Integrin shedding as a mechanism of cellular adaptation during cardiac growth

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Submitted 25 October 2002; accepted in final form 3 February 2003

Integrin shedding as a mechanism of cellular adaptation during cardiac growth. Am J Physiol Heart Circ Physiol 284: H2227–H2234, 2003. First published February 6, 2003; 10.1152/ajpheart.00920.2002.—Integrin-mediated cell-extraacellular matrix (ECM) interactions are essential for multiple cellular processes; however, little is known regarding integrin turnover during these events. Recent studies have demonstrated shedding of cell surface molecules and suggested this as a potential mechanism for integrin turnover. Confocal microscopy of mouse hearts under different physiological conditions demonstrated the presence of β1-integrin-immunoreactive material in the interstitium. Culture media from neonatal rat cardiac myocytes and fibroblasts contained a 55-kDa fragment of β1-integrin. Attachment to ECM components, response to phorbol 12-myristate 13-acetate stimulation, and matrix metalloproteinase inhibition assays demonstrated that fibroblasts responded differently to the fragment compared with myocytes. The β1-integrin fragment stimulated myocyte attachment to collagen and the fragment itself bound a variety of ECM proteins. These studies indicate that as myocytes and fibroblasts change size and shape, cellular contacts with the ECM are altered, resulting in the liberation of a β1-integrin fragment from the cell surface. Integrin shedding may represent a novel mechanism of rapidly modifying cell-ECM contacts during various cellular processes.

integrins; hypertrophy; extracellular matrix; metalloproteinases

THE EXTRACELLULAR MATRIX (ECM) in the heart consists of interstitial collagens, proteoglycans, glycoproteins, and proteases that are arranged in a precise, three-dimensional network associated with myocytes and capillaries (4). Of these components, the arrangement of interstitial collagen has received the most attention because of the important roles of the collagen network in main-
and would provide a focused mechanism for regulating specific functions. In addition, the shed fragment could bind to cells or ECM components or be involved in signaling biological events involved in cellular growth and remodeling.

Increasing biochemical and morphological evidence of ectodomain shedding from membrane-anchored proteins indicates that this may be a relatively common process (21, 26, 27). However, the physiological role of many of these shed ectodomains remains to be defined. The ectodomains can be detected in fluids associated with tissue injury and repair, which has led to the hypothesis that these proteins can function in wound healing, host defense, arthritis, and development (11).

Extracellular proteinases may potentially be responsible for proteolytic cleavage of membrane proteins; however, only a few specific types have been identified. Recent studies indicate that matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase (ADAM) are potential candidates (2, 3, 19, 27). These studies, together with recent immunohistochemical data (10), have lead to the hypothesis that integrin shedding resulting from proteolysis is critical to cell growth. The goal of this study is to characterize the β1-integrin shed fragment observed to be associated with the ECM (10) and to begin experiments aimed at defining a physiological role for the shed fragment.

MATERIALS AND METHODS

Antibodies. Multiple monoclonal and polyclonal (AB314, AB1292, AB1134, and AB1501) antibodies were used to examine integrin shedding (Table 1). To confirm that the 55-kDa fragment was indeed a portion of β1-integrin, two mouse anti-human β1-integrin monoclonal antibodies (MAB2251 and MAB2252, Chemicon, Temecula, CA) that recognize amino acids 648–670 and 15–54, respectively, in the extracellular region of β1-integrin were used in Western blotting.

Confocal microscopy and image analysis. The degree of β1-integrin staining in the ECM of hearts undergoing hypertrophy and dilatation was assessed using confocal fluorescence microscopy as previously described (10). Briefly, 100-μm vibratome sections from mouse hearts subjected to aortic stenosis (AS) (10) or hearts from tropomodulin overexpressing transgenic (TOT) mice (24) were stained with AB1134 (diluted 1:200) (16). Sections were rinsed with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, and 2 mM KH2PO4; pH 7.4) and allowed to polymerize for 1.5 h at 37°C, after which time serum-free medium (10 ml) was collected and frozen in one uniform direction. Gels containing 1 ml of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4) were prepared on 60-mm2 culture dishes as previously described (6) and cultured in DMEM supplemented with 10% newborn bovine serum, 5% fetal bovine serum (Atlanta Biological; Atlanta, GA), 100 U/ml penicillin G, 100 μg/ml streptomycin, 1 μg/ml amphotericin B, and 10 μg/ml gentamicin (Sigma) until 80% confluent. Cells were washed twice in Moscona’s buffer and serum starved for 24 h in DMEM/F-12 media (Sigma) before being used in experiments. All fibroblasts used in the experiments described below were between passages 2 and 4.

Collagen substrate preparation. Thin, aligned collagen gels were prepared on 60-mm2 culture dishes as previously described (22). A neutral collagen solution was prepared by combining 200 mM HEPES (pH 9), 10× minimal essential medium (Sigma), and bovine dermal collagen type I (Cohesion Technology; Palo Alto, CA) [1:1:8 (vol/vol/vol)] and placed on ice. The collagen stock solution was coated on dish surfaces and allowed to flow in one uniform direction. Gels were tilted at an angle in the direction of the collagen flow and allowed to polymerize for 1.5 h at 37°C, after which time they were rinsed with Moscona’s buffer and dialyzed overnight.

Immunoprecipitation and purification of the β1-integrin fragment. Serum-free medium (10 ml) was collected and concentrated to a final volume of 2 ml, followed by immunoprecipitation with 5 μl/ml AB314 and 100 μl/ml Protein G and were collected on a Bio-Rad MRC 1024 confocal scanning laser microscope (Hercules, CA).

Table 1. Anti-β1-integrin antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Antigen/Epitope</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB314</td>
<td>Polyclonal goat anti-β1-integrin</td>
<td>Rat β1-integrin (whole molecule)</td>
</tr>
<tr>
<td>AB1292</td>
<td>Polyclonal rabbit anti-β1-integrin</td>
<td>Rat β1-integrin (whole molecule)</td>
</tr>
<tr>
<td>AB1134</td>
<td>Polyclonal rabbit anti-β1-integrin</td>
<td>Rat β1-integrin (whole molecule)</td>
</tr>
<tr>
<td>AB1501</td>
<td>Polyclonal rabbit anti-55-kDa β1-integrin shed fragment</td>
<td>Purified rat 55-kDa β1-integrin fragment</td>
</tr>
<tr>
<td>MAB2251 (Chemicon)</td>
<td>Mouse anti-human β1-integrin monoclonal</td>
<td>Human β1-integrin/amino acids 648–670</td>
</tr>
<tr>
<td>MAB2252 (Chemicon)</td>
<td>Mouse anti-human β1-integrin monoclonal</td>
<td>Human β1-integrin/amino acids 15–54</td>
</tr>
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</table>
Sepharose (Amersham Pharmacia Biotech; Piscataway, NJ) before analysis by SDS-PAGE and Western blotting. Media from both myocytes (n = 3 dishes) and fibroblast (n = 3 dishes) cultures were collected and subjected to centrifugation at 3,000 rpm using a JA-10 rotor for 15 min at 4°C to remove any cell debris. Media were then concentrated by dialyzing against polyethylene glycol (Sigma) and protein concentrations were determined using the bicinchoninic acid (BCA) assay (Fierce Chemical; Rockford, IL). A total of 50 mg of media protein was lyophilized, resuspended in PBS, and subjected to preparative SDS-PAGE using Bio-Rad 490 Prep Cell. Fractions were screened for the 55-kDa protein believed to be the shed portion of β1-integrin. All fractions containing the fragment were combined and dialyzed in PBS containing 0.01% sodium azide and concentrated using Centricron-plus 20 protein concentrators (30,000 MWCO, Fisher Scientific; Springfield, NJ). Protein concentrations were determined as described above. Protein samples were separated on 10% SDS-PAGE gels and either silver stained or transferred to nitrocellulose membranes (0.45 μm, Bio-Rad) for Western blotting. To confirm that the 55-kDa fragment was indeed a portion of β1-integrin, two mouse anti-human β1-integrin antibodies (MAB2251 and MAB2252, Chemicon) that recognize amino acids 648–670 and 15–54, respectively, in the extracellular region of β1-integrin were used in Western blotting.

Sequence analysis of the shed fragment was performed using previously described procedures (28). Briefly, the shed fragment and concentrated media were run on two-dimensional SDS-PAGE, transferred to nitrocellulose, and Western blotted with AB314. Tryptic digestion and peptide mass analysis were performed on spots and compared with predicted masses for trypsin-digested β1-integrin using SwissProt.

Phorbol ester perturbation. To determine the extent to which integrin shedding can be stimulated, myocytes and fibroblasts were plated on 60-mm² dishes and incubated for 24–48 h. Cells were serum starved for 24 h at 37°C before experiments. For both myocytes and fibroblasts, serum-free media were treated with phorbol 12-myristate 13-acetate (PMA; 100, 250, and 500 ng/ml, Sigma) for 24 and 48 h at 37°C. Controls consisted of treatment with only the vehicle. Media were collected as described above and analyzed for the shed fragment by Western blotting with AB314. The resulting bands were quantified using the GelDoc system (Bio-Rad) and changes in shedding are expressed as a percentage of the control.

MMP inhibition. To determine the extent to which integrin shedding can be inhibited, myocytes and fibroblasts were plated on 60-mm² dishes and incubated for 24–48 h. Cells were serum starved for 24 h at 37°C before experiments. For both myocytes and fibroblasts, the MMP inhibitor GM6001 (Chemicon) was added to serum-free media at concentrations of 5 nM, 50 nM, 500 nM, and 5 μM. After 24 h of culture, media were collected and analyzed for the shed fragment as described above.

Isolation of membrane proteins from AS mouse hearts. Atria were removed from freshly isolated hearts, and membrane proteins were isolated as previously described (16). Membrane proteins were solubilized in PBS containing 1% Triton X-100, 1 mM MnCl₂, and CompleteMini protease inhibitor (Roche; Mannheim, Germany). Protein concentrations were determined using the BCA assay described above. Equal amounts of protein (10 μg/sample) were loaded onto 4–15% gradient gels (Bio-Rad) and subjected to SDS-PAGE. For Western blotting, proteins were transferred to 0.45-μm nitrocellulose membranes (Bio-Rad) for analysis of β1-integrin using AB1292.

Antibody production and purification. Polyclonal antibodies directed against the isolated 55-kDa protein were generated as previously described (25). New Zealand White rabbits were first bled to obtain preimmune antibodies and subsequently injected with 100 μg of the isolated protein. Antibodies were purified using a Protein A-Sepharose (Amersham Pharmacia Biotech) affinity column and the antibody concentration was determined spectrophotometrically.

To confirm antibody reactivity, nitrocellulose membranes bearing the 55-kDa fragment were incubated in blocking buffer (5% wt/vol fat-free milk in PBS-0.1% Tween 20 (PBS-T)) for 2 h at room temperature. Membranes were incubated for 1 h with various antibody bleeds (diluted 1:500 in blocking buffer) to test antibody specificity. After being washed with PBS-T, membranes were incubated in donkey anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase (Amersham Pharmacia Biotech) diluted 1:2,000 in PBS-T. Membranes were washed in PBS-T and bands were visualized using enhanced chemiluminescence detection (Amersham Pharmacia Biotech).

Adhesion assay. Adhesion assays were used to measure the attachment of myocytes and fibroblasts to attach to various ECM components (6). Wells of a 24-well plate were coated with ECM proteins (50 μg/ml collagen, 10 μg/ml laminin, and 10 μg/ml fibronectin) by incubation at 4°C overnight and subsequently rinsed with buffer 3 (137 mM NaCl, 5 mM KCl, 0.6 mM MgSO₄, 2 mM CaCl₂, and 10 mM HEPES; pH 7.4). Before wells were seeded, each well was incubated in 2 mg/ml BSA in buffer 3 for 1 h at 37°C to prevent nonspecific attachment. Myocytes and fibroblasts were preincubated with 100 μg/ml of the 55-kDa β1-integrin fragment for 30 min at 4°C before being plated. Plates were rinsed again with buffer 3 and, cells (500,000 myocytes and 200,000 fibroblasts) were added to each well and allowed to attach for 1 h at 37°C. Nonattached cells were removed by rinsing with buffer 3, after which 500 μl were added to each well. A standard curve was generated by plating 10,000–500,000 myocytes and 10,000–200,000 fibroblasts into wells. Substrate buffer (50 mM sodium citrate (pH 5.0), 0.25% Triton X-100, 4 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide; Sigma) was added to each well and the plate was incubated for 3.5 h at 37°C (1.5 h for fibroblasts). After incubation, aliquots were transferred to wells in a 96-well plate and development/stop buffer (50 mM glycine and 5 mM EDTA; pH 10.4) was added. The absorbance at 405 nm was read immediately using a Bio-Rad Benchmark microtiter plate reader. The number of cells attached was determined by comparison to the standard curve and expressed as the total number of cells attached.

ELISA to examine 55-kDa fragment binding to the ECM. The wells of a 96-well plate were coated with various ECM proteins (50 μg/ml collagen, 10 μg/ml laminin, and 10 μg/ml fibronectin) overnight at 4°C, followed by PBS-T washes and blocking in 2 mg/ml casein for 1 h at 37°C. The 55-kDa shed fragment (100 μg/ml) was added and incubated with ECM substrates for 1 h at 37°C. The wells were rinsed with PBS-T and incubated with AB1501 diluted 1:250 in PBS-T for 1 h at 37°C. Wells were washed twice with PBS-T and incubated with donkey anti-rabbit IgG antibody conjugated to horseradish peroxidase (1:5,000, Amersham Pharmacia Biotech) for 1 h at 37°C. Wells were washed twice in PBS-T, and detection was accomplished using 2,2’-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) tablets (Sigma) dissolved in citrate-phosphate buffer (pH 5.0). Absorbance was read at 405 nm using the Benchmark microtiter plate reader (Bio-Rad).

Statistical methods. Statistical significance was determined using Student’s t-test with significance at P < 0.05. All values are reported as means ± SE. AJP-Heart Circ Physiol • VOL 284 • JUNE 2003 • www.ajpheart.org
RESULTS

Confocal microscopy demonstrating \( \beta_1 \)-integrin immunoreactive material in the ECM. By confocal microscopy, \( \beta_1 \)-integrin staining was observed at the Z lines on cardiac myocytes and in the extracellular space of mouse hearts from two different models (Fig. 1). Stacked Z-series images of hearts stained with AB1134 suggest that the immunoreactive material in the extracellular space is not bound to any cell surface. An increase in staining was observed in mouse hearts that developed cardiac hypertrophy in response to AS (10) (Fig. 1, A and B). In the early stages of hypertrophy (4 wk post-AS), \( \beta_1 \)-integrin localization was increased in the extracellular space compared with control animals (data not shown). During this time, myocyte size has been previously shown to increase compared with control (10). After 8 wk post-AS with continued hypertrophy, increased staining was observed prominently around areas of myocyte branching (Fig. 1A) and the intensity of staining in the extracellular space was visibly greater than age-matched controls (Fig. 1B). The amount of immunoreactive material detected in the ECM of 8 wk post-AS animals was greater than that observed at 4 wk post-AS (Table 2).

In TOT mice, the heart becomes dilated and the myocytes become long and slender compared with control hearts (23, 24). Hearts from TOT animals show a thinning of the ventricular wall beginning by day 8 of neonatal development. Staining of 3-wk hearts with AB1134 showed slightly decreased staining in TOT animals compared with transgene-negative littermate controls (Fig. 1, C and D). Clear differences can be seen in the size of the myocytes and in the distribution of the \( \beta_1 \)-integrin immunoreactive material in the extracellular space. The myocytes in the TOT animals (Fig. 1C) were shown to be thinner and more elongated compared with age-matched controls (Fig. 1D).

Table 2. Quantitation of \( \beta_1 \)-integrin shedding during cardiac disease

<table>
<thead>
<tr>
<th></th>
<th>Pixels</th>
<th>Fold Increase</th>
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<tbody>
<tr>
<td>AS mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,900</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>7,036</td>
<td>3.7</td>
</tr>
<tr>
<td>4 wk</td>
<td>15,559</td>
<td>2.2</td>
</tr>
<tr>
<td>8 wk</td>
<td>31,541</td>
<td>4.5</td>
</tr>
<tr>
<td>TOT mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>110,000</td>
<td>0</td>
</tr>
<tr>
<td>3-wk dilatation</td>
<td>71,000</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Controls for aortic stenosis (AS) mice were age-matched animals, whereas shams were age-matched control animals subjected to mock surgery; AS mice were studied at 4 and 8 wk after surgery. Controls for tropomodulin-overexpressing transgenic (TOT) mice were age-matched nontransgenic littermates.
demonstrated a more diffuse surface-associated β₁-integrin staining pattern compared with controls (Fig. 1D).

Quantitative analysis of the immunoreactive material detected by AB1134 in the hypertrophic and dilated hearts showed distinct differences compared with controls and shams (Table 2). In the AS model, a small amount of β₁-integrin immunoreactive material was evident in the interstitium of control and sham animals with increases in staining evident in 4 and 8 wk post-AS animals. In the TOT model, there was a decrease in the β₁-integrin staining in the interstitium of the 3-wk animals. This correlates to a period of myocyte elongation (Table 2). These data clearly document the quantitative as well as qualitative changes in the amount of β₁-integrin immunoreactive material in the ECM as the myocytes changed shape in vivo.

Biochemical characterization of the shed β₁-integrin fragment. By Western blotting with AB1292, a low-molecular-mass β₁-integrin positive material was observed in the initial pellet of membrane preparations from 8-wk AS hearts, whereas only intact integrins were associated with the membrane fraction (Fig. 2A). To examine integrin shedding in vitro, the media from cultures of neonatal cardiac fibroblasts and myocytes were immunoprecipitated with AB314 to isolate the shed β₁-integrin fragment. Media from myocytes grown on aligned collagen or from fibroblasts showed the presence of a β₁-integrin immunoreactive component with a relative molecular mass (Mr) of 55 kDa under nonreducing conditions (Fig. 2B). This 55-kDa material was detected with AB1292 prepared against intact β₁-integrin and AB1501 against the 55-kDa fragment (Fig. 2B). The higher-molecular-mass proteins observed in these blots are attributed to intact β₁-integrin. To confirm that the 55-kDa fragment was shed from β₁-integrin, commercial monoclonal antibodies with defined epitopes at the NH₂-terminal (amino acids 15–54, MAB2252) and COOH-terminal (amino acids 648–670, MAB2251) regions of the β₁-integrin extracellular domain were used (Fig. 2C). Both antibodies recognized the purified shed fragment in Western blotting, indicating that this fragment corresponded to the extracellular region of rat β₁-integrin. In addition, two-dimensional SDS-PAGE, followed by Western blotting with AB314 was also used to confirm the 55-kDa fragment was derived from β₁-integrin (Fig. 2D). β₁-Integrin fragments from two-dimensional gels were subsequently subjected to trypsin digestion and mass analysis, producing molecular masses that would be expected for β₁-integrin (data not shown).

The regulation and function of shedding is likely to be different in fibroblasts and myocytes because fibroblasts are motile cells that change position and hence change contact with the ECM more than stationary myocytes. To determine whether integrins were shed in response to growth stimuli, myocytes and fibroblasts were cultured separately in the presence of PMA (Fig. 3, A and B), which has been shown to induce cardiomyocyte hypertrophy. Neonatal cardiac myocytes showed a significant (P < 0.05) increase in shedding in response to PMA stimulation (Fig. 3A), whereas PMA had no significant affect on cardiac fibroblasts (Fig. 3B). These data clearly showed that as myocytes underwent hypertrophic growth, in vitro shedding was significantly increased. PMA does not affect fibroblas-

![Fig. 2. Western blot analysis for shed β₁-integrin. A: Western blot of membrane preparation fractions from AS and control mice using AB1292 demonstrates the presence of a low-molecular-mass β₁-integrin fragment in the pellet fraction not observed in the membrane fraction. B: Myocyte and fibroblast culture media were subjected to immunoprecipitation with AB314 and subjected to Western blot analysis for the shed β₁-integrin fragment. C: Western blotting of the purified 55-kDa shed fragment using commercial monoclonal antibodies that recognize amino acids 15–54 (MAB2252) or 648–670 (MAB2251) in the extracellular domain of human β₁-integrin. D: Western blot of shed fragment subjected to two-dimensional SDS-PAGE with AB314 detected one spot with an approximate molecular mass of 55 kDa.](http://ajpheart.physiology.org/doi/10.1152/ajpheart.00148.2002)
tic growth in the same manner and little change was observed in the amount of shedding.

To determine whether shedding was affected by inhibition of MMPs, separate cultures of neonatal myocytes and fibroblasts were treated with various concentrations of the MMP inhibitor GM6001, which has previously been shown to block shedding of other receptors (14). Over 24 h, neonatal myocytes showed no change in shedding (Fig. 4A); however, fibroblasts showed a clear dose-dependent inhibition of shedding (Fig. 4B). These data support the suggestion that fibroblasts may have a different mechanism responsible for shedding than neonatal myocytes.

Attachment of myocytes and fibroblasts to ECM components in the heart is required to maintain cell function and survival. Adhesion assays were used to ascertain whether the 55-kDa β1-integrin fragment could interfere with the attachment of these cells to ECM components. In adhesion assays, addition of the shed fragment promoted attachment of myocytes to collagen but had no effect on attachment to fibronectin or laminin (Fig. 5A). However, in fibroblasts, adhesion appeared to decrease on all substrates tested in the presence of the fragment, although these changes were not statistically significant compared with controls (Fig. 5B). These data suggest that the β1-integrin fragment may alter cellular interactions with the ECM either by binding of the fragment to ECM components, therefore blocking cell surface integrins from binding, or by fragment binding to cell surface proteins, perhaps integrins, and preventing interaction with the ECM directly. An ELISA was used to determine whether the fragment could preferentially bind particular ECM components (Fig. 6). The 55-kDa fragment demonstrated the greatest interaction with collagen, followed by laminin and fibronectin. This result clearly indicates that the shed β1-integrin fragment maintains the ability to bind ECM proteins and may therefore modulate cellular behavior through a blocking mechanism.

DISCUSSION

While receptor-mediated cellular interactions with the ECM are dynamic events, the orderly movement and organization of cells during development and cellular adaptation to pathophysiological signals requires that cell-ECM contacts change in a very concerted manner; however, the mechanisms that regulate such behavior are unknown. Recent studies (10) suggest that the loss of integrins from the cell surface may provide one mechanism to modulate cell-ECM interactions. The data presented here demonstrate that the extracellular domain of β1-integrin can be shed into the extracellular environment both in vivo and in vitro. Both cardiac fibroblasts and myocytes demonstrate shedding in vitro; however, the regulation and function...
of the shed ectodomain may be different in each cell type.

During cardiac disease, myocytes undergo shape and size changes. Interstitial collagen attaches to the cell at or near the Z line (8), and for the cell to change in size or shape these sites of collagen attachment must be modified. Analysis of the confocal data presented indicates a significant difference in the amount of shed integrin in the extracellular space surrounding hypertrophying myocytes (AS model) compared with elongated myocytes from the TOT model (Table 2). During cardiac hypertrophy, integrin expression increases (25) and, although these cells are changing in size, the increased amount of integrin present on the cell surface could accommodate the increased shedding of β₁-integrin observed in the AS model. As myocytes elongate during cardiac dilation, the surface area of these cells increases (unpublished observations), which could spatially segregate integrins from cell surface enzymes that may be responsible for integrin shedding. This protein segregation would lead to the reduced levels of integrin shedding observed in the TOT model. The data presented demonstrate that integrins are shed into the ECM, allowing for modulation of cell shape (Figs. 1 and 2). These data indicate that myocyte cell growth does not involve total cellular release from the ECM, but in fact these cells maintain some degree of constant contact with the ECM. This contact with the ECM is necessary for cell viability as release from the ECM can lead to anoikis, an anchorage-dependent form of apoptosis (13). It has recently been proposed that anoikis may be responsible for the slight increases in myocyte apoptosis observed in the late stages of cardiac hypertrophy when abnormal myocyte connections with the ECM were observed (10).

The enzyme(s) responsible for β₁-integrin shedding remains unknown. Two protein families believed to be essential in shedding are MMPs and ADAMs. Several members of both families have been shown to be present in the heart, but which enzymes are involved in integrin shedding remain unclear. Fibroblast shedding of the ectodomain was significantly reduced by GM6001, which inhibits MMP-1, -2, -3, -8, and -9, whereas myocyte shedding was not affected (Fig. 4, A and B). This would allow each cell type to respond differently to the same signal. Further experimentation is clearly necessary to determine the role of specific proteases on each cell type. Recently, ADAM-12-mediated cleavage of heparin-binding epidermal growth factor has been demonstrated to have a role in cardiac hypertrophy (1). Although integrins have been shown to associate with specific members of the ADAM protein family through the disintegrin domain (9), no evidence for cleavage of an integrin by ADAM proteins has yet been reported. A related metalloproteinase containing a disintegrin domain, jararhagin, has been shown to directly bind α₂β₁-integrin and specifically cleave the β₁-integrin subunit (20).

The function of the 55-kDa shed β₁-integrin fragment remains unclear. Potentially, the fragment may provide a feedback signal to regulate receptor distribution on the cell surface. Another potential function supported by our data is that the shed fragment binds to specific ECM components in turn enhancing or inhibiting some cellular functions. The data presented demonstrate that, whereas the 55-kDa β₁-integrin fragment slightly increased myo-
cyte adhesion to collagen, this fragment has no significant affect on fibroblast adhesion to ECM proteins (Fig. 5, A and B). These observed differential effects of the shed fragment may be due to the presence of different ECM receptors on the cell surfaces of myocytes and fibroblasts through which the fragment may be altering cellular adhesion. The increase in myocyte attachment may be due to the 55-kDa β1-integrin fragment binding collagen and then interacting with other receptors on the surface of myocytes, therefore increasing the number of cell-ECM interactions. In addition, the shed β1-integrin fragment is capable of interacting with collagen, laminin, and fibronectin (Fig. 6) independent of association with other proteins. Previous studies examining ADAM-17-mediated shedding of TNF-α demonstrated that as a result of shedding and binding to fibronectin, ECM-bound TNF-α was capable of inhibiting chemotactic stimulated migration of T cells (12). Whether similar mechanisms occur that regulate fibroblast movement or some aspect of myocyte-ECM interactions remains unclear.

Integrin shedding represents an exciting, novel mechanism by which cell-ECM interactions can be modulated. The data presented demonstrate potential functional roles that the shed β1-integrin fragment may exert on both cardiac myocytes and fibroblasts. Further experiments are necessary to clarify the functional role of shed integrins in the modulation of myocyte and fibroblast interactions with the ECM, to determine whether specific α-chains are involved in the shedding process and to identify the enzyme(s) responsible for β1-integrin shedding.

This work was supported by National Institutes of Health Grants HL-37669 (to E. C. Goldsmith, W. Carver, R. L. Price, and T. K. Borg), P20-RR-16434 (to E. C. Goldsmith and T. K. Borg), HL-38189 (to B. H. Lorell), HL-58224-02, HL-66035-01, and HL-67245 and American Heart Association Established Investigator Award 0040051N (to M. Loell), and the University of South Carolina Faculty Exchange Program (to J. Goldsmith).

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