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Upmodulation of αvβ1 Integrin Expression on Human Tumor Cells by Human Interleukin for DA Cells/Leukemia Inhibitory Factor and Oncostatin M: Correlation With Increased Cell Adhesion on Fibronectin

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Abstract Integrins belong to a large family of heterodimeric membrane glycoproteins which mediate cell-cell or cell–extracellular matrix interactions. These interactions could play a major role during the migration of tumor cells across the extracellular matrix and vascular endothelium and would thus appear to be requisite for the metastatic process. Pretreatment of the Foss human melanoma cell line with HILDA/LIF or OSM, two cytokines involved in acute-phase response, increased the expression of membrane αvβ1 1.5–2-fold. The same phenomenon was observed on the SK-N-SH human neuroblastoma cell line. αvβ1 upmodulation was concomitant with improved tumor cells attachment to the fibronectin matrix. This greater adhesion of tumor cells to fibronectin was inhibited by specific monoclonal antibodies against αv or β1 integrin subunits. Similar results were obtained after TNF-α treatment. Our findings demonstrate the ability of HILDA/LIF and OSM to modulate tumor cell capacity to adhere to the matrix component, suggesting a potential role for these cytokines in modulation of tumoral progression. © 1995 Wiley-Liss, Inc.

Key words: αvβ1, HILDA/LIF, oncostatin M, TNF-α, melanoma, neuroblastoma, fibronectin adhesion

The process of tumor invasion requires complex changes in normal cell–extracellular matrix protein interactions [Liotta, 1986; Nicolson, 1987; Juliano, 1987]. The surface receptors mediating these adhesive events belong to the integrin family and are thus likely to be important in tumor invasion and metastasis [Ruoslahti and Giancotti, 1989; Buck and Horwitz, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Albelda, 1993]. Structurally, each integrin is a heterodimer consisting of an α subunit noncovalently associated with a β subunit. There are 15 α and 8 β subunits known to date [Ruoslahti et al., 1994]. While several observations in vitro and in vivo have indicated changes in the biosynthesis, localization, and level of β1 subfamily expression upon neoplastic transformation [Akiyama et al., 1990; Plantfaber and Hynes, 1989], little correlation has been found between the altered pattern of integrin expression and tumorigenesis [Albelda et al., 1990]. However, functional studies suggest that integrins are important in the metastatic process. Thus, it has been determined that synthetic peptides containing the RGD aminoacid sequence that blocks the binding of many integrins to their extracellular matrix ligands [Pierschbacher and Roaslahti, 1984; Yamada and Kennedy, 1984] reduce the number of metastatic nodules found within the lungs of mice given B16-F10 melanoma cells [Humphries et al., 1986, 1988; Saïki et al., 1988]. In addition, αvβ3 integrin expression has been associated with invasive melanoma in vitro and in vivo [Albelda et al., 1990;

Abbreviations used: HILDA/LIF, human interleukin for DA cells/leukemia inhibitory factor; ICAM-1, intercellular adhesion molecule-1; LAK, lymphokine activated killer; MFI, mean fluorescence intensity; OSM, oncostatin M; TNF-α, tumor necrosis factor-alpha.

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Felding-Habermann et al., 1992; Gehlsen et al., 1992] through increased induction of the matrix-degrading protease, type IV collagenase [Seftor et al., 1992]. Similarly, α3β1 and α2β1 integrin expression has been correlated with the tumor progression of melanoma [Natali et al., 1993; Eberhard-Klein et al., 1991].

Cytokines play a role in regulating cell-matrix and cell-cell interactions by influencing the expression of integrins during inflammation and remodeling of damaged tissues. In fact, TGF-β increases the expression of extracellular matrix receptors in fibroblasts [Heino et al., 1989; Ignotz et al., 1989], whereas its effects are dualistic on human osteosarcoma since it decreases α3β1 expression while increasing α2β1 and α5β1 expression [Heino and Massagué, 1989]. The inflammatory cytokines IL-1β and TNF-α can upregulate the expression of α1β1 integrin on a human osteosarcoma cell line, increasing its adhesion to laminin [Santala and Heino, 1991]. Similarly, they can enhance the expression of α5β1 on a melanoma cell line, increasing its adhesion to fibronectin [Mortarini et al., 1991]. In contrast, these cytokines downregulate the α6β1 integrin in human endothelial cells [Defilippi et al., 1992].

We recently showed that HILDA/LIF and OSM, two IL-6 family cytokines [Rose and Bruce, 1991] involved in acute-phase response [Baumann and Wong, 1989; Baumann et al., 1993], upregulate the expression of melanoma cell surface ICAM-1 without inducing the shedding of the soluble form [Heymann et al., in press, 1995], as in the case of TNF-α and IL-1β [Dustin et al., 1986; Becker et al., 1991; Giavazzi et al., 1992]. Thus, cells treated with these cytokines become more sensitive to lysis by LAK cells. The present study shows that HILDA/LIF and OSM also upregulate the expression of αvβ1 integrin, inducing increased melanoma cell adhesion to fibronectin. The upregulation of this integrin was also observed on SK-N-SH, a neuroblastoma cell line which adheres to fibronectin via αvβ1 integrin [Dedhar and Gray, 1990].

MATERIALS AND METHODS

Reagents and Antibodies

Human rTNF-α (from yeast; specific activity 10^8 units/mg) was purchased from Boehringer Mannheim (Penzberg, Germany) and human rOSM (0.33–0.66 × 10^7 units/mg) from British Biotechnology Products (Oxon, UK). rHILDA/LIF (specific activity 4 × 10^7 units/mg) was purified from serum-free conditioned medium of CHO cells transfected with full-length cDNA, as described in Godard et al. [1992]. The following monoclonal antibodies (mAbs) to integrin subunits were used: 1) rat mAb GoH3 to α6 [Sonnenberg et al., 1987], a gift from Dr. Sonnenberg (Cancer Institute, Amsterdam, The Netherlands); 2) mouse mAbs B-5G10 to α4 [Hemler et al., 1987] and TS2/7 to α1 [Hemler et al., 1984], a gift from Dr. Hemler (Dana Farber Institute, Boston, MA); 3) rat mAbs AII-B2 to β1 and BII-G2 to α5 [Brown et al., 1989], a gift from Dr. Damsky (University of California, San Francisco, CA); 4) mouse MAbs specific for αvβ5 (P1F6) and for αvβ6 (R6G9) [Weinacker et al., 1994], a gift from Dr. Sheppard (Lung Biology Center, San Francisco, CA); 5) mouse mAbs Gi9 to α2, M-Kid2 to α3, AMF7 to αv, and K20 to β1, purchased from Immunotech (Marseille, France); and 6) mouse mAb VNR3 to β3, obtained from Takara Shuzo (Shiga, Japan).

Cell Cultures

Two human tumor cell lines were studied: Foss melanoma cells and SK-N-SH neuroblastoma cells which exhibit specific binding sites for HILDA/LIF [Godard et al., 1992; Gearing et al., 1994]. The Foss melanoma cell line, derived by Dr. B.C. Giovanella at the Stekling Foundation (Houston, TX), was provided by J.Y. Douillard (Nantes, France), and the SK-N-SH neuroblastoma cell line was purchased from the American Culture Type Collection (ATCC, Rockville, MD). These tumor cells were cultured in RPMI 1640 supplemented with 10% FCS. Tumor cells (0.7 × 10^6) were seeded in a 25 cm^3 flask in 5 ml of RPMI 1640 containing 2.5% FCS. After 48 h, monolayers were washed with medium, and 5 ml of fresh medium with or without cytokines (HILDA/LIF, OSM, TNF-α) was added. In all experiments, treatment with cytokines began on cells in subconfluent state. After 24 or 48 h incubation at 37°C, cells were collected to analyze integrin membrane expression.

Flow Cytometry Analysis

Integrin expression on cells was determined by indirect immunofluorescence using a FACScan (Becton Dickinson, San Jose, CA). Briefly, 2 × 10^5 cells were first incubated with the appropriate primary antibody (antiintegrin antibody) for 45 min at 4°C (or the same volume with PBS-gelatin 0.1% for the control) and then

washed twice with PBS-gelatin 0.1% and incubated with an affinity-purified fluorescein isothiocyanate-labeled goat antimouse or -rat $F(ab')_2$ fragment (Sigma, La Verpillière, France) diluted to 1:200 for 45 min at 4°C. Results are expressed by linear mean fluorescence intensity after background subtraction of the control (cells incubated only with FITC antibody).

Cell Surface Iodination

Ten million tumor cells were washed three times with PBS, suspended in 0.5 ml of PBS, and surface-iodinated in glass vials coated with 100 μg of iodogen by adding 0.5 mCi of Na ^{125}I (New England Nuclear, Boston, MA). The reaction was performed at room temperature for 10 min with frequent agitation. The free Na ^{125}I was then eliminated by washing the cells three times with PBS.

Immunoprecipitation

The integrins were immunoprecipitated from the iodinated cells as described by Akiyama and Yamada [1987] using antibodies against human integrins. Briefly, ^{125}I-labeled cells were solubilized with 0.5 ml ice-cold 2% Triton X-100 in PBS supplemented with 1 mM PMSF and 10 μg/ml leupeptin for 10 min. The extract was centrifugated at 12,500*g*. The supernatant solution was preadsorbed on a 50% suspension (v/v) of antimouse IgG agarose (Sigma). The agarose beads were pelleted by centrifugation and discarded. The supernatant was then incubated with antibodies with PBS and constant end-over-end mixing for 60 min at 4°C. The immune complexes were precipitated by adding to the mixture 25 μl of antimouse IgG agarose preadsorbed with unlabeled Triton X-100 cell extract and incubated for 60 min at 4°C. After centrifugation, the supernatants were used for sequential immunoprecipitation. The IgG agarose bound antibody-antigen complexes were washed with 1% Triton X-100 in PBS and then extracted from agarose beads by adding solubilizing buffer without β-mercaptoethanol and heating for 3 min at 100°C.

Gel Electrophoresis

SDS-polyacrilamide slab gel electrophoresis was performed by the method of Laemmli [1970] using 4% stacking gel and 7.5% resolving gel or 3–9% gradient gel. The proteins were visualized by exposing hyperfilm-MP (Les Vlis, France) to dried gels at −70°C. Prestained standard molecular weights used were purchased from Bio-rad (Richmond, CA).

Cell Attachment Assays

Assays were performed on 96-well microtiter plates (flat bottom). Wells were coated with 100 μl of a dilution of fibronectin in PBS for 18 h at 4°C. After coating, the supernatant was eliminated, and the nonspecific attachment sites were saturated by incubation for 1 h at room temperature with 100 μl of BSA 1% heat-denatured at 80°C for 1 min [Yamada and Kennedy, 1984]. The plates were then washed with PBS.

Twenty-four hour cytokine-treated cells were grown for 18 h at 37°C in RPMI 1640 supplemented with 2.5% FCS containing 1 μCi/ml [^3H] thymidine (New England Nuclear). Cells were washed with PBS, detached with trypsin-EDTA, and resuspended in the binding buffer (20 mM Tris, 135 mM NaCl, 5 mM KCl, 1.8 mM D-glucose, 2 mM glutamine, 1% BSA, 1 mM MnCl2, pH 7.4) [Elices et al., 1991] at 5×10^5 cells/ml.

Next, 5×10^4 cells/well were added to the plates coated with fibronectin and allowed to attach for 90 min in a 95% air, 5% CO_2 humidified atmosphere. Unbound cells were removed by gentle aspiration and washed three times with PBS. The wells were then solubilized by addition of 0.1 M NaOH for 10 min at 37°C, and radioactivity was measured using a Beckman LS2800 beta counter. Assays were performed in triplicate, and the percentage of adherent cells was determined as the ratio bound of total radioactivity added.

Adhesion inhibition assays were conducted in the presence of 4 μg/ml of monoclonal antibody against the αv (AMF7) or β1 integrin subunit (K20). Tumor cells were incubated with the antibody for 30 min at 37°C before being added to the plates coated with fibronectin. A mouse isotype (IgG2a) antibody was used as negative control.

RESULTS

Stimulation of αvβ1 Integrin Expression on Human Foss Melanoma Cell Surface by HILDA/LIF, OSM, and TNF-α

Figure 1 shows the expression of integrin subunits on Foss melanoma cell surface analyzed by flow cytometry. Thus α3, α4, α6, αv, β1, β3, and β6 subunits were expressed on these cells but not α1, α2, and α5 subunits (Table I).

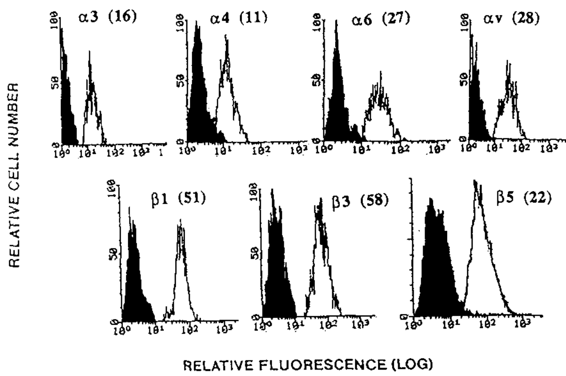

Fig. 1. Flow cytometry analysis of immunofluorescence-labeled Foss cells. As described in Materials and Methods, 2 × 10^5 cells were incubated with specific monoclonal antibodies against integrin subunits (empty histograms). Control cells (black histograms) were incubated only with the second FITC-labeled antibody.

TABLE I. Integrin Subunit Pattern on Human Foss Melanoma Cells*

Integrin subunit	Expression	Regulation by HILDA/LIF, OSM, or TNF-α
α1	No	–
α2	No	–
α3	+	No
α4	+	No
α5	No	–
α6	++	No
αv	+	Yes
β1	++	Yes
β3	++	No
β5	+	No
β6[a]	No	–

*After cytokine treatment, cells were analyzed for their integrin expression by flow cytometry. +, MFI ≤30 linear arbitrary units; ++, MFI ≥30 linear arbitrary units).
[a]Analyzed by immunoprecipitation.

After 24 h treatment, HILDA/LIF and TNF-α upregulated the β1 integrin subunit on Foss cell membrane in a dose-dependent manner. This increase reached a plateau at 2.5 ng/ml for HILDA/LIF (Fig. 2a, left) but not for TNF-α even at 10 ng/ml (Fig. 2b, left). The maximum stimulation level was 1.9- and 1.6-fold compared to the control after treatment with 10 ng/ml of HILDA/LIF and TNF-α, respectively. The upregulation level observed did not increase after a longer period (48 h) of treatment with these cytokines (data not shown).

The modulation study of different α integrin subunits indicated that only αv was upregulated by both cytokines on Foss cell membrane (Fig. 3; Table I). Thus, after 24 h treatment with 2.5 ng/ml of HILDA/LIF or TNF-α, αv subunit expression was increased 1.5-fold compared to the control (Fig. 3).

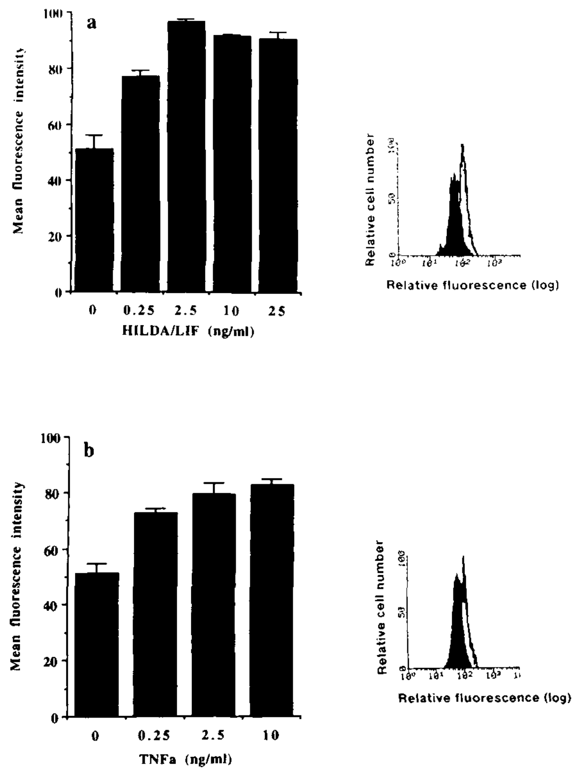

Fig. 2. Upmodulation of β1 integrin subunit expression on human Foss melanoma cells by HILDA/LIF and TNF-α. Cells were cultured for 24 h in RPMI 1640 supplemented with 2.5% FCS and HILDA/LIF (a) or TNF-α (b) cytokines at different concentrations (left figures) prior to analysis of cell-surface β1 integrin expression by flow cytometry. Results were expressed as mean ± SD from three independent experiments. Right figures are FACs profiles corresponding to the β1 modulation by 2.5 ng/ml of both cytokines. Without cytokine, black histograms; with cytokines, empty histograms.

As αv subunit could be associated with four different β subunits (β1, β3, β5, and β6), immunoprecipitation experiments were realized in the aim of ensuring the effective association of αvβ1 integrin on Foss melanoma cells (Fig. 4). Monoclonal antibodies against αv immunoprecipitated three bands which had a Mr about 150, 110, and 90 Kd corresponding to αv, β1, and β3 subunits, respectively (Fig. 4, lanes 1,2). The use of an anti-β3 antibody confirmed that the 90 Kd band was a β3 subunit (data not shown). Sequential immunoprecipitation with β1 immunoprecitated the other α(s)β1 integrins (Fig. 4, lane 3). Similarly, when we depleted cell lysates with anti-β1 antibody, only two bands were ob-

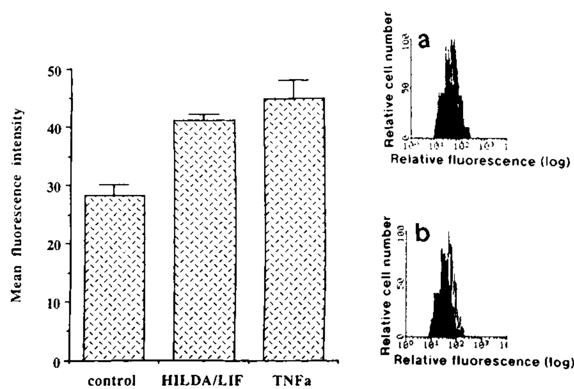

Fig. 3. Upmodulation of the membrane αv integrin subunit expression on human Foss melanoma cells by HILDA/LIF and TNF-α. After 24 h treatment with 2.5 ng/ml of HILDA/LIF or TNF-α, Foss cells were analyzed for αv integrin expression by flow cytometry. Results are expressed as mean ± SD from three independent experiments. **a,b:** FACs profiles corresponding to the αv modulation by 2.5 ng/ml of HILDA/LIF or TNF-α, respectively. Without cytokine, black histograms; with cytokines, empty histograms.

served corresponding to β1 and α(s) subunits (Fig. 4, lanes 4,5), and sequential immunoprecipitation with antibody against αv specifically precipitated the αvβ3 integrin (Fig. 4, lane 6). Moreover, antibodies against β6 subunit indicated that αvβ6 integrin was not expressed on Foss melanoma cells (Fig. 4, lane 7). All these results showed that αvβ1 was well expressed on Foss cell surface. Moreover, although expressed, the β3 and β5 integrin subunits were not regulated on Foss melanoma cells after treatment with both cytokines (Table I).

HILDA/LIF belongs to the IL-6 cytokine family which shares common binding receptors on membrane cells. Thus, HILDA/LIF shares two binding chains with OSM, a gp190 chain binding HILDA/LIF but not OSM with low affinity and a gp130 chain which confers high-affinity binding of both HILDA/LIF and OSM when expressed with the low-affinity receptor [Godard et al., 1992; Gearing et al., 1992; Gearing and Bruce, 1992]. To determine whether the same effect of HILDA/LIF on αvβ1 integrin could be observed with OSM, we performed experiments with this cytokine at 2.5 ng/ml, the concentration giving the highest response with HILDA/LIF. At this concentration, OSM increased β1 integrin expression on Foss cell membrane to a level similar to that obtained with HILDA/LIF (1.8-fold) (Fig. 5a). In the same conditions, OSM only modulated αv integrin expression (1.4-fold compared to the control) (Fig. 5b) without affecting the α1–α6 subunits on Foss melanoma cells (data not shown).

Moreover, treatment of cells with both HILDA/LIF and OSM did not induce a synergestic effect in modulating the expression of these integrins. Similarly, TNF-α did not synergize with HILDA/LIF or OSM (data not shown).

Upregulation of Membrane αvβ1 Integrin Expression Correlates With Foss Melanoma Cell Attachment on Fibronectin

Foss cells adhered to fibronectin in a dose-dependent manner (0.5–5 μg/ml) (data not shown). For further experiments, we chose to perform adhesion assays using 2 μg/ml of fibronectin which gave a medium attachment level. One of the functional effects of αvβ1 *integrin* upmodulation on Foss cells was their increasing capacity to adhere to fibronectin. Twenty-four hour treatment of cells with 2.5 ng/ml of all three cytokines increased the percentage of specific cell attachment on plates coated with fibronectin (Fig. 6). Thus, 31% of specific cell attachment was observed for HILDA/LIF, 39% for OSM, and 32% for TNF-α vs. 21% for the control.

This upregulation of specific cell attachment on fibronectin after cytokine treatment was inhibited by specific monoclonal antibodies against αv or β1 integrin subunits, and the adhesion level was reduced to that observed with the control without cytokine treatment (Fig. 6). Thus, after HILDA/LIF and TNF-α treatment, the percentage of cell-attachment inhibition in the presence of 4 μg/ml of monoclonal antibody against the αv or β1 subunit was nearly the same (42%) but higher than that obtained with OSM-treated cells (32%) (Fig. 6). This inhibition was specific since a mouse isotype antibody (IgG_{2a}) did not induce the same effect. In all cases, these antibodies did not completely reduce cell adhesion to fibronectin, which suggests that other molecules could be involved in the basal adhesion mechanism of Foss cells to fibronectin.

Upregulation of αvβ1 Integrin on Human SK-N-SH Neuroblastoma Cells by HILDA/LIF, OSM, and TNF-α Correlates With Cell Attachment on Fibronectin

To investigate whether HILDA/LIF, OSM, and TNF-α would have the same effect on αvβ1 integrin expression in another cell line, we chose SK-N-SH, a human neuroblastoma cell line

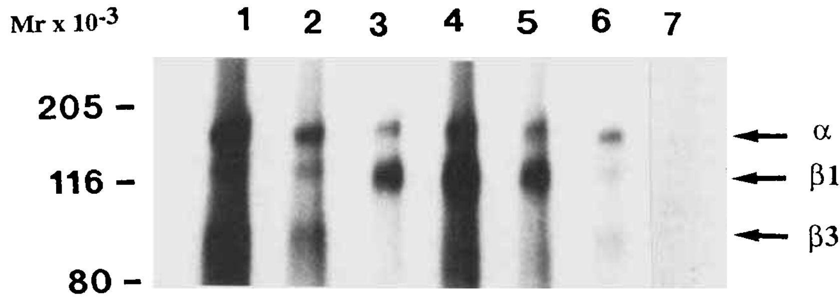

Fig. 4. Immunoprecipitation of integrins expressed on Foss melanoma cells. Cells were ^{125}I-labeled, extracted with Triton X-100, and immunoprecipitated as described in Materials and Methods. Cell lysates were immunoprecipitated one- (**lanes 1,4**) or twofold (**lanes 2,5**) by anti-αv (**lanes 1,2**) or anti-β1 antibody (**lanes 4,5**). Twice-depleted cell lysates were immunoprecipitated by anti-β1 (**lane 3**) or anti-αv antibody (**lane 6**). **Lane 7:** Immunoprecipitation with anti-β6 antibody. The immune complexes were separated by SDS polyacrylamide gradient gel (3–9%).

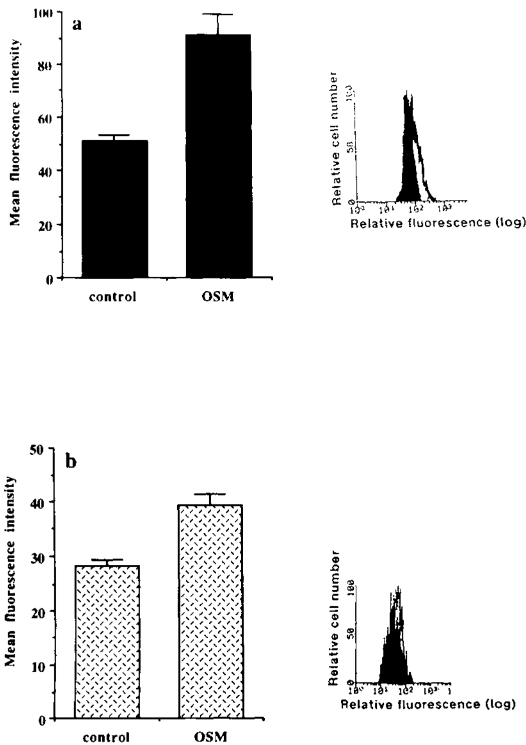

Fig. 5. Effect of OSM on αvβ1 expression on Foss melanoma cells. After 24 h treatment with 2.5 ng/ml of OSM, flow cytometry analysis was conducted to test β1 (**a**) or αv (**b**) integrin subunit expression. Results are expressed as mean ± SD from three independent experiments. Without cytokine: black histograms; with cytokine: empty histograms.

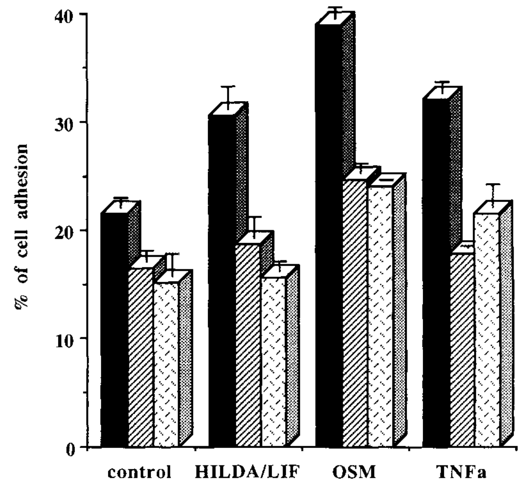

Fig. 6. Effect of cytokines on Foss melanoma cell attachment to fibronectin substratum. After treatment of Foss melanoma cells with 2.5 ng/ml of each cytokine during 24 h, cells were labeled for 18 h with medium (2.5% FCS) containing 1 μCi/ml [^{3}H] thymidine at 37°C. Then 5 × 10^4 cells/well were added to the plate coated with 2 μg/ml of fibronectin. Cell adhesion was tested as described in Materials and Methods. Adhesion inhibition assays were conducted with cells pretreated for 30 min at 37°C with 4 μg/ml of monoclonal antibodies against αv (AMF7) or β1 (K20) integrin subunits. All experiments were done in triplicate, and results are means ± SD from two independent experiments. ■, control cells; ▨, pretreated cells with AMF7; ▩, pretreated cells with K20.

which exhibits specific binding sites for HILDA/LIF and OSM [Gearing et al., 1994] and adheres to fibronectin via αvβ1 integrin [Dedhar and Gray, 1990].

As in the case of Foss melanoma cells, sequential immunoprecipitation with antibodies against αv or β1 indicated that αvβ1 was structurally associated on cell membrane (Fig. 7). With 2.5 ng/ml of these three cytokines, a nearly 1.3-fold upmodulation of β1 and αv subunit expression was observed by flow cytometry (Fig. 8). This upregulation on SK-N-SH cell membrane was correlated with increased cell attachment to fibronectin. Thus, 33, 54, and 38% increases in cell adhesion were observed after cell treatment with HILDA/LIF, OSM, and TNF-α, respectively (Fig. 9). This upmodulation was inhibited by specific anti-αv and -β1 monoclonal antibodies. The adhesion-inhibition level in the pres-

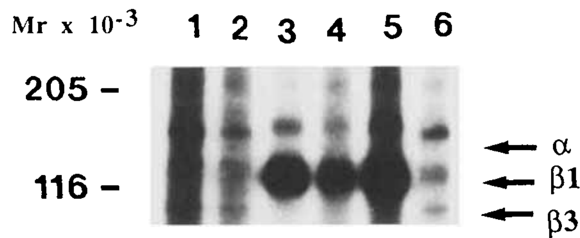

Fig. 7. Immunoprecipitation of integrins expressed on SK-N-SH neuroblastoma cells. Cells were ^{125}I-labeled, extracted with Triton X-100, and immunoprecipitated as described in Materials and Methods. Cell lysates were immunoprecipitated one- (**lanes 1,5**) or twofold (**lanes 2,4**) by anti-αv (**lanes 1,2**) or anti-β1 (**lanes 4,5**) antibody. Twice-depleted cell lysates were immunoprecipitated by anti-β1 (**lane 3**) or anti-αv antibody (lane 6). The immunoprecipitation complexes were separated by a SDS polyacrilamide gel (7.5%).

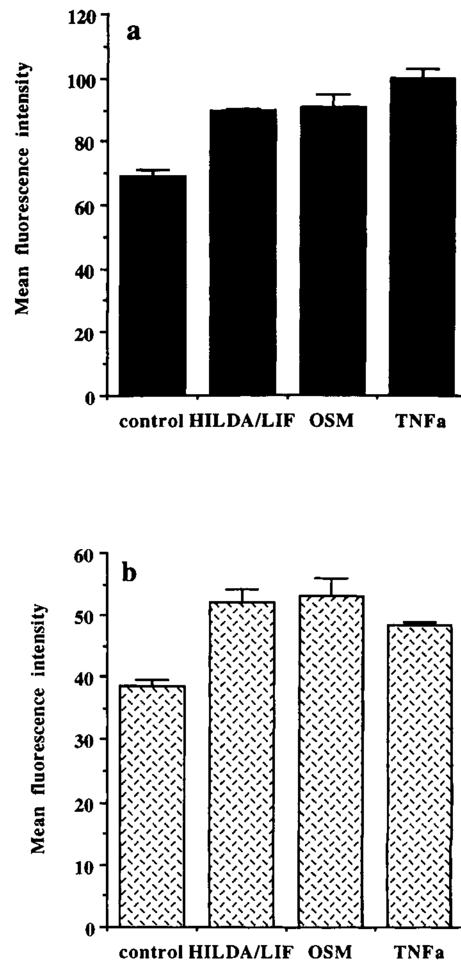

Fig. 8. Modulation of αvβ1 integrins by HILDA/LIF, OSM, or TNF-α on human SK-N-SH neuroblastoma cells. After 24 h treatment with 2.5 ng/ml of each cytokine, the expression of the β1 (a) or αv (b) integrin subunit was analyzed by flow cytometry. Results are expressed as means ± SD from three independent experiments.

ence of 4 μg/ml of monoclonal antibody against αv or β1 was higher on cells treated with HILDA/LIF and TNF-α (71 and 63%, respectively) than with OSM (54%) (Fig. 9).

DISCUSSION

It has been suggested that tumor cell interactions with extracellular matrix proteins play a critical role during embryogenesis (development and differentiation) and in the malignant process [Albelda, 1993]. Specific integrin receptors may facilitate such interactions and provide a common mechanism for tumor dissemination. Several reports have described various changes in integrin expression and functions in cultured cells [Plantfaber and Hynes, 1989; Dedhar and Saulnier, 1990; Giancotti and Ruoslahti, 1990]. Moreover, αvβ3 or α3β1 integrin expression has been correlated with the invasive properties of human melanoma cells [Gehlsen et al., 1992; Nip et al., 1992; Natali et al., 1993; Felding-Habermann et al., 1992], and α1β1, α2β1, or α3β1 integrins have been involved during human neuroblastoma cell differentiation [Rozzo et al., 1993]. Cytokines such as Il-1β, TNF-α, IFN-γ, or TGF-β have been shown to regulate integrin expression on different tumor cells [Mortarini et al., 1991; Rozzo et al., 1993; Santala and Heino, 1991], thus playing an important role in tumoral progression.

In the present study, we demonstrated for the first time that two IL-6 family cytokines, HILDA/LIF and OSM, specifically upregulate the expression of αvβ1 integrin on human tumor cell surface. This upmodulation was about 1.5–2-fold higher than constitutive expression on human Foss melanoma cells. A similar effect was obtained after treatment of a neuroblastoma cell line (SK-N-SH) with both cytokines. This upregulation of the αvβ1 integrin but not other integrins from β1, β3, β5, and β6 families was also induced at a similar level after treatment of both cell lines with TNF-α. These latter results differ from those obtained on another melanoma cell line (Me10538) in which TNF-α upregulated α5β1 and downregulated α4β1 integrins [Mortarini et al., 1991], so that β1 integrin expression was less increased than in our study. This effect could be due to the extremely high heterogeneity of integrin distribution among different melanoma cell lines [Marshall et al., 1991; Martin-Padura et al., 1991; Mortarini et al., 1991]. Thus, interactions between melanoma

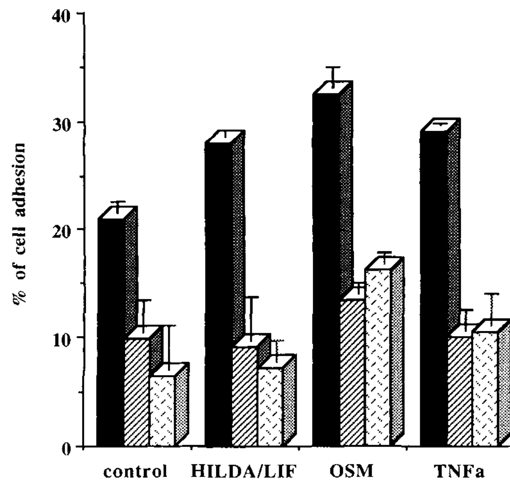

Fig. 9. Effect of cytokines on SK-N-SH cell attachment to fibronectin substratum. After treatment of SK-N-SH neuroblastoma cells with 2.5 ng/ml of each cytokine during 24 h, cells were labeled for 18 h with medium (2.5% FCS) containing 1 μCi/ml [^{3}H] thymidine at 37°C. Then 5 × 10^4 cells/well were added to the plate coated with 2 μg/ml of fibronectin. Adhesion inhibition assays were conducted with cells pretreated for 30 min at 37°C with 4 μg/ml of monoclonal antibodies against the αv (AMF7) or β1 (K20) integrin subunits. All experiments were done in triplicate, and results are means ± SD from two independent experiments. ■, control cells; ▨, pretreated cells with AMF7; ▩, pretreated cells with K20.

cell lines and extracellular matrix proteins could be supported by different integrins. In Foss melanoma cells, α5β1 was not expressed or modulated by cytokine treatment, whereas this integrin is the major fibronectin receptor in the Me10538 cell line [Mortarini et al., 1991]. Similarly, the existence of alternative forms of the vitronectin receptor (αvβ1 or αvβ3) on melanoma cells of common origin is considered to be the cause of diversity in receptor function since a correlation has been found between tumorigenic capacity in athymic nude mice and αvβ3 levels [Marshall et al., 1991]. Recently, Dedhar et al. [1991] showed that retinoic acid strongly induced the expression of αvβ1 and also, to a smaller extent, the expression of αvβ3 on murine embryonnal carcinoma cells concomitantly to the neuronal differentiation of these cells. This upmodulation by retinoic acid could be indirect via the induction of cytokine secretion such as cholinergic neuronal differentiation factor (CNDF) which had been identified as HILDA/LIF [Yamamori et al., 1989].

We also noted the increased ability of cells to attach to a fibronectin substratum after treatment with the three cytokines. Although OSM did not produce a higher expression of αvβ1 integrin than that observed with HILDA/LIF or TNF-α, it induced greater adhesion to fibronectin (Figs. 6, 9). This suggests that an additional mechanism is involved in fibronectin adhesion after OSM cell treatment. The fact that antibodies against αv or β1 caused less inhibition of adhesion to fibronectin of cells treated with this cytokine reinforces this notion. Thus, OSM could upregulate other cell surface receptors involved in the interaction with fibronectin, such as surface proteoglycans [Ruoslahti, 1988]. Another explanation is that the slight upmodulation by OSM or downregulation by HILDA/LIF or TNF-α of other integrins involved in cell attachment to fibronectin (α3β1, α5β1, α4β1, αvβ3, αvβ6) [Ruoslahti et al., 1994] may not be detectable by flow cytometry analysis. However, the additional effect of this regulation could account for the difference in adhesion between cells treated with OSM and HILDA/LIF or TNF-α.

The incomplete inhibition of adhesion to fibronectin observed with αv or β1 antibodies on control cells (as well as on cytokine-treated cells) suggests that the basal adhesion to fibronectin is also mediated by receptors distinct from αvβ1 (αvβ3, αvβ5, αvβ6) and probably by additional fibronectin receptors belonging or not to the integrin β1 family. However, the three cytokines only upregulated αvβ1 and concomitantly the adhesion to fibronectin. Thus, antibodies against αv or β1 integrin abolished the increasing of adhesion αvβ1-dependent and maintained the level of basal adhesion. The percentage of adhesion inhibition being higher on SK-N-SH (61%) than on Foss tumor cells (23%) suggests that the integrins involved in the basal adhesion to fibronectin are different in Foss and SK-N-SH cell lines and confirms that SK-N-SH adheres primarly to fibronectin via αvβ1 as previously reported by Dedhar and Gray [1990].

The αvβ1 integrin was shown to be a fibronectin attachment receptor [Vogel et al., 1990] that does not support either fibronectin matrix assembly nor all migration on component when expressed in CHO cells [Zhang et al., 1993]. The fact that HILDA/LIF and OSM induce αvβ1 expression on surface tumor cells could enhance cell attachment to extracellular matrix proteins, thus reducing cell motility, tumor cell dissemination, and the metastastic process. This lower metastatic phenotype induced by HILDA/LIF and OSM could be increased by the higher expression of ICAM-1 on melanoma cells, leading

to better cell lysis by lymphocytes (LAK cells) after treatment with both cytokines [Heymann et al., in press]. In this context, HILDA/LIF and OSM could be of interest for the regulation of tumor progression since they may both act on tumor cell recognition by the immune system and on tumor cell interaction with the extracellular matrix, two mechanisms involved in tumor regression or progression.

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