The Role of α1β1 Integrin in Wound Contraction

A QUANTITATIVE ANALYSIS OF LIVER MYOFIBROBLASTS IN VIVO AND IN PRIMARY CULTURE

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Wound repair is a multi-step process in which contraction is an important element (1). In its initial phase, mesenchymal cells are drawn to the injury site, where they deposit a collagen-rich extracellular matrix (ECM). The ECM undergoes organization, with alignment of collagen fibrils. Myofibroblasts attach via matrix-binding surface receptors, which provide points of traction as cells shorten. The contractile action compacts the ECM lattice, promoting wound closure and restoring tissue integrity. Although this process is vital to host defense, it has a negative side. In epithelial tissues, repetitive injury leads to contractility. In vitro studies of wound repair is the availability of methods for preparing mass isolates of stellate cells. By analysis of isolates at various time points during the evolution of the injury, direct information on gene expression and function is obtained, representing a profile of in vivo integrin expression. Amplification of cells in culture, which routinely results in phenotypic change (10, 11), is avoided. The isolated cells are suitable also for direct in vitro assessment of their ECM binding activity and contractility.

EXPERIMENTAL PROCEDURES

Antibodies—Monoclonal blocking antibodies against rat α1 (clone Ha31/8), α2 (clone Ha1/29), β1 (clone Ha2/11), and murine α1 (clone 3A3) integrin subunits were gifts from V. Kotelniansky (Biogen Inc., Boston, MA) (12–14); clone 3A3 cross-reacts with the rat protein (4). MA2 polyclonal antibody against the cytoplasmic domain of murine α2 integrin subunit was a gift from S. A. Santoro (St. Louis, MO) (15, 16). Polyclonal antibodies against the cytoplasmic domains of human integrins α1 and α2 subunits were gifts of G. Tarone (Torino, Italy). Monoclonal antibody against murine α1 integrin subunit was obtained from Pharmingen (San Diego, CA).

cDNA Probes—The probe for α1 integrin subunit was a 479-base pair cDNA (nucleotides 1871–2350) subcloned into pGEM4Z (Promega, Madison, WI) from a full-length cDNA provided by M. J. Ignatius (Berkeley, CA) (17). The probe for the α2 integrin subunit was an

An unresolved question in wound contraction concerns the identity of integrins mediating the attachment of tissue myofibroblasts to matrix in the injury site. Previous studies with cell lines have focussed on α1β1 and α2β1, the principal collagen-binding integrins, but have yielded conflicting data. We have examined this issue in wound healing in the liver, isolating the myofibroblast population (activated stellate cells) and quantitating expression of the α1 and α2 integrin subunits during the in vivo injury. Normal stellate cells displayed α1 but no detectable α2. During injury, α1 expression was maintained; α2 became detectable at the mRNA level but at all times was <8% of α1 mRNA. Contraction of collagen lattices, studied with 24-h cultured cells and initiated by endothelin 1, was blocked 70% by anti-α1 and 30% by anti-α2 (both significant, p < 0.05). The inhibition by anti-α2, which was unexpected, was attributable to culture-induced change in integrin expression; both the mRNA and protein for α2 increased strikingly within 24 h of plating stellate cells on a collagen gel. We conclude that α1β1 is the sole integrin utilized by contracting myofibroblasts in vivo. Although α2β1 is capable of mediating contraction, its expression by myofibroblasts occurs largely, if not exclusively, in response to culture.
843-base pair cDNA (nucleotides 1456–2299) subclone in pBluescript II SK+ kindly provided by S. A. Santore (St. Louis, MO) (15).

Animal Models of Wound Repair—Hepatic injury was induced in male Sprague-Dawley rats (500–600 g) by ligation of the biliary duct. Bile duct ligation is well characterized with respect to the time course and extent of fibrogenesis (18, 19). In this model, bile duct manipulation without ligation. Animals were maintained postoperatively on food and water with ad libitum. In some studies, hepatic fibrosis was induced by intraperitoneal injection of dimethylnitrosamine in a dose of 1 μl (diluted 1:100 in 0.15 m NaCl)/100 g body weight given on the first 3 days of each week (20).

Cell Isolation and Culture—Stellate cells were isolated as described (21) by liver perfusion with Pronase (Boehringer Mannheim) and collagenase (Crescent Serva, Hauppauge, NY) followed by ultracentrifugation on a discontinuous gradient of Accudense® (8.2 and 15.6% w/v) (Accurate Chemicals, Westbury, NY). The top interface contained stellate cells, which were collected and washed twice in culture medium to remove debris. Stellate cells were identified by their intrinsic vitamin A autofluorescence (22) and by staining for the intermediate filament, desmin (9). Their purity was >95%. They were used fresh or plated in a modified medium 199 (23) containing 20% serum (10% horse, 10% calf; Life Technologies, Inc.).

RNase Protection Assay—Total RNA was extracted from stellate cells using TRI Reagent® (Molecular Research Center Inc., Cincinnati, OH) (24). The RNA was assessed by electrophoresis on a Modified 5% agarose gel containing 200 μg/ml (all as purified IgG).

Flow Cytometry—Isolated cells were washed with PBS containing Ca2+ and Mg2+, and 500,000–500,000 cells were incubated in a blocking solution consisting of 10% goat serum in PBS for 15 min at 37 °C. The primary antibody (clone Ha31/8 for α1, clone Ha1/29 for α2, and clone Ha2/11 for β1) was added to the cell sample and incubated for 25 min on ice. After washing with PBS, the cells were resuspended in phycoerythrin-conjugated anti-hamster IgG diluted in PBS (10 μg/106 cells) (Caltag, South San Francisco, CA), incubated for 20 min on ice, and then washed. The cells were analyzed for fluorescence on a FACStarPLUS Flow Cytometer (Becton Dickinson, San Jose, CA). For control samples, the primary antibody was replaced by non-immune IgG.

Immunohistochemistry on Liver Sections—The liver was perfused under low pressure in situ with PBS via the portal vein until free of blood and then removed and cut into small pieces, which were snap-frozen in isopentane precooled in liquid nitrogen and stored at −80 °C. Immunohistology was performed on 8-μm thick cryostat sections fixed for 10 min in −20 °C acetone (15, 16). Sections were incubated 15–30 min in a blocking solution containing 1% fish gelatin (Sigma) in PBS and then incubated with either 3A3 anti-α1 monoclonal antibody (1:500 dilution in blocking solution) (14) or with MA2 anti-α2 polyclonal antibody (1:200 dilution) (15, 16). A biotinylated sheep anti-mouse IgG (1:200) (Amersham Corp.) or a biotinylated goat anti-rabbit IgG (1:200) (Vector Laboratories, Burlingame, CA) was added. After further washes, the sections were exposed to streptavidin–linked Texas Red (Amersham Corp.) (1:100) for 1 h, washed with PBS, and mounted in glycerol for fluorescence microscopy (Dako Corp., Carpinteria, CA).

Adhesion Assay—Cell attachment to collagen was performed as described (26). Briefly, untreated polylysine 96-well flat-bottom microtiter plates (Linbro/Titertek, Flow Laboratories, McLean, VA) were coated with collagen type I or IV (10 μg/ml in PBS) (Sigma) or with 1% bovine serum albumin (Sigma) in PBS. Plates were washed with PBS and then blocked with 1% bovine serum albumin for 1 h. Cells were plated at a density of 100,000 cells/well in 200 μl of serum-free medium. For blocking experiments, they were preincubated with antibodies for 15 min at 4 °C before plating. After a 2-h incubation at 37 °C in a humidified 5% CO2 incubator, non-adherent cells were removed by three washes with PBS. The attached cells were fixed and stained with a solution containing 3% formaldehyde, 10% methanol, and 0.5% crystal violet for 1 h. After washing with PBS to eliminate excess dye, the wells were drained, and the absorbance at 595 nm was measured in a microplate reader (Bio-Rad). Values were corrected for background defined as adhesion to bovine serum albumin alone. Each assay was performed in triplicate.

Collagen Gel Contraction Assay—Contraction of stellate cells on collagen lattices was examined in 24-well flat-bottom tissue culture plates (Corning Glass Works, Corning, NY) as described previously (27). Briefly, culture vessels were preincubated with PBS containing 1% bovine serum albumin (Sigma) (500 μl per well) for at least 1 h at 37 °C and then washed twice with PBS and air dried. The gel mixture consisted of 8 parts Vitrogen (Celltrix Corp., Santa Clara, CA), 1 part (10 ×) minimal essential medium (Life Technologies, Inc.), and 1 part 0.2 mM HEPES, pH 9.0, which resulted in a final collagen concentration of 2.4 mg/ml. It was prepared at 4 °C, added to the culture vessel, and incubated for 1 h at 37 °C to allow gelation. Stellate cells isolated from animals 6 days after bile duct ligation were plated on top of the gels. After cell attachment for 24 h, serum-free conditions were introduced into the culture vessels. Two superfuse containing 1% collagen gel (Sigma) were added to elicit contraction (27). Contraction was monitored as the change in lattice area over time. For integrin-blocking experiments, the appropriate antibodies (clones Ha1/38 for α1, Ha1/29 for α2 and Ha2/11 for β1) were added at plating. The antibody concentration was one that produced 95–100% of maximal inhibition, as determined in preliminary experiments. For anti-α1, this was 217 μg/ml; for anti-α2 it was 223 μg/ml, and for anti-β1 it was 235 μg/ml (all as purified IgG).

Metabolic Labeling and Immunoprecipitation—Stellate cells were metabolically labeled with [35S]methionine (5 μCi/100-mm plate; ICN, Irvine, CA) overnight in medium without methionine and cysteine. Cellular proteins were solubilized in immunoprecipitation buffer (100 mM Tris/HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 0.1% N-dodecyl P-40, 2 mM phenylmethylsulfonyl fluoride, 20 μg of aprotinin, 20 μg of leupeptin) for 30 min at 4 °C. The cell lysate was centrifuged 10 min at 10,000 g, and the supernatant was preclared by incubation with protein A-Sepharose CL-4B beads (1:1 v/v, slurry in immunoprecipitation buffer; Pharmacia Biotech Inc.) for 45 min at 4 °C. Incubation with appropriate monoclonal antibody (clone Ha31/8 for α1, clone Ha1/29 for α2, and clone Ha2/11 for β1) was carried out overnight at 4 °C. Rabbit anti-hamster IgG (Pierce) was added 1 h before the end of the incubation, followed by addition of protein A-Sepharose beads. After a 45-min incubation, the beads with attached immune complexes were washed five times with immunoprecipitation buffer and then eluted by boiling for 5 min in 2 × Laemli loading buffer and resolved in an 8% SDS-polyacrylamide gel. Dried gels were exposed to X-Omat AR-5 film (Kodak).

Confocal Microscopy—For studies of cellular localization of α1 and α2, double labeling experiments were performed on stellate cells after 3 days in primary culture. Cells were fixed with 1.5% paraformaldehyde, 0.1% Triton X-100 for 10 min and then immunostained as described above (see “Immunohistochemistry on Liver Sections”). For evaluating co-localization of α1 or α2 and the actin cytoskeleton, double labeling using anti-talin antibody (1:100) (Amersham Corp.) was performed. Staining was analyzed with a confocal microscope (Meridian ACAS 570, Ohemos, MI). Generated images were corrected for compensation and threshold.

Statistical Analysis—Values are expressed as mean ± S.E. Statistics were performed with one-way analysis of variance with multiple comparisons. Statistical significance was assigned at probability value less than 0.05.

RESULTS

Expression of α1 and α2 Integrin mRNA by Stellate Cells in Vivo—The expression of α1 and α2 mRNA was examined in freshly isolated stellate cells and at various time points after bile duct ligation or dimethylnitrosamine treatment. In stellate cells from normal liver, α1 mRNA was readily detected (Fig. 1, A and B), and after bile duct ligation, it remained essentially constant over the 6-day period of observation (Fig. 1, A and C). In contrast, α2 mRNA was undetectable in normal stellate cells (Fig. 1, A and B). Although measurable after bile duct ligation
In immunohistology of normal liver, a detectable despite the observed, albeit small, increase in (Fig. 1, a specific probes for the a1 and a2 integrin subunits and for the ribosomal protein S14. The same RNA extract was used for all probes at individual time points. A and B, autoradiograms; arrows indicate specific bands. C, densitometry quantification of the autoradiogram presented in A and B showing the relative expression of a1 and a2 mRNA after correction for S14. Mean ± S.E. for each group (*p < 0.05 compared with normal). ■, a1 mRNA; □, a2 mRNA.

(Fig. 1, A and C), at all time points it was less than 8% of a1 mRNA. To test whether these findings were peculiar to bile duct ligation, which causes periporal injury (28), we carried out a similar study in rats treated for 1 week with dimethylsulfoxide. This chemical causes midzonal and pericentral necrosis (29). The profiles of a1 and a2 expression were entirely similar (Fig. 1, B and C).

Surface Expression of a1, a2, and β1 by Stellate Cells—Expression at the protein level was assessed with specific antibodies to a1, a2, and β1 subunits on stellate cells freshly isolated as above. Bound antibody was quantitated by flow cytometry. To ensure that the integrin subunits of interest were not altered by the proteases used in cell isolation, we carried out control studies with WKY cells, a smooth muscle line that is known to express both a1 and a2 integrins and for the ribosomal protein S14. The same RNA extract was used for all probes at individual time points. a1 and a2 subunits on stellate cells freshly isolated from normal livers or at different time points after bile duct ligation (1 (w) week (DMN)) (B), with three animals in each group. Total RNA was extracted and analyzed by RNase protection assay with specific probes for the a1 and a2 integrin subunits and for the ribosomal protein S14. The same RNA extract was used for all probes at individual time points. A and B, autoradiograms; arrows indicate specific bands. C, densitometry quantification of the autoradiogram presented in A and B showing the relative expression of a1 and a2 mRNA after correction for S14. Mean ± S.E. for each group (*p < 0.05 compared with normal). ■, a1 mRNA; □, a2 mRNA.

Immunofluorescence Detection of a1 and a2 in Liver Tissue during Injury—In immunohistology of normal liver, a1 appeared as a sharp linear stain along the sinusoids consistent with its localization to stellate or endothelial cells. At 6 days after bile duct ligation, staining was unchanged. In neither setting was a2 detectable, in agreement with the fluorescence-activated cell sorter analysis. The reactivity of the a2 antibody in immunohistology was verified by positive staining of rat footpad skin (30; data not shown).

Stellate Cell Binding to Collagen I and IV—Stellate cells were isolated 6 days after bile duct ligation, and their adhesion to collagen was tested. Antibody to α1 integrin reduced adhesion to collagen type I by 90% and to collagen type IV by 95% (Fig. 3). Antibody to a2 had no effect, and the combination of anti-a1 and anti-a2 had effects similar to those of anti-a1 alone. Anti-β1 inhibited 55% of the binding to collagen type I and 80% of the binding to collagen type IV. Although the effect of anti-β1 was on average less than that of anti-a1, the difference was not significant. Anti-αv had no effect on binding to either type of collagen. From these results, and studies at both the mRNA and the protein levels, we conclude that expression of a2 integrin is negligible both in normal liver and in the setting of wound healing.

Role of α1β1 and α2β1 Integrins in Stellate Cell Contraction—For studies of contraction, stellate cells were isolated 6 days after bile duct ligation at which time they exhibit myofibroblast characteristics, attaching rapidly to collagen gels. Contraction was elicited by endothelin 1 (27, 31, 32), and the decrease in lattice area was monitored in the presence or absence of blocking antibodies to a1, a2, or β1 integrin subunits. Base-line contraction (indicated as 100% contraction) was established in plates treated with non-immune IgG and exposed to endothelin 1 (Fig. 4); the gel area was 40–50% that of non-contracted controls. Anti-a1 blocked contraction significantly. Unexpectedly, the effect of anti-a2 also was significant although less than that of anti-a1. Given together, anti-a1 and anti-a2 were additive, completely inhibiting contraction, and anti-β1 was similarly effective. Antibody to αv, which does not bind collagen, had no effect. When the morphology of cultures on collagen gels was examined, the effect of an individual antibody on cell processes correlated closely with its effect on contraction. Processes were numerous in control cultures or in cultures exposed to anti-αv (Fig. 5, a and f). By contrast, in cultures exposed to anti-a1 or anti-a2 (Fig. 5, b and c), processes were reduced, and they were virtually eliminated in cultures containing both anti-a1 and anti-a2 or anti-β1 (Fig. 5, d and e). None of the antibodies altered cell attachment to the gel.
Regulation of $\alpha_{2}\beta_1$ Expression in Stellate Cells Cultured on Collagen Gels—A salient difference between these and the preceding experiments is the fact that the flow cytometry and collagen-binding studies were conducted with fresh isolates, whereas the contraction studies required a minimum period in culture (about 24 h) for attachment of the cells to the gel. Because phenotypic adaptation to culture is known to occur within this time frame (10, 11), we evaluated $\alpha_2$ integrin mRNA expression early after cell plating. Cells from 6-day bile duct ligated liver were isolated and placed on collagen gels, and $\alpha_1$ and $\alpha_2$ mRNA were quantitated. As shown (Table I), $\alpha_2$ mRNA increased strikingly after just 24 h of culture, and synthesis of its protein was readily detected by immunoprecipitation after metabolic labeling. By contrast, $\alpha_1$ expression did not change either at the mRNA or protein level during 24 h of culture on collagen gels (Fig. 6). The rapid up-regulation of $\alpha_2\beta_1$ in culture appears to explain its participation in contraction.

Distribution of the $\alpha_1$ or $\alpha_2$ Integrin Subunits in Cultured Stellate Cells and Their Co-localization with the Actin Cytoskeleton—Double labeling experiments on 3-day cultured stellate cells revealed a striking difference in the distribution of $\alpha_1$ and $\alpha_2$ integrins (Fig. 7). $\alpha_1$ was present on processes and at the periphery of cells to a much greater extent than was $\alpha_2$; the latter was concentrated over and around the nucleus, suggesting...
Expression of the 24 hours in culture. RNase protection assay was used to quantify the remaining cells were plated on top of collagen I gels and harvested after ligation. One-half of the harvest was taken for RNA extraction. The presence of either anti-α1 or anti-α2 and nearly abolished by the combination of anti-α1 and anti-α2 or by anti-β1.

FIG. 5. Effect of blocking antibody to α1, α2, or β1 integrin subunits on stellate cell morphology. Hepatic stellate cells isolated from livers 6 days after bile duct ligation were plated on top of collagen I gels in the absence or presence of specific monoclonal antibodies. After 24 h, the morphology of living cells was examined by phase-contrast microscopy. a, no antibody; b, anti-α1; c, anti-α2; d, anti-α1 + anti-α2; e, anti-β1; f, anti-αv. In the control and in cultures treated with anti-αv, numerous cell processes are present (arrows); these are reduced in the presence of either anti-α1 or anti-α2 and nearly abolished by the combination of anti-α1 and anti-α2 or by anti-β1.

TABLE I

Regulation of α1 and α2 mRNA expression by activated stellate cells in culture for 24 hours on collagen gels

Hepatic stellate cells were isolated from livers 6 days after bile-duct ligation. One-half of the harvest was taken for RNA extraction. The remaining cells were plated on top of collagen I gels and harvested after 24 hours in culture. RNase protection assay was used to quantify expression of the α1 and α2 integrin subunits and the ribosomal protein S14. Data are densitometric units after correction for the amount of S14 and represent the mean ± S.E. for each group (n = 7).

|       | Fresh isolates | 24-h culture | Fold increase at 24 h |
|-------|----------------|--------------|-----------------------|
| α1 mRNA | 5982 ± 907     | 5255 ± 545   | 0.88                  |
| α2 mRNA | 449 ± 61       | 3465 ± 1515* | 7.71                  |

*p < 0.05 compared to fresh isolates.

FIG. 6. Synthesis of α1β1 and α2β1 integrins by hepatic stellate cells in culture. Hepatic stellate cells in culture were labeled overnight with [35S]methionine, lysed as described under "Experimental Procedures" and immunoprecipitated with monoclonal antibody to α1, α2, or β1 integrins. Immunoprecipitates were resolved by SDS-polyacrylamide gel electrophoresis and autoradiography. Controls consisted of extracts processed in parallel with nonspecific antibody.

**DISCUSSION**

From studies with individual cell lines, it is clear that both α1β1 and α2β1 may exist on the same cell type and that either or both can provide the anchorage needed for contraction (3–7). On the other hand, expression of the respective integrins in the mature organism in vivo appears to be restricted by cell type with little overlap between the two. The separation appears to be maintained even in mutant mice lacking α1, in which there is no evidence of a compensatory increase in α2 expression (33).
α1 is expressed on smooth muscle, microvascular endothelium, glomerular mesangium, mammary myoepithelial cells, and chondrocytes (33). α2 appears on fibroblasts, but its predominant distribution is to epithelium, particularly at sites of proliferation (15, 30). In normal human liver, it is reportedly on the biliary epithelium, although α1 is present on hepatocytes and sinusoidal lining cells (34, 35). In the present study, α1 mRNA was found on hepatic stellate cells, whereas α2 was not detectable even with a sensitive RNase protection assay. After injury, α2 mRNA was detectable although its protein was not, even on the biliary epithelium. The discrepancy with the α2 data from human liver may be species-related or reflect a difference in the relative affinity of the anti-human and antimerine antibodies.

To ensure that the finding of α2 mRNA was not peculiar to the biliary ligation model, we performed a similar evaluation of injury induced by the toxin dimethylsulfoximine, with essentially identical results. Although the data confirm that liver myofibroblasts may co-express these integrins (36), the marked predominance of α1β1 indicates that, of the two, it is functionally the most important. This conclusion is clearly supported by the binding studies, which indicate that essentially all stellate cell binding to collagen I or IV is mediated by α1β1 (Fig. 4). The data differ from those of Schiro and colleagues (7), who evaluated the contractile function of a human fibroblast cell line and of transfected rhabdomyosarcoma cells, concluding that only α2 is important. Although the cells reportedly expressed both α1 and α2 (7), the relative level of these integrins was not assessed nor was the binding activity of α1 examined. It is known that a cell line expressing α1 only is capable of contracting a collagen lattice (4). Thus, it appears that both integrins can subserve contraction and that the predominance of one or the other in a specific cell type or physiological circumstance will reflect its relative expression on the cell surface, apart from considerations of receptor activation and signaling pathways. Our data indicate that in vivo the expression of α1 on myofibroblasts far exceeds that of α2.

The role of α1 and α2 integrins in contraction of collagen gels by activated stellate cells was examined with direct experiments in vitro, in which cells were plated with or without the appropriate monoclonal blocking antibody. Neither anti-α1 nor anti-α2 (or both together) reduced cell attachment to the gels, indicating the presence of other receptor(s). The principal fibronectin receptor, α5β1, is well expressed by stellate cells,2 and activated stellate cells produce significant fibronectin (21), which may add to a collagenous substrate through its well-characterized collagen-binding domain (37, 38), thus providing a ligand for α5β1. By a similar mechanism involving other secreted ECM proteins, a variety of receptors (integrins and others) could substitute for α1β1, mediating the attachment of cells to collagen gels.

Although not altering attachment, the antibodies to α1 and α2 nonetheless had well-defined effects on morphology, causing retraction of cell processes. Qualitatively, such effects closely paralleled the ability of these same antibodies to inhibit contraction. The morphological change suggests that both integrins occupy the periphery and cell processes, as would be expected for attachments that provide traction. Confocal microscopy confirms that this is the case for α1. The localization of α2 differed in being more central than peripheral, although the limited expression on cell processes still may be sufficient to account for the small but significant effect of anti-α2 on stellate cell contraction. If the change in α2 expression is persistent, as seems likely, the data provide an explanation for the fact that α2 integrin commonly is present on smooth muscle cell lines despite its absence on this cell type in vivo (15, 30).

This has implications not only for the use of myofibroblast lines in modeling contraction but also for primary culture. Several studies point to the fact that normal stellate cells respond to culture with a program of gene expression that mimics activation in vivo (10). The up-regulation of α2β1 in early primary cells, while demonstrating the latent capacity for expression of this integrin, indicates that the culture model deviates significantly from in vivo reality, highlighting the necessity for routinely validating with intact tissue the findings from culture models.

The relatively constant expression of α1β1 in normal liver and during wound healing is at variance with data from other tissues in which this integrin increases with injury and fluctuations during development, as judged by immunohistology (39, 40). For example, it is undetectable in the normal rat carotid but clearly present in the neointima after balloon injury (33), suggesting that its level and timing may be critical to contraction. In this respect, the injury response of epithelia may differ from that of the vasculature.

The findings suggest that in liver the level of α1 expression does not govern the contractile response. If not α1, then what? The principal contractile agonists for activated stellate cells are endothelins 1 and 3. Interestingly, expression of their receptors is prominent on normal stellate cells and, like α1 integrin, unaffected by injury (41). Expression of endothelin 1 by stellate cells, however, is up-regulated in injury (41, 42). If regulation of contraction by endothelin is autocrine, then this is significant. Another potentially regulatory factor is the ECM. Although collagen IV is a ligand for α1β1 and is found normally in the subendothelial space, contraction may require organized fibrillar collagen, which are sparse in normal liver. Collagen I is confined largely to vascular branch points where it may serve as a substrate for vasoregulatory stellate cells (43). Similarly, contraction in hepatic wound healing may require deposition of the appropriate substrate in the form of fibrillar collagen (types I, III, V, and VI) at the injury site. A final variable is the rate at which stellate cells in the injured liver acquire the necessary contractile apparatus, which must be synthesized de novo; this process is mirrored by expression of smooth muscle α-actin (8, 9, 11), which increases in parallel with contractility (44).

In summary, contraction of hepatic myofibroblasts in wound healing utilizes the α1β1 integrin. We find no evidence for a role of α2β1. Because α1β1 is expressed quasi-constitutively, contractility may depend primarily on formation of a fibrillar ECM at the injury site, coupled with cellular expression of contractile proteins and local (autocrine) elaboration of the appropriate agonist in the form of endothelins 1 and 3. Given that these events are co-temporal, the possibility exists that they are coordinated by signaling via α1β1. This is a topic for further studies.

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