. To avoid autofluorescence in experiments involving fluorescein microscopy, we used phenol red-free DMEM (Invitrogen, Paisley, UK) and replaced 24-well culture dishes with 13-mm glass-bottomed 24-well plates (MatTek, Ashland, MA).

For mast cell-ASM cocultures, 3-D gels were cast in the same fashion with the addition of HMC-1 cells $(2 \times 10^5$/ml). For identification of specific cell types in cocultures, ASM and HMC-1 cells were labeled with CellTracker or Hoechst dyes. Cells were labeled by incubation with the appropriate CellTracker dye at 5 μM (in DMEM with 10% FBS) for 1 h at 37°C and then washed three times in serum-free medium before use. CellTracker dyes were obtained from Invitrogen; rat tail collagen I and other tissue culture reagents were obtained from Sigma (Gillingham, UK).

**Immunocytochemistry.** After the appropriate experimental treatment, cells were fixed in situ using 4% paraformaldehyde for 24 h, permeabilized with 0.2% Triton X-100, incubated with monoclonal mouse antibodies against vimentin (1:50 dilution; Abcam, Cambridge, UK) and α-SMA (1:100 dilution; Sigma), and then labeled with an Alexa 488 secondary anti-mouse antibody (1:200 dilution; Invitrogen). Nuclei were stained with 4',6-diamidino-2-phenylindole (1:600 dilution; Sigma). Images were captured using laser scanning confocal microscopy with a Zeiss Axiosvert 100 inverted microscope equipped with a Zeiss LSM510 UV combi confocal lens (Carl Zeiss, Hertfordshire, UK).

**Western blotting.** Extraction of ASM cells was accomplished by incubation of 3-D gels with collagenase I (200 U/ml; Sigma). To allow for an effect of the culture condition on cell number and to avoid confounding of protein assessment by collagen I, we counted the cells manually using a hemocytometer. Protein lysates from equal numbers of cells were obtained by treatment of cell pellets with SDS-PAGE running buffer, separation on a 10% SDS-PAGE gel, and transfer to a polyvinylidene difluoride membrane by semidy blotting. Membranes were nonspecifically blocked with dry milk powder and then incubated with anti-α-SMA antibody (1:100 dilution; Sigma) or anti-vimentin primary antibody (1:50 dilution; Adcam, Cambridge, UK). A secondary polyclonal goat anti-mouse IgG-horseradish peroxidase conjugate was then applied (Dakocytomation, Ely, Cambridge, UK). Filters were stripped and reprobed with an antitubulin antibody (1:500 dilution; Sigma) to ensure equal protein loading.

**Cell proliferation.** Cell proliferation within the 3-D gels was examined using Ki67 staining. After the appropriate experimental treatment, at 24 h, cells were fixed and incubated with anti-Ki67 (1:75 dilution; Dakocytomation) and labeled with Alexa 488. Nuclei were stained using propidium iodide (PI). Cells were examined using a Zeiss Axio Observer D1 microscope, and six fields at ×200 magnification were counted manually. Ki67-positive cells were expressed as a percentage of the total number of cells determined by PI staining. Experiments of three replicates were performed on at least four occasions, and results are expressed as percentage (mean ± SE) of control conditions. To avoid inclusion of mast cells in ASM counts in cocultures, the nuclei of HMC-1 cells were labeled with Hoechst 33342 (Invitrogen) at 5 μg/ml for 1 h before coculture.

**Gelatin zymography.** Media were sampled from cultures every 24 h. In some experiments, media were changed completely every 24 h. Harvested media were loaded in equal volumes into Novex precast 10% gelatin and run at 125 V for 90 min. Gels were then developed according to the manufacturer’s instructions. MMP species were identified by molecular weight and incorporation of recombinant MMP standards (R & D Systems, Abington, UK). For quantification of MMP expression between experiments, band density was compared with control values from a minimum of three independent experiments by densitometry. All reagents were purchased from Invitrogen. Densitometry was performed using Image J software (http://rsb.info.nih.gov/ij/).

**Gel contraction.** Gel contraction was used as an assay of ECM remodeling (1). 3-D gels were cast as described above. After polymerization, gels were released from the 24-well plate by gentle insertion of a 10-μl pipette tip between the gel and tissue culture plastic and transferred to a six-well plate submerged in DMEM containing 10% FCS, as a positive control for gel contraction (28). 10 μM ilomastat, an MMP inhibitor (Chemicon, Hampshire, UK), or serum-free DMEM containing an equal concentration of the vehicle DMSO. Images of gel contraction were obtained every 24 h over a 7-day time course with a Genesnap camera (Genetools, Syngene, Cambridgeshire, UK), and gel contraction was measured using Image J software (Image Solutions, Chereley, Lancs, UK).

**Analysis.** Data were tested for normality using a Q-Q plot. Results of normally distributed data were compared by one-way ANOVA, with Bonferroni’s post hoc correction used for multiple comparisons. For analysis of nonnormally distributed data, including differences in MMP-2 production, Mann-Whitney U-test and Kruskal-Wallis H-test were used as appropriate. Time trends were analyzed using a
Fig. 1. Morphological characteristics of airway smooth muscle (ASM) cultured on standard tissue culture plastic (plastic), on a layer of collagen [2-dimensional (2-D) collagen], or in a collagen matrix [3-dimensional (3-D) collagen]. Phase-contrast (phase) and epifluorescence microscopy for α-smooth muscle actin (α-SMA) shows that cells in 3-D collagen gel are more spindle shaped with long cytoplasmic projections. Confocal microscopy shows that actin and vimentin expression (green staining) is less prominent in the spindle-shaped cells of the 3-D collagen cultures. Nuclei were stained with 4',6-diamidino-2-phenylindole (blue). Magnification ×200 for phase-contrast and epifluorescent images and ×400 for confocal images.
RESULTS

Optimization of 3-D ASM culture. We observed that ASM 3-D gel cultures contract over time when seeded at high cell densities, particularly in the presence of serum. Although this is a useful method for study of contractile events and ECM turnover (25), our aim was to produce a 3-D culture system where individual cells and cell-cell interactions could be directly visualized over days. We therefore developed culture conditions that avoided gel contraction over this period. In initial experiments, we examined the effect of cell density on gel contraction in the presence and absence of serum (data not shown). We found that a seeding density of $1 \times 10^5$ ASM cells/ml in serum-free conditions allowed for 7 days of culture without shrinkage or distortion of gels when the gel cultures were left in contact with the culture dishes. Gels contracted spontaneously when they were separated from the walls of their culture dishes or cultured in serum or when higher cell densities were used (data not shown).

Effect of culture method on ASM phenotype. Inasmuch as tissue culture conditions, particularly ECM substrate, can affect cellular phenotype, we studied the effect of culture system on the morphology of our cells and the expression of contractile proteins. Using microscopy, confocal microscopy, and Western blotting, we compared standard tissue culture on plastic with 2-D culture on collagen type I and 3-D culture on collagen I. ASM cells cultured on plastic adopted the classic hill-and-valley appearance, with a large rounded body dominated by the nucleus. α-SMA immunostaining was strongest in the perinuclear area and following the spindly projections along the longitudinal axis of the cell. When cultured in the collagen-coated wells (2-D), the ASM cells tended to organize themselves into small groups, as shown previously (17). Cells on the 2-D collagen cultures had morphological characteristics similar to those of cells growing on plastic, with cytoplasmic projections extending along their longitudinal axis. In contrast, cells embedded within the 3-D gel had more spindle-shaped cytoplasmic projections, which fanned out from the cell body in all directions. These cell bodies lost their round shape and became more elongated, with the nucleus in these cells occupying the majority of the cell body (Fig. 1).

Expression of contractile proteins, a key feature of the ASM phenotype, was studied semiquantitatively by Western blotting. Consistent with the findings from confocal microscopy, expression of α-SMA and vimentin was reduced at 24 h in cells cultured in 2-D and 3-D collagen compared with cells grown on plastic (Fig. 2). These differences were more marked at 7 days, particularly for vimentin. In cells grown on 3-D collagen, expression of both proteins was lowest at 24 h and 7 days, although differences were only significant for vimentin (Fig. 2).

Low rates of proliferation in ASM cells grown in 3-D culture. Increase in ASM cell number is a key feature of airway remodeling; to examine the effect of culture conditions on ASM proliferation in our model, we used the proliferation marker Ki67. Ki67 allowed for the selective measurement of ASM proliferation in coculture studies that would not be possible with other techniques, such as thymidine incorporation or cell counting. To validate this method in the 3-D system, we examined the effect of two well-characterized ASM mitogens, FBS (10%) and platelet-derived growth factor-BB (10 ng/ml), on proliferation compared with cells growing in serum-free conditions after 24 h of serum withdrawal. In the 3-D model, ASM nuclei were clearly visualized using PI, and proliferating cells were labeled with Ki67 (Fig. 3A). In serum-free conditions, 12% of nuclei were dual labeled with Ki67 and PI. In response to FBS and platelet-derived growth factor, there was a significant increase of 2.4- and 2.5-fold,
respectively (Fig. 3B). We then compared the effect of culture system on rate of proliferation. The rate of proliferation of Ki67-PI dual-labeled nuclei was highest in cells cultured on plastic. Cells on 2-D collagen were less proliferative, whereas the rates of proliferation were lowest in cells cultured on 3-D collagen (Fig. 3C).

Increase in ASM proliferation in ASM-mast cell coculture. Consistent with our primary aim of devising more accurate systems with which to study airway remodeling, we made 3-D cocultures of ASM and mast cells. Addition of HMC-1 cells to the 3-D collagen gels did not affect gel viability: gels retained their structure and synthetic activity over this period. Mast cells were visualized in gels by differential labeling of ASM and HMC-1 cells with CellTracker green and red dyes, respectively (Fig. 4A). Mast cells remained viable in the 3-D cocultures for ≥7 days: they continued to retain the CellTracker dye and affect ASM function over this period. To determine the effect of mast cell coculture on ASM cells, we initially examined ASM proliferation. Coculture of ASM with mast cells over 24 h led to a significant increase in ASM proliferation. There was a trend toward a further increase when the mast cells had been preactivated (Fig. 4B). Interestingly, this effect was not seen with the addition of mast cell supernatants to the 3-D ASM cultures, where a nonsignificant increase of 22 ± 3.9% was seen in Ki67 labeling after the addition of serum-free medium incubated with mast cells for 24 h.

Effect of 3-D culture and mast cell coculture on ASM MMP-2 secretion. We previously showed that ASM cells constitutively produce pro-MMP-2, which can be activated by collagen (16). To examine the effect of coculture on ASM synthetic activity, we studied the effect of 3-D coculture on MMP-2 production and activation. Using gelatin zymography, we examined MMP-2 secretion in ASM cells on standard tissue culture plastic, in 2-D culture on collagen, and in 3-D collagen. In addition, we examined mast cell-derived MMP production and studied the effect of cocultures on MMP-2 secretion and activation. In all culture conditions, ASM cells produced similar amounts of pro-MMP-2. Significantly more active MMP-2 was produced in cells cultured on 3-D collagen than on plastic or 2-D collagen (P = 0.009). Over 7 days, the production of pro-MMP-2 fell steadily in plastic and 2-D cultures (P = 0.017). In 3-D cultures, there was no fall in MMP production and a trend toward increasing MMP-2 activation over the 7-day period (P = 0.055; Fig. 5). Mast cells did...
not produce MMP-2 under standard or 3-D conditions. Interestingly, coculture of ASM and mast cells on plastic reduced MMP-2 secretion. In 2-D or 3-D collagen cocultures, this was not seen with increasing amounts of active MMP-2 produced over time (Fig. 5).

To study how ASM cell-derived proteases modify the ECM scaffold of these cultures, we examined gel contraction, a marker of ECM turnover (8). Gels in these experiments were identical to those described, except they had been separated from the walls of their tissue culture plates to allow contraction. Untreated gels contracted to <10% of their original area over 7 days (Fig. 6, A and B). To examine the role of ASM-derived MMPs in ECM remodeling in these cultures, we used the broad-spectrum MMP inhibitor ilomastat. Ilomastat (10 \( \mu \)mol) had no effect on gel contraction in the first 24 h but significantly reduced gel contraction at 4 and 7 days (Fig. 6, A and B). We next examined the effect of mast cells on ECM remodeling at 7 days. Incorporation of mast cells into 3-D ASM cultures was associated with a trend toward an increase in gel contraction, although this was not significant. Mast cell activation had no effect on gel contraction (Fig. 6C), although in all cases ilomastat (10 \( \mu \)mol) inhibited gel contraction at 7 days (Fig. 6D).

Fig. 5. 3-D ASM culture enhances long-term production and activation of matrix metalloproteinase (MMP)-2 in ASM cells grown on plastic, 2-D, and 3-D culture. Medium was replaced daily, and gelatinase activity was assessed by gelatin zymography. HMC-1 cells were cultured under the same conditions, and ASM-HMC-1 cocultures were also performed and analyzed in the same way. Daily production of pro-MMP-2 and active (act) MMP-2 was quantitated by densitometry. HASM, human ASM cells. Values are means ± SE of 3 independent experiments.

Fig. 6. 3-D gel contraction is MMP-2 dependent. A: contraction of 3-D collagen gels was measured over 7 days (gel edges are highlighted for clarity). B: ilomastat had no effect on gel contraction at 24 h but reduced contraction compared with vehicle over 4 and 7 days. Values are means ± SE of 5 gels from a representative experiment repeated 3 times. **\( P < 0.01 \). C: contraction at day 7 in ASM-HMC-1 cocultures. Values are means ± SE of 4 independent experiments, each repeated 3 times. Gel contraction in ASM monocultures in serum-free conditions or in the presence of 10% FBS are shown as negative and positive controls, respectively. ASM-HMC-1 coculture was associated with a trend toward increased gel contraction. Activation of mast cells by PMA/calcium ionophore had no effect on gel contraction. D: ilomastat (ilo, 10 \( \mu \)mol) inhibits gel contraction at 7 days independent of mast cell activation. Veh, vehicle. Values are means ± SE. *\( P < 0.05 \).
DISCUSSION

We have developed a 3-D culture system with which to study the interaction of ASM cells with their airway environment. Our system shows differences in morphology, contractile protein expression, rates of proliferation, and synthetic function between ASM cells in 3-D culture and those grown in 2-D on tissue culture plastic or collagen I. In proof of concept studies of ASM-mast cell coculture, we have shown that the presence of mast cells in the ASM gels led to an increase in ASM proliferation and a change in synthetic function, as shown by an increase in the production of active MMP-2 over 7 days.

The change in ASM phenotype was the adoption of spindle-shaped morphology compared with cells grown under 2-D conditions. This spindle-shaped morphology reflects the majority of cells in human airways (14) but is only observed in 2-D cultures under conditions of long-term serum deprivation in canine tracheal myocytes and growth of human ASM on homologous cell substrate (12, 35). In our 3-D cultures, the elongated morphology was associated with reduced expression of α-SMA and vimentin over the culture period of up to 7 days. Our findings are consistent with those of others who showed that expression of α-SMA (but not vimentin) is reduced in cells grown on a collagen I substrate in 2-D cultures (17) and that the contractile protein expression of bovine tracheal strips is reduced after collagen I treatment (6).

In the airway wall of healthy subjects, the smooth muscle layer consists mainly of differentiated ASM cells, which are characterized by low proliferation rates (18). To measure proliferation in the 3-D system and to allow selective measurement of ASM proliferation in cocultures, we used Ki67 staining of ASM cell nuclei. In validation experiments, this technique was reproducible and gave clear signals in response to ASM mitogens. Rates of proliferation were significantly lower for cells in 3-D collagen than in 2-D cultures in the absence of mitogens. We also found that, in the 3-D environment, the elongated appearance of ASM cells was coupled with reduced contractile protein expression. Significant differences exist in 2-D cultures, where 1) collagen I enhances proliferation in response to mitogens (17) and 2) a spindle-shaped morphology is associated with enhanced contractile protein expression and lower proliferation rates (13, 37). Our findings may be consistent with the notion that the larger integrin (or other collagen receptor) stimulus from the 3-D collagen I environment has a paradoxical inhibitory effect on proliferation or, more likely, that the effect of collagen I on proliferation may be overridden by other signals, such as cytoskeletal rearrangement in the 3-D environment, leading to changes in proliferation. Our findings also suggest that proliferation, morphology, and contractile protein expression are regulated independently. To test the significance of our observations in future studies, it will be important to examine how gene expression in 2-D and 3-D cultures compares with that in ASM in human airways in vivo to understand which culture techniques are more representative of human airways.

This model can be used to study mast cell-ASM interactions. Inasmuch as ASM hyperplasia and mast cell infiltration are recognized features of the airways of patients with asthma (3), in proof of concept experiments, we initially studied the effect of mast cells on ASM proliferation in the 3-D cultures. Coculture with mast cells was associated with a 40% increase in nuclear Ki67 staining of ASM cells. When mast cells had been preactivated, there was a greater than twofold increase in nuclear Ki67 staining. In cultures, mast cells were closely associated with ASM cells, and mast cell supernatants alone had no effect on ASM proliferation, suggesting that direct cell-cell contact may be required for this effect. We next examined the effect of 3-D culture and mast cell coculture on ASM synthetic function. We previously showed that ASM cells constitutively produce pro-MMP-2, which can be activated in the presence of collagen (16). We showed that ASM cells in the 3-D system continued to produce MMP-2, whereas in 2-D culture, with and without collagen, MMP-2 expression fell over time. Others have shown that αβ1-integrin can dose dependently regulate ASM synthetic function on collagen (5): it is possible that the 3-D culture provides a greater stimulus to cell surface integrin receptors, resulting in increased synthetic function. It is also possible that MMP-2 production is dependent on a different cytoskeletal arrangement in the 3-D cultures.

A shortcoming of this system is the reliance on one ECM protein as the scaffold. Although more physiological biosynthesized matrix can be derived from cell preparations that can be used in 2-D systems (21, 36), this approach is impractical for the 3-D system because of the quantities required. The culture model does allow incorporation of a mixture of ECM proteins into the gel scaffold, and, additionally, over time, ASM cells will secrete their own matrix components into the gel. We used collagen I, inasmuch as it is present in human airways and is increased in airway remodeling. Furthermore, collagen I has been used in airway fibroblast 3-D culture (25) and, in our experiments, supported 3-D ASM growth; however, in preliminary experiments, Matrigel did not.

In conclusion, our initial description of this model system has shown that ASM cells in 3-D culture adopt a spindle-shaped morphology, with reduced contractile protein expression and a low basal rate of proliferation and a change in synthetic function. 3-D cultures can be used to study ASM-ECM mast cell interactions.

ACKNOWLEDGMENTS

We are grateful to Dr. Neil Henderson and Tim Self for technical assistance and Dr. John Blakey for statistical analysis.

GRANTS

This study was funded by Asthma UK Grant 05/007.

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