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

C. Eberhard Klein,* Daniela Dressel,* Thomas Steinmayer,* Cornelia Mauch,§ Beate Eckes,§ Thomas Krieg,§ Richard B. Bankert,‡ and Lutz Weber

*Department of Dermatology, University of Ulm, Ulm, Germany; ‡Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, New York 14263; and §Department of Dermatology, Ludwig-Maximilians-University Munich, Munich, Germany

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 α_2 and β_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-α_2 in conjunction with anti-β_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)[1] receptors on the fibroblast surface for this function. Recently, evidence has been provided that polyclonal antisera directed against the β_1 chain of integrins may interfere with gel contraction (Gullberg et al., 1990). Among the β_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-α_2- in conjunction with anti-β_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 aminoacids, 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). Melanocyte 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 fetal 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, Academish Ziekenhuis, University of Nijmwegen). 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 subjected to hybridization tests using ^{3}H-labeled mycoplasma DNA (Mycoplasma TC Gen Probe Inc., San Diego, CA) and were negative.

Monoclonal antibodies

Serum or ascites of hybridoma bearing mice or tissue culture supernatant was the source of mAbs: mAb TS2/7 binds to the α_1-chain of integrin $\alpha_1\beta_1$ (VLA-1) (Hemler et al., 1985), mAbs 10G11 (Hemler et al., 1988), A-1-43 (Klein et al., 1991), Gil4 (Santoso et al., 1989), 5E8 (Zylstra et al., 1986; Takada et al., 1989), and P1E6 (Wayner et al., 1988) detect the α_2 chain of $\alpha_2\beta_1$, mAbs J143 (Kantor et al., 1987), and P1B5 (Wayner and Carter, 1987) define the α_3 chain of $\alpha_3\beta_1$. mAbs B5G10 (Hemler et al., 1987) and P4G9 (Wayner et al., 1989) recognize the α_4 chain of $\alpha_4\beta_1$; mAbs 16 (Akyama et al., 1989) and P1D6 (Wayner et al., 1988) bind to the α_5 chain; mAbs GoH3 (Sonnenberg et al., 1989) and MT78 (Klein et al., 1990) detect the α_6 chain; mAb LM142 (Cheresh and Spiro, 1987) recognizes the α_v 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 β_1 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. Akayama (Howard University Cancer Center, Washington, DC). mAbs J143 and Aj2 were kindly provided by L. J. Old (Memorial Sloan Kettering Cancer Center, New York) mAbs GoH3 and 10G11 were gifts from A. Sonnenberg (Central Blood Bank of the Netherlands, Amsterdam). mAb LM142 was kindly provided by D. Cheresh (Scripps Clinic, La Jolla, CA). mAbs P1E6, P1D6, P4G9, and 4B4 were purchased from Telios Pharmaceuticals Inc. (San Diego, CA) and Coulter Corporation (Hialeah, FL), respectively. The function blocking mAbs used in this study are listed in Table I. For inhibition studies, mAbs were 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 (Mauch 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, 1.85 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 and carefully mixed by circular movements. Then, 1×10^6 cells suspended in 0.5 ml Mc Coy's medium containing 20% FBS were added and the solution (5 ml) was mixed again. Gel formation occurred within the first two hours of culture at 37°C and 5% CO_2. For experiments in 35-mm bacteriological petri dishes, gels of 2-ml vol containing 4×10^5 cells were prepared. For inhibition studies, purified mAbs were added during gel preparation. The mAb concentration in the stock solutions added ranged from 0.6 to 1.6 mg/ml (0.1 M Tris, pH 8).

Radioimmunoprecipitation

Cells in monolayer or gel culture were metabolically labeled with [^{35}S]methionine (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-lysis 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 with NP-40 lysis buffer, scraped off the tissue culture plastic ware and then treated equally as the gel culture cell 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.

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

Polypeptide	mAb	Reference
α_2 chain	5E8	Zylstra et al., 1986
		Takada et al., 1989
	P1E6	Wayner et al., 1988
α_3 chain	P1B5	Wayner et al., 1987
α_5 chain	16	Akyama et al., 1989
β_1 chain	13	Akyama et al., 1989
	4B4	Shimizu et al., 1990
		Morimoto et al., 1985

RNA Isolation and Northern Blot Hybridization

Total RNA was isolated from fibroblasts as described previously (Mauch et al., 1988). Briefly, cells were homogenized in 4 M guanidinium isothiocyanate using a Potter-Elvehjem and extracted with phenol/chloroform to remove collagenous debris. The supernatants were then centrifuged 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 prime-labeled radioactive cDNA probes specific for the VLA α_2 and β_1 chain as well as for β tubulin. Densitometric scans were performed from autoradiographs to quantify the intensity of hybridization (Hirschmann Elscript 400). cDNA's for VLA α_2 (clone 2.72L) (Takada and Hemler, 1989) were obtained from Dr. M. E. Hemler (Dana Farber Cancer Center, Boston, MA) for VLA β_1 (clone p GEM1-P32) (Argraves et al., 1987) from Dr. E. Ruoslahti (La Jolla Cancer Research Institute, La Jolla, CA) and for β-tubulin (clone Dβ_1) (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 reorganize 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 2×10^5 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

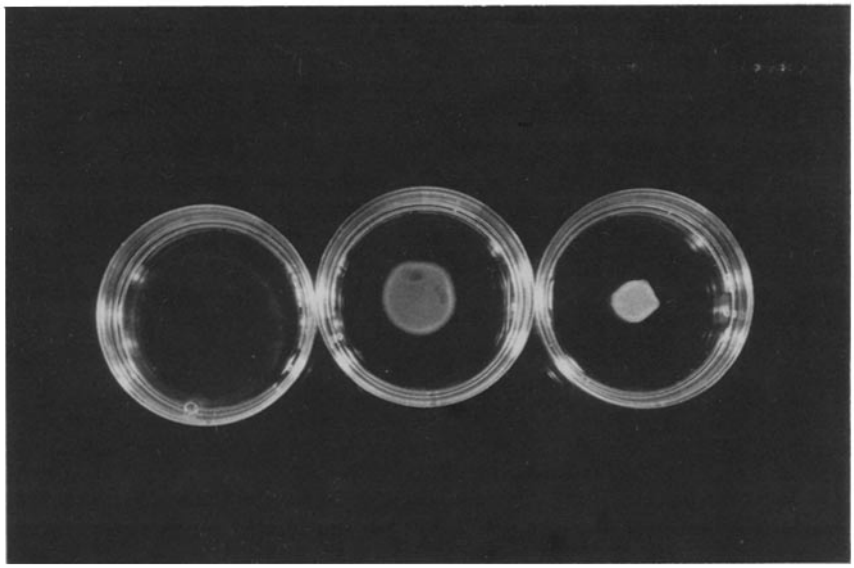

Figure 1. Contraction of collagen I gels by human adult fibroblasts (H-45). Three collagen I gel cultures (2 ml) of 2 × 10^5 human adult fibroblasts (H-45) were prepared in 35-mm bacteriological petri dishes. Cultures were photographed at different times after culture initiation. From left to right: 6, 18, 48 h.

[^{35}S]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 α_1-, α_2-, α_3-, α_4-, α_5-, α_6-, α_v, and β_1 chain of integrins were used.

Fibroblasts contracting collagen gels showed 10- to 14-fold higher synthetic levels of $\alpha_2\beta_1$ than monolayer cultures, whereas all other integrins studied were unchanged ($\alpha_1\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_x$) or reduced ($\alpha_4\beta_1$) (Figs. 2 and 3). It was remarkable that the two other β_1 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 $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$, the results were further confirmed by comparative immunoprecipitations from monolayer and gel cultures using three different mAbs (10G11,

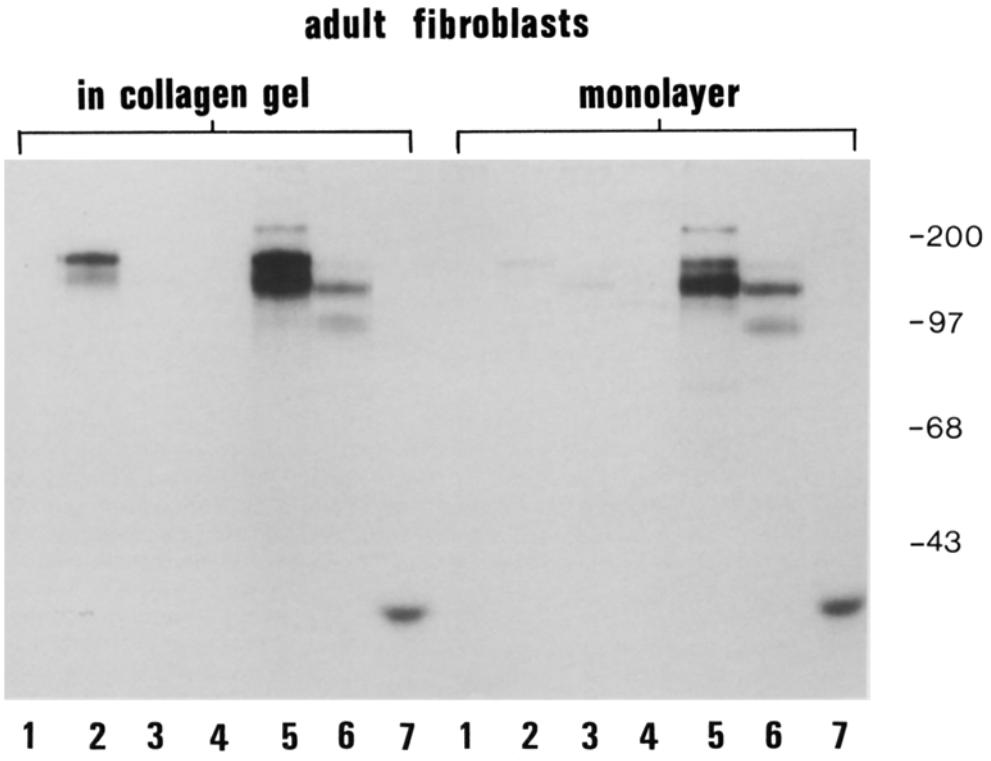

Figure 2. Synthesis of integrins in collagen I gel and monolayer cultures of normal human fibroblasts. Con A–bound fractions of NP-40 cell lysates from [^{35}S]methionine-labeled parallel cultures (H-45) were analyzed by immunoprecipitation and SDS-PAGE. The fluorograph shows immunoprecipitates obtained with different mAbs: (lane *1*) control, normal mouse serum; (lane *2*) 10G11, anti-α_2; (lane *3*) J143, anti-α_3; (lane *4*) GoH3, anti-α_6; (lane *5*) Aj2, anti-β_1; (lane *6*) LM 142, anti-α_v; (lane *7*) W6/32, anti-HLA class I.

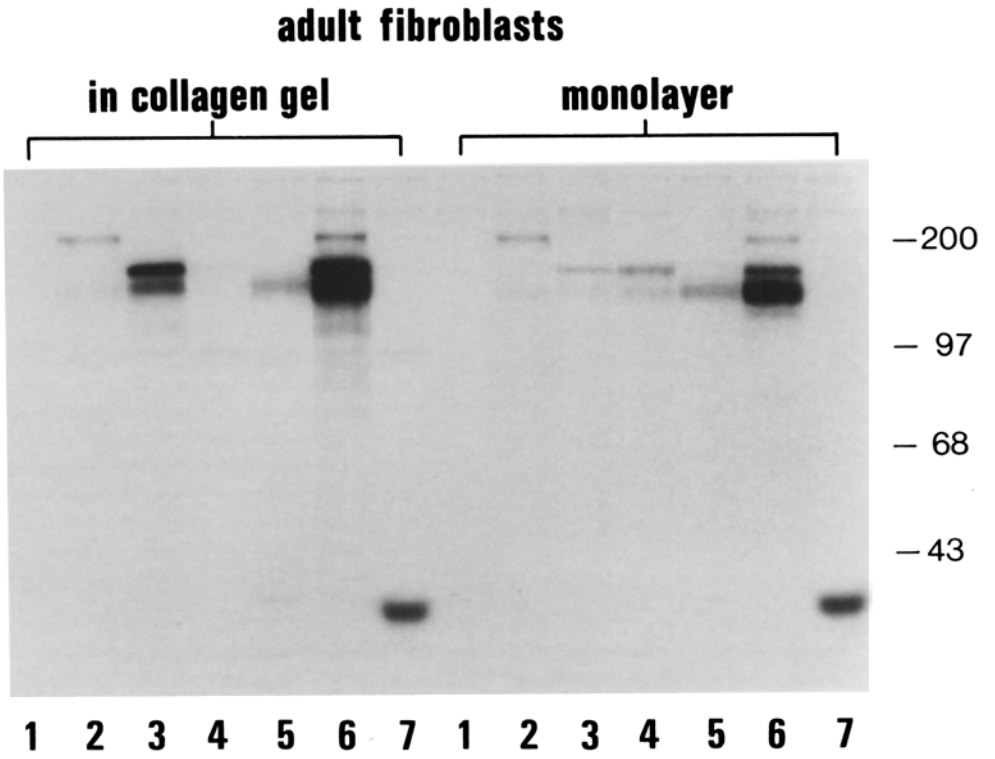

Figure 3. Synthesis of integrins in collagen I gel and monolayer cultures of normal human fibroblasts. Con A-bound fractions of NP-40 cell lysates from [^{35}S]methionine-labeled parallel cultures (H-45) were analyzed by immunoprecipitation and SDS-PAGE. The fluorograph shows immunoprecipitates with different mAbs: normal mouse serum (lane *1*); Ts2/7, anti-α_1 (lane *2*); 10G11, anti-α_2 (lane *3*); B5G10, anti-α_4 (lane *4*); P1D6, anti-α_5 (lane *5*); Aj2, anti-β_1 (lane *6*); and W6/32, anti-HLA class I (lane *7*).

Gi14, and 5E8). These mAbs define at least two nonoverlapping epitopes on the α_2 subunit (unpublished data). To insure that the differences of integrin synthesis were not caused by other experimental artifacts, we also compared the synthesis of HLA class I proteins using mAb W6/32 which defines a monomorphic determinant of the molecules. No change of the synthetic levels of this glycoprotein was noticeable under both culture conditions (Figs. 2 and 3). The selective induction of $\alpha_2\beta_1$ synthesis was also seen when fetal skin fibroblasts were seeded into collagen lattices. Here, the synthetic levels of $\alpha_2\beta_1$ differed in monolayer and gel culture by the same ratio (1:10–14) as found for adult fibroblasts (not shown).

Time course studies were performed to further analyze the upregulation of $\alpha_2\beta_1$. Adult fibroblasts in "gel culture" were metabolically labeled for 6 h at different times after culture initiation: it was found that $\alpha_2\beta_1$ synthesis was already increased when cells were labeled 6 h after initiation of the cultures. High levels of $\alpha_2\beta_1$ synthesis were seen at 6–12, 24–30, and 48–54 h with the peak level between 24–30 or 48–54 h. Interestingly, at day 5 when gel contraction had subsided, the synthetic activity of $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$ synthesis already declined 24 h after culture initiation. From these ex-

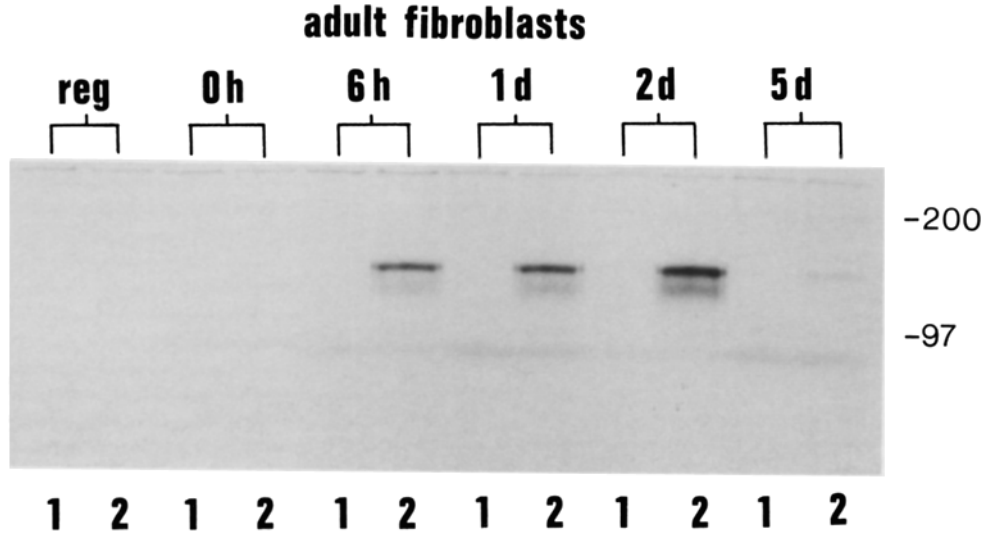

Figure 4. Synthesis of integrin $\alpha_2\beta_1$ by fibroblasts in gel cultures. Adult fibroblasts (H-45) in collagen I gel culture were metabolically labeled for 6 h at different times after culture initiation (0, 6, 24, 48 h, and 5 d). The synthetic levels of $\alpha_2\beta_1$ were then compared with 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-α_2 chain.

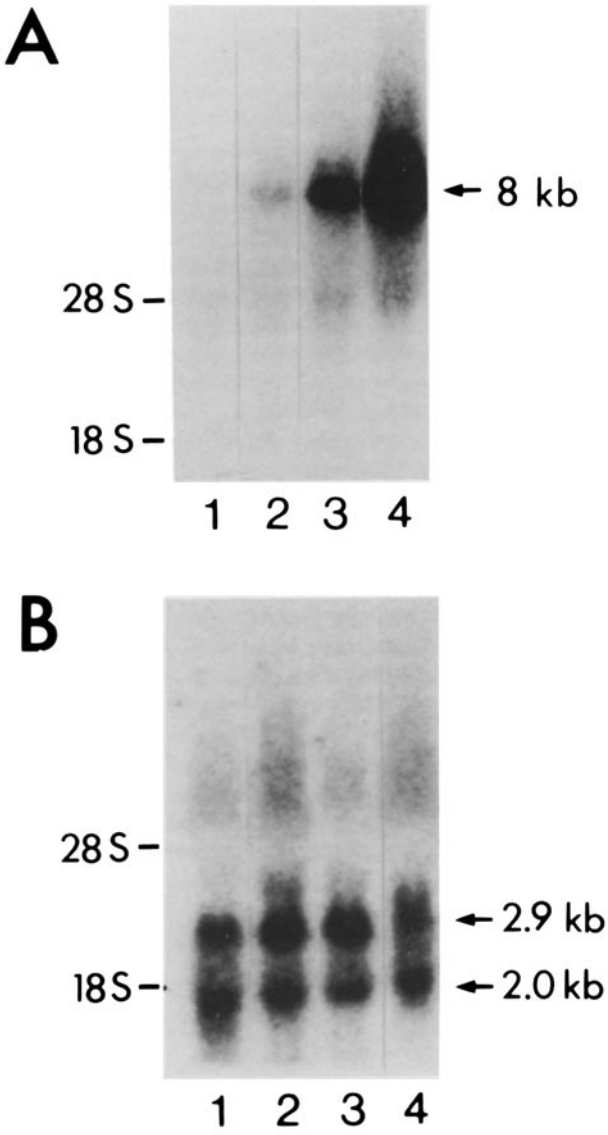

Figure 5. Expression of VLA-α_2 by cells in collagen gels and monolayers. Total RNA was isolated from fibroblasts (NH-1) grown for different times in collagen gels (*2–4*) and as monolayers (*1*). Total RNA was separated in a denaturing agarose gel containing 1% formaldehyde, blotted and hybridized with the radioactively labeled cDNA probe specific for VLA-α_2 (*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).

periments it was concluded that high levels of $\alpha_2\beta_1$ synthesis are coincidentally associated with the process of gel contraction. We then analyzed the transcriptional regulation of α_2 and β_1 chain expression. mRNA was extracted from fibroblast gel cultures at 6, 12, and 18 h of culture and was compared with mRNA of monolayer cultures. The transcription rate of the α_2 chain was highly increased after initiation of gel contraction (Fig. 5). mRNA levels of the β_1 chain were also elevated under the gel culture condition (not shown). The difference of the β_1 chain transcription rates, however, under monolayer and gel culture condition was significantly smaller than that of the α_2 chain.

To address the question of whether the selective induction of $\alpha_2\beta_1$ has functional relevance for gel contraction, we performed inhibition studies using function blocking mAb's directed against the α_2, α_3, α_5, and β_1 chain of integrins (see Table I). mAb W6/32 directed to HLA class I molecules was used as a control antibody. The mAbs were added to the gel culture when the fibroblasts were seeded into the gels.

MAbs 13 and 4B4 which are directed to the β_1 chain partially inhibited gel contraction (see also Table II). Titration experiments using antibody concentrations between 0.1 and 50 μg/ml revealed that the full inhibitory effect of each of the β_1 chain antibodies was already reached at concentrations of 0.6 μg/ml gel. When α_2, α_3, and α_5 chain mAbs or mAb W6/32 were alone added to the gels (concentrations ranging from 1 to 20 μg/ml), no inhibition of gel contraction was observed. Also, the combination of the α_2 chain mAb 5E8 with α_3 chain mAb P1B5 revealed no inhibitory effect.

We then studied the possibility of synergistic effects between β_1 and α chain mAbs. For this purpose, β_1 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 α_2 chain mAb 5E8 in conjunction with one of the anti-β_1 chain reagents further augmented the partial inhibition of gel contraction which was exerted by the β_1 chain mAbs alone. This synergistic effect could also be demonstrated when another α_2 chain mAb (P1E6) was used. The α_3 chain mAb P1B5, the α_5 chain mAb 16, however, and mAb W6/32 had no augmentory effect in combination with β_1 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

	Gel diameter (area)*		
mAb	18 h	24 h	48 h
0	8 (0,50)	7 (0,38)	7 (0,38)
HLA class I	8 (0,50)	7 (0,38)	7 (0,38)
α_5 chain	9 (0,64)	8 (0,50)	8 (0,50)
α_2 chain	8 (0,50)	7 (0,38)	7 (0,38)
β_1 chain (low conc)	10 (0,79)	9 (0,64)	0 (0,64)
β_1 chain plus HLA class I	10 (0,79)	10 (0,79)	10 (0,79)
β_1 chain plus α_5 chain	14 (1,54)	10 (0,79)	9 (0,64)
β_1 chain plus α_2 chain	25 (4,91)	16 (2,01)	15 (1,77)
β_1 chain (high conc)	27 (5,72)	15 (1,77)	15 (1,77)
β_1 chain plus HLA class I	23 (4,15)	15 (1,77)	15 (1,77)
β_1 chain plus α_5 chain	22 (3,80)	15 (1,77)	15 (1,77)
β_1 chain plus α_2 chain	29 (6,60)	20 (3,14)	20 (3,14)
α_2 chain plus α_5 chain	11 (0,95)	8 (0,50)	nd
α_2 chain plus HLA class I	10 (0,79)	8 (0,50)	nd

Experiments were performed in 35-mm Petri dishes. 4×10^5 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 gel for all mAbs except the function blocking β_1 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_5 chain mAb, which blocks function (mAb16); anti-α_2 chain mAb, which blocks function (5E8). nd, not done; 0, no mAb was added.
* gel diameter (area) in mm (cm^2).

*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.	34 (9,07)
	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 cm^2). *n.c.*, no contraction. 1.2×10^6 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 (cm^2).

concluded that the $\alpha_2\beta_1$ complex is of functional relevance for the gel contraction by fibroblasts.

To further study the involvement of $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$ 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 I 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 $\alpha_2\beta_1$, $\alpha_3\beta_1$, and $\alpha_4\beta_1$ than 530 (Fig. 6). $\alpha_5\beta_1$ and $\alpha_6\beta_1$ were expressed at very low and intermediate levels, respectively, in both lines (Fig. 6) whereas $\alpha_1\beta_1$ was not detectable (not shown). When seeded in collagen gels, MV3 cells revealed a significant induction of $\alpha_2\beta_1$ synthesis in comparison to the monolayer cultures (Fig. 6). Density scanning of the fluorographs showed a four- to eightfold increase. Other integrins were unchanged ($\alpha_5\beta_1$, $\alpha_v\beta_x$) or reduced ($\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_6\beta_1$). The same results were obtained when the other "contracting" cell line BLM was studied. In contrast, upregulation of $\alpha_2\beta_1$ synthesis in gel culture was not seen in the "noncontracting" line 530 (Fig. 6).

To further link collagen gel contraction to $\alpha_2\beta_1$ function, we tried to block the contraction by MV3 cells with anti-$\alpha_2\beta_1$ mAbs. Again, function blocking mAbs directed to the α_2, α_3, and α_5 chain of integrins as well as the control mAb W6/32 were used alone or in combination with blocking mAbs directed to the β_1 chain. Here, it was found that the anti-α_2 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-β_1 chain mAbs alone (4B4 or 13) achieved full inhibition for a short time period only (Table IV). The anti-α_3 and α_5 chain mAbs P1B5 and 16, respectively, as well as control mAb W6/32 had no effect. However, when mAb 5E8 (anti-α_2) (2.5 μg/ml) was added to the gel culture in combination with mAbs 4B4 or 13 (anti-β_1) 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 [^{35}S]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-α_2 and β_1 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.

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 β_1 subfamily ($\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$) only $\alpha_2\beta_1$ was strongly upregulated in both cell types during gel contraction. The elevation in $\alpha_2\beta_1$ synthesis was closely associated with the contraction process and returned to baseline levels after contraction had subsided. Moreover, the induction of $\alpha_2\beta_1$ was also demonstrable in the transcriptional level.

Inhibition studies using mAbs which block ligand binding or function of β_1 integrins, revealed that the combination of anti-α_2 chain with anti-β_1 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 $\alpha_2\beta_1$ or collagen receptor independent mechanisms contribute to this process. For instance, the $\alpha_1\beta_1$ receptor which was synthesized in fibroblasts at low to intermediate levels could be involved. Since function blocking anti-α_1 chain mAbs were not available to us we could not exclude this possibility. In contrast to $\alpha_2\beta_1$, however, $\alpha_1\beta_1$ synthesis was not increased during gel contraction. The third collagen

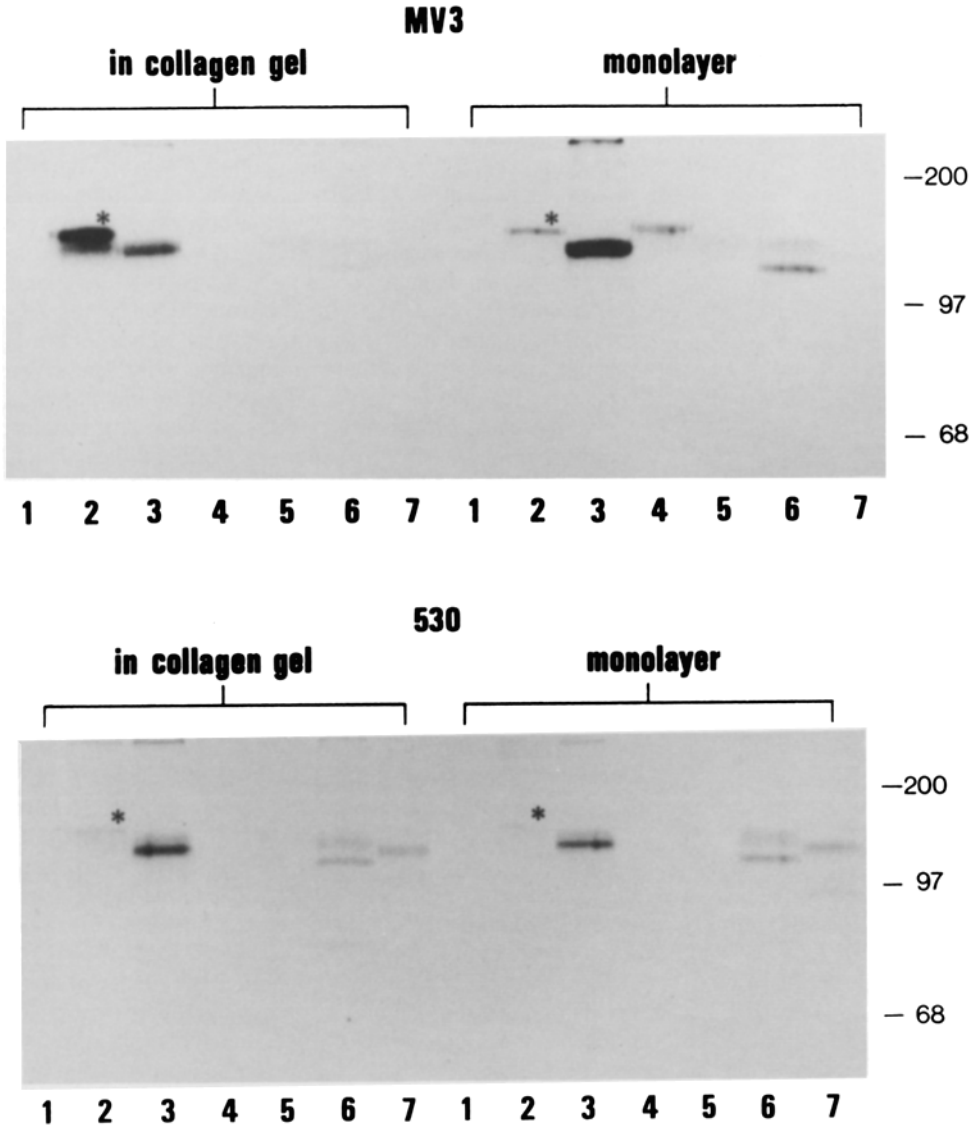

Figure 6. Synthesis of integrins in collagen I gel and monolayer cultures of two melanoma cell lines MV3 and 530. Con A–bound fractions of NP-40 cell lysates from [^{35}S]methionine-labeled parallel cultures were analyzed by immunoprecipitation and SDS-PAGE. The fluorographs show immunoprecipitates obtained with different mAbs: normal mouse serum (lane *1*); A-1-43, anti-α_2 (lane *2*); J143, anti-α_3 (lane *3*); P4G9, anti-α_4 (lane *4*); P1D6, anti-α_5 (lane *5*); GoH3 anti-α_6 (lane *6*); and LM142, anti-α_v (lane *7*). Asterisk indicate the $\alpha_2\beta_1$ immunoprecipitate. Note, that $\alpha_2\beta_1$ synthesis is strongly upregulated in gel cultures of MV3 cells which are able to contract the gels but not in 530 cells.

binding integrin $\alpha_3\beta_1$ appears not involved in gel contraction of fibroblasts. This is based on our observation that $\alpha_3\beta_1$ was synthesized at low levels and was not upregulated under gel culture conditions. More importantly, the anti-α_3 chain mAb P1B5 did not interfere with the contraction process.

In melanoma cells, gel contraction was completely blocked by the combination of anti-α_2 and anti-β_1 chain mAbs suggesting that $\alpha_2\beta_1$ is indispensable for the function in this cell type. In contrast to fibroblasts in which anti-α_2 chain mAbs alone had no inhibitory effect on gel contraction, the addition of anti-α_2 chain mAbs to melanoma cells resulted in partial inhibition of gel contraction. Full inhibition, however, could not be achieved with anti-α_2 chain mAbs alone even at high mAb concentrations. Interestingly, also anti-β_1 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 α_2 and one on the β_1 chain are involved in this function. Since the binding of cells to collagen can efficiently be blocked by the anti-α_2 chain mAbs used (5E8, Bankert, unpublished results, and P1E6, Wayner et al., 1988), the requirement of a second epitope on the β_1 chain for full inhibition possibly indicates that the prevention of collagen binding to $\alpha_2\beta_1$ is not the only crucial aspect of integrin function in this process.

The view that $\alpha_2\beta_1$ is the collagen-binding integrin primarily involved in collagen gel reorganization by melanoma cells is further underscored by our observations: (*a*) that $\alpha_1\beta_1$ is not synthesized by the melanoma lines which were used in gel contraction assays; (*b*) that the synthetic levels of $\alpha_3\beta_1$ are reduced during gel culture; (*c*) and that the function-blocking anti-α_3 chain mAb P1B5 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 $\alpha_5\beta_1$, the integrin

Table IV. Influence of mAb on Collagen I Gel Contraction by Human Melanoma Cells

mAb	Gel diameter (area)*		
	18 h	24 h	48 h
0	21 (3.46)	13 (1.33)	10 (0.79)
HLA class I	24 (4.52)	12 (1.13)	10 (0.79)
α_5 chain	25 (4.91)	13 (1.33)	10 (0.79)
α_2 chain	30 (7.07)	30 (7.07)	15 (1.77)
β_1 chain (low conc.)	30 (7.07)	20 (3.14)	10 (0.79)
β_1 chain plus HLA class I	28 (6.15)	20 (3.14)	10 (0.79)
β_1 chain plus α_5 chain	27 (5.72)	18 (2.54)	10 (0.79)
β_1 chain plus α_2 chain	30 (7.07)	30 (7.07)	30 (7.07)
β_1 chain (high conc.)	30 (7.07)	20 (3.14)	12 (1.13)
β_1 chain plus HLA class I	30 (7.07)	27 (5.72)	12 (1.13)
β_1 chain plus α_5 chain	30 (7.07)	26 (5.31)	12 (1.13)
β_1 chain plus α_2 chain	30 (7.07)	30 (7.07)	30 (7.07)

Experiments were performed in 35-nm Petri dishes. 4×10^5 MV3 melanoma cells 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 unconcentrated collagen gel was 7.07 cm^2. Different mAbs were added to the gels during preparation. The mAb concentration was 2.5 μg/ml gel for all mAbs except the function blocking β_1 chain mAb 4B4, which was used at a low concentration resulting in marginal inhibition (0.4 μg/ml gel) or at the high concentration providing optimal inhibition (2.5 μg/ml gel). HLA class I mAb (W6/32); α_5 chain mAb, which blocks function (mAb16); α_2 chain mAb, which blocks function (5E8). *0*, no mAb was added.

* Gel diameter (area) in mm (cm^2).

$\alpha_4\beta_1$ which binds to the CS-1 region of fibronectin (Wayner et al., 1989), and the "multifunctional" receptor $\alpha_3\beta_1$. An involvement of $\alpha_4\beta_1$ is unlikely, because it was clearly downregulated during gel contraction. Also $\alpha_5\beta_1$ and $\alpha_3\beta_1$ are probably not involved since mAbs interfering with α_5 and α_3 chain function showed no inhibitory effect on gel contraction. Because interference with $\alpha_2\beta_1$ led to partial inhibition of collagen gel contraction and because there is no direct evidence for the involvement of other collagen receptors, one has to take into consideration that collagen receptor independent mechanisms mediated by cFN take a substantial part in the reorganization of collagen I by fibroblasts.

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 $\alpha_2\beta_1$ is upregulated during this process now directs attention to a possible role for wound healing in vivo. Here, immunohistological studies of healing wounds will be helpful in addressing this issue. Moreover, the availability of function blocking mAbs directed to the α_2 and β_1 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_5 and β_1 chain expression in healing porcine wounds led to the observation that the fibronectin receptor $\alpha_5\beta_1$ 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 $\alpha_5\beta_1$ 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—$\alpha_5\beta_1$ function is irrelevant for collagen gel contraction. This is based on the observations that anti-α_5 chain mAbs did not influence the gel contraction rate of fibroblasts and that the synthetic levels of $\alpha_5\beta_1$ 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 $\alpha_2\beta_1$ 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 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 $\alpha_2\beta_1$ when seeded into collagen I gels. The view that the upregulation of $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$ is associated with malignant transformation and that $\alpha_2\beta_1$-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 $\alpha_2\beta_1$ (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 α_2 chain message than normal adult lung tissue. Furthermore, Yamada et al. (1990) reported that mAbs directed to the α_2 chain of integrins strongly inhibited migration of tumor cells in three-dimensional collagen gels. Moreover, transfection and overexpression of the human $\alpha_2\beta_1$ 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 $\alpha_2\beta_1$ 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.

We thank Hilde Heim for expert secretarial assistance.

This work was supported in part by grants from the German Research Foundation DFG KL 510/2-2 to C. E. Klein and DFG Kr 558/6-1 to T. Krieg.

Received for publication 15 April 1991 and in revised form 19 July 1991.

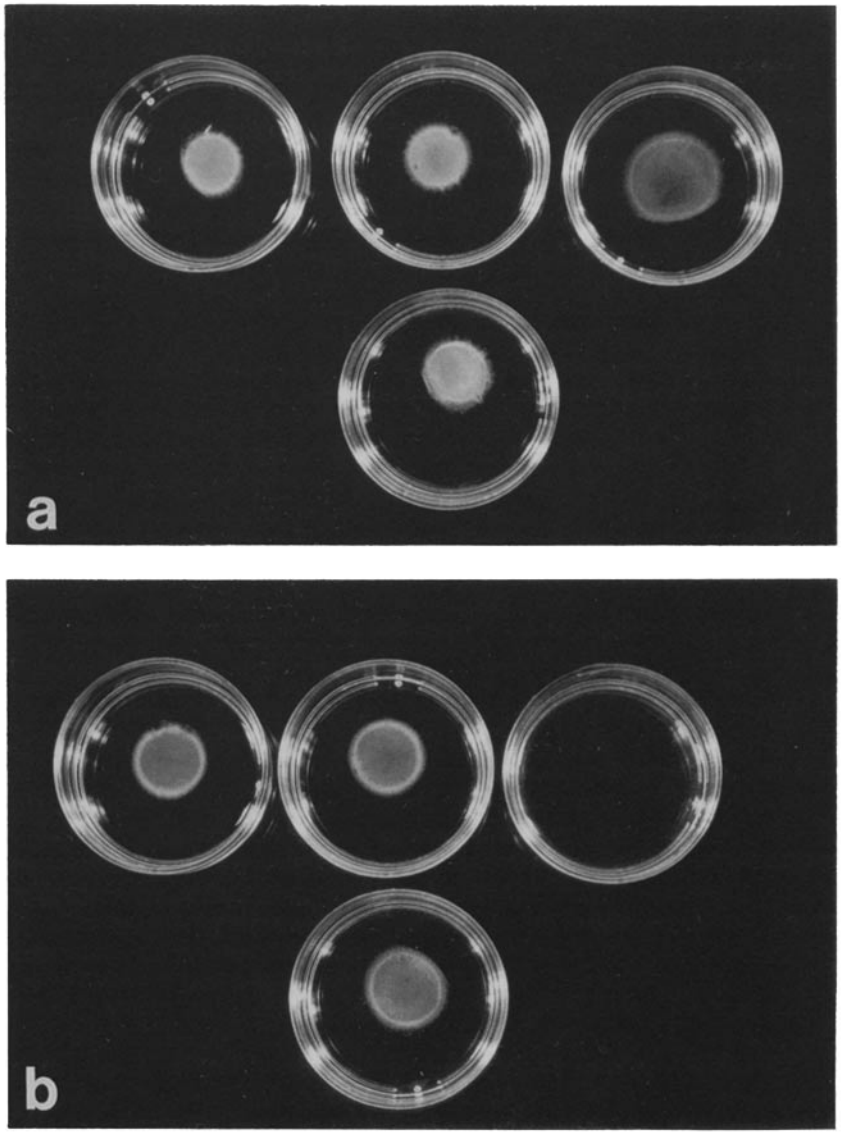

Figure 7. Inhibition of collagen I gel contraction by MV3 melanoma cells by anti-α_2 and β_1 chain mAbs. Collagen I gel cultures (2 ml) containing 4×10^5 MV3 melanoma cells were prepared in 35-mm petri dishes. mAbs were added during preparation of the gels. Photographs were taken after 30 h of culture. (*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-α_5 chain); 5E8 (anti-α_2 chain). (Lower row, single dish): no antibody. (*B*) Each dish contained 0.04 μg/ml mAb 4B4 (anti-β_1 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-α_5 chain); 5E8 (anti-α_2 chain). (Lower row, single dish): mAb 4B4 only.

References

Akyama, S. K., S. S. Yamada, W. T. Chen, and K. M. Yamada. 1989. Analysis of fibronectin receptor function with monoclonal antibodies: roles in cell adhesion, migration, matrix assembly and cytoskeletal organization. *J. Cell Biol.* 109:863–875.

Argraves, W. S., S. Suzuki, H. Avai, K. Thompson, M. D. Pierschbacher, and E. Ruoslahti. 1987. Amino acid sequence of the human fibronectin receptor. *J. Cell Biol.* 105:1183–1190.

Asaga, H., S. Kikuchi, and K. Yoshizato. 1991. Collagen gel contraction by fibroblasts requires cellular fibronectin but not plasma fibronectin. *Exp. Cell Res.* 193:167–174.

Belkin, V. M., A. M. Belkin, and V. E. Koteliansky. 1990. Human smooth muscle VLA-1 integrin: purification, substrate specificity, localization in aorta, and expression during development. *J. Cell Biol.* 111:2159–2170.

Bell, E., B. Ivarsson, and C. Merill. 1979. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc. Natl. Acad. Sci. USA.* 76:1274–1278.

Chan, B. M. C., N. Matsuura, Y. Takada, B. R. Zetter, and M. E. Hemler. 1991. In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. *Science (Wash. DC).* 251:1600–1602.

Chen, F. A., E. A. Repasky, and R. B. Bankert. 1991. Human lung tumor associated antigen identified as an extracellular matrix adhesion molecule. *J. Exp. Med.* 173:1111–1119.

Cheresh, D. A., and R. C. Spiro. 1987. Biosynthetic and functional properties of an Arg-Gly-Asp-directed receptor involved in human melanoma cell attachment to vitronectin, fibrinogen and von Willebrand Factor. *J. Biol. Chem.* 262:17703–17711.

Clark, R. A. F. 1990. Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. *J. Invest. Dermatol.* 94:128S–134S.

Dedhar, S., and R. Saulnier. 1990. Alterations in integrin receptor expression on chemically transformed human cells: specific enhancement of laminin and collagen receptor complexes. *J. Cell Biol.* 110:481–489.

Eisinger, M., and O. Marko. 1982. Selective proliferation of normal human melanocytes in vitro in the presence of phorbol ester and cholera toxin. *Proc. Natl. Acad. Sci. USA.* 79:2018–2022.

Guidry, C., and F. Grinell. 1985. Studies on the mechanism of hydrated collagen gel reorganization by human skin fibroblasts. *J. Cell Sci.* 79:67–81.

Gullberg, D., A. Tingström, C. Thuresson, L. Olsson, L. Terracio, T. K. Borg, and K. Rubin. 1990. β_1 Integrin-mediated collagen gel contraction is stimulated by PDGF. *Exp. Cell Res.* 186:264–272.

Halaban, R., S. Gosh, P. Duray, J. M. Kirkwood, and A. B. Lerner. 1986. Human melanocytes cultured from nevi and melanomas. *J. Invest. Dermatol.* 87:95–101.

Hemler, M. E., J. G. Jacobson, and J. L. Strominger. 1985. Biochemical characterization of VLA-1 and VLA-2 cell surface heterodimers on activated T cells. *J. Biol. Chem.* 260:15246–15252.

Hemler, M. E., C. Huang, and Y. Takada. 1987. Characterization of the cell surface heterodimer VLA-4 and related peptides. *J. Biol. Chem.* 262:11478–11485.

Hemler, M. E., C. Crouse, Y. Takada, and A. Sonnenberg. 1988. Multiple very late antigen (VLA) heterodimers on platelets. Evidence for distinct VLA-2, VLA-5 (fibronectin receptor) and VLA-6 structures. *J. Biol. Chem.* 263:7660–7665.

Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549–554.

Kantor, R. R. S., M. J. Mattes, and K. O. Lloyd. 1987. Biochemical analysis of two cell surface glycoprotein complexes, very common antigen 1 and very

common antigen 2. Relationship to very late activation T cell antigens. *J. Biol. Chem.* 262:15158–15165.

Kaufman, R., D. Frösch, C. Westphal, L. Weber, and C. E. Klein. 1989. Integrin VLA-3: Ultrastructural localization at cell-cell contact sites of human cell cultures. *J. Cell Biol.* 109:1807–1815.

Kirchhofer, D., L. R. Languino, E. Ruoslahti, and M. D. Pierschbacher. 1990. $\alpha_2\beta_1$-integrins from different cell types show different binding specificities. *J. Biol. Chem.* 265:615–618.

Klein, C. E. 1988. A transformation associated 130 kD cell surface glycoprotein is growth controlled in normal human cells. *J. Exp. Med.* 167:1684–1696.

Klein, C. E., T. Steinmayer, J. Mattes, R. Kaufmann, and L. Weber. 1990. Integrins of normal human epidermis: differential expression, synthesis and molecular structure. *Br. J. Dermatol.* 123:171–178.

Klein, C. E., T. Steinmayer, D. Kaufmann, and L. Weber. 1991. Identification of a melanoma progression antigen as integrin VLA-2. *J. Invest. Dermatol.* 96:281–284.

Kramer, R. H., K. A. McDonald, and M. P. Vu. 1989. Human melanoma cells express a novel integrin receptor for laminin. *J. Biol. Chem.* 264:15642–15649.

Lloyd, K. O., J. Ng, and W. G. Dippold. 1981. Analysis of the biosynthesis of HLA-DR glycoprotein in human malignant melanoma cell lines. *J. Immunol.* 126:2408–2413.

Mauch, C. 1986. Ph.D. thesis. Zell-Matrix-Wechselwirkungen von Fibroblasten. Physiologische und pathophysiologische Aspekte. Naturwissenschaftliche Fakultät III der Universität Regensburg, Regensburg, Germany.

Mauch, C., A. Hatamochi, K. Scharfetter, and T. Krieg. 1988. Regulation of collagen synthesis in fibroblasts within a three dimensional collagen gel. *Exp. Cell Res.* 178:493–503.

Morimoto, C., N. L. Letvin, J. A. Distaso, W. R. Aldrich, and S. F. Schlossman. 1985. The isolation and characterization of the human suppressor inducer T Cell subset. *J. Immunol.* 134:1508–1515.

Muijen van, G. N. P., I. M. H. A. Cornelissen, C. F. J. Jansen, and D. J. Ruiter. 1989. Progression markers in metastasizing human melanoma cells xenografted to nude mice. *Anticancer Res.* 9:879–884.

Muijen van, G. N. P., C. F. J. Jansen, L. M. H. A. Cornelissen, J. L. M. Beck, and D. J. Ruiter. 1991. Establishment and characterization of a human melanoma cell line (MV3) which is highly metastatic in nude mice. *Int. J. Cancer.* 48:85–91.

Nakagawa, S., P. Pawelek, and F. Grinnell. 1989. Extracellular matrix organization modulates fibroblast growth and growth factor responsiveness. *Exp. Cell Res.* 182:572–582.

Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion: RDG and integrins. *Science (Wash. DC).* 238:491–497.

Shimizu, Y., G. A. van Serenter, K. J. Horgan, and S. Shaw. 1990. Regulated expression and binding of three VLA (β_1) integrin receptors on T cells. *Nature (Lond.).* 345:250–253.

Santoso, S., V. Kiefel, and C. Müller-Eckhardt. 1989. Immunochemical characterization of the new platelet alloantigen system Br^a/Br^b. *Br. J. Haematol.* 72:191–198.

Sonnenberg, A., P. W. Modderman, and F. Hogervorst. 1988. Laminin receptor on platelets is the integrin VLA-6. *Nature (Lond.).* 336:487–489.

Takada, Y., and M. E. Hemler. 1989. The primary structure of the VLA-2/collagen receptor α_2-subunit (platelet GPIa): homology to other integrins and the presence of a possible collagen-binding domain. *J. Cell Biol.* 109:397–407.

Tiollier, J., H. Dumas, M. Tardy, and J.-L. Tayot. 1990. Fibroblast behavior on gels of type I, II and IV human placental collagens. *Exp. Cell Res.* 191: 95–104.

Tomasek, J. J., E. D. Hay, and K. Fujiwara. 1982. Collagen modulates cell shape and cytoskeleton of embryonic corneal and fibroma fibroblasts: Distribution of actin, α-Actinin and myosin. *Dev. Biol.* 92:107–122.

Versteeg, R., I. O. A. Noordermeer, M. Krüse-Wolters, D. J. Ruiter, and P. I. Schrier. 1988. C-myc down-regulates class I HLA expression in human melanomas. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1023–1029.

Wayner, E. A., and W. G. Carter. 1987. Identification of multiple cell adhesion receptors for type VI collagen and fibronectin in human fibrosarcoma cells possessing unique α and common β-subunits. *J. Cell Biol.* 105:1873–1884.

Wayner, E. A., W. G. Carter, R. S. Pistrowicz, and T. J. Kunicki. 1988. The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J. Cell Biol.* 107:1881–1891.

Wayner, E. A., A. Garcia-Pardo, M. J. Humphries, J. A. McDonald, and W. G. Carter. 1989. Identification and characterization of the T-lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. *J. Cell Biol.* 109:1321–1330.

Welch, M. P., G. F. Odland, and A. F. Clark. 1990. Temporal relationships of F-Actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction. *J. Cell Biol.* 110: 133–145.

Yamada, K. M., D. W. Kennedy, S. S. Yamada, H. Gralnick, W. T. Chen, and S. K. Akiyama. 1990. Monoclonal antibody and synthetic peptide inhibitors of human tumor cell migration. *Cancer Res.* 50:4485–4496.

Zylstra, S., F. A. Chen, S. K. Ghosh, E.A. Repasky, U. Rao, H. Takita, and R. B. Bankert. 1986. Membrane-associated glycoprotein (gp 160) identified on human lung tumors by a monoclonal antibody. *Cancer Res.* 46:6446–6451.