Responsiveness of Aortic Smooth Muscle Cells to Soluble Growth Mediators Is Influenced by Cell-Matrix Contact

Michael Thie, Bärbel Harrach, Elke Schönherr, Hans Kresse, Horst Robenek, and Jürgen Rauterberg

Excessive proliferation and overexpression of collagens by smooth muscle cells (SMCs) are important features of atherogenesis. To understand the role of the extracellular matrix in the regulation of these processes, we examined proliferation and protein/collagen synthesis of SMCs in contact with a collagen matrix. Adult pig SMCs were isolated from the aortic media by collagenase digestion, subcultured as monolayers, and then embedded into a three-dimensional network of type I collagen, ie, a collagen lattice. Cells were subsequently exposed to growth-promoting media, and their behavior was observed in comparison with monolayer cultures on plastic. Treatment of monolayers with increasing concentrations of fetal calf serum resulted in activation of the cell cycle, onset of cell proliferation, and increased protein/collagen synthesis. In contrast, similar treatment of collagen lattice-cultured SMCs failed to influence cell proliferation and protein/collagen synthesis. However, stimulation of proliferation of lattice-cultured SMCs by platelet-derived growth factor–A/B was feasible; nevertheless, the rate of proliferation was modest compared with monolayers. In addition, the onset of proliferation was accompanied by a decrease in collagen synthesis of the cells. Thus, a collagenous matrix appears to suppress the responsiveness of SMCs to soluble growth mediators. It is speculated that interactions between SMCs and the extracellular matrix may modify proliferation and protein/collagen synthesis of cells not only in vitro but also in vivo during atherogenesis by making and breaking binding sites between extracellular collagen and matrix receptors. (Arteriosclerosis and Thrombosis 1993;13:994-1004)

KEY WORDS • arteriosclerosis • atherosclerosis • smooth muscle cells • collagen synthesis • proliferation • growth factors • extracellular matrix • cell-matrix contact

Atherosclerosis is a complex disorder of the arterial vessel wall in which the behavior of the tissue resident smooth muscle cells (SMCs) is of central importance. During pathogenesis of atherosclerosis, SMCs modulate from the contractile state characteristic of the healthy vessel to a synthetic state typical of the atherosclerotic vessel. This modulation is accompanied by an increasing proliferation of cells and excessive synthesis and deposition of extracellular matrix proteins, in particular, collagens. Although the precise mechanisms underlying these changes are far from clear, the mutual interaction between the tissue resident SMCs and the extracellular matrix itself is claimed to play a role in regulating cell behavior.

When SMCs are disaggregated from the healthy vascular matrix and further cultured in a matrix-free system, ie, as a monolayer on plastic, the cells undergo a radical change in their ultrastructural and functional character. Electron microscopic studies have shown that modulation from the contractile to the synthetic phenotype is characterized by a loss of myofilament bundles and the appearance of extensive rough endoplasmic reticulum and a large Golgi complex. Biochemical studies have indicated enhanced synthesis of matrix proteins, in particular, both type I and type III collagen. In contrast, when monolayer-cultured SMCs are reintegrated into a tissue-like matrix, ie, a collagen lattice, the cells are reinfluenced toward those of the healthy vessel. We previously demonstrated that total protein synthesis and collagen synthesis are reduced and proliferation is suppressed. Thus, the extracellular matrix may also act in vivo as a biologically active dimension by exerting control over cellular functions.

To understand the role of the extracellular matrix in the regulation of biosynthetic activity, we assess in this report the potential of SMCs to proliferate and to synthesize protein when in contact with a matrix of type I collagen. We embedded monolayer-cultured SMCs into a collagen lattice, then exposed cells to growth-promoting media, and observed their behavior in comparison with cell cultures on plastic. Our present results show that SMCs do not respond to stimulation with fetal calf serum (FCS) when they are in contact with the collagenous matrix; protein/collagen synthesis and proliferation of collagen lattice–cultured SMCs are not affected on serum stimulation. Nevertheless, the stimulation of proliferation of collagen lattice–cultured...
SMCs by means of platelet-derived growth factor–A/B (PDGF-A/B) is feasible. However, response to this growth factor, ie, onset of proliferation and decrease in collagen synthesis, is modest. The involvement of collagen-receptor interactions in the control of SMC growth and secretion is discussed.

Methods

Materials

Medium 199, FCS, penicillin, streptomycin, L-glutamine, and trypsin-EDTA were obtained from Boehringer, Mannheim, FRG. Collagenase CLS (Worthington, 204 U/mg) was purchased from Biochrom KG, Berlin, FRG. Elastase type III (62 U/mg) was obtained from Sigma, Deisenhofen, FRG. Chondroitin ABC lyase (5 U/vial) and chondroitin AC II lyase (5 U/vial) were obtained from Seikagaku Kogyo Co Ltd, Tokyo, Japan. Heparitinase (200 to 600 U/mg) was obtained from Sigma. Human recombinant PDGF-A/B was a product of Boehringer and handled as a solution of 50 μg/mL of phosphate-buffered saline (PBS), pH 6.4, containing bovine serum albumin (1 mg/mL). L-(3)H proline (9.69 GBq/mmol) was obtained from New England Nuclear, Dreieich, FRG. 33 S-sulfate, carrier free, (0.9 to 1.5 TBq/mg of sulfur) was obtained from Amersham-Buchler, Braunschweig, FRG. The cycle TEST DNA reagent kit for flow-cytometric analysis of nuclear DNA was obtained from Becton Dickinson, Erembodegem, Belgium. Resin type M71 was purchased from Beckman, München, FRG. DEAE-Trisacryl was obtained from Serva, Heidelberg, FRG. Nitrocellulose was obtained from Schleicher & Schüll, Dassel, FRG. All other chemicals were of analytical grade and were obtained from Merck, Darmstadt, FRG, or Sigma.

Cell Culture

Thoracic aortas from healthy 6- to 8-month-old female pigs were used for the preparation of SMCs. Cells were released from the tunica media by collagenase digestion.13 Briefly, immediately after bleeding, the thoracic aorta was excised and the tunica adventitia removed. The aorta was opened longitudinally and the remaining tunica media was cut into small pieces. Samples of 0.3 g wet weight were first incubated with collagenase (3 mg/mL) and afterward with elastase (0.5 mg/mL) in serum-free medium 199 at 37°C for 1 hour each. Isolated SMCs were obtained by a second collagenase treatment (5 mg/mL serum-free medium 199) in a moist atmosphere of 5% CO2 and 95% air at 37°C until the tissue was completely dispersed. The final digest was passed through a filter (mesh size, 40x40 μm), and the freed cells were recovered by centrifugation at 200g for 10 minutes. The cells were resuspended in medium 199 supplemented with 10% FCS, 4 mmol/L L-glutamine, and antibiotics and seeded at a density of 1.5x10^4 cells/cm^2. The growth medium was changed every 2 days, and cells were subcultured by trypsinization when they became confluent. In all experiments, SMCs were used at the fourth passage.

Culture Systems

SMCs were cultivated within hydrated collagen lattices from type I collagen and as monolayers on plastic.

Collagen lattices were prepared in 35-mm tissue-culture dishes as described previously12 by mixing 2.5x10^6 cells in 150 μL medium and 1350 μL neutralized type I collagen solution consisting of 690 μL of a 1.76-fold concentrated medium, 450 μL type I collagen solution (3.3 mg type I collagen purified from acid-extracted calf skin collagen by fractionated salt precipitation per milliliter of 0.1% acetic acid), 75 μL 0.1N NaOH, and 135 μL FCS. After lattice formation the collagen lattice was supplemented with 1 mL of culture medium supplemented with either 0.5% or 10% FCS and cultured up to 6 days. The medium was changed every 2 days. On day 4 of culture, some lattices were switched to growth medium supplemented with 20 ng PDGF-A/B per milliliter of medium. Then cells were further incubated up to day 6.

For monolayer cultures, SMCs were plated on 35-mm tissue-culture dishes (6x10^4 cells/dish) and grown in 1 mL culture medium before use. Cells cultivated on plastic dishes were treated for experiments as described above. This treatment of cell cultures produced proliferating and quiescent cells, which generally did not grow as monolayers. Despite this fact, we considered cell cultures on plastic dishes as monolayer cultures.

The cells in collagen lattices and in monolayer cultures were counted by using an electronic Coulter counter (CASY 1, Schärfe System, Reutlingen, FRG). Cells from collagen lattices were isolated by incubating the lattices in 2.5 mg collagenase per milliliter of PBS and 1 mmol/L CaCl2, pH 7.4, at 37°C for 30 minutes. The suspension was centrifuged at 200g for 10 minutes at 4°C and the cell number determined. Monolayer cultures were trypsinized, centrifuged, and examined as described above.

Flow-Cytometric Analysis

Collagen lattice–cultured SMCs were isolated by incubating the lattices in collagenase as described above. The free cells were centrifuged at 200g for 10 minutes at 4°C, and the pellet was washed in culture medium without FCS. Further disaggregation with trypsin, enzymatic digestion of RNA, and staining of isolated nuclei with propidium iodide were carried out by using a set of reagents (Cycle TEST Kit) according to the instructions of the manufacturer. DNA measurement was carried out by using the FACSScan flow cytometer (Becton Dickinson). The percentage of cells in each phase of the cell cycle was estimated with an analysis model calculating the S phase from a rectangle (RFIT model). Monolayer cultures were trypsinized, washed, and examined as described above.

Collagen and Total Protein Syntheses

Collagen and total protein syntheses were measured after preparation of cultures as previously described.14 Briefly, 0.37 MBq/mL of 3H-proline and 50 μg/mL ascorbic acid were added to growth medium of collagen lattices as well as to monolayer cultures. After 24 hours in a moist atmosphere of 5% CO2,95% air at 37°C, the medium was collected. The collagen lattices and cells were washed three times with 1 mL PBS at room temperature for 30 minutes, and the washing solutions were added to the collected media. Collagen lattices were frozen in liquid nitrogen, pulverized, lyophilized, and resuspended in 3 mL buffer. Cells grown in mono-
layers were removed by scraping. Cell suspensions were sonicated for 2 minutes. Samples were exhaustively dialyzed against 0.5% acetic acid, hydrolyzed for 24 hours at 110°C with 6N HCl, and subjected to ion-exchange chromatography for separation of proline and hydroxyproline. Collagen synthesis was calculated according to Krieg et al. Total protein synthesis was calculated by determining the amount of labeled proline and hydroxyproline. Results were normalized to the number of recovered cells.

**PDGF-A/B Binding to Proteoglycans Extracted From Collagen Lattices**

After lattice formation, collagen lattices were incubated with 3.7 MBq 35S-sulfate per milliliter of culture medium containing 10% FCS. The incubation medium was changed after 2 days. On day 4 of culture, three lattices were extracted with 6 mol/L guanidinium hydrochloride in buffer A (20 mmol/L Tris[hydroxymethyl]aminomethane [Tris] hydrochloride, pH 7.4, 0.1% Triton X-100, 100 mmol/L aminocaproic acid, 10 mmol/L EDTA, 5 mmol/L benzamidinium hydrochloride, and 150 mmol/L NaCl) for 2 hours at room temperature. The extract (1 mL) was diluted with 29 mL buffer A and passed over a DEAE-Trisacryl column (1 mL) equilibrated with buffer A. The column was washed exhaustively with buffer A with 150 and 300 mmol/L NaCl to remove contaminating glycoproteins. Proteoglycans were elut with buffer A containing 1 mol/L NaCl, and the 35S-sulfate content was determined by liquid scintillation counting. The eluate was dialyzed against Tris-buffered saline (TBS; 20 mmol/L Tris hydrochloride, pH 7.4, and 150 mmol/L NaCl) and stored at -20°C.

Equal aliquots of 35S-sulfate–labeled proteoglycans (20,000 cpm) were treated with chondroitin ABC or ACII lyase, heparitinase, or enzyme-free buffer before sodium dodecyl sulfate–polyacrylamide gradient gel electrophoreses and fluorography.

PDGF-A/B (0, 1, 2, 4, or 10 ng) in culture medium was spotted onto activated nitrocellulose, and the filter was blocked with TBS and 3% bovine serum albumin. 35S-sulfate–labeled proteoglycans (1 x 10⁶ cpm) were treated with chondroitin ABC or ACII lyase, heparitinase, or enzyme-free buffer before sodium dodecyl sulfate–polyacrylamide gradient gel electrophoreses and fluorography.

**Fig 1.** Bar graphs of effects of low (0.5%) and high (10%) concentrations of fetal calf serum (FCS) on total protein synthesis (panels A and B) and collagen synthesis (panels C through F) in smooth muscle cells cultured as monolayers (monolayer) or within collagen lattices (lattice). Cells from three animals (a, b, and c) were cultured in growth medium supplemented with either 0.5% or 10% FCS for 5 days. On day 4, cultures were pulsed with 3H-proline for 24 hours. Total protein synthesis, expressed as counts per minute x 10⁶ per cells x 10⁶, was determined by measuring the radioactivity of nondialyzable proline and hydroxyproline in the combined cell layer/substratum and liquid medium. Total amount of synthesized collagen, expressed as counts per minute x 10⁶ per cells x 10⁶, was determined by measuring the radioactivity of hydroxyproline in the combined cell layer/substratum and liquid medium (panels C and D). Collagen synthesis is also expressed as a percentage of total protein synthesized in cells (panels E and F). Values expressed are the mean±SEM of triplicate determinations from three separate cultures (ie, nine measurements). Results are normalized to the number of recovered cells, ie, 66 000±3000 (a), 78 000±8000 (b), and 89 000±6000 (c) cells per 0.5% FCS per monolayer culture; 120 000±7000 (a), 157 000±9000 (b), and 422 000±4000 (c) cells per 10% FCS per monolayer culture; 251 000±6000 (a), 279 000±8000 (b), and 244 000±7000 (c) cells per 0.5% FCS per lattice culture; and 404 000±7000 (a), 265 000±4000 (b), and 380 000±8000 (c) cells per 10% FCS per lattice culture.
Thie et al

SMCs in Collagen Lattice Culture 997

FIG 2. Line plots of effects of low (0.5%) and high (10%) concentrations of fetal calf serum (FCS) on cell-cycle distribution of smooth muscle cells cultured as monolayers (monolayer) or within collagen lattices (lattice). Cells were cultured in growth medium supplemented with either 0.5% or 10% FCS for 6 days. Flow-cytometric analysis was performed on days 4 (d4), 5 (d5), and 6 (d6) of culture. Cell-cycle distribution, according to propidium iodide fluorescence, is expressed as a percentage of the total cell population. Values expressed are the mean±SEM of triplicate determinations from three separate cultures from each of three animals (ie, 27 measurements). Dashed line, G0/M phase; solid line, S phase; dotted line, G2/M phase.

Applied in 1 mL TBS and 3% bovine serum albumin for 6 hours at room temperature. Then the filter was washed five times for 5 minutes with TBS. The dried filter was exposed to Kodak X-OMAT AR film for up to 2 weeks.

Preparation of Samples for Electron Microscopy

Cell cultures were fixed with Karnovsky's reagent, postfixed with OsO4, dehydrated in ethanol, cleared in propylene oxide, and embedded in Epon 812 as described previously. Ultrathin sections were doubly stained with uranyl acetate and lead citrate and examined with a Philips electron microscope 410 at 60 kV.

Results

Stimulation of Lattice-Cultured SMCs by FCS

Total protein and collagen synthesis. The influence of low (0.5%) and high (10%) FCS concentrations on protein and collagen synthesis in SMCs was tested in collagen lattice cultures and compared with monolayers (Fig 1). The response of cells to serum differed markedly in the two culture systems. Whereas monolayer cultures could be manipulated by serum, collagen lattice cultures could not.

Lattice-cultured SMCs and monolayer-cultured SMCs synthesized total protein under low and high serum concentrations. Cells cultured as monolayers (Fig 1A) showed an increase in values of total protein with increasing serum concentrations (0.73±0.25 cpm×10^6/cells×10^6 versus 2.6±1.0 cpm×10^6/cells×10^6; mean of three animals). Cells within a collagen lattice (Fig 1B), however, showed no differences in values of total protein synthesis when cultured either under low (0.55±0.24 cpm×10^6/cells×10^6; mean of three animals) or high (0.85±0.39 cpm×10^6/cells×10^6; mean of three animals) serum concentrations. Lattice cultures showed comparable values of total protein synthesis with monolayer cultures supplemented with 0.5% FCS.

The amount of collagen synthesized was estimated from the radioactivity associated with nondialyzable hydroxyproline in the cells, in the collagen lattices, and in the medium. Lattice-cultured SMCs and monolayer-cultured SMCs synthesized collagen under low and high serum concentration. Monolayer cultures (Fig 1C) showed an increase in collagen synthesis with an increase in serum concentration (1.55±0.53 cpm×10^5/cells×10^6 versus 2.71±1.14 cpm×10^5/cells×10^6; mean of three animals). SMCs within collagen lattices (Fig 1D) showed no differences in collagen synthesis when cultivated either under low (0.70±0.52 cpm×10^5/cells×10^6; mean of three animals) or high (0.70±0.37...
FIG 3. Line plots of effects of platelet-derived growth factor-A/B (PDGF) in the presence of low (0.5%) and high (10%) concentrations of fetal calf serum (FCS) on cell-cycle distribution of smooth muscle cells cultured as monolayers (monolayer) or within collagen lattices (lattice). Cells were cultured in growth medium supplemented with either 0.5% or 10% FCS for 6 days. On day 4 of culture, growth medium was further supplemented with 20 ng PDGF-A/B per milliliter for 48 hours. Flow-cytometric analysis was performed on days 4 (d4), 5 (d5), and 6 (d6) of culture. Cell-cycle distribution, according to propidium iodide fluorescence, is expressed as a percentage of the total cell population. Values expressed are the mean±SEM of triplicate determinations from three separate cultures from each of three animals (ie, 27 measurements). Dashed line, G0/G1 phase; solid line, S phase; dotted line, G2/M phase.

Stimulation of Lattice-Cultured SMCs by PDGF-A/B

Cell-cycle distribution. The mitogenic response of SMCs to PDGF-A/B was tested in collagen lattice cultures under low and high serum concentrations (Fig 3). In general, SMCs responded to PDGF-A/B stimulation. Compared with controls (Fig 2), an increase in the proportion of cells in the S phase and a decrease in the proportion of cells in the G0/G1 phase was observed. The proportion of cells in the G2/M phase remained unchanged. However, collagen lattice cultures responded less to PDGF-A/B treatment than did monolayer cultures.

The cell-cycle distribution was influenced by PDGF-A/B during the entire period of treatment. Effects reached a peak, however, 24 hours after supplementation with PDGF. With regard to the fraction of cells in the S phase, the increase in monolayer-cultured cells was greater under 0.5% FCS (8.1-fold of control on day...
Thie et al. SMCs in Collagen Lattice Culture

FIG 4. Bar graphs of effects of platelet-derived growth factor-A/B in the presence of low (0.5%) and high (10%) concentrations of fetal calf serum (FCS) on cell proliferation of smooth muscle cells cultured as monolayers (monolayer) or within collagen lattices (lattice). Cells were cultured in growth medium supplemented with either 0.5% or 10% FCS for 6 days. On day 4 of culture, growth medium was further supplemented with 20 ng PDGF-A/B per milliliter for 48 hours (striped columns). On days 4 (d4), 5 (d5), and 6 (d6), cells were counted with a Coulter counter. Data represent the multiple of cell number of day 4 of cultures, ie, 69 000±5000 (0.5% FCS per monolayer culture), 178 000±8000 (10% FCS per monolayer culture), 261 000±7000 (0.5% FCS per lattice culture), and 311 000±4000 (10% FCS per lattice culture) cells per culture. Values expressed are the mean±SEM of triplicate determinations from three separate cultures from each of three animals (ie, 27 measurements).

5 of culture) than under 10% FCS (2.1-fold of control on day 5 of culture). In contrast, in collagen lattice-cultured SMCs, the increase in the S phase was greater under 10% FCS (6.0-fold of control on day 5 of culture) than under 0.5% FCS (3.1-fold of control on day 5 of culture).

Proliferative response. With prolonged culture time, a proliferative response of SMCs to PDGF-A/B was observed (Fig 4). However, collagen lattice-cultured cells proliferated to a lower degree than did monolayer-cultured cells.

When cells were cultured under low serum concentrations, a first increase in cell number was observed 48 hours after PDGF-A/B supplementation. In contrast, the cell number increased 24 hours after PDGF-A/B supplementation when cells were cultured under high serum concentrations. Analysis of cell numbers of cultures 48 hours after PDGF supplementation, ie, on day 6 of cell culture, revealed that monolayer and lattice cultures tended to show a slightly higher proliferative response under low serum concentrations (2.3-fold of controls in monolayers and 1.4-fold of controls in lattices, respectively) compared with cultures under high serum concentrations (2.1-fold of controls in monolayers and 1.2-fold of controls in lattices, respectively).

Total protein and collagen synthesis. Induction of proliferation was accompanied by changes in protein and collagen synthesis of SMCs (Fig 5). Lattice-cultured cells, however, reacted less intensely than did monolayer cultures.

With low serum concentrations, monolayer-cultured cells (Fig 5A) and lattice-cultured cells (Fig 5B) responded to PDGF-A/B in a similar way. Compared with controls, values of total protein synthesis increased 24 hours after PDGF-A/B supplementation and decreased 48 hours after PDGF supplementation. However, the decrease in lattice cultures was not significant. With high serum concentrations, monolayer-cultured cells (Fig 5A) and lattice-cultured cells (Fig 5B) showed an inverse pattern of response. Twenty-four hours as well as 48 hours after PDGF supplementation, values of protein synthesis decreased in monolayer cultures and increased in lattice cultures. However, the increase in lattice cultures 24 hours after PDGF supplementation was not significant.

With regard to newly synthesized collagen, SMCs reduced collagen synthesis on prolonged PDGF-A/B treatment, irrespective of the culture system used (Figs 5C and 5D). Only 24 hours after PDGF-A/B treatment and under low serum concentrations were values for collagen synthesis increased.

With regard to the proportions of collagen in synthesized protein, monolayer-cultured SMCs (Fig 5E) showed a specific reduction of collagen synthesis only on day 5 of culture, ie, 24 hours after PDGF supplementation. In contrast, lattice-cultured SMCs (Fig 5F) showed a specific reduction of collagen synthesis on days 5 and 6 of culture, ie, throughout the period of PDGF-A/B treatment.

PDGF-A/B binding to matrix-associated proteoglycans. To determine whether proteoglycans that accu-
mulate in collagen lattices before PDGF-A/B treatment can bind this growth factor, cell cultures were labeled with $^{35}$S-sulfate, and matrix-bound proteoglycans were partially analyzed. Two small proteoglycans (one ≈200 kD, presumably decorin, and the other ≈300 kD, presumably biglycan) and a large proteoglycan (in the 3% polyacrylamide stacking gel) containing chondroitin or dermatan sulfate chains were found. Heparan sulfate proteoglycans were only a minor component (Fig 6A). None of the $^{35}$S-sulfate–labeled material bound significantly to PDGF-A/B under the chosen conditions (Fig 6B).

Ultrastructural Features of Cultured SMCs

SMCs subcultured as monolayers or within collagen lattices in the presence of either 0.5% or 10% FCS showed no differences in their ultrastructure, which was that of a synthetic phenotype as described. The cytoplasm was filled with elements of the endoplasmic reticulum, Golgi complexes, and a large number of mitochondria. Myofilament bundles were lacking. Compared with monolayer cultures, lattice-cultured SMCs were completely surrounded by a matrix of collagen fibrils, which were in close contact with the cell membrane (Fig 7). These ultrastructural...
features of cells were stable with prolonged culture time and were also maintained in the presence of PDGF-A/B.

**Discussion**

In the present study, we have demonstrated that the extracellular matrix appears to be a critical dimension in regulating the cellular functions of SMCs. A reconstituted extracellular matrix, ie, a three-dimensional matrix of type I collagen, appears to be capable of modulating responsiveness of SMCs to soluble mediators of growth. This is shown by the failure of growth stimulation of collagen lattice–cultured cells by the full complement of growth factors as present in FCS. When SMCs are cultured in the presence of extracellular matrix, ie, within a collagen lattice, treatment with serum affects neither DNA synthesis and cell proliferation nor levels of collagen synthesis and total protein synthesis. SMCs remain in a state of metabolic quiescence, ie, arrest of the cell cycle in the G_0/G_1 phase and suppression of collagen synthesis and total protein synthesis. In contrast, when SMCs are cultured in the absence of extracellular matrix, ie, as a monolayer on plastic, stimulation of cells with increasing concentrations of serum results in activation of the cell cycle, onset of proliferative activity, increase in total protein synthesis, and increase in collagen synthesis.

We used cultures of SMCs on plastic, in the presence of serum, as a control for cultures in collagen lattices. The idea for using monolayer cultures on uncoated plastic dishes was to compare a minimum of cell-matrix contacts with a maximum of cell-matrix contacts present in collagen lattices. Nevertheless, we are aware of the fact that cell behavior in this monolayer model is mediated by serum-derived matrix proteins. For example, fibronectin favors attachment of SMCs; vitronectin supports attachment and spreading, as well as migration of cultured cells; and thrombospondin is involved in the regulation of proliferation. For a review, see Reference 26.

With regard to ultrastructure, SMCs in both lattice culture and monolayer culture exhibit the synthetic phenotype. Thus, the nonresponsiveness to serum stimulation of SMCs seems to be an effect of the collagen lattice culture. From the data presented here, it could be speculated that the contact of collagen fibrils to the cell surface might contribute to the regulation of proliferation and protein/collagen synthesis. Cells bind to extracellular matrix via specific cell-surface receptors. SMCs express multiple integrin receptors with different ligand specificities, mediating cell interactions with components of the extracellular matrix. Very late acting antigen–1 is described as being the main collagen receptor in SMCs. Furthermore, cells can upregulate and downregulate integrin receptors, depending on the actual conditions in their environment. As a consequence of the contact between the cells and the extracellular matrix, intracellular cytoskeletal organization, cell shape, and growth rate might be influenced as described for fibroblasts. Therefore, as SMCs express matrix receptors and as they contact collagen fibrils, cells may subsequently mediate the regulation of processes in the growth-stimulatory pathway. Thus, despite serum support, proliferation and protein/collagen synthesis of SMCs are suppressed and remain low, so long as the cells contact the collagen fibrils via specific receptors on the cell plasma membrane.

The fact that the growth factor–dependent "machinery" necessary for growth regulation remains intact in lattice-cultured cells is shown by growth stimulation with PDGF-A/B. On treatment with PDGF, SMCs show an increase in the proportion of cells in the S phase, followed by an increase in the number of cells. The fact that PDGF-A/B, but not serum, activated the proliferation of SMCs that were growth-arrested in collagen lattices might be due to differences in the pathway of signal transduction. PDGF-induced growth might be associated with the specific breakdown of phosphatidylinositol-4,5-bisphosphate (PIP2), whereas serum-induced growth might require other systems of second messengers as suggested for fibroblasts. The latter systems, but not the PIP2 pathway, may be inactive when cells are grown in collagen lattices. The PIP2 pathway, however, appears also to be influenced to a certain degree by extracellular matrix, as a response to PDGF-A/B in lattice cultures is strongly suppressed compared with that of monolayer cultures. The poor response to growth factor might be due to downregulation of the cell-surface receptor for PDGF-A/B when the cells are cultured within a collagen lattice. Furthermore, the poor response to PDGF-A/B could be due to immobilization and/or inactivation of the growth factor by matrix-associated proteoglycans, eg, heparan sulfate proteoglycans. To prove this possibility, we examined the secreted proteoglycans that accumulated in the type I collagen matrix and tested the binding of...
the growth factor. We demonstrated the presence of two small proteoglycans, presumably decorin and biglycan, and of a large proteoglycan containing chondroitin or dermatan sulfate chains. Heparan sulfate represented only a minor proportion of the secreted proteoglycans. Using a nitrocellulose membrane technique, we were unable to detect any binding of PDGF-A/B to matrix-extracted proteoglycans. Although we failed to show growth-factor binding to matrix-associated proteoglycans, others have demonstrated interactions between PDGF polypeptide chains with membrane- and matrix-associated heparin-like glycosaminoglycans. These interactions have been shown to regulate the mitogenic effect of PDGF, and thus, they might be involved in growth regulation of collagen lattice–cultured SMCs, too.

Induction of proliferation by PDGF-A/B, i.e., the transition of cells from the G_0/G_1 phase to the S phase, is accompanied by changes in collagen synthesis of SMCs. Collagen synthesis of PDGF-A/B–stimulated...
cells decreases in lattice cultures as well as in monolayer cultures. In addition, lattice-cultured cells show lower values of collagen synthesis than do monolayer cultures. Thus, the rate of collagen synthesis appears to be affected by both the proliferative activity of SMCs and the presence of extracellular matrix. SMCs in monolayer culture are described as showing an increase in collagen gene expression as the rate of growth decreases.42–45 Using PDGF for growth stimulation, we have demonstrated transformation of cells into the cycling phase with the subsequent onset of proliferation and a decrease in collagen synthesis. These features are evident in monolayer cultures as well as in lattice cultures and are in good compliance with the aforementioned data. However, with the use of serum for stimulation, ie, the full complement of growth factors, cells in monolayer cultures show increases in proliferation rate and collagen synthesis. In contrast, cells in lattice cultures do not respond to serum, as already mentioned. This cell behavior indicates the importance of mutual interactions of several growth factors and extracellular matrix in cellular functions.46–48

The observations reported here show an altered response to growth-promoting media in cultured SMCs when cultivated within a type I collagen lattice. The results suggest that cell-matrix contact might be involved in overlapping control of the cell cycle, proliferation, and protein/collagen synthesis. Therefore, perturbation of the interactions between cells and the extracellular matrix, ie, an imbalance of the making and breaking of binding sites between extracellular collagen and cell-surface receptors, might lead to some of the changes typical of atherogenesis.

Acknowledgments
This work was supported by the funds from the Deutsche Forschungsgemeinschaft (SFB 223/SFB 310). The skillful technical assistance of C. Fabritius, R. Fischer, M. Opalka, and S. Otter is gratefully acknowledged.

References


Responsiveness of aortic smooth muscle cells to soluble growth mediators is influenced by cell-matrix contact.

M Thie, B Harrach, E Schönherr, H Kresse, H Robenek and J Rauterberg

doi: 10.1161/01.ATV.13.7.994

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/13/7/994

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/