Induction of Tissue Inhibitor and Matrix Metalloproteinase by Serum in Human Heart-Derived Fibroblast and Endomyocardial Endothelial Cells

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Abstract   To understand the regulatory mechanisms of extracellular matrix (ECM) turnover and proteinase expression in human cardiovascular tissue, we have isolated and characterized human heart fibroblast (HHF) and human heart endothelial (HHE) cells from endomyocardial biopsy specimens. HHE cell in culture exhibited the typical cobblestone growth pattern and positive immunofluorescent staining for factor VIII related antigen. HHF demonstrated the typical spindle shape during culture and were positive for vimentin. Both cell types were negative for α-actin, indicating that these cells were of nonmuscle origin. Cell growth studies revealed significant growth when maintained in limiting serum concentration, suggesting mitogenic activity of these cells, and demonstrated growth inhibitory activity when grown in serum-free medium. Serum-dependent matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) expression was measured by zymography, immunoblot, and Northern blot analysis. Results indicated that serum induces both the MMP and TIMP expression at the mRNA and protein levels in a dose-dependent manner. This induction was inhibited by actinomycin D and cycloheximide, suggesting transcriptional and translational regulation of MMP and TIMP. Indirect immunofluorescence labeling indicated expression of MMP and TIMP in HHF and HHE cells. These results suggested that the serum induces proliferation as well as expression of MMP and TIMP in HHE and HHF cells. The growth inhibitory activity of these cell cultures will enable us to explore further the nature of this response and compare this phenomenon with other growth inhibitors and growth promoters identified in other normal and transformed cells.   © 1995 Wiley-Liss, Inc.

Key words: myocardial fibroblast, endothelial, collagenase, tissue inhibitor of metalloproteinase, matrix metalloproteinase, serum induction, gene regulation

Human myocardium consists of mainly three components: (1) muscle (myocytes); (2) vasculature; and (3) interstitium. Cardiac myocytes (muscle) occupy ~90% of the cardiac mass. Ninety to 95% of the nonmyocyte fraction of cardiac cells consists of fibroblasts (interstitial cells) [1,2]. Ventricular function of the heart relies largely on autoregulatory mechanisms effected by cell-cell interactions [3]. We have shown in a coculture experiment that bovine endothelial cells secrete a factor that induces matrix metalloproteinases (MMPs) activity in rat cardiac fibroblasts [4]. Autoregulation is accomplished through intrinsic feedback of each cardiomyocyte and cardiac fibroblasts in the interstitium [5] and extrinsic feedback mediated by the circulatory hormones through endothelial cells which are in continuous contact with blood [6]. The endocardial endothelium is the innermost structure of the heart and vessels and is lined by a monolayer of endothelial cells. It occupies a unique position in the cardiovascular system [7]. The autoregulatory mechanism may involve neurohormonal factors released from endothelial cells which in turn may induce fibroblast proliferation or fibroblast growth inhibition [3,4]. Therefore, understanding of the regulatory mechanism of cell-cell interaction by human fibroblast and endothelial cells is of great importance.

Coronary vasculature is unique with local structural and functional characteristics associated with blood circulation. However, to date, only limited study has been undertaken to ascertain coronary vascular components from normal

heart. We have isolated endothelial and fibroblast cells from endomyocardial biopsy specimens obtained from transplanted hearts of patients (with renal failure) for long-term in vitro culture. In this report we describe the conditions required for the isolation, adaptation, and growth of long-term serial cultures of human heart-derived endothelial and fibroblast cell populations. We also show that these cells express MMPs and tissue inhibitors of metalloproteinases (TIMPs) in response to serum.

Previously we have demonstrated that MMPs are localized in endomyocardium, subendomyocardial, and interstitial space and can be activated under various physiological conditions [8]. These MMPs, which can be induced by serum in these cells, may play a major role in chamber dilatation and remodeling following myocardial infarction, dilated cardiomyopathy, and valvular heart diseases [9,10]. Collagen degradation depends on the balance of MMP and its inhibitor. We show evidence that in myocardium the control mechanisms of proteolysis are complicated by the simultaneous expression of TIMP and the sources of this MMP and TIMP activity are human heart fibroblasts and endothelial cells.

MATERIALS AND METHODS
Materials

Fetal calf serum (FCS), normal rabbit serum, minimum essential medium with Earle's salts (MEM), collagen and laminin coated culture plates, and Hanks' balanced salt solution were obtained from Collaborative Research (Bedford, MA). Trypsin was obtained from GIBCO BRL (Gaithersburg, MD). Collagenase was from Worthington Biochemical Corp. (Freehold, NJ). Antibodies to MMP-1 and TIMP-1 were kind gifts of Dr. Hideaki Nagase, Department of Biochemistry, Kansas University Medical Center, KS. Anti-sheep IgG alkaline phosphatase, gelatin (porcine skin, 300 bloom), casein, Triton X-100, SDS, actinomycin D, cycloheximide and 1,10 phenanthroline (Phen) were purchased from Sigma (St. Louis, MO). Prestained electrophoresis protein standards were obtained from Bio-Rad (Richmond, CA).

Tissue Sources

Tissue biopsy samples (5 mm^3) from posttransplanted patients were obtained from the University Medical Center at Missouri University, Co-lumbia. Normal and abnormal (diseased) tissue was identified by measuring MMP activity. Previously we have demonstrated that in a normal heart most of the MMP was in latent form and very little ($\sim 5\%$) was in active form [11,12] whereas in diseased tissue most of the MMP was in active form. Tissue samples were washed with saline and either quickly frozen in liquid nitrogen for storage or placed directly onto ice in culture medium to isolate cells.

Cell Isolation and Culture

Normal cells were isolated from 20 biopsy tissue samples. Human heart endothelial (HHE) cells were harvested by trypsin (0.1%) and collagenase digestion (200 U). This treatment detached endothelial cells from basement membrane without disturbing interstitium [13]. These cells were further recovered to homogeneity into one major band (mostly endothelial) by centrifugation on Ficoll-Paque (Pharmacia, Biotech, Piscataway, NJ). HHE were sorted out from non-endothelial cells based on their "cobblestone" characteristics and by the presence of factor VIII antigen. HHE were maintained at 37°C in a humidified 5% CO_2 atmosphere in MEM supplemented with 20% FCS, containing 2 mM 1-glutamine and glucose. Isolated HHE were cultured on collagen-coated plates in medium supplemented with 10% normal rabbit serum, 5% fetal calf serum, and 2 mM glutamine. Human heart fibroblast (HHF) cells were isolated from the same tissue specimens as for the HHE cells by excising and mincing the washed tissue and treated with 0.1% trypsin and 100 U of collagenase per ml for 10 min at 37°C. Isolated cells were plated at the end of several 10-min digestion periods on 100-mm culture dishes in MEM containing 10–15% FCS, and incubated for 2 h at 37°C in an incubator humidified with 90% $O_2/10\%CO_2$. At the end of that period, unattached cells were discarded and attached cells were grown in MEM with 20% FCS. Cultures were routinely checked for the presence of mycoplasma (14) which has been shown to stimulate MMP levels (15).

It has been demonstrated that cells cultured in 2-D (plastic surface) are metabolically different than cells cultured in 3-D (more like in vivo situations, i.e., plates coated with collagen or laminin) and cultured in matrix suspension [16]. In order to overcome this experimental problem, we have cultured HHF and HHE on collagen-coated and uncoated plates in medium supple-

mented with 20% normal rabbit serum, 0.1% collagen suspension (Vitrogen 100, Celtrix, Santa Clara, CA), 5% fetal bovine serum, 4.5 mg/ml glucose, gentamycin and fungizone (10 µg/ml), and 2 mM glutamine. For most experiments, cells were washed two times with serum-free Dulbecco's modified Eagle's medium (MEM) prior to the experimental treatment.

Immunolabeling of HHE and HHF

For cell characterization studies, confluent cells were grown on cover slips in medium containing serum. Cell layers were washed twice with phosphate-buffered saline (PBS) and permeabilized in absolute methanol for 7 min. Staining of cell layers was performed as described [17]. Briefly, cells were incubated with a 1:50 dilution of monoclonal antibodies to muscle-specific actin or smooth muscle-specific actin (Sigma), polyclonal rabbit anti-human factor VIII (Beohringer-Mannheim Biochemicals, Indianapolis, IN), rabbit anti-desmin (Calbiochem, La Jolla, CA), or a polyclonal rabbit anti-vimentin antibody (Sigma) for 1 h and washed with PBS. For a negative control, IgGs prepared from mouse or rabbit preimmune serums were used. Fluorescein isothiocyanate-conjugated goat anti-rabbit and anti-mouse IgGs were used as secondary antibodies.

Serum-induced matrix metalloproteinase and tissue inhibitor of metalloproteinase expression in HHE and HHF were ascertained with specific antibodies to proMMP-1 and TIMP-1 raised in sheep. Fluorescein staining was viewed with a Nikon-light microscope equipped with a HBO mercury lamp. Photographs were taken on Ilford HP-5, 400 ASA film using epi-illumination on a Zeiss (Thornwood, NY) photomicroscope III.

Serum Induction of MMP and TIMP

HHE and HHF were cultured until confluent in MEM and 20% FCS on Lab-Tek Permanox chamber slides (Nunc, Inc., Naperville, IL) or on 60-mm culture dishes. Confluent cells were deprived of serum for 48 h. Cells were cultured without and with 20, 10, and 5% serum in the medium for 24 h. Serum was removed and cells were kept in serum-free MEM for 24, 48, 72, and 96 h, and MMP activity released in the MEM was detected by zymography and TIMP was measured by immunoblot analysis.

To measure induction at the mRNA and protein synthesis levels, cells in 20% serum were incubated in the presence of 0.5 mM actinomycin D and 0.1 mM cycloheximide, respectively.

Electrophoresis

Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) was performed with or without reduction by the method of Laemmli [18]. After electrophoresis proteins were stained with silver nitrate or Coomassie Brilliant Blue R 250.

Zymographic Analysis for MMPs Activity

Myocardial matrix metalloproteinase activity in the gel was measured as described previously [11] using type I gelatin as substrate.

Immunoblot Analysis of TIMP-1

Following electrophoresis, under reducing conditions, gels were equilibrated in transfer buffer. Proteins in the gel were transferred to nitrocellulose paper using a Bio-Rad Trans Blot apparatus. Before probing, the nitrocellulose paper was blocked in PBS containing 5% fat-free milk for 2 h and then probed in fresh blocking buffer containing anti-TIMP-1 antibody and 0.5% fat-free milk. After extensive washing with PBS containing 0.05% Tween-20, alkaline phosphatase-conjugated secondary antibody was added and incubated for 1 h. Following a final wash, nitrocellulose paper was stained with buffer containing 5-bromo-4-chloro-3-indolyl phosphate (p-toluidine salt) and nitro blue tetrazolium chloride.

Northern Blot Analysis of MMP, TIMP, and Actin

Total RNA was isolated from 1×10^5 of cells using 4 M Guanidine thiocyanate buffer [19]. RNA was quantitated by absorbance at 260 nm. The purity of total RNA was assessed by absorbance ratio (260/280 nm) of 1.9. Twenty micrograms of total RNA was denatured in a formamide/formaldehyde solution at 65°C for 15 min, and samples resolved on denaturing 1% agarose gel. The gel was transferred to nitrocellulose filter, where it was prehybridized in a buffer containing 50% formamide, 5 × SSC, 0.1% SDS, 1 × Denhardt's, 50 mM $NaHPO_4$, and 100 µg/ml denatured sperm DNA at 42°C for 4 h. Blots were then hybridized for 16 h at 42°C with [α-^{32}P]-dCTP random prime labelled

cDNA. The plasmid containing fibroblast collage-nase (MMP-1) cDNA was obtained from American Type Culture Collection (Rockville, MD). MMP-1 probe was 2.05 kb Hind III and Sma I fragment from human MMP-1 cDNA. The plasmid containing TIMP cDNA probe was obtained from Synergen Corp, Boulder, CO. TIMP-1 probe was 0.7 kb EcoRI fragment of a human TIMP-1 cDNA. A 1.1 kb EcoRI fragment from human β-actin cDNA was used as an internal control. The membrane was washed in 0.1 standard saline citrate plus 0.1% SDS at 42°C for 1 h, then exposed to X-ray films at −70°C for 24 h. Scan values for TIMP-1, MMP-1, and β-actin mRNA signals were expressed in arbitrary units.

RESULTS

Isolation and Characterization of HHE and HHF Cells

Tissue obtained from endomyocardial biopsy specimens from transplanted hearts was used to isolate HHE and HHF. Normality of the tissue was assessed by measuring MMP activity. HHE and HHF were isolated by trypsin and collage-nase treatment of tissue containing minimum intrinsic MMP activity. The cells seeded on culture dishes began to attach and flatten out by 2–3 days. Rapidly adhering cells to culture dishes grew in small colonies of polygonal cells. These cells grew closely apposed to each other without overlapping and were characterized to be endothelial cells. Based on phase-contrast micrograph, HHE-parent monolayer cell culture at early passage (p5) exhibited the typical "cobblestone" appearance (Fig. 1). HHE displayed morphologic characteristics of endothelial cells and were further identified as endothelial cells by factor VIII immunofluorescent staining (Fig. 2). Variability of intensity of the reaction from cell to cell was noted; however, most cells exhibited some degree of specific staining when compared with controls. These cells were negative for actin and suggested that HHE are of non-muscle origin. Contamination by nonspecific esterase-

Human heart endothelial cells

Human heart firbroblast cells

A
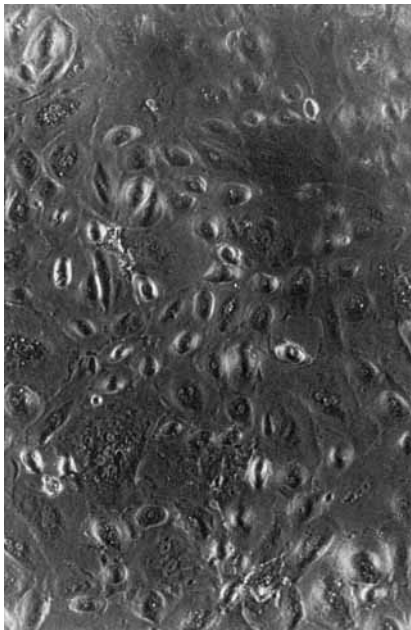
B

Fig. 1. A: Phase-contrast micrograph of phenotypically distinct endothelial cell colonies isolated from endomyocardial biopsies from normal hearts: Seven days in primary culture colonies exhibit well-defined endothelial appearing cells growing in close opposition to one another. The cell population exhibits the typical cobblestone appearance (×200). B: Phase-contrast micrograph (×200) of slow adhering cell population consisting of several spindle-shaped fibroblastic cells 11 days in primary culture: Isolated from myocardial biopsies. The cell population exhibits the typical multilayered hill and valley appearance characteristics of fibroblastic cells (34).
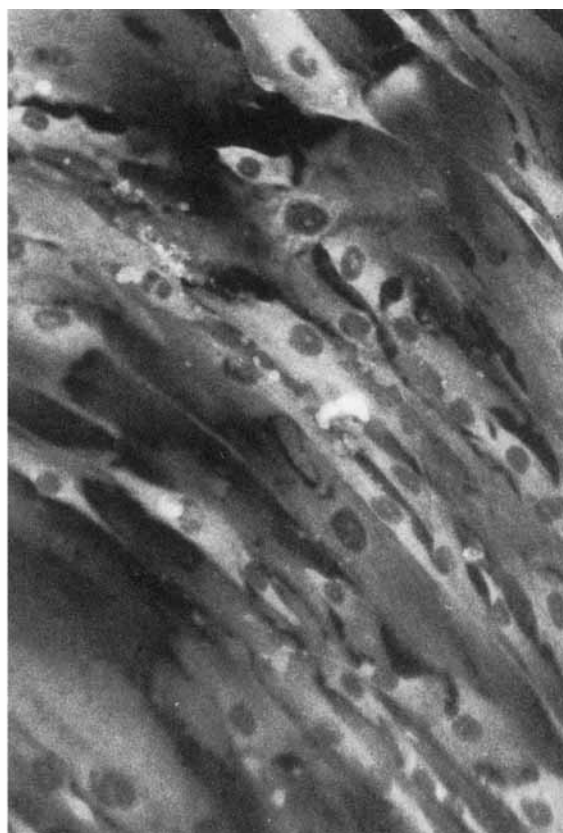
Human Heart Endothelial Cells Positive for Factor VIII

Staining without primary antibody   ## Staining with primary antibody

A   B

Fig. 2.   Indirect immunofluorescent staining for endothelial specific factor VIII-related antigen in endothelial culture (×200). A: Staining with non-immune IgG. B: Staining with primary antibody. Factor VIII stain appears bright and crisp in the cytoplasm of endothelial cells.

positive cells or cells with morphologic features and growth characteristics of monocytes, macrophages, or smooth muscle cells was not observed in our cell culture preparation.

By 1 week, HHF cells seeded on culture dishes showed adhering cell populations consisting of large polygonal spindle-shaped cells under phase-contrast microscopy (Fig. 1) with little or no tendency to grow as tight clusters. The fibroblastic nature of confluent cells as well as their purity were determined by immunofluorescence staining with anti-desmin and antivimentin antibodies. We observed that in a confluent HHF cell preparation only 1–2% of cells were positive with either anti-desmin or α-actin. All other cells were stained positively with antivimentin antibody (Fig. 3) indicating the presence of vi-

mentin-like intermediate filaments in HHF. Our results indicate that the isolated fibroblasts are negative for desmin and α-actin but positive for vimentin. Identification and characterization of these cells were carried out by studying their culture behavior, and by phase contrast microscopy and specific immunostaining.

Maintenance and Cell Growth

The primary cultures of HHF continued to proliferate and by 3 weeks (Fig. 4) confluent monolayers were obtained that exhibited a multilayered hill and valley appearance typical of fibroblast cultures. These HHF were routinely maintained and subcultured at split ratios 1:3 at weekly intervals. The comparative growth curves of early passage (p5) of HHF-parent culture are

Human Heart Fibroblast Cells Positive for Vimentin

Staining without primary antibody   Staining with primary antibody

A   B

Fig. 3. Indirect immunofluorescent staining for vimentin in early passage-HHF parent cell culture (×200). **A:** Staining with non-immune IgG. **B:** Staining with primary antibody. Vimentin appears in the cytoskeletal of the fibroblast cells.

shown in Figure 4. Culture was maintained with and without varying concentrations of FCS. Culture maintained with either 5, 10, or 20% FCS grew at comparable rates. All cultures seemed to have a lag in growth over the first 5–6 days but finally by day 12–13 attained growth levels comparable to each other. Cultures maintained without serum did not achieve efficient confluent levels. Although they failed to grow in serum-free medium, these cells were capable of being maintained for the duration of the experiment. These early passage HHF-parent cultures expressed a population doubling time of 52 h and saturation density of 1×10^5 cells.

Similar growth curves were obtained for HHE. Comparable growth levels were achieved by each HHE culture grown in medium containing 5, 10, and 20% FCS. However, HHE-parent cells exhibited a significant variance in growth level when maintained in medium with no serum or low serum (0.4%) compared with HHF. Interestingly, significant growth levels were achieved in all cultures. HHE-parent cell cultures expressed a noticeably faster population doubling time of
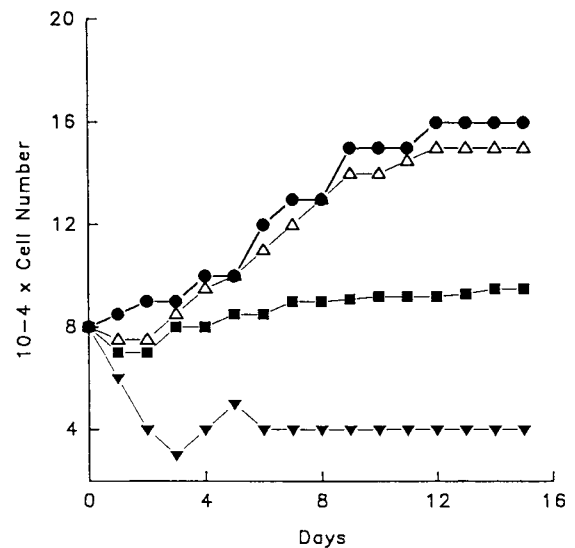

Fig. 4. Growth curves. Cell growth kinetic patterns of early passage (p5) of HHF: Parent cell culture grown in MEM containing 0% (▲—▲), 5% (■—■), 10% (△—△), and 20% (●—●) FCS. Cultures were seeded at an initial concentration of 8×10^4 cells/well. Each point represents the average number of cells obtained from three independent experiments. Culture in serum-free MEM demonstrated growth inhibitory activity.

30 h and higher saturation density of 1×10^5 cells. Twelve to 14 days after initiation of HHE in primary culture the developing colonies were of sufficient size to permit selective experimentation. HHE grew to confluence faster than HHF.

Serum Induction of MMP and TIMP

Cell cultures were capable of supporting growth in limiting serum concentrations, suggesting that these cells produce growth factors in response to serum for cellular proliferation. However, cultures maintained with MEM alone showed a slight decrease in cell number over the 7 days. This suggested that these cells also produce growth inhibitory factors (Fig. 4). To identify serum response to MMP expression in HHF, secreted MMP activity was measured in the medium, and obtained from the cells induced by 0, 5, 10, and 20% serum. Results indicate a dose dependent induction of MMP expression by serum (Fig. 5). This also suggested that the proliferating HHF and HHE are expressing active MMPs. Control cells in serum-free medium show basal activity.

To determine whether serum induces MMP, TIMP, and β-actin expression at the mRNA level, we analyzed MMP-1, TIMP-1, and actin mRNA (Fig. 6). Results suggested that TIMP, MMP, and actin levels were increased ~ 3–4-fold by 20% when compared with 0% serum. This serum induction was inhibited by actinomycin D (a DNA intercolator and transcription elongation inhibitor), suggesting induction at the transcription level. Similar results were obtained in HHF cells.

In order to evaluate the induction of mRNA in relation to the levels of protein synthesis, we carried out Western blot analysis. Based on an immunoblot analysis, apparent TIMP-1 level was found to be increased by serum treatment of HHF (Fig. 7) and this induction was inhibited by cycloheximide. Similar results were observed with HHE cells. Serum-induced TIMP-1 expression was inhibited by cycloheximide (a protein synthesis inhibitor), suggesting the contribution of translational events in TIMP-1 expression. These results suggested transcriptional and translational induction of MMP, TIMP, and actin by serum.

Collagen suspension provides cells a more in vivo like attachment to the basement and therefore produces normal protein expression. We have also cultured HHF attached to collagen

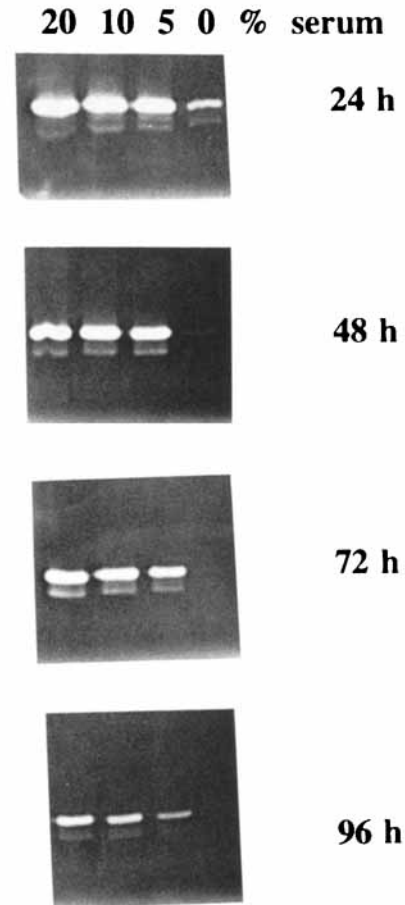## Serum-induced MMPs Expression in Human Heart Fibroblasts

Fig. 5. Zymographic analysis of serum-induced MMP expression: HHF (1×10^5 cells) were grown to confluency and starved in serum-free MEM for 48 h. Serum was added back to the cells at 20, 10, 5, and 0% concentrations. After 24 h, serum was removed and MEM was added. Secreted MMP activity in MEM after 24, 48, 72, and 96 h was detected. Identical total amount of protein was loaded onto each lane.

matrix and in suspension (Vitrogen-100) in culture dishes. This attachment is possible through intgrin molecules, as reported in the literature, mimicking an in vivo situation. We observed similar expression of MMP as obtained with the cell cultured on a plastic surface. This suggested that serum-induced expression of MMPs is not altered by extracellular collagen matrix in our experiments.

To examine the effect of serum with respect to normal cell growth and MMP expression, serum was added to normal subconfluent cultures of

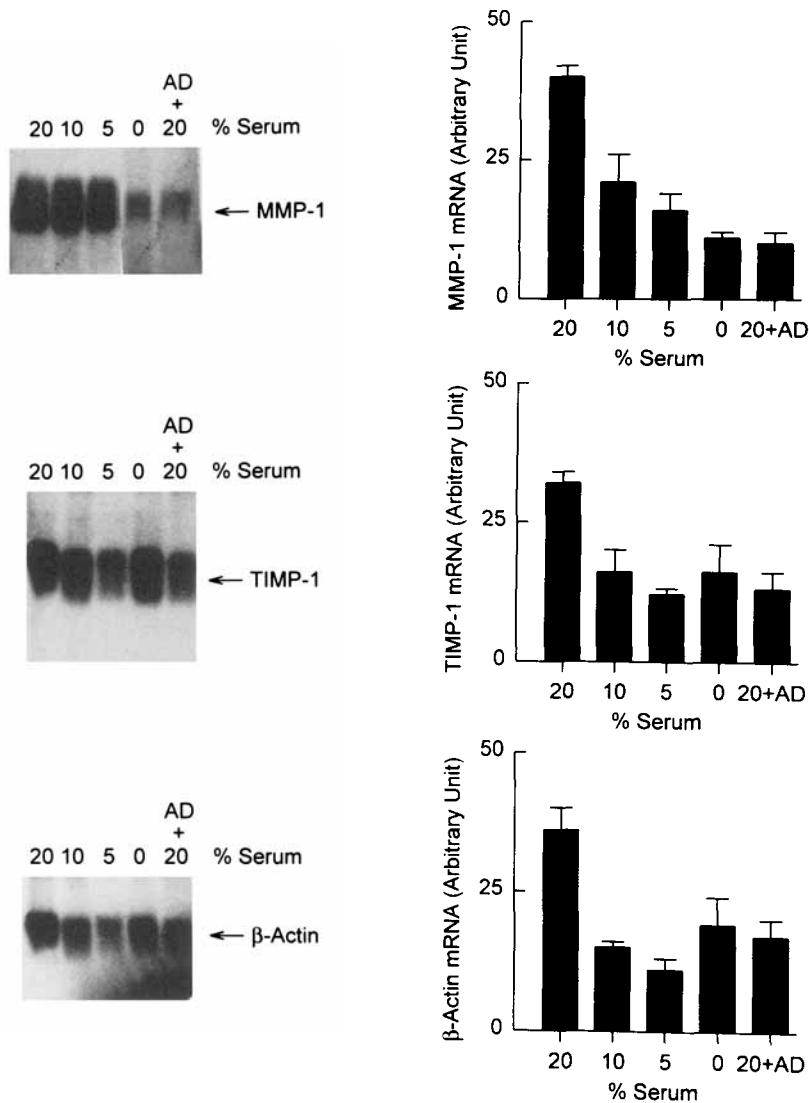## MMP and TIMP Expression in Human Heart Endothelial Cells

Fig. 6. Northern blot (mRNA) analysis of MMP, TIMP, and β-actin induction by serum: HHE (1 × 10⁶ cells) were grown to confluency and starved in serum-free MEM for 24 h. Serum was added back to the cells at 20, 10, 5, and 0% concentrations. Cells were incubated with 20% serum plus 0.5 mM actinomycin D (AD) to inhibit serum-induction. After 24 h cells were removed. Total RNA was isolated and separated by agarose gel, transferred to nitrocellulose membrane, and hybridized with cDNA probe for MMP-1, TIMP-1, and β-actin. Identical amount of total RNA was loaded onto each lane. Histographic representation of the scanned data (arbitrary unit) is shown. Results suggest dose-dependent induction of the MMP, TIMP, and β-actin expression at the mRNA level by serum and this induction was inhibited by actinomycin D. Mean value ± SD of triplicates are reported.

HHF and HHE. Indirect immunofluorescent staining of these cells with proMMP-1 and TIMP-1 antibodies was carried out. The staining exhibited similar patterns of bright fluorescence throughout the cell cytoplasm (Figs. 8 and 9). This suggested a continuous pool of MMP and TIMP from cell to the medium. No fluorescent staining was noted without primary antibodies, suggesting specific labeling by the antibodies. These results indicate serum-induced expression of MMP and TIMP in the cytoplasm of HHE and HHF.

DISCUSSION

To study the specific regulation of human cardiovascular matrix turnover at the cellular
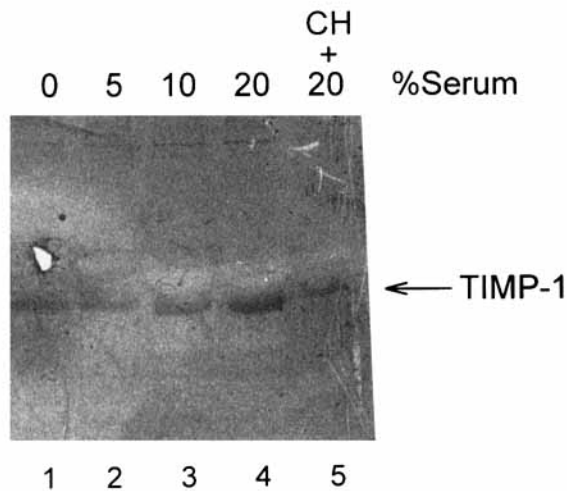
Immuno-blot Analysis of TIMP

Fig. 7. Immunoblot analysis of TIMP induction by serum: HHF (1 × 10^6 cells) were grown to confluency and starved in serum-free MEM for 24 h. Serum was added back to the cells at 20, 10, 5, and 0% concentrations. Cells were incubated with 20% serum plus 0.1 mM cycloheximide (CH) to inhibit serum-induction. After 24 h serum was removed and MEM was added. Secreted proteins in MEM after 24 h were analyzed for TIMP-1. An identical amount of total protein was loaded onto each lane in SDS-PAGE (10%) prior to transfer onto nitrocellulose membrane for immunoblot. The apparent level of TIMP was increased by serum induction and inhibited by cycloheximide.

level, we have isolated HHE and HHF cells. HHF were positive for vimentin but negative for actin and HHE were positive for factor VIII. We show evidence that serum induces expression of MMP and TIMP in HHF and HHE at the mRNA and at the protein levels (Figs. 6 and 7). MMP and TIMP were coexpressed and localized in the cytoplasm of these cells in response to serum (Figs. 8 and 9). Secretion of these proteins was also increased in response to serum induction (Fig. 5). However, in the absence of serum these cell produce growth inhibitory factors (Fig. 4).

Cultures of endothelial cells and fibroblasts from endomyocardium are important in vitro models for studying cardiovascular cell functions. It is well recognized that there are functional differences between vascular cells derived from different tissues (17,20–22). Previously, we have shown enhanced endomyocardial MMP activity in dilated cardiomyopathy compared to normal tissue and proposed MMP activation as a marker of ventricle dilatation (12). By employing similar criteria of MMP activation to identify normal and disease tissue, we isolated cells

from normal tissue. This report is the first to describe HHE and HHF cell populations derived from human endomyocardium. These cell cultures were characterized as endothelial and fibroblasts by their in vitro growth behavior and immunohistochemical criteria.

HHF and HHE cell cultures maintained with varying serum concentrations were found to exhibit similar growth patterns (Fig. 4). These data suggest that HHE and HHF cell cultures produce factors in response to serum that can support growth of these cells. Also, the data shown in Figure 4 suggest the presence of growth inhibitory factors in serum-free HHF-derived condition medium which may inhibit cell growth and proliferation. Our observation is in agreement with similar studies reported by Gajdusek et al. [23–25], on cardiovascular cells. These authors have shown that cells secrete not only factor(s) that are capable of supporting growth in limiting serum concentration, but also have additional factor(s) which are mitogenic to these cells. Diglio et al. [17] have indicated that mitogenic activity was more pronounced in resistant vessel cells than aortic cells, suggesting a selective regional cellular response in cardiovasculature. Therefore, our cell culture from endomyocardium and interstitium may be a useful tool to study such regional responses in the myocardium.

Serum can induce early and late gene expression [26,27]. The gene for tissue factor, a primary initiator of the proteinase cascades in circulation, was shown to be induced by serum through the serum responsive element in fibroblasts and endothelial cells [28]. Serum-starved cells were shown to increase procoagulant activity by increasing tissue factor activity by the addition of serum as a source of growth factors. These authors have suggested a new role of tissue factor in cell growth [28]. Serum can also induce cardiac specific genes by increasing transcription factor activity to bind serum responsive element (SRE) sequences in the DNA [29,30]. Collagenase, gelatinase, and stromelysin synthesized by human skin fibroblasts cultured on three models of tridimensional matrix: native collagen sponge, native collagen complexed with glycosaminoglycans sponge, and acellular sarcoid matrix complex prepared from human sarcoid granulomas were differentially stimulated [31] by serum. The same amount of collagenase or stromelysin was secreted when

Human Heart Endothelial Cells Expressing Matrix Metalloproteinases [MMPs] and Tissue Inhibitors of Metalloproteinases [TIMPs]

Staining without Primary Antibody	Staining with Primary Antibody to ProMMP-1	Staining with Primary Antibody to TIMP-1

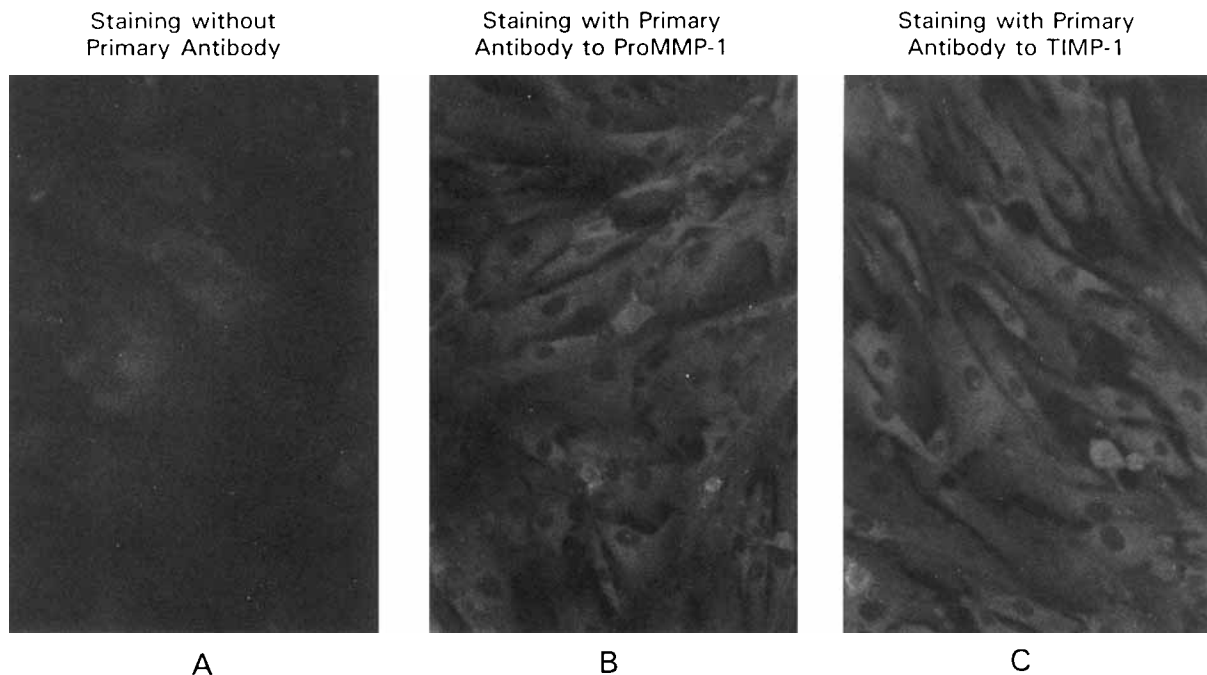A   B   C

Fig. 8. Immunofluorescent photomicrographs of human endomyocardial endothelial cells. Confluent quiescent cardiac endothelial cells in 20% serum plus MEM were washed with PBS two times and fixed and permeabilized with absolute methanol for 7 min. Cells were then stained with antibody (1:50 dilution) to proMMP-1 and TIMP-1. A: staining with preimmune serum. B: proMMP-1 expression after 24 h treatment with FCS. C: TIMP-1 expression after 24 h treatment with FCS (×200). Cultured HHE cells expressing MMP and TIMP.

cells were cultured on collagen gel matrix [31]. These results suggest that collagenase and stromelysin synthesis are coregulated while gelatinase production was controlled by a different mechanism. Brenner et al. [32] have demonstrated that genes for extracellular matrix (ECM) degrading MMPs and their inhibitor, TIMP, are expressed during early mammalian development. Ostrowski et al. [33] have shown gene expression pattern for secretion of MMP during late stages of tumor progression. However, it is not known whether serum can induce MMP and TIMP in cardiac fibroblasts. We show evidence that serum can not only induce growth and proliferation of HHE and HHF but can also induce the synthesis and coexpression of MMPs/TIMPs at the mRNA and protein levels (Figs. 5–7). These proteins were identified in cytoplasm and in the secretion of these cells.

In summary, examination of regional cardiovasculature is important since cardiovascular cells derived from different species and also from different sites are likely to exhibit morphologic, functional, biochemical, and molecular heterogeneity. Our culture preparations provide the opportunity to explore the experimentation of the coculture derived from the same organ (i.e., cardiovasculature) and further the biology of cardiovascular cell-cell interactions. In addition, the demonstration of mitogenic and growth inhibitory activity expressed in HHE and HHF cell cultures will enable us to explore the nature and the regulation of this response.

ACKNOWLEDGMENTS

Human Heart Fibroblast Cells Expressing Matrix Metalloproteinases [MMPs] and Tissue Inhibitors of Metalloproteinases [TIMPs]

Staining without Primary Antibody	Staining with Primary Antibody to ProMMP-1	Staining with Primary Antibody to TIMP-1

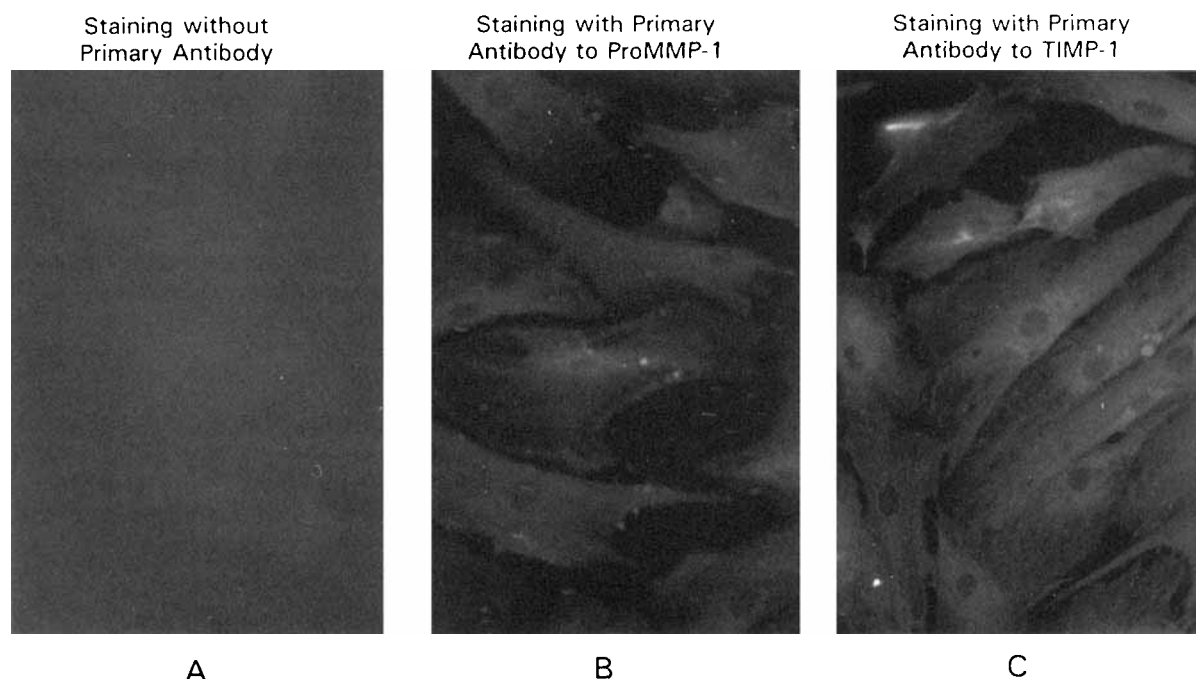A   B   C

Fig. 9.   Immunofluorescent photomicrographs of human cardiac fibroblasts. Confluent quiescent cardiac fibroblasts in 20% serum plus MEM were washed with PBS two times and fixed and permeabilized with absolute methanol for 7 min. Cells were then stained with antibody (1:50 dilution) to proMMP-1 and TIMP-1. A: Staining with preimmune serum. B: ProMMP-1 expression after 24 h treatment with FCS. C: TIMP-1 expression after 24 h treatment with FCS (×200). Cultured HHF cells expressing MMP and TIMP.

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