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Research Article

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The α1β1 Integrin Is Expressed during Neointima Formation in Rat Arteries and Mediates Collagen Matrix Reorganization

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Abstract

Remodeling of the extracellular matrix by activated mesenchymal cells (myofibroblasts) is a critical aspect of wound repair in all adult organs. Collagen-dependent gel contraction, a process requiring integrin function, is an established in vitro assay thought to mimic in vivo matrix remodeling. Numerous data have implicated the α2β1 integrin in various cell types as the primary collagen receptor responsible for collagen gel contraction. However, evidence from the literature suggests that the major collagen binding integrin expressed on mesenchymally derived cells in situ is the α1β1 integrin, not the α2β1 integrin. In this report, we use a rat vascular injury model to illustrate that the α1β1 integrin is the major collagen receptor expressed on vascular smooth muscle cells after injury. Using two smooth muscle cell lines, expressing either the α1β1 integrin alone or both the α1β1 and α2β1 integrins, along with Chinese hamster ovary cells transfected with the α1 integrin, we demonstrate that α1β1 supports not only collagen-dependent adhesion and migration, but also gel contraction. These data suggest that in vivo the α1β1 integrin is a critical collagen receptor on mesenchymally derived cells potentially involved in matrix remodeling after injury. (*J. Clin. Invest.* 1996. 97:2469–2477.) Key words: integrin • smooth muscle cell • neointima • gel contraction • collagen

Introduction

Remodeling of the extracellular matrix (ECM)[1] is a critical component of wound healing and related pathophysiological events (1–3). Response to tissue injury results in the activation of resident mesenchymal cells or myofibroblasts (4) which synthesize and secrete molecules necessary for the remodeling of the ECM. The molecular phenotype shared by these activated mesenchymal cells is the constitutive or transient expression of smooth muscle specific genes (4, 5). Examples include: dermal myofibroblasts, vascular smooth muscle cells (SMC), lipocytes in the liver; mesangial cells in the kidney; and stromal myofibroblasts in different carcinomas (4). Interaction between these cells and the surrounding ECM is critical for the repair response.

Integrins, heterodimeric transmembrane cell surface receptors, are required for both movement through and communication with the ECM (6), and are thought to be a key component of wound healing (2). The major cell surface receptors for collagens, components of both basement membranes and interstitial matrices, are the α1β1 and α2β1 integrins (7–9).

Collagen gel contraction, an integrin-dependent process (10), is an accepted in vitro model of collagenous tissue remodeling. Tensile forces imparted by fibroblasts that develop during gel contraction are thought to be similar to those forces generated during wound contraction (11, 12). Integrin-dependent collagen gel contraction has been attributed to the α2β1 integrin (13–15). By extension, this integrin has been implicated in a number of pathophysiological processes which involve the production and remodeling of extracellular matrices (16, 17). However, evidence from the literature suggests that in adult tissue the α2β1 integrin is expressed primarily on epithelial cells (18) while α1β1 integrin is expressed primarily on mesenchymally derived cells (19, 20) suggesting a role for α1β1 integrin in myofibroblast function during wound repair. Therefore, we investigated the possibility that collagen-mediated in vitro behaviors, which may reflect in vivo function, could be assigned to the α1β1 integrin.

We have begun to examine the role of the α1β1 integrin using a rat vascular injury model and demonstrate that the α1β1, but not the α2β1, integrin is expressed on neointimal SMC. Using two SMC lines, expressing either the α1β1 integrin alone or both the α1β1 and α2β1 integrin, along with Chinese hamster ovary (CHO) cells transfected with the α1 integrin subunit, we demonstrate that α1β1 integrin supports not only collagen-dependent adhesion and migration, but also gel contraction. These data suggest that in vivo the α1β1 integrin is likely to be a critical collagen receptor involved in matrix remodeling by mesenchymally derived cells, and that improper regulation of this integrin may result in certain pathological conditions.

Methods

Antibodies and adhesive proteins. Antibodies against rat α1 (clone Ha31/8), α2 (clone Ha21/9), and β1 (clone Ha2/11) integrin subunits were gifts from Dr. Donna Mendrick (21, 22) (Boston, MA). Antibody against the rat α1 integrin subunit (clone 3A3) was a gift from

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1. *Abbreviations used in this paper:* CHO, Chinese hamster ovary; ECM, extracellular matrix; SMC, smooth muscle cell.

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Dr. Salvatore Carbonetto (23) (Montreal, Quebec). Polyclonal antibodies against the cytoplasmic domains of human integrins α1, α2, α5, and αv subunits were gifts from Dr. Guido Tarone (Torino, Italy); and mAb against VLA-4 (HP1/2) was a gift from Dr. Roy Lobb (Cambridge, MA). Antibodies to vinculin (24), calponin (25), and phosphoglucomutase (26) were described previously. Antibody to smooth muscle α-actin was purchased from Sigma Immunochemicals (St. Louis, MO).

Rat fibronectin and bovine vitronectin were purchased from GIBCO BRL (Gaithersburg, MD); type I rat tail collagen was purchased from Collaborative Research Inc. (Bedford, MA); and type IV collagen and laminin were gifts from Dr. Hynda Kleinman (Bethesda, MD).

Arterial injury model. Male Sprague-Dawley rats (400 grams, 3–4-mo old; Bantin and Kingman, Inc., Edmonds, WA) were used in all experiments. All surgical procedures were carried out under general anesthesia. The left common carotid artery and the aorta were denuded with a 2F balloon catheter as described (27). Deendothelialized segments of arteries were identified by intravenous injection of Evans blue (0.3 ml in 5% saline solution) 10 min before killing. All animals were perfusion-fixed at 8, 14, and 28 d after balloon catheter denudation (three rats per time point) and both carotid arteries and the aorta were excised. For immunostaining on frozen sections, tissue was embedded in OCT compound (Miles, Kankakee, IL). Immunostaining of luminal cells was carried out on en face preparations as described (28). In situ hybridization was performed on en face preparations as well as on paraffin-embedded sections.

Immunostaining. Immunostaining was carried out on cross-sections and on en face preparations as described (29) using mouse mAb 3A3 (2 μg/ml) (23); rabbit antisera raised to the cytoplasmic domains of the human α1 or α2 integrin subunits; hamster mAb Ha21/9 (10 μg/ml) (22); or matching concentrations of normal mouse IgG and normal rabbit serum as controls.

In situ hybridization. Sense and antisense probes were generated from a fragment of pHβApr-α1 (described below) subcloned into pBluescriptSK$^+$ (Stratagene, La Jolla, CA). In situ hybridization was carried out on sections and on en face preparations of vessel segments as described (29, 30). The Häutchen procedure for en face preparations was carried out after hybridizations (31).

Cell culture. Rat pulmonary artery SMC (PAC1) (32) were maintained in M199 (GIBCO BRL), and rat aortic SMC (WKY) (33) were maintained in D–MEM (GIBCO BRL). Both media contained 10% FCS (JRH Biosciences, Lenexa, KS), 100 U/ml penicillin and streptomycin, and 2 mM L-glutamine (BioWhittaker, Inc., Walkersville, MD). CHO cells were maintained in α-MEM (GIBCO BRL) supplemented with 10% FCS and 4 mM L-glutamine. All cultures were maintained in a humidified incubator with 5% CO_2/95% air at 37°C.

Flow cytometry. Cells were incubated with primary antibody, washed, resuspended in the appropriate FITC-conjugated secondary antibody (Caltag Laboratories, San Francisco, CA; or Amersham, Arlington Heights, IL), and analyzed for fluorescence on a FACStar$^{PLUS®}$ flow cytometer (Becton Dickinson, San Jose, CA). *x* and *y* axes represent log fluorescent intensity and cell number, respectively. CHO cells selected for high α1 integrin expression were collected sterilely and maintained in selective medium.

Western blot analysis. Samples were electrophoresed, transferred to nitrocellulose (MSI, Westborough, MA), and incubated at room temperature as follows. Nonspecific sites were blocked with blocking buffer (2% BSA, 0.3% gelatin, 150 mM NaCl, 0.05% Tween-20, 25 mM Tris-HCl, pH 7.6) for 1 h. Antibodies appropriately diluted in blocking buffer were added to the filter and incubated for 2 h. The filter was washed three times with 0.3% Tween-20 in TBS (150 mM NaCl, 10 mm Tris-HCl, pH 7.5), and subsequently incubated with ^{125}I anti-IgG (Amersham) at 1–2 × 10^5 cpm/ml in blocking buffer. After extensive washing, the filter was blotted, wrapped in plastic, and exposed to XAR-5 film (Kodak, Rochester, NY).

Transfection. Plasmid pHβAPr-α1, which contains a neomycin-resistance gene and a full-length α1 integrin cDNA expressed from a β-actin promoter, was a gift from Dr. Michael Ignatious (Berkeley, CA). A control plasmid lacking the α1 integrin cDNA (pHβAPr) was prepared by releasing the α1 integrin cDNA insert and religating the vector.

10^7 CHO cells, resuspended in 20 mM Hepes, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2PO_4, 6 mM dextrose containing 100 μg of either pHβAPr-α1 or pHβAPr, and 100 μg salmon sperm DNA, were electroporated for 10 s at 0.29 V in a gene pulser (Bio-Rad, Richmond, CA). Cells were washed once in media, plated, and allowed to recover for 72 h. Cells were then transferred to media containing 0.5 mg/ml geneticin (GIBCO BRL) and maintained in selective medium. Cells expressing high levels of the α1 integrin were selected by two sequential FACS® sortings (see above).

Adhesion assay. The minimum concentration of each adhesive molecule required to give maximum cell adhesion was determined: collagen type I (5 μg/ml), collagen type IV (5 μg/ml), laminin (25 μg/ml), fibronectin (2.5 μg/ml), and vitronectin (1 μg/ml). 96-well MaxiSorp plates (Nunc, Naperville, IL) were coated overnight at 4°C with 50 μl of adhesive substrates diluted in PBS to the stated concentrations. Wells were washed twice with PBS, and nonspecific binding sites were blocked with 1% BSA in PBS for 1 h at room temperature. Control wells were treated with BSA alone. 3 × 10^4 cells, labeled with 2 μM BCECF [2′,7′-bis(carboxyethyl)-5(6) carboxyl fluorescein penta acetoxymethylester] or Calcein-AM (Molecular Probes, Eugene, OR), in 100 μl, untreated or treated with appropriate neutralizing antibodies, were plated in each well for 60 min at 37°C. Unbound cells were removed by washing wells three times with 0.25% BSA in DMEM. Adhesion was quantified by measuring the fluorescence in a CytoFluor 2350 fluorescent plate reader (Millipore, Bedford, MA). Percent adhesion was calculated by dividing the fluorescence in an experimental well by fluorescence of input cells and multiplying by 100. Background values due to cell adhesion on wells coated with BSA alone were subtracted from those values due to adhesion on the various ECM proteins.

Migration assay. Migration assays were performed using a modified Boyden chamber as described previously (34). 400 μl of 4 μg/ml type I collagen, diluted in D–MEM containing 0.25% BSA, experimentally determined to be the optimal concentration for migration, was added to the lower chamber of an MBA96 microtiter plate (Neuro Probe Inc., Cabin John, MD). Control wells contained no collagen. 6–7.5 × 10^4 cells, labeled with Calcein-AM, and either untreated or treated with appropriate antibodies for 15 min at 37°C, were added to the top wells. Migration was allowed to proceed for 4 h at 37°C. Migration was quantified by measuring the fluorescence index in a CytoFluor 2350 fluorescent plate reader (Millipore). The fluorescent index is proportional to cell number over a range of 250 to 3.5 × 10^4 cells. Background values due to cell migration to wells containing no collagen were subtracted from those values due to collagen-dependent migration.

Gel contraction assay. Collagen gel contraction assays were performed as described (35). Briefly, 2-ml aliquots of D–MEM containing 1 mg/ml neutralized type I collagen, 10% FCS, and 1.5–3 × 10^5 cells were pipetted into wells of a 24-well plate (Corning, Corning, NY), previously coated with 1% BSA in PBS. For antibody blocking experiments, the appropriate antibodies were added to the cells in media before the addition of collagen and incubated for 15 min at 37°C. Gels were allowed to contract for 8–48 h at 37°C in a humidified cell culture incubator. The contracted gels were photographed and the area of the gel was determined. In some experiments, the kinetics of contraction was determined by measuring the area of the gel at defined time intervals.

Results

The α1β1 integrin is expressed by intimal SMC in response to vessel injury. The α2β1 integrin has been implicated in pathophysiological events after vessel injury based on in vitro stud-

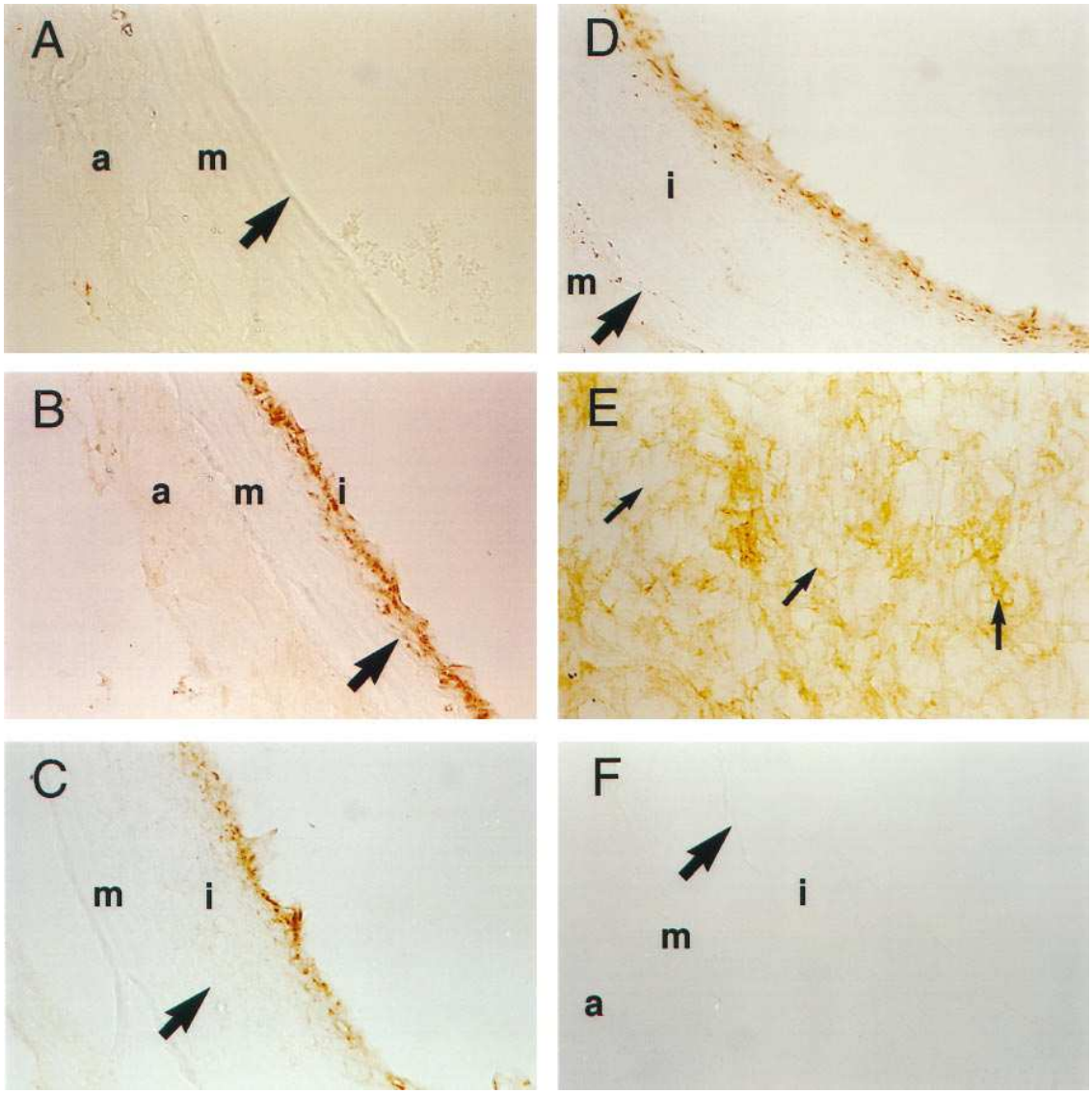

Figure 1. Expression of the α1β1 and α2β1 integrins in uninjured and injured rat carotid artery. Cross-sections (*A–D*, *F*) or en face preparation (*E*) of a normal rat carotid artery (*A*) or 8 (*B*), 14 (*C*, *E*, *F*), or 28 (*D*) d after injury immunostained with rabbit serum generated to the human α1 (*A–E*) or α2(*F*) integrin cytoplasmic domains. Immunostaining with mAb 3A3 (anti–rat α1 integrin) gave results identical to the rabbit serum. Note that α1 integrin immunostaining is stronger near cell borders (*E, narrow arrows*). Broad arrows mark the internal elastic lamina. *a*, adventitia; *m*, media; *i*, neointima. ×400.

ies using human vascular SMC lines (17, 36). α2β1 integrin expression has not been detected on vascular SMC in the vessel wall (36), and no assessment of either α1β1 or α2β1 integrin expression in injured vessels has been made. Therefore, we examined the distribution of both α2β1 and α1β1 integrins in the rat carotid artery after balloon injury. Uninjured vessels express little or no α2β1 or α1β1 integrin (Fig. 1 *A*). Neointimal SMC express both α1 mRNA (Fig. 2 *A*) and protein (Fig. 1 *B*) by day 8 after injury. En face preparations demonstrate that immunostaining is primarily localized at the cell borders, consistent with cell surface expression (Fig. 1 *E*). Expression of the α1β1 integrin persists for at least an additional 3 wk after injury but is largely confined to the luminal three to four layers of neointimal SMC (Fig. 1, *C* and *D*), and, therefore, may be indicative of replicating or migrating SMC. There is also less intensive, but identifiable, α1β1 integrin staining in the adventitia (Fig. 1 *B*). This staining may reflect expression by adventitial myofibroblasts, which have been implicated in vascular remodeling associated with injury (37). There is no evidence that the α2β1 integrin is expressed on either medial or neointimal SMC (Fig. 1 *F*). However, α2β1 is expressed on regenerating endothelium (data not shown). Neither regenerating nor quiescent endothelial cells express the α1 integrin (Fig. 2 *B*). These data suggest that, at least in rat, the major collagen binding integrin on vascular SMC that could be involved in events after vascular injury is α1β1.

Identification of a vascular SMC line that expresses α1β1, but not α2β1 integrin. To study the function of α1β1 integrin on vascular SMC, we characterized the expression of integrins and SMC markers in two rat vascular SMC lines. Vascular

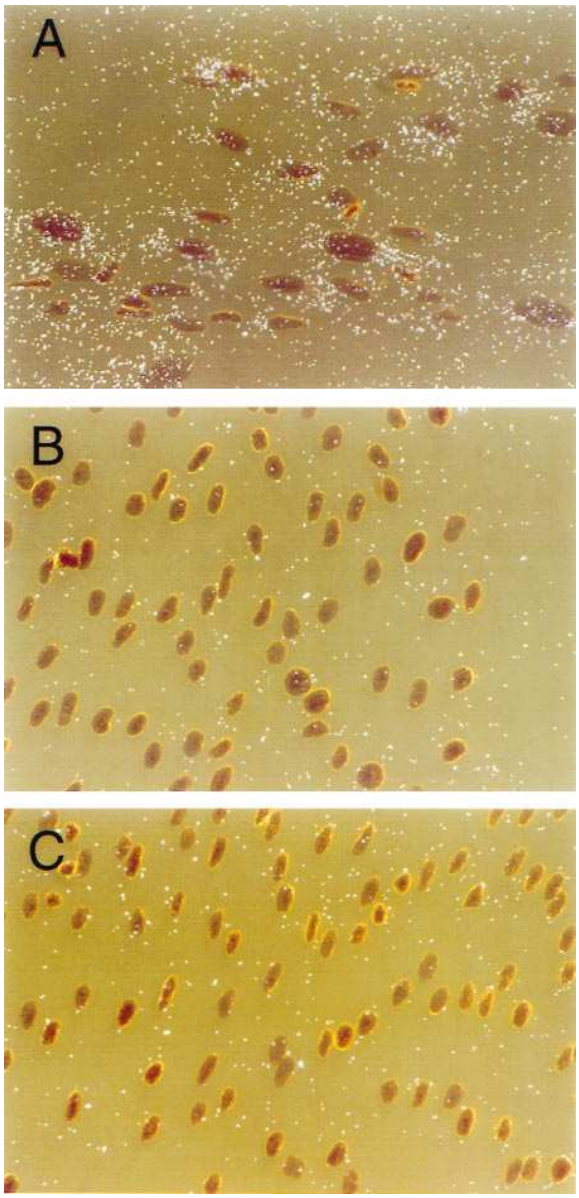

Figure 2. α1 integrin mRNA expression after rat carotid artery injury. Sections were counterstained with hematoxylin to identify nuclei. An antisense α1 integrin probe hybridizes to intimal SMC (*A*), but not to regenerating endothelial cells (*B*) on the luminal surface of a carotid artery 8 d after injury. En face preparation of a normal carotid artery hybridized with an α1 integrin sense probe shows low background hybridization (*C*). ×630.

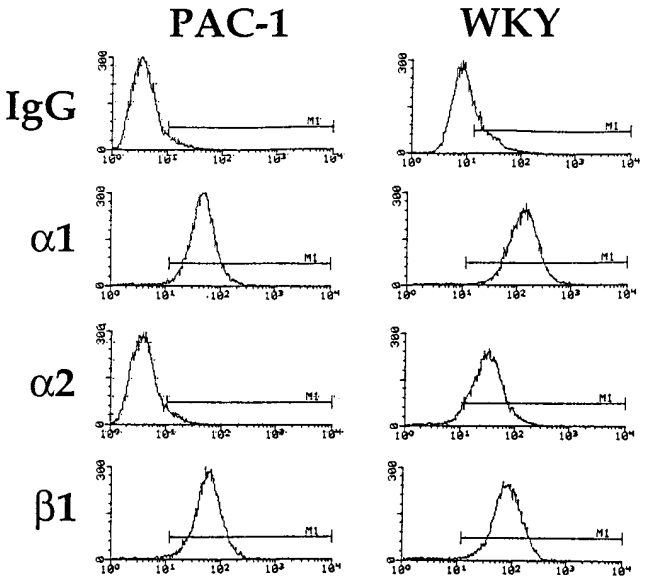

Figure 3. Expression of the α1β1 and α2β1 integrins on the surface on PAC1 and WKY cells. Cells were fixed and incubated with either control IgG, Ha31/8 (anti-α1), Ha21/9 (anti-α2), and Ha 2/11 (anti-β1). An FITC-conjugated secondary antibody was applied and surface expression was analyzed by flow cytometry. The *x* axis reflects log fluorescence intensity, and the *y* axis reflects cell number.

SMC typically express the α1β1 integrin as the major collagen binding integrin in vivo (36, 38, 39), while cultured vascular SMC downregulate α1β1 and express α2β1 integrin (36). The rat pulmonary SMC line PAC1 is unique among cultured vascular SMC cell lines analyzed to date in that it expresses high levels of α1β1, but not α2β1 integrin (Fig. 3). In contrast, the rat aortic vascular SMC line WKY is more typical of vascular SMC in culture in that it exhibits both α1β1 and α2β1 integrin surface expression (Fig. 3). Both cell lines express the α3, α5, αv, and β1 integrin subunits (Table I). The α4 subunit is expressed in PAC1 cells, but not WKY cells. Of interest, PAC1 express the α8 subunit which, in adult mammalian tissues, is expressed predominantly in smooth muscle and smooth muscle–like contractile cells (40). In contrast, WKY cells express limited amounts of the α8 integrin subunit.

PAC1 expression of the α1 and α8 integrin suggested that this cell line might bear a greater resemblance to vascular SMC in vivo than many other cultured SMC lines. Therefore, we compared the PAC1 and WKY cells for a variety of SMC markers (41) which identify vascular SMC in vivo. PAC1 cells, known to express a set of smooth muscle–specific cytoskeletal proteins (32), were also found to express high levels of the SMC markers smooth muscle α-actin, meta-vinculin, phosphoglucomutase, and calponin (38) by Western blot and immunohistochemistry (Table II). For comparison, WKY cells express limited amounts of smooth muscle α-actin and none of the other SMC markers (Table II). Typically, vascular SMC rapidly change phenotype when they are placed in culture losing expression of the α1 integrin and SMC-specific cytoskeletal proteins. However, the PAC1 cell line retains expression of SMC markers and may be representative of vascular SMC in vivo.

PAC1 cell adhesion to and migration on collagen are α1β1 integrin dependent. To assess the functionality of the α1β1 in-

Table I. Expression of Selected Integrins on PAC1 and WKY Cells

	α1	α2	α3	α4	α5	α8	αv	β1
PAC1	+	−	+	+	+	+	+	+
WKY	+	+	+	−	+	±	+	+

Table II. Expression of SMC Markers on PAC1 and WKY Cells

	α-sm	m-vn	ppg	cpn
PAC1	++	+	++	++
WKY	±	−	−	−

α-sm, smooth muscle a-actin; *m-vn*, meta-vinculin; *ppg*, phosphoglucomutase; and *cpn*, calponin.

tegrin on vascular SMC, we tested PAC1 cells for their ability to migrate on type I collagen in a modified Boyden chamber assay and for their ability to adhere to collagen (types I and IV) and laminin. Collagen-dependent PAC1 cell migration is completely inhibited by antibodies against either the α1 or the β1 integrin subunit (Fig. 4 *A*) demonstrating that migration on collagen is completely α1β1 integrin dependent. WKY cells migrate more efficiently than PAC1 cells (30 vs. 15%). Antibodies against both the α1 and the α2 integrin subunits are required to block WKY migration on collagen (Fig. 4 *B*), consistent with the integrin repertoire on these cells.

PAC1 cells adhere to both type IV and type I collagen, and laminin. Adhesion to these substrates can be completely blocked by antibodies to either the α1 or β1 integrin subunits (Fig. 5 *A*). For comparison, WKY cells also adhere to type I and type IV collagen, and laminin, but antibodies directed against both the α1 and α2 integrin subunits are required to completely inhibit adhesion (Fig. 5 *B*). These data demonstrate that the α1β1 integrin is the only functional collagen/laminin receptor on the surface of PAC1 cells, while WKY cells use both the α1β1 and the α2β1 integrins as collagen/laminin receptors. PAC1 is the first mesenchymal cell line identified which expresses the α1β1 integrin in the absence of α2β1 expression and should prove useful for functional studies of the α1β1 integrin.

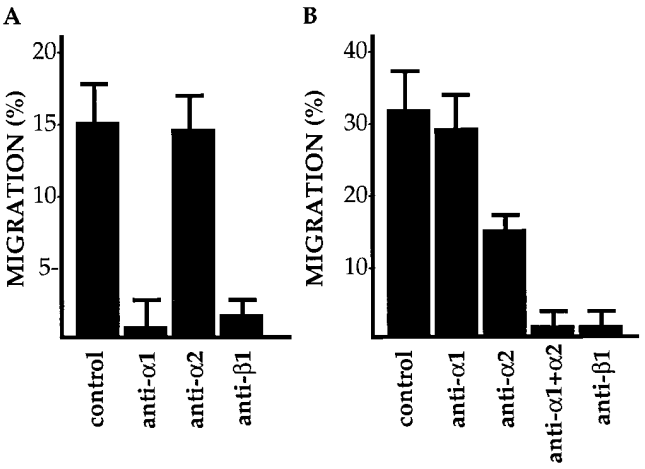

Figure 4. Collagen-mediated PAC1 and WKY cell migration. 6–7.5 × 10^4 PAC1 (*A*) or WKY (*B*) cells were labeled with Calcein-AM, left untreated or treated with the indicated antibodies, and added to the upper well of a modified Boyden chamber containing a membrane coated on the lower side with 4 μg/ml type I collagen. Cells, untreated or treated with 2 (PAC1) or 4 (WKY) μg/ml mAb Ha31/8, or 2 (PAC1) or 4 (WKY) μg/ml mAb Ha21/9, 4 μg/ml mAb Ha31/8 + 4 μg/ml mAb Ha21/9, 5 (PAC1) or 10 (WKY) μg/ml mAb Ha2/11 as indicated, were allowed to migrate for 4 h at 37°C in a tissue culture incubator. Each experiment contained quadruplicate samples, and measurements were calculated from two to four independent experiments. Migration is expressed as mean percentage of total input cells ±SEM.

The α1β1 integrin mediates collagen matrix reorganization. Hydrated collagen gel contraction is a well established in vitro model of matrix remodeling during wound contraction (2, 12). If the α1β1 integrin plays a role in the remodeling of the ECM after tissue injury, one would expect it to mediate collagen matrix reorganization in vitro. We compared the relative abilities of both PAC1 and WKY cells to contract hydrated collagen gels. WKY cells efficiently contract collagen gels in a β1 integrin–dependent manner (Fig. 6), and both the α1 and the α2 integrin chains appear to play a role. Contraction after 8 h in culture is completely blocked by antibodies against both the α1 and α2 chains used in combination, but not when the antibodies are used independently (Fig. 6). The observation that the α2 integrin can mediate collagen gel contraction is consistent with data in the literature (13, 15–17). PAC1 cells also contract collagen gels efficiently, but in an α1β1 integrin–dependent manner. Antibodies directed against the α1 integrin chain or the β1 integrin chain, but not the α2 integrin chain, completely block matrix reorganization (Fig. 7). Because the α2β1 integrin is upregulated in certain cell types during gel contraction (15), we tested by FACS®, but failed to detect, α2 integrin expression during PAC1 gel contraction (data not shown), consistent with the antibody blocking results.

CHO cells express no collagen binding integrins and do not mediate gel contraction. To directly test whether the α1β1 integrin can mediate matrix reorganization, we transfected CHO cells with a cDNA encoding the full-length rat α1 integrin (pHβAPr-α1). Selected pools of cells expressing high levels of the α1 integrin (Fig. 8 *A*) were compared with cells transfected with pHβAPr alone for their ability to contract collagen gels. Only those cells expressing the α1β1 integrin were able to contract gels, and contraction was completely blocked by two different monoclonal antibodies directed against the α1 integrin, but not by an anti–α2 integrin antibody (Fig. 8 *B*). These data directly demonstrate that the α1 integrin, both in the context of vascular SMC and a heterologous cell type, is able to mediate collagen matrix reorganization.

Discussion

Integrins have emerged as critical cell surface receptors in a wide variety of developmental, physiological, and pathological processes. Using a vessel injury model, we have begun to investigate those integrins expressed on vascular SMC that may play a role in events associated with wound repair. We have focused specifically on the collagen binding integrins α2β1 and α1β1 because collagens are associated with pathological events in response to injury.

The α2β1 collagen binding integrin has been implicated in processes associated with tissue repair, in general, and vessel injury specifically based on its in vitro expression on vascular SMC and the ability of the α2β1 integrin to mediate vascular SMC adhesion (36), migration (36), and collagen matrix reor-

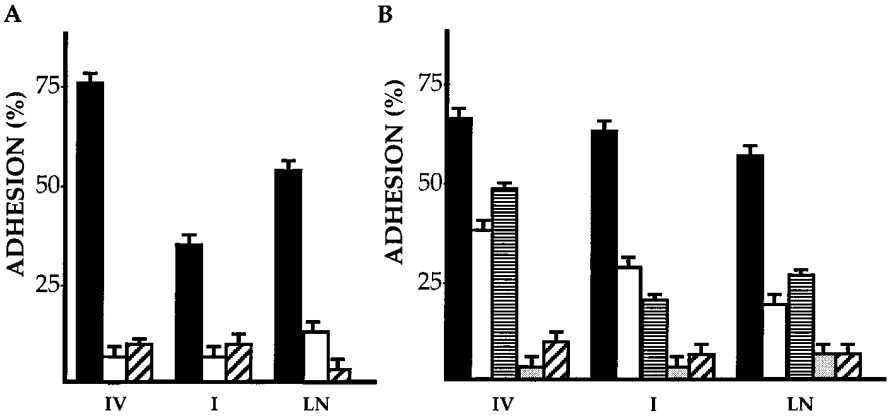

Figure 5. Integrin-dependent binding of PAC1 and WKY cells to collagen and laminin. Wells were coated with 5 μg/ml collagen type IV (*IV*), 5 μg/ml collagen type I (*I*), and 25 μg/ml laminin (*LN*). 3×10^4 PAC1 (*A*) or WKY (*B*) cells, labeled with Calcein-AM, and untreated or treated with indicated antibodies for 15 min before plating, were added to coated wells and incubated for 60 min. Percent adhesion was calculated by dividing the fluorescence in an experimental well by fluorescence of input cells, and multiplying by 100. Background values due to cell adhesion on wells coated with BSA alone were subtracted from those values due to adhesion on the various ECM molecules. Adhesion assays were done in triplicate and at least three independent experiments were performed. Data are expressed as mean±SEM. *Black bars*, no antibody; *white bars*, 2 μg/ml mAb Ha31/8; *horizontally striped bars*, 2 μg/ml mAb Ha21/9; *stippled bars*, 2 μg/ml mAb Ha31/8 + 2 μg/ml mAb Ha21/9; *diagonally striped bars*, 5 μg/ml mAb Ha2/11.

ganization (13, 16, 17). However, there is little or no evidence demonstrating that α2β1 is expressed on either vascular SMC in the vessel wall or activated mesenchymal cells in other tissues (20, 36). On the other hand, the α1β1 integrin is expressed on many mesenchymally derived cells in adult tissue including vascular SMC (20, 39). Furthermore, the α1β1 integrin, but not the α2β1 integrin, is expressed on myofibroblasts resident in tumor stroma(5), and therefore could be involved in repair events after injury. In this report, we demonstrate that the α1β1 integrin has both appropriate expression in a rat model of vessel injury and appropriate in vitro function in the context of vascular SMC to be involved in pathophysiological events after injury.

α1β1 integrin expression after vessel injury. Because there are no data directly addressing which collagen binding integrins are expressed on vascular SMC after vessel injury, we investigated the expression of both the α1β1 and α2β1 integrins after

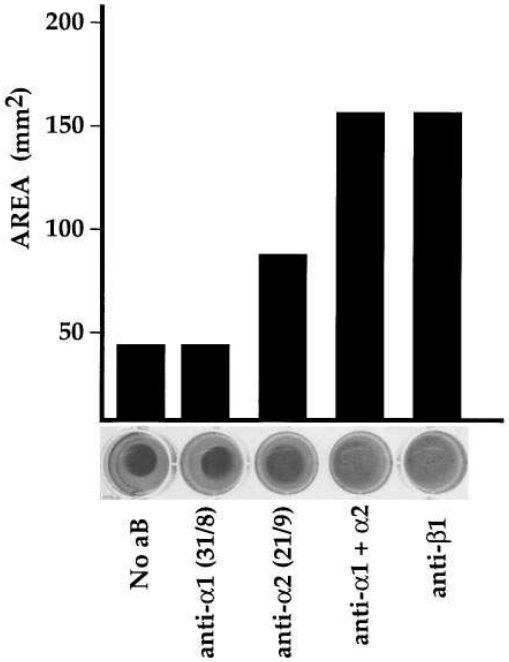

Figure 6. Collagen gel contraction is mediated by the α1β1 and α2β1 integrins in WKY cells. 1.5×10^6 WKY cells, untreated or treated with 4 μg/ml mAb Ha31/8, or 4 μg/ml mAb Ha21/9, 4 μg/ml mAb Ha31/8 + 4 μg/ml mAb Ha21/9, 10 μg/ml mAb Ha2/11 as indicated, were suspended in 3.0 ml of hydrated collagen solution and added to BSA-coated wells of a 24-well dish. Contraction was allowed to proceed for 8 h, after which gels were photographed and measured. A representative experiment is shown. Data are expressed as area of gel (mm^2) after contraction.

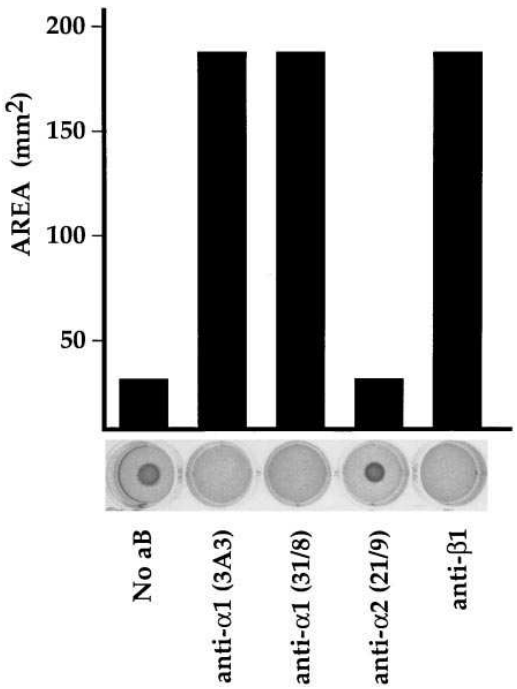

Figure 7. Collagen gel contraction is mediated by the α1β1 integrin in PAC1 cells. 3.0×10^6 PAC1 cells, untreated or treated with 2 μg/ml mAb 3A3, 2 μg/ml mAb Ha31/8, or 2 μg/ml mAb Ha21/9, 5 μg/ml mAb Ha2/11 as indicated, were suspended in 3.0 ml of hydrated collagen solution and added to BSA-coated wells of a 24-well dish. Contraction was allowed to proceed for 24 h, after which gels were photographed and measured. A representative experiment is shown. Data are expressed as area of gel (mm^2) after contraction.

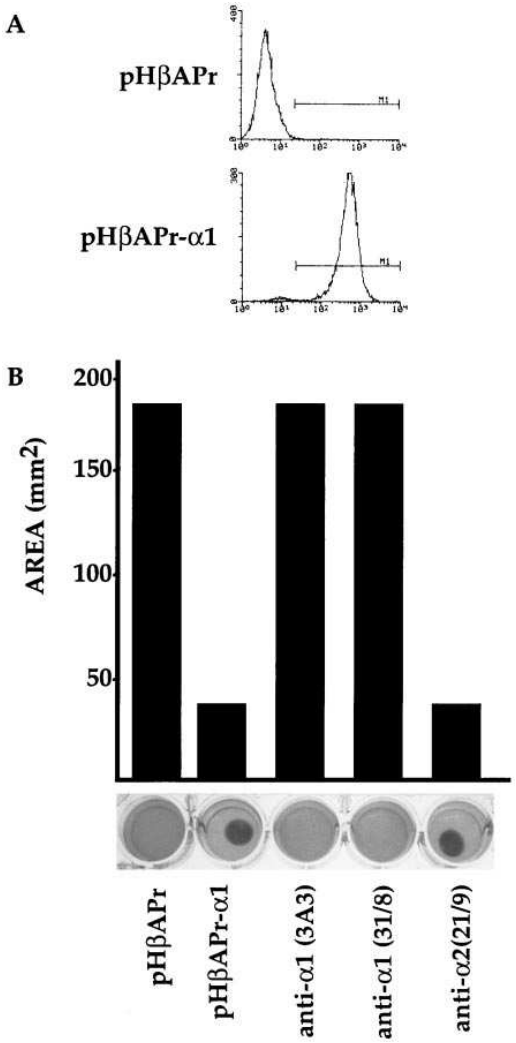

Figure 8. CHO cells transfected with the α1 integrin mediate collagen gel contraction. (*A*) CHO cells transfected with the α1 integrin (pHβAPr-α1) were selected for α1 integrin surface expression by flow cytometry using mAb 3A3. A representative FACS® scan compares the expression of α1 integrin in pHβApr-α1–transfected cells with those cells transfected with pHβAPr alone. The *x* axis reflects log fluorescence intensity, and the *y* axis reflects cell number. (*B*) CHO cells transfected with pHβAPr or with pHβApr-α1,untreated or treated with 2 μg/ml mAb 3A3, 2 μg/ml mAb Ha31/8, or 2 μg/ml mAb Ha21/9 as indicated, were used in collagen gel contraction assays as outlined in the legend to Fig. 6.

balloon injury to the rat carotid artery. Uninjured carotid and aortic arteries express neither the α1β1 nor α2β1 integrins. However, SMC of smaller vessels such as small muscular arteries do express α1β1 (Lindner, V., unpublished observations). By 8 d after injury, the α1β1, but not the α2β1, integrin is expressed in neointimal cells and expression persists for at least an additional 3 wk. α1β1 expression is confined largely to the three to four most luminal layers of the developing neointima correlating with sites of SMC involved in processes affecting vessel repair such as replication, migration, and contraction. SMC appear in the neointima of the carotid artery as early as 4 d after injury, followed by replication and collagen matrix deposition over the following weeks (27, 42). Therefore, the α1β1 integrin is expressed both on the proper cell type as well as at the correct time to be involved with pathophysiological events associated with repair processes after vessel injury.

We were surprised to find that rat medial SMC in large vessels expressed little or no α1 integrin because both human and chicken medial SMC express high levels of this integrin (39, 43). The medial SMC of large vessels in mouse also do not express the α1 integrin (Gardner, H., and V. Koteliansky, unpublished observation) suggesting an evolutionary change in the regulation of α1 integrin expression. Regardless of medial SMC expression, neointimal SMC in both humans and rat express the α1 integrin subunit which is consistent with this integrin being involved in the response to injury.

PAC1 cells express molecular markers typical of vascular SMC in vivo. The study of vascular SMC in vitro is made difficult by the fact that most cultured SMC are phenotypically very different from SMC in vivo (41). Primary cultures of vascular SMC rapidly loose the expression of smooth muscle specific cytoskeletal proteins, de-differentiate, and change from a contractile to a synthetic state. In an effort to identify an immortalized cell line that resembles vascular SMC in vivo, we screened a number of vascular SMC lines with antibodies to a battery of SMC markers and cell surface integrins and found a uniquely representative rat SMC line, PAC1.

PAC1 cells express high levels of the α1β1 and α8β1 integrins, typical of SMC in vivo. In most cultured SMC lines such as WKY, expression of α8β1 is lost, while α1β1 is often supplemented or replaced by α2β1. PAC1 cells also express a set of SMC specific cytoskeletal proteins including smooth muscle α-actin, myosin heavy chain, myosin regulatory light chain, and α-tropomyosin (32). We have extended this work to show that these cells also express SMC markers meta-vinculin, calponin, and phosphoglucomutase. In contrast, WKY cells express only limited amounts of smooth muscle α-actin and are more typical of cultured SMC. Based on both the integrin and SMC marker expression patterns, we believe that the PAC1 cells are phenotypically related to vascular SMC in situ and relevant for the study of vascular SMC function.

α1β1 mediates collagen matrix reorganization. The ability of cells to contract and organize collagen matrices is a critical component of any wound healing response. After damage to the skin, for instance, myofibroblasts at the site of injury contract loose granulation tissue to close the wound and form a strong scar (1–3). Similarly, after vascular injury, intimal SMC (44, 45) as well as adventitial myofibroblasts (37) contribute to vascular remodeling by reorganizing interstitial collagen matrices. A dense collagenous matrix is often seen in atherosclerotic plaques (46). In vitro collagen gel contraction has long been thought to mimic the physical forces generated during wound contraction (12). Contraction is β1 integrin dependent (10), and numerous studies have demonstrated that this process can be mediated through the α2 chain (13, 15–17). If the α1β1 integrin is involved in wound healing events after vessel injury, one would expect it to mediate collagen gel contraction. Three lines of evidence demonstrate this to be the case. PAC1 cells express the α1β1, but not the α2β1, integrin on their surface, and contract collagen gels in a completely α1β1-dependent manner. WKY SMC express both the α1β1 and α2β1 integrins and require function blocking antibodies against both α chains to completely block contraction. Finally, CHO cells transfected with α1 integrin reorganize collagen matrices in an α1β1-dependent manner. Therefore, the α1β1 integrin can

confer gel contraction behavior. It has also been shown recently that rat cardiac fibroblasts, which express both the α1β1 and α2β1 integrins, are at least partially dependent on the α1β1 integrin for collagen gel contraction (47).

Lee et al. (17) concluded from blocking antibody studies that the α1β1 integrin did not play a role in collagen gel contraction by human SMC. We believe there are a number of possibilities for the discrepancy between our data. Lee et al. note that the anti-β1 integrin antibody TS2/16 blocks gel contraction but stimulates adhesion, demonstrating that antibody effects are assay dependent. One cannot assume that antibodies that block α1-dependent adhesion to collagen in two dimensions will necessarily block three-dimensional collagen gel contraction. The antibodies used in our studies happen to block both processes. Second, the primary human SMC that Lee et al. use express both the α1 and the α2 integrins, although the relative amounts are not clear. We know from our studies that cells that express both these integrins can use either one for contraction. Under the experimental conditions used by Lee et al., there may be no blocking effect of the anti-α1 integrin antibody because the action of α2 is simply masking that of α1.

Finally, Lee et al. do not control for the possibility that the primary human SMC which they use may lose α1 integrin expression during gel contraction. It is clear that the surface integrin expression of primary SMC can rapidly change due to environmental condition (36, 39). Klein et al. (15) have demonstrated that α2β1 integrin can be upregulated during gel contraction. Note that we controlled for the possible upregulation of α2 in our studies.

The data presented here demonstrate that the α1β1 integrin is not only expressed on the proper cell type at the proper time, but also has in vitro functions expected for a collagen receptor involved in vascular wound healing. These data, in concert with observations from the literature demonstrating that myofibroblast-like cells express α1β1 integrin as the predominant collagen binding integrin, suggest that this receptor may be involved in both the normal and pathophysiological responses to tissue injury.

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