Integrin $\alpha_2\beta_1$ Is Upregulated in Fibroblasts and Highly Aggressive Melanoma Cells in Three-Dimensional Collagen Lattices and Mediates the Reorganization of Collagen I Fibrils

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Abstract. The ability of cultured human fibroblasts to reorganize and contract three-dimensional collagen I gels is regarded as an in vitro model for the reorganization of connective tissue during wound healing. We investigated whether adhesion receptors of the integrin family are involved. It was found that synthesis and transcription of the $\alpha_2\beta_1$ integrin (but not of $\alpha_1\beta_1$ or $\alpha_3\beta_1$) is selectively upregulated when fibroblasts are seeded into type I collagen gels. Time course experiments revealed that high synthetic levels of $\alpha_2\beta_1$ parallel the gel contraction process and return to "baseline" levels after the contraction has subsided. Furthermore, function-blocking mAbs directed to the $\alpha_2$ and $\beta_1$ chain of integrins inhibited gel contraction.

Remodelling of connective tissue can be important for tumor cells during invasion and formation of metastases. Therefore, we tested human melanoma cell lines for this function. Five out of nine melanoma lines contracted collagen gels in vitro. Among these, two highly aggressive melanoma cell lines (MV3 and BLM) most efficiently contracted gels almost reaching the rate of normal adult fibroblasts. In these cells, synthesis of $\alpha_2\beta_1$ was also significantly upregulated when seeded into collagen I gels. Moreover, function blocking anti-$\alpha_2$ in conjunction with anti-$\beta_1$ chain mAbs completely inhibited gel contraction for several days. Other melanoma cells (530) with lower metastatic potential which were not able to contract gels, showed no induction of $\alpha_2\beta_1$ synthesis in gel culture. Our results suggest an important role of integrin $\alpha_2\beta_1$ in the contraction of collagen I by normal diploid fibroblasts during wound healing and in the reorganization of collagen matrices by highly aggressive human melanoma cells.

The reorganization of collagen by fibroblasts is an important function in wound healing which leads to wound contraction and finally helps to reestablish organ integrity. The ability of cultured fibroblasts to reorganize and contract three-dimensional collagen I gels (Bell et al., 1979) is considered as an in vitro model for wound contraction. Previous studies have described in detail the influence of cytokines (Gullberg et al., 1990), the requirement of protein synthesis and of an intact cytoskeleton for this process (Mauch, 1986; Guidry and Grinnell, 1985). Seeding of fibroblasts into a three-dimensional collagen lattice results in major changes of their morphology (Tomasek et al., 1982), their protein and collagen metabolism (Mauch et al., 1988) as well as in their response to cytokines (Nagakawa et al., 1989). However, little is known, so far, about the role of extracellular matrix (ECM) receptors on the fibroblast surface for this function. Recently, evidence has been provided that polyclonal antisera directed against the $\beta_1$ chain of integrins may interfere with gel contraction (Gullberg et al., 1990). Among the $\beta_1$ subgroup of integrins, at least three receptors ($\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$) are known to interact with collagen (Wayner and Carter, 1987; Belkin et al., 1990; Kirchhofer et al., 1990).

In this study, our first aim was to identify single integrin receptors involved in this process. We show that $\alpha_2\beta_1$ is the only integrin which is strongly upregulated when fibroblasts start contracting collagen I gels. Furthermore, we demonstrate that function blocking anti-$\alpha_2$ in conjunction with anti-$\beta_1$-chain mAbs most efficiently inhibit gel contraction.

The capability to reorganize collagen may also be advantageous for tumor cells during tissue invasion. Recent studies revealed that $\alpha_2\beta_1$ is involved in the migration of tumor cells within collagenous matrices (Yamada et al., 1990) and that it is expressed at increased frequency during tumor progression in human melanoma (Klein et al., 1991). Therefore, we also tested human melanoma cell lines for their ability to reorganize collagen I lattices and investigated the role of $\alpha_2\beta_1$ in this function.

1. Abbreviations used in this paper: CFN, cellular fibronectin; ECM, extracellular matrix.
Material and Methods

Cells and Culture Conditions

Cell lines were cultured in RPMI 1640 supplemented with 10% FBS, 2 mM glutamine, 1% nonessential amino acids, 100 U/ml penicillin, and 100 U/ml streptomycin. Cultures of normal fibroblasts and melanocytes were established and maintained as previously described (Klein et al., 1988; Eisinger and Marko, 1982; Halaban et al., 1986). Melanoocyte cultures (M.LM, p-5; M.SD, p-12; M.RF, p-6; and M.HF, p-6) established from the foreskin of young children or young adults were kindly provided by Dr. D. Kaufman (Department of Human Genetics, University of Ulm, Ulm, Germany). The foreskin fibroblast cell line F 135-60-86-skin was originally obtained from Dr. J. Fogh's cell bank at the Memorial Sloan Kettering Cancer Center, New York. The melanoma cell lines SK-MEL-13, -19, -29, and -113 were gifts from Dr. L. J. Old (Memorial Sloan Kettering Cancer Center, New York). The melanoma cell lines 530 (Versteeg et al., 1988), BLM (van Muijen et al., 1989), and MV3 (van Muijen et al., 1991) were kindly provided by Dr. G. van Muijen (Department of Pathology, Academisch Ziekenhuis, University of Nijmegen). The squamous carcinoma cell lines SCL1 and -2 (Tilgen et al., 1986) were gifts from Dr. N. E. Fusenig (Department of Biochemistry, German Cancer Research Center, Heidelberg, Germany). All cell lines were repeatedly subject to hybridization tests using β2-labeled mycosplasma DNA (Mycosplasma TC Gen Probe Inc., San Diego, CA) and were negative.

Monoclonal antibodies

Serum or ascites of a hybridoma bearing mice or tissue culture supernatant was the source of mAbs: mAb TS2/7 binds to the α1-chain of integrin α5β1 (VLA-4) (Hemler et al., 1985), mAbs 10G11 (Hemler et al., 1988), A-143 (Klein et al., 1991), GI4 (Santoso et al., 1989), SEB (Zystra et al., 1986; Takada et al., 1989), and P1E6 (Wayner et al., 1988) detect the α2-chain of α2β1, mAbs J143 (Kantor et al., 1987), and P1B5 (Wayner and Carter, 1987) define the α5 chain of α5β1, mAbs B5G10 (Hemler et al., 1987) and P4G9 (Wayner et al., 1989) recognize the α4 chain of α4β1; mAbs 16 (Akyama et al., 1989) and P6D6 (Wayner et al., 1988) bind to the α3 chain; mAbs GoH3 (Sonnenberg et al., 1989) and MT78 (Klein et al., 1990) detect the α6 chain; mAb LM142 (Cheresh and Spiro, 1987) recognizes the β1 chain. mAbs Aj2 (Kantor et al., 1987), 13 (Akyama et al., 1989), and 4B4 (Morimoto et al., 1985; Shimizu et al., 1990) are directed to the β1 chain. mAbs TS2/7 and B5G10 were kindly provided by M. Hemler (Dana Farber Cancer Center, Boston, MA); the purified mAbs 16 and 13 were gifts from S. A. Akayama (Howard University Cancer Center, Washington, DC). mAbs J143 and A2 were kindly provided by L. J. Old (Memorial Sloan Kettering Cancer Center, New York) mAbs GoH3 and 10G11 were gifts from S. Sonnenberg (Central Blood Bank of the Netherlands, Amsterdam). mAb LM142 was kindly provided by D. Cheresh (Scripps Clinic, La Jolla, CA). mAbs P1E6, P6D6, P4G9, and 4B4 were purchased from Telios Pharmaceuticals Inc. (San Diego, CA) and Coulter Corporation (Hialeah, FL), respectively. The function blocking mAbs used in the experiments listed in Table I. For inhibition studies, mAbs were affinity-purified by ammonium sulfate precipitation and subsequent affinity chromatography on PA-Sepharose Columns using standard procedures.

Preparation of Collagen Gels (Hydrated Collagen I Lattices)

Collagen I was extracted from rat tail tendons and stored lyophilized as previously described (Mausch et al., 1988). Collagen I (2 mg/ml) was dissolved in 0.1% acetic acid and stored at 4°C as stock solution. For gel preparation, 2.5 ml Mc Coy's medium (1.95-fold concentrated), 0.9 ml FBS, 0.25 ml 0.1 N NaOH was added to 1.5 ml collagen I stock solution in 60-mm bacteriological petri dishes. The supernatants were then centrifuged to remove collagenous debris. The supernatants were then centrifuged at 9000 g for 10 min. The RNA pellet was dissolved in water, treated with phenol/chloroform, precipitated with 0.3 M sodium acetate, through a 5.7 M CsCl cushion. The RNA pellet was dissolved in water, treated with phenol/chloroform, precipitated with 0.3 M sodium acetate, and 2.5 vol of ethanol. For Northern blot hybridization, 5 μg of total RNA was separated by electrophoresis on a 1% formaldehyde agarose gel and transferred to Gene Screen hybridization transfer membranes (New England Nuclear Research Products, Boston, MA). The filters were cross-linked by UV (Stratagene) and hybridized with primer-labeled radioactive cDNA probes specific for the VLA α2 and β1 chain as well as β tubulin. Densitometric scans were performed from autoradiographs to quantify the intensity of hybridization (Hirschmann Elsper 400). CDNA's for VLA α2 (clone 2.72L) (Takada and Hemler, 1989) were obtained from Dr. M. E. Hemler (Dana Farber Cancer Center, Boston, MA) for VLA β1 (clone pGEMI-P32) (Argraves et al., 1987) from Dr. E. Ruoslahti (La Jolla Cancer Research Institute, La Jolla, CA) and for β-tubulin (clone D21) (Hall et al., 1983) from Dr. D. W. Hall (Dept. of Biochemistry, New York University, New York).

Results

When seeded into collagen I gels, fibroblasts contract and organize the collagen leading to the formation of a dense "interstitial connective tissue." For our studies, adult human dermal fibroblasts were seeded at a density of 2 × 104 cells/ml into gels containing 0.6 mg/ml collagen I which had been purified from rat tail tendons. Time course studies showed that under this condition contraction of the gels was first noticeable after 6-7 h. Most of the contraction, however, occurred between 12 and 48 h and after 72-80 h the process was completed. A representative experiment is shown in Fig. 1.

First, we compared the synthesis of integrins in fibroblast monolayer cultures with that of split cultures which had been seeded into the gels. Cells were metabolically labeled with "H]-thymidine (60-200 μCi/ml; New England Nuclear, Boston, MA) for 6 or 16 h in methionine-free medium containing 10% dialyzed FBS. After metabolic labeling, the collagen gels were immersed into NP40-lys buffer (0.5% NP-40, 0.015 M NaCl, 0.01 M Tris pH 7.5, 0.002 M PMSF, and aprotinin), minced into fine pieces, repeatedly aspirated into syringes, and forced through needles with decreasing diameter. Monolayer cultures were incubated in NP-40 lys buffer, scraped off the tissue culture plastic ware and then treated equally as the gel culture lysate. Glycoproteins were isolated from NP-40 solubilized cell extracts by adsorption to Con A Sepharose (Pharmacia Inc., Uppsala, Sweden) (Lloyd et al., 1981). Immunoprecipitations were carried out as previously described (Klein et al., 1988). To compare glycoprotein synthesis under different culture conditions, equal numbers of counts of the Con A-bound fractions were immunoprecipitated. The amounts of precipitated glycoproteins were determined after SDS-PAGE by quantitative density scanning of the fluorographs.

RNA Isolation and Northern Blot Hybridization

Table I. Function Blocking mAbs Directed to Integrins Used in This Study

| Integrin Chain | mAb | Reference |
|----------------|-----|-----------|
| α2 chain       | 5E8 | Zylstra et al., 1986 |
|                |     | Takada et al., 1989 |
|                | P1E6| Wayner et al., 1988 |
|                | P1B5| Wayner et al., 1987 |
| α3 chain       | 16  | Akyama et al., 1989 |
| β1 chain       | 13  | Akyama et al., 1989 |
|                | 4B4 | Shimizu et al., 1990 |
|                |     | Morimoto et al., 1985 |

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[35S]methionine for 16 h, starting 24 h after culture initiation. Synthesis of integrin receptors was assessed by immunoprecipitation from the Con A–bound fractions of NP-40 cell lysates. mAbs directed to the α1, α2, α5, α1, α4, αβ, and β1 chain of integrins were used.

Fibroblasts contracting collagen gels showed 10- to 14-fold higher synthetic levels of α2β1 than monolayer cultures, whereas all other integrins studied were unchanged (α1β1, α2β1, α5β1, α6β1, αβ) or reduced (α4β1) (Figs. 2 and 3). It was remarkable that the two other β1 integrins which are known to also bind collagen were unchanged. To exclude the possibility that changes in the glycosylation of integrins led to their differential binding to Con A we also immunoprecipitated the seven receptors from whole cell lysates of the two culture conditions without adsorption to Con A and found the same relationship as between the immunoprecipitates from the Con A–bound glycoproteins (not shown). Particularly, the same 10- to 14-fold increase of the α2β1 immunoprecipitate in fibroblasts contracting collagen gels was observed. Furthermore, the comparison of the immunoprecipitates from whole cell lysates and from the Con A–bound fractions by SDS-PAGE revealed the same electrophoretic mobility suggesting that there are no major differences in glycosylation. In respect to α2β1, the results were further confirmed by comparative immunoprecipitations from monolayer and gel cultures using three different mAbs (10G11, 1429).

**Figure 1.** Contraction of collagen I gels by human adult fibroblasts (H-45). Three collagen I gel cultures (2 ml) of 2 x 10⁵ human adult fibroblasts (H-45) were prepared in 35-mm bacteriological petri dishes. Cultures were photographed at different times after culture initiation. From left to right: 6, 18, 48 h.

**Figure 2.** Synthesis of integrins in collagen I gel and monolayer cultures of normal human fibroblasts. Con A–bound fractions of NP-40 cell lysates from [35S]methionine-labeled parallel cultures (H-45) were analyzed by immunoprecipitation and SDS-PAGE. The fluorograph shows immunoprecipitates obtained with different mAbs: (lane 1) control, normal mouse serum; (lane 2) 10G11, anti-α2; (lane 3) J143, anti-α5; (lane 4) GoH3, anti-α6; (lane 5) Aj2, anti-β1; (lane 6) LM 142, anti-α4; (lane 7) W6/32, anti-HLA class I.
upregulation of α2β1. Adult fibroblasts in “gel culture” were metabolically labeled for 6 h at different times after culture initiation; it was found that the synthesis of α2β1 was already increased when cells were labeled at 6 h after initiation of the cultures. High levels of α2β1 synthesis were seen at 6–12, 24–30, and 48–54 h with a peak level between 24–30 or 48–54 h. Interestingly, at day 5 when gel contraction had subsided, the synthetic activity of α2β1 was decreased to the “baseline” level of monolayer fibroblasts (Fig. 4). HLA class I antigens (mAb W6/32) were analyzed in parallel for each condition and no change of their synthetic levels was noted (not shown). In some fibroblast cell lines, which showed a particular high contraction rate, the level of α2β1 synthesis already declined 24 h after culture initiation. From these ex-

Figure 3. Synthesis of integrins in collagen I gel and monolayer cultures of normal human fibroblasts. Con A-bound fractions of NP-40 cell lysates from [35S]methionine-labeled parallel cultures (H-45) were analyzed by immunoprecipitation and SDS-PAGE. The fluorograph shows immunoprecipitates with different mAbs: normal mouse serum (lane 1); Ts2/7, anti-α1 (lane 2); 10G11, α2 (lane 3); B5G10, anti-α4 (lane 4); P1D6, anti-α3 (lane 5); A2, anti-β1 (lane 6); and W6/32, anti-HLA class I (lane 7).

Figure 4. Synthesis of integrin α2β1 by fibroblasts in gel cultures. Adult fibroblasts (H-45) in collagen I gel culture were metabolically labeled for 6 h at different times after culture initiation (0, 6, 24, 48, and 5 d). The synthetic levels of α2β1 were then compared with that of fibroblast monolayer cultures (reg). Cell lysates were processed for immunoprecipitation as described in Materials and Methods. (lane 1) normal mouse serum, control; (lane 2) mAb 10G11, anti-α2 chain.
Figure 5. Expression of VLA-α2 by cells in collagen gels and monolayers. Total RNA was isolated from fibroblasts (NH-1) grown for different times in collagen gels (2-4) and as monolayers (1). Total RNA was separated in a denaturing agarose gel containing 1% formaldehyde, blotted and hybridized with the radioactively labeled cDNA probe specific for VLA-α2 (A) and β-tubulin (B). Positions of 18S and 28S RNA markers are indicated. Lane 1 represents mRNA from fibroblasts grown for 24 h as monolayers, lanes 2-4 mRNA from fibroblasts grown for different times in collagen gels (6, 12, and 18 h).

We then studied the possibility of synergistic effects between β1 and α chain mAbs. For this purpose, β1 chain mAbs were used either at a suboptimal concentration of 0.4 µg/ml or at an optimal inhibitory concentration of 2.5 µg/ml and were combined with different α chain mAbs (2.5 µg/ml) or mAb W6/32 (2.5 µg/ml). The α2 chain mAb 5E8 in conjunction with one of the anti-β1 chain reagents further augmented the partial inhibition of gel contraction which was exerted by the β1 chain mAbs alone. This synergistic effect could also be demonstrated when another α; chain mAb (P1B5) was used. The α5 chain mAb PIB5, the α chain mAb 16, however, and mAb W6/32 had no augmentory effect in combination with β1 chain mAbs. A representative experiment is shown in Table II. From these experiments we

Table II. Influence of mAbs on Collagen I Gel Contraction by Adult Human Skin Fibroblasts

| mAb                          | Gel diameter (area) |
|------------------------------|---------------------|
| 0                            | 18 h 24 h 48 h      |
| HLA class I                  | 8 (0.50) 7 (0.38) 7 (0.38) |
| α2 chain                     | 9 (0.64) 8 (0.50) 8 (0.50) |
| α2 chain                     | 8 (0.50) 7 (0.38) 7 (0.38) |
| β1 chain (low conc)          | 10 (0.79) 9 (0.64) 0 (0.64) |
| β1 chain plus HLA class I    | 10 (0.79) 10 (0.79) 10 (0.79) |
| β1 chain plus α; chain       | 14 (1.54) 10 (0.79) 9 (0.64) |
| β1 chain plus α2 chain       | 25 (4.91) 16 (2.01) 15 (1.77) |
| β1 chain (high conc)         | 27 (5.72) 15 (1.77) 15 (1.77) |
| β1 chain plus HLA class I    | 23 (4.15) 15 (1.77) 15 (1.77) |
| β1 chain plus α; chain       | 22 (3.80) 15 (1.77) 15 (1.77) |
| β1 chain plus α2 chain       | 29 (6.60) 20 (3.14) 20 (3.14) |
| α; chain plus α; chain       | 11 (0.95) 8 (0.50) nd |
| α; chain plus HLA class I    | 10 (0.79) 8 (0.50) nd |

Experiments were performed in 35-mm Petri dishes. 4 x 10^5 fibroblasts (H-45) were seeded into 2 ml of gel containing 1.2 mg collagen I. The inner diameter of the dishes was 30 mm. Gel diameters were measured with a ruler. The area of an uncontracted collagen gel was 7.07 cm^2. Different mAbs were added to the gels during preparation. The mAb concentration was 2.5 µg/ml for all mAbs except the function blocking β1 chain antibody 4B4, which was used either at a low concentration resulting in a marginal suboptimal inhibitory effect (0.4 µg/ml gel) or at a high concentration providing optimal inhibition (2.5 µg/ml gel). HLA class I mAb (W6/32); anti-α; chain mAb, which blocks function (mAb16); anti-α2 chain mAb, which blocks function (5E8), nd, not done; 0, no mAb was added.

* gel diameter (area) in mm (cm²).
concluded that the α2β1 complex is of functional relevance for the gel contraction by fibroblasts.

To further study the involvement of α2β1 in the reorganization of collagen I fibrils, we chose another cell system and tested human melanoma cell lines and normal melanocyte cultures for their ability to contract collagen gels. This cell type appeared particularly interesting, since it has recently been found that α2β1 is differentially expressed in normal and transformed melanocytic cells in vitro and is associated with tumor progression in vivo (Klein et al., 1991). Five out of nine melanoma cell lines were able to contract collagen gels, whereas normal melanocytes were not (Table III). The contraction rate was significantly lower than that of diploid fibroblasts with the exception of the lines MV3 and BLM which contracted the gels with almost the same efficiency as fibroblasts. Two melanoma lines, MV3 and 530, were selected for further study and served as examples for contracting and noncontracting cells, respectively. First, we compared the synthesis of several integrins in monolayer culture. MV3 cells showed higher synthetic levels of α2β1, α3β1, and α6β1 than 530 (Fig. 6). α2β1 and α6β1 were expressed at very low and intermediate levels, respectively, in both lines (Fig. 6) whereas α3β1 was not detectable (not shown). When seeded in collagen gels, MV3 cells revealed a significant induction of α2β1 synthesis in comparison to the monolayer cultures (Fig. 6). Density scanning of the fluorographs showed a four- to eightfold increase. Other integrins were unchanged (α2β1, α3β1) or reduced (α2β1, α3β1, α6β1). The same results were obtained when the other “contracting” cell line BLM was studied. In contrast, upregulation of α2β1 synthesis in gel culture was not seen in the “noncontracting” line 530 (Fig. 6).

To further link collagen gel contraction to α2β1 function, we tried to block the contraction by MV3 cells with anti-α2β1 mAbs. Again, function blocking mAbs directed to the α2, α3, and α6 chain of integrins as well as the control mAb W6/32 were used alone or in combination with blocking mAbs directed to the β1 chain. Here, it was found that the anti-α2 chain mAb 5E8 alone inhibited gel contraction (Table IV and Fig. 7). However, complete inhibition was transient and the cells had partially overcome the inhibition after 48 h. Long lasting complete inhibition was not accomplished even at antibody concentrations of 50 μg/ml gel. Also, anti-β1 chain mAbs alone (4B4 or 13) achieved full inhibition for a short time period only (Table IV). The anti-α3 and α6 chain mAbs P1B5 and 16, respectively, as well as control mAb W6/32 had no effect. However, when mAb 5E8 (anti-α2) (2.5 μg/ml) was added to the gel culture in combination with mAbs 4B4 or 13 (anti-β1) a long lasting complete inhibition of gel contraction was observed (Table IV and Fig. 7). The inhibition could not be overcome by the cells even after a culture period of 5 d without any further addition of mAbs. In normal diploid fibroblasts, complete inhibition had not been observed. Metabolic labeling of the “long-term inhibited” melanoma cultures with [35S]methionine showed that the cells had retained their metabolic activity with protein synthesis levels as high as in 24 h gel cultures (not shown). The differential inhibitory effect of function blocking anti-α3 and β1 chain mAbs was confirmed in five experiments in which the influence of the mAbs on the contraction by fibroblasts and melanoma cells was studied in parallel.

**Table III. Contraction of Hydrated Collagen I Lattices by Human Melanoma Cells, Normal Melanocytes and Fibroblasts**

|                     | 48 h  | 96 h  |
|---------------------|-------|-------|
| **Melanomas**       |       |       |
| MV3                 | 12(1,13)± | 10 (0,79) |
| BLM                 | 13 (1,33) | 10 (0,79) |
| 530                 | n.c.  | n.c.  |
| IF6                 | n.c.  | n.c.  |
| Mewo                | n.c.  | n.c.  |
| SK-Mel-13           | 40 (12,56) | 33 (8,55) |
| SK-Mel-19           | n.c.  | n.c.  |
| SK-Mel-29           | n.c.  | 40 (12,56) |
| SK-Mel-113          | n.c.  | n.c.  |
| **Melanocyte cultures** |   |       |
| M.LM                | n.c.  | n.c.  |
| M.SD                | n.c.  | n.c.  |
| M.RF                | n.c.  | n.c.  |
| M.HF                | n.c.  | n.c.  |
| **Fibroblasts**     |       |       |
| H-45                | 12 (1,13) | 10 (0,79) |
| H-EK                | 10 (0,79) | 10 (0,79) |
| H-50                | 12 (1,13) | 11 (0,95) |
| H-63                | 9 (0,64)  | 9 (0,64)  |
| NH-1                | 8 (0,50)  | 8 (0,50)  |
| MU-2                | 8 (0,50)  | 8 (0,50)  |
| F135-60-86 skin     | 8 (0,50)  | 8 (0,50)  |

* Experiments were performed in 60-mm petri dishes (inner area 23.7 cm²). n.c., no contraction. 1.2 x 10⁵ fibroblasts were seeded into 5 ml of gel containing 3 mg collagen I. The experiments were read after 48 and 96 h. Gel diameters were measured with a ruler. ± Gel diameter (area) in mm (cm²).

**Discussion**

In this study, we investigated the involvement of integrin receptors in the reorganization of collagen I by human fibroblasts and melanoma cells. Among the three known collagen-binding integrins of the β1 subfamily (αβ1, αβ1, and αβ1) only αβ1 was strongly upregulated in both cell types during gel contraction. The elevation in αβ1 synthesis was closely associated with the contraction process and returned to baseline levels after contraction had subsided. Moreover, the induction of αβ1 was also demonstrable in the transcriptional level.

Inhibition studies using mAbs which block ligand binding or function of β1 integrins, revealed that the combination of anti-α3 chain with anti-β1 chain mAbs was most effective in inhibiting gel contraction. The comparison of the two cell types in respect to the inhibitory effect showed that these mAbs can block gel retraction of normal fibroblasts only partially whereas full- and long-lasting inhibition can be achieved in some melanoma cell lines (MV3 and BLM). This finding suggests that in fibroblasts, collagen receptors other than αβ1 or collagen receptor independent mechanisms contribute to this process. For instance, the αβ1 receptor which was synthesized in fibroblasts at low to intermediate levels could be involved. Since function blocking anti-α3 chain mAbs were not available to us we could not exclude this possibility. In contrast to αβ1, however, αβ1 synthesis was not increased during gel contraction. The third collagen
binding integrin αβ1 appears not involved in gel contraction of fibroblasts. This is based on our observation that αβ1 was synthesized at low levels and was not upregulated under gel culture conditions. More importantly, the anti-α3 chain mAb PIB5 did not interfere with the contraction process.

In melanoma cells, gel contraction was completely blocked by the combination of anti-α2 and anti-β1 chain mAbs suggesting that αβ1 is indispensable for the function in this cell type. In contrast to fibroblasts in which anti-α2 chain mAbs alone had no inhibitory effect on gel contraction, the addition of anti-α2 chain mAbs to melanoma cells resulted in partial inhibition of gel contraction. Full inhibition, however, could not be achieved with anti-α2 chain mAbs alone even at high mAb concentrations. Interestingly, also anti-β1 chain mAbs were alone incapable of a full- and long-lasting inhibitory effect on gel retraction by melanoma cells. This indicates that at least two extracellular domains, one on the α2 and one on the β1 chain are involved in this function. Since the binding of cells to collagen can efficiently be blocked by the anti-α2 chain mAbs used (5E8, Bankert, unpublished results, and PIE6, Wayner et al., 1988), the requirement of a second epitope on the β1 chain for full inhibition possibly indicates that the prevention of collagen binding to αβ1 is not the only crucial aspect of integrin function in this process.

The view that αβ1 is the collagen-binding integrin primarily involved in collagen gel reorganization by melanoma cells is further underscored by our observations: (a) that αβ1 is not synthesized by the melanoma lines which were used in gel contraction assays; (b) that the synthetic levels of αβ1 are reduced during gel culture; (c) and that the function-blocking anti-α3 chain mAb PIB5 has no inhibitory effect.

Very recently, evidence has been provided that cellular fibronectin (cFN) has an important role for the reorganization of collagen I gels by fibroblasts (Asaga et al., 1991). It was suggested that cFN on the cell surface mediates gel contraction by binding to collagen I via its collagen binding domain. No evidence, however, was provided, which cellular receptor binds cFN under this condition. Possible candidates are the "classical" fibronectin receptor α5β1, the integrin

Figure 6. Synthesis of integrins in collagen I gel and monolayer cultures of two melanoma cell lines MV3 and 530. Con A–bound fractions of NP-40 cell lysates from [35S]methionine-labeled parallel cultures were analyzed by immunoprecipitation and SDS-PAGE. The fluorographs show immunoprecipitates obtained with different mAbs: normal mouse serum (lane 1); A-1-43, anti-α2 (lane 2); J143, anti-α2 (lane 3); P4G9, anti-α5 (lane 4); PID6, anti-α5 (lane 5); GoH3 anti-α5 (lane 6); and LM142, anti-α5 (lane 7). Asterisk indicates the α2β1 immunoprecipitate. Note, that α2β1 synthesis is strongly upregulated in gel cultures of MV3 cells which are able to contract the gels but not in 530 cells.
trix protein provided a means in which collagen I is the only extracellular matrix in animals, the concept was put forward that α5β1 is functionally mediated stage of wound healing just before wound contraction and α5β1 is expressed by fibroblasts during an inter-al model. The antibodies interfere with wound healing processes in animal species will enable scientists to analyze whether directed to the α chain 30 1 chain plus HLA class 1 27 5.72 10 0.79

Moreover, the availability of function-blocking mAbs of healing wounds will be helpful in addressing this issue. The contraction of collagen gels by cultured fibroblasts is considered to reflect an important aspect of the wound healing process. Our finding that the human α5β1 is upregulated during this process now directs attention to a possible role for wound healing in vivo. Here, immunohistochemical studies of healing wounds will be helpful in addressing this issue. Moreover, the availability of function blocking mAbs directed to the α and β1 chains of mouse integrins or other mammalian species will enable scientists to analyze whether the antibodies interfere with wound healing processes in animal models.

Recent studies of α and β1 chain expression in healing porcine wounds led to the observation that the fibronectin receptor α5β1 is expressed by fibroblasts during an intermediate stage of wound healing just before wound contraction (Welch et al., 1989; Clark, 1990). Based on these findings, the concept was put forward that α5β1 is functionally relevant for wound contraction in vivo. The results of the study presented here suggest that—at least under in vitro conditions in which collagen I is the only extracellular matrix protein provided—α5β1 function is irrelevant for collagen gel contraction. This is based on the observations that anti-α chain mAbs did not influence the gel contraction rate of fibroblasts and that the synthetic levels of αβ1 were unchanged.

In the second part of this study we investigated the ability of melanoma cell lines to contract collagen gels and the role of α5β1 in respect to this process. Five out of nine melanoma cell lines contracted collagen gels. Most of these were relatively ineffective in contracting gels when compared to normal fetal or adult fibroblasts. However, two melanoma cell lines (MV3 and BLM) demonstrated contraction efficiencies comparable to that of fibroblasts whereas normal melanocytes were not able to reorganize these gels. These two lines were recently established in an effort to obtain human melanoma cells, which are highly aggressive after transplantation in immunocompromised mice. Both cell lines generated metastases at high frequency in nu/nu mice after subcutaneous inoculation (van Muijen et al., 1989, 1991). We have found that these cells strongly upregulate α5β1 when seeded into collagen I gels. The view that the upregulation of α5β1 is a critical prerequisite for gel contraction is further supported by our observation that the melanoma line 530 which was not capable to contract gels, did not increase α5β1 synthesis in gel culture. Interestingly, 530 was also not capable of forming lung metastases in nu/nu mice (van Muijen, personal communication). These observations point to an interesting association of the ability of melanoma cells to contract collagen I gels with their metastatic potential. The link between the two phenomena possibly represents the ability to upregulate α5β1 expression. Furthermore, it is likely, that the ability of remodelling connective tissue in itself provides substantial advantages for melanoma cells during tumor progression.

Several recent observations strengthen the concept that increased expression of α5β1 is associated with malignant transformation and that α5β1-mediated functions favor tumor progression. We have recently demonstrated that a previously characterized tumor progression antigen, which is expressed at increased frequency in primary melanomas and melanoma metastases relative to benign melanocytic lesions is identical to the integrin α5β1 (Klein et al., 1991). F.A. Chen et al. (1991) found that human lung tumors (non-small cell lung cancer) express at least twenty times more integrin α5 chain message than normal adult lung tissue. Furthermore, Yamada et al. (1990) reported that mAbs directed to the α5 chain of integrins strongly inhibited migration of tumor cells in three-dimensional collagen gels. Moreover, transfection and overexpression of the human α5β1 integrin leads to an increased metastatic potential of the recipient cells (Chan et al., 1991).

The results of our present study further support the assumption that the ability to express and upregulate α5β1 is an important feature in the reorganization of the connective tissue during wound healing but also plays a critical role for tissue invasion and metastasis of tumor cells.
Figure 7. Inhibition of collagen I gel contraction by MV3 melanoma cells by anti-α2 and β1 chain mAbs. Collagen I gel cultures (2 ml) containing 4 x 10^5 MV3 melanoma cells were prepared in 35-mm petri dishes. mAbs were added during preparation of the gels. Photographs were taken after 30 h of culture. (A) The following mAbs (2.5 μg/ml) were added to the cultures. (Upper row, from left to right): W6/32 (anti-HLA class I); 16 (anti-α5 chain); 5E8 (anti-α2 chain). (Lower row, single dish): no antibody. (B) Each dish contained 0.04 μg/ml mAb 4B4 (anti-β1 chain). To study synergistic effects with 4B4, the following mAbs (2.5 μg/ml) had been added: (Upper row from left to right): W6/32 (anti-HLA class I); 16 (anti-α5 chain); 5E8 (anti-α2 chain). (Lower row, single dish): mAb 4B4 only.

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