Insulin-like Growth Factor Receptor Cooperates With Integrin αvβ5 to Promote Tumor Cell Dissemination In Vivo

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Abstract

Tumor cell interactions with adhesion proteins and growth factors likely contribute to the metastatic cascade. Evidence is provided that insulin or insulin-like growth factor–mediated signals cooperate with the commonly expressed integrin αvβ5 to promote spontaneous pulmonary metastasis of multiple tumor cell types in both the chick embryo and severe combined immune deficiency mouse/human chimeric models. Expression of αvβ5 in tumor cells promoted their adhesion to vitronectin in vitro. However, cell motility required cytokine stimulation, which caused redistribution of α-actinin to membrane-adhesive sites containing αvβ5. Significantly, ligation of αvβ5 and cytokine receptors were both required for spontaneous pulmonary metastasis of multiple tumor types even though it was not necessary for primary tumor growth. Thus, tumor cell metastasis can be regulated by a functional cooperation between cytokine signaling events and the adhesion receptor αvβ5 in a manner independent of tumor cell growth. These findings provide evidence that integrin ligation, in conjunction with cytokine activation, plays an important role in the dissemination of malignant tumor cells. (*J. Clin. Invest.* 1997. 99:1390–1398.)
Key words: adhesion • invasion • motility • cytokine • α-actinin

Introduction

The spread of malignant tumor cells to secondary sites continues to be a major obstacle for the treatment of neoplastic disease. Recent reports implicate both cytokines and adhesion receptors in tumor cell invasion and metastasis (1–5). In fact, a correlation has been established between specific integrins and metastatic behavior in vivo (6–8).

Adhesion receptors of the integrin family promote cell attachment to proteins within the extracellular matrix and potentiate cellular migration and invasion (9–11). Integrin ligation typically leads to organization of the actin cytoskeleton by direct or indirect association of integrin cytoplasmic domains with several cytoplasmic proteins including α-actinin, talin, and focal adhesion kinase (12–15). However, little is known about how these molecular events regulate tumor cell motility and invasion. While integrin-mediated adhesion may be necessary for tumor cell motility and invasion, it is not sufficient. For example, human tumor cells typically express the integrin αvβ5, facilitating attachment to vitronectin. However, cells expressing αvβ5 require a tyrosine kinase receptor–mediated signaling event for motility on vitronectin (16). Interestingly, tumor cells expressing αvβ3 migrate in vitro and metastasize in vivo without the need for exogenous cytokine stimulation (17).

Recent studies demonstrate that cytokines such as insulin or insulin-like growth factor-1 potentiate tumor cell migration in vitro (18, 19). In fact, tumor cells can secrete these cytokines, which may lead to autocrine stimulation of tumor cell growth and/or motility (20–23). Thus, it appears that cytokine receptors and adhesion molecules may cooperate functionally to promote cell motility in vitro and perhaps invasion and metastasis in vivo. In this report, evidence is provided that spontaneous tumor cell metastasis can be induced in a manner dependent on both cytokine stimulation and ligation of integrin αvβ5. These findings provide the first direct evidence that an integrin can functionally cooperate with a cytokine receptor to promote spontaneous tumor cell invasion and dissemination in vivo.

Methods

Antibodies, chemicals, and reagents. Monoclonal antibodies LM609 anti–αvβ3, LM142 anti–human αV, P1F6 anti–αvβ5, and 661 antivitronectin have been described previously (24). mAb W6/32 anti–MHC-I was obtained from American Type Culture Collection (Rockville, MD). mAb 7E2, specific for hamster β1 integrin, was graciously provided by Dr. Rudolph Juliano (University of North Carolina, Chapel Hill, NC). mAb αIR3, anti–IGF-1 receptor was obtained from Oncogene Science Inc., (Cambridge, MA). Phycoeurythrin-labeled goat anti–mouse IgG was obtained from Southern Biotechnology Associates Inc. (Birmingham, AL). Insulin was obtained from Boehringer Mannheim GmbH (Mannheim, Germany). Insulin-like growth factor-1 was obtained from Genzyme Corp. (Cambridge, MA). Laminin was obtained from Upstate Biotechnology Inc. (Lake Placid, NY). Vitronectin was purified as previously described (25). Bacterial collagenase was obtained from Worthington Biochemical Corp. (Freehold, NJ).

Cell lines and tissue culture. Hamster melanoma cell line CS-1 was obtained from Dr. Caroline Damsky (University of California at San Francisco, San Francisco, CA). CS-1 cells were selected for the lack of functional expression of both αvβ3 and αvβ5. β5CS-1 cell line was generated by transfection of full length cDNA encoding the β5 integrin subunit and has been described previously (26). FG human pancreatic carcinoma cells and MCF-7PB human breast carcinoma cells, both of which express integrin αvβ5 but not αvβ3, have been described previously (16, 27). All cell lines were cultured in RPMI-1640, Gibco Laboratories (Grand Island, NY), supplemented with 5% fetal bovine serum, 2 mM L-glutamine, and 50 μg/ml gentamicin (Sigma Chemical Co., St. Louis, MO). Fresh human neonatal foreskins were obtained from the Cooperative Human Tissue Network

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(Cleveland, OH) and stored in sterile RPMI-1640 media supplemented with 2% FBS and 1% gentamycin.

Cell migration assays. Cell migration assays were performed using Transwell migration chambers as previously described (16) with minor modifications. Briefly, the under surface of the membranes (8-μm pores) were coated with either vitronectin or laminin at a concentration of 10 μg/ml. The lower chamber was filled with 0.5 ml of Fibroblast Basal Medium (FBM) containing 0.5% bovine serum albumin. CS-1 melanoma cells were serum starved for 16 h, and then treated with insulin (0.1–20 μg/ml) for 30 min. CS-1 melanoma cells (10^5) in 0.1 ml of FBM-BSA were added to the upper wells of the Transwell chambers and allowed to migrate for 16 h. Similar experiments were performed in the presence of IGF-1 (20 ng/ml). For antibody inhibition experiments, cells were allowed to migrate in the presence of either mAbs P1F6 (anti–αvβ5), 661 (antivitronectin), or LM609 (anti–αvβ3) (25 μg/ml). Cells migrating to the under side of the membrane were stained with 1% crystal violet. Migration was quantified by counting the number of stained cells per 40× field with an inverted microscope (BX-60; Olympus Corp., Lake Success, NY).

Immunofluorescence analysis of β5CS-1 cells stimulated with cytokine. CS-1 melanoma cells expressing integrin αvβ5 (β5CS-1) were stimulated with either insulin (10 μg/ml) or IGF-1 (20 ng/ml) for 30 min, and then allowed to attach to vitronectin-coated glass cover slips for 30 min at 37°C. Growth factors were again added to the attached cells for an additional 15 min. Attached cells were extracted to remove non–cytoskeletal-associated cell material as previously described (28). Fixed cells were stained with both anti–αvβ5 antibody P1F6 (20 μg/ml) and rabbit polyclonal anti–α-actinin (1:100) dilution for 1 h at 37°C. Two-color staining was detected by incubation with both sheep anti–mouse FITC and goat anti–rabbit rhodamine-labeled secondary antibodies. Cell fluorescence was analyzed with a laser confocal microscope (Carl Zeiss Inc., Thornwood, NY) focused at the cell substratum interface (200 nm Z sections).

Chick embryo metastasis assay. The chick embryo metastasis assay was performed as described previously with some modifications (17, 29). CS-1, β5CS-1, or FG human pancreatic carcinoma cells were stimulated 24 h before with 10 μg/ml of insulin, 20 ng/ml IGF-1, or unstimulated. Tumor cells ($5–10 \times 10^6$) were inoculated on the chorioallantoic membrane (CAM)[1] of 9- or 10-d-old chick embryos in a total volume of 40 μl RPMI. The embryos were allowed to incubate

1. *Abbreviations used in this paper:* CAM, chorioallantoic membrane; SCID, severe combined immune deficiency.

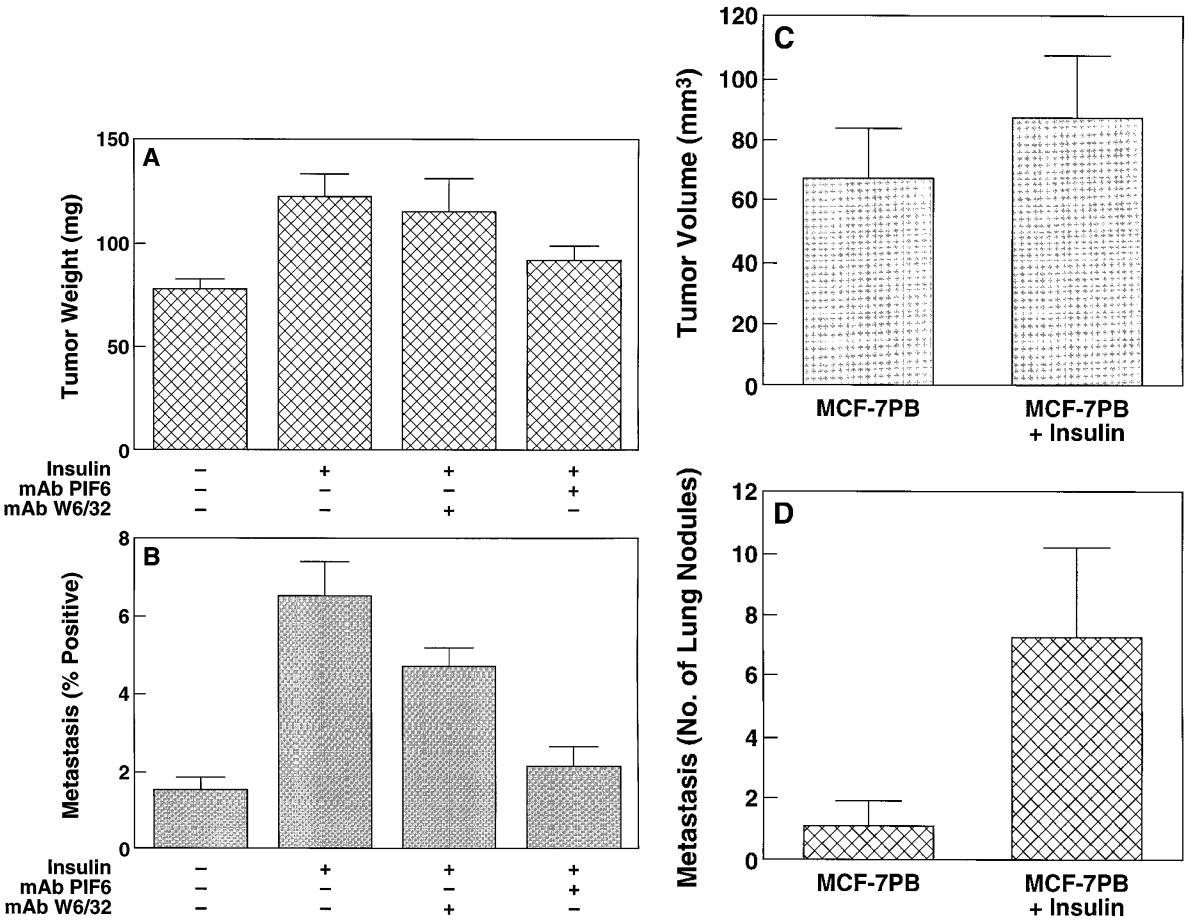

Figure 1. Cytokine stimulation of tumor cell metastasis in vivo. FG human pancreatic carcinoma cells and MCF-7PB human breast carcinoma cells expressing integrin αvβ5 but not αvβ3 were incubated in the presence or absence of insulin (10 μg/ml) and analyzed for their metastatic ability in two models of spontaneous metastasis. (*A* and *B*) FG cells were inoculated onto the chorioallantoic membrane (CAM) of 9-d-old chick embryos and tumors were allowed to grow for 9 d. (*C* and *D*) MCF-7PB cells were injected intradermally into full thickness human skin transplanted on SCID mice and tumors were allowed to grow for 10 wk. (*A*) White hatched bars represent the mean±SEM of FG pancreatic carcinoma tumor weights. (*B*) Grey bars represent the mean±SEM of the relative percentage of FG tumor cells in the lungs of chick embryo. (*C*) Solid grey bars represent the mean±SEM of MCF-7PB breast carcinoma tumor volumes. (*D*) Hatched bars indicate mean±SEM of the number of surface lung tumor nodules. P1F6 (mAb directed to integrin αvβ5). W6/32 (mAb directed to MHC-I). Assays were performed twice with 5–10 animals per condition.

for a total of 7 to 9 d, at which time the embryos were killed. Tumors that formed at the primary site were excised, trimmed free of surrounding CAM tissue, and weights were determined. Pulmonary metastasis was assessed by determining the percentage of either hamster melanoma or human carcinoma cells present in a single cell suspension of whole lung tissue by flow cytometry. Single cell suspensions were prepared by resecting the chick lungs, mincing the tissue, and resuspending the tissue in 0.25% bacterial collagenase in PBS for 2 h at 37°C with occasional vortexing. Cell suspensions were washed 4× with 2.5% BSA in PBS and fixed in 1.0% paraformaldyde for 15 min. Cell suspensions were washed as before and incubated in 2.5% BSA, 0.02% sodium azide in PBS for 24 h at 4°C to block nonspecific binding sites. Flow cytometry was performed as previously described (17) using mAb 7E2, specific for hamster β1 integrin or mAb LM142 directed to human αV integrin. For antibody inhibition experiments, embryos were injected intravenously with purified mAbs P1F6 or W6/32 (300 μg/100 μl), 24 h after initiation of tumor growth as previously described (29).

Severe combined immune deficiency mouse/human chimeric model. Transplantation of human skin was performed as described previously (30). Briefly, severe combined immune deficiency (SCID) mice were anesthetized and fresh full thickness human neonatal foreskin was sutured into place. The skin grafts were allowed to heal for 4 wk. CS-1, β5CS-1, or MCF-7PB human breast carcinoma cells were preincubated for 24 h with either insulin (10 μg/ml) or IGF-1 (20 ng/ml), and then injected intradermally (2–4 × 10^6 per animal) into the human skin. The tumors were allowed to grow for a total of 4 wk (melanoma tumors) or 10 wk (carcinoma tumors), at which time they were resected and wet weights and/or caliper measurements were determined. Lungs from tumor-bearing mice were dissected, washed 3× in sterile PBS, and incubated in Bouin's fixative for 8 h. Pulmonary metastasis was quantified by counting the number of surface lung nodules in a double blind fashion. Lungs were further analyzed by histological analysis. Briefly, 4-μm sections of paraffin-embedded lungs were stained with hematoxylin and eosin as previously described (31). Tissue sections were examined with a BX60 compound microscope.

Statistical analysis. Statistical analysis was performed with a Stat Works program for Macintosh computers, Cricket Software Inc. (Philadelphia, PA). Data was analyzed for statistical significance with Student's *t* test.

Results

Cytokine stimulation of CS-1 cell migration. Tumor cell metastasis depends on the migration and invasive properties of individual tumor cells. This process may be enhanced by cytokines

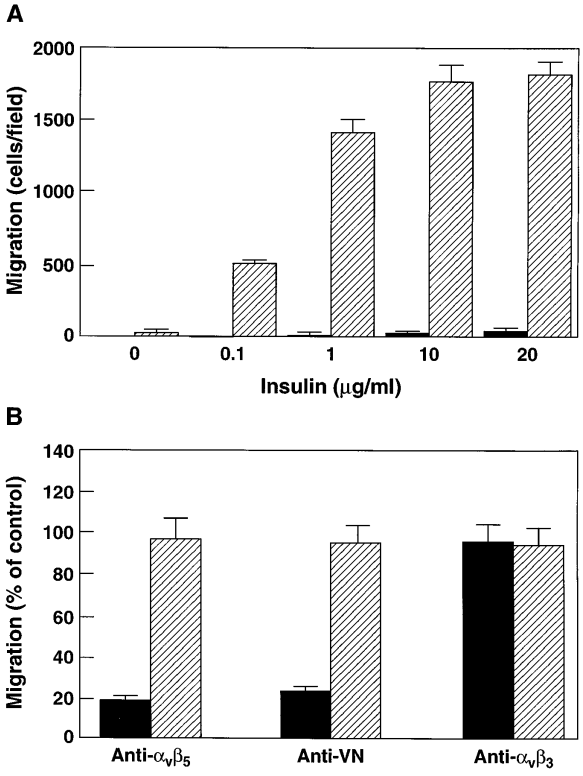

Figure 2. Effect of cytokine stimulation on CS-1 cell migration on vitronectin and laminin. Cell migration assays were performed using Transwell migration chambers coated with either purified vitronectin or laminin. (*A*) CS-1 or β5CS-1 cells were pretreated with insulin (0.1–20.0 μg/ml). Black bars represent the mean(±SD) of control CS-1 cell migration on vitronectin. Hatched bars represent the mean±SD of β5CS-1 cell migration on vitronectin. (*B*) β5CS-1 cells were pretreated with insulin (10 μg/ml) and allowed to migrate toward either vitronectin or laminin. Black bars represent the mean±SD β5CS-1 cell migration on vitronectin. Hatched bars represent the mean±SD β5CS-1 cell migration on laminin. Anti–αvβ5 (mAb P1F6 directed to integrin αvβ5), anti–VN (mAb 661 directed to vitronectin), and anti–αvβ3 (mAb LM609 directed to integrin αvβ3). Experiments were performed three times with triplicate samples.

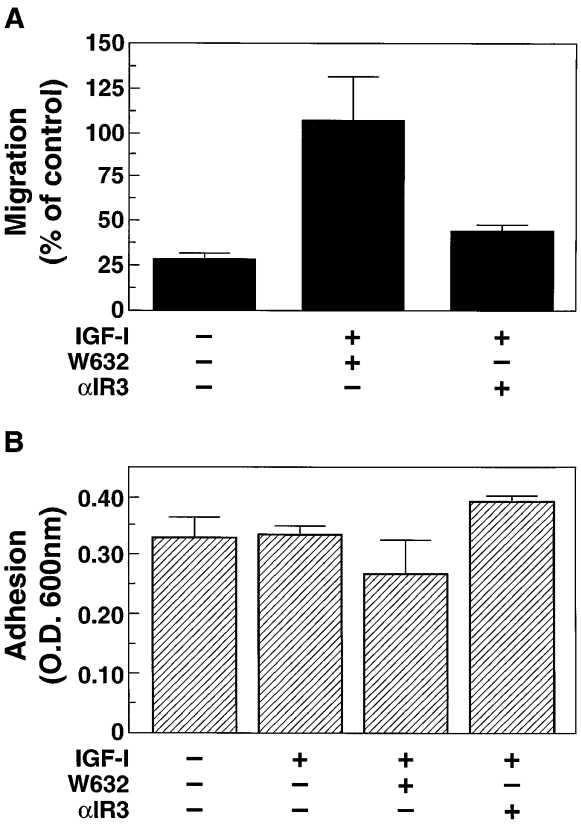

Figure 3. Effects of anti–IGF-1 receptor antibody on β5CS-1 cell migration and adhesion. Cell migration and adhesion assays were performed on vitronectin-coated surfaces with αvβ5 expressing CS-1 cells (β5CS-1) treated with or without IGF-1 (20 ng/ml), W6/32 anti–MHC (1.0 μg/ml), or αIR3 anti–IGF-1 receptor (1.0 μg/ml). (*A*) Black bars represent mean β5CS-1 cell migration to vitronectin. (*B*) Hatched bars represent mean β5CS-1 cell adhesion to vitronectin. Error bars represent standard deviation of triplicate samples from three independent experiments.

such as IGF-1, which can potentiate cell motility in vitro. In fact, we recently observed that cells expressing integrin αvβ5 could attach to vitronectin, but failed to migrate without exogenous growth factor stimulation (16). Recent reports also suggest that cytokine stimulation of human breast carcinoma cells can promote vitronectin-dependent migration in vitro (32). To investigate the biological relevance of cytokine-dependent αvβ5-directed motility on spontaneous metastasis in vivo, we examined the invasive and metastatic ability of both human pancreatic carcinoma (FG) and human breast carcinoma cells (MCF-7PB) in vivo. FG and MCF-7PB carcinoma cells were treated with or without insulin (10 μg/ml) for 24 h. FG human pancreatic carcinoma cells were implanted onto the CAMs of 9-d-old chick embryos. 24 h after tumor cell implantation, embryos were injected intravenously with either mAb P1F6 (anti–αvβ5) or W6/32 (anti–MHC-I). The tumors were allowed to grow for a total of 9 d. FG human pancreatic carcinoma cells formed well defined solid tumors in the chick embryo with similar mean tumor weights irrespective of cytokine stimulation or antibody treatment (Fig. 1 *A*). In contrast,

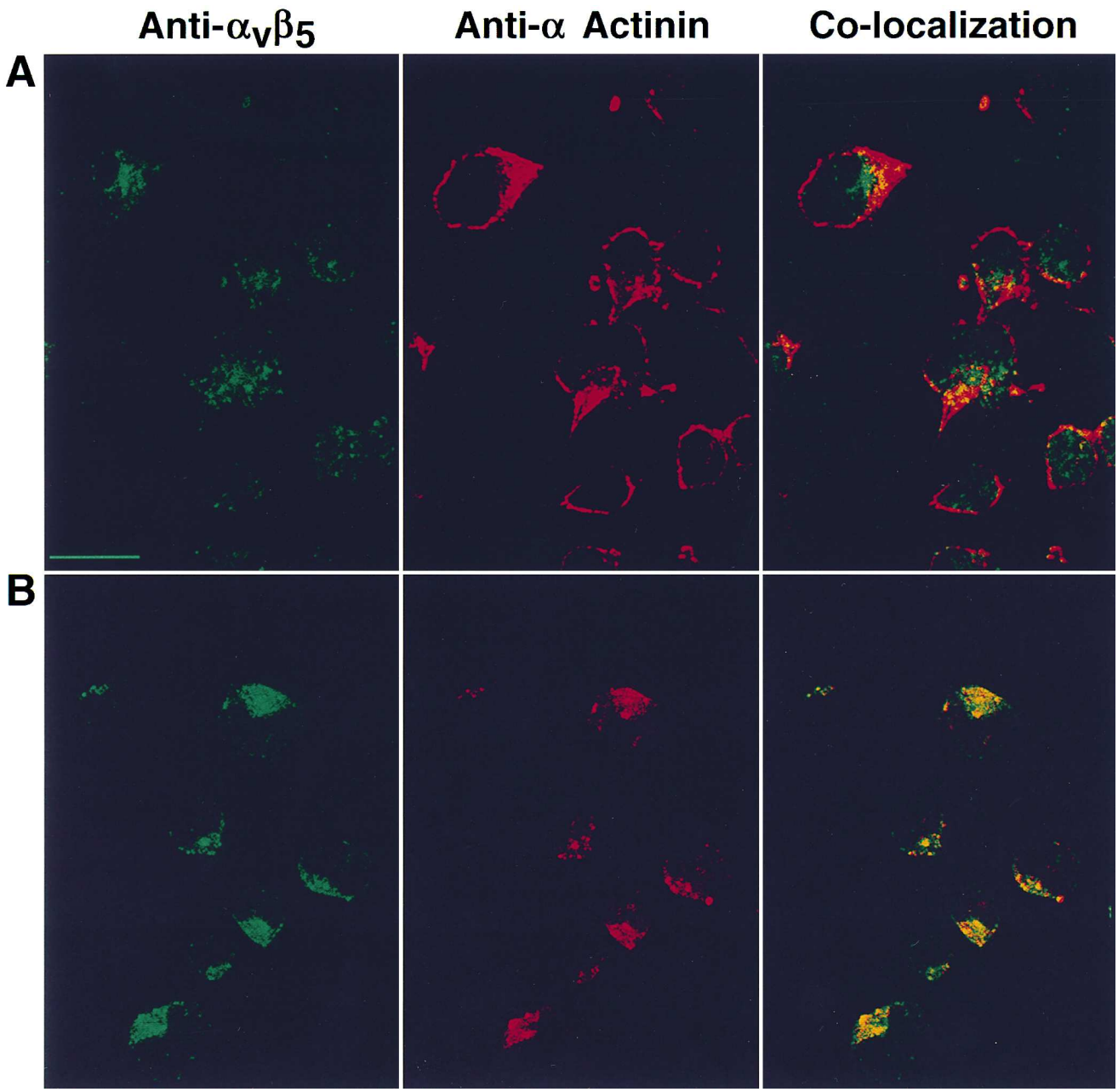

Figure 4. Effect of cytokine on localization of integrin αvβ5 and α-actinin. Unstimulated or insulin- (10 μg/ml) stimulated β5CS-1 melanoma cells attached to vitronectin-coated glass cover slips were fixed, permeabilized, and costained with both anti–αvβ5 antibody P1F6 and rabbit polyclonal anti–α-actinin. Two-color staining was detected by incubation with both sheep anti–mouse FITC and goat anti–rabbit rhodamine-labeled secondary antibodies. (*A*) Unstimulated β5CS-1 cells. (*B*) Insulin (10 μg/ml) stimulated β5CS-1 cells. Photographs were taken with a Zeiss laser confocal microscope focused at the cell substratum interface (594×). Green, integrin αvβ5; red, α-actinin at the cell substrate interface (200 nm Z section); and yellow, colocalization between integrin αvβ5 and α-actinin. Identical results were obtained after IGF-1 stimulation (20 ng/ml).

insulin stimulation of FG cells caused a fourfold increase in pulmonary metastasis that was not only dependent on cytokine stimulation, but also appeared to require integrin αvβ5, since an antibody directed to αvβ5 (P1F6) significantly reduced this activity (Fig. 1 *B*). Interestingly, similar findings were obtained with human breast carcinoma MCF-7PB cells in the human/mouse chimeric model of metastasis. While insulin apeared to have little effect on the size of human breast carcinoma tumors grown within full thickness human skin, this treatment caused a three- to fourfold increase in pulmonary metastasis (Fig. 1, *C* and *D*).

To further define the cooperative interaction between integrin αvβ5 and ligation of insulin or IGF-I receptors during invasive cell behavior, we examined the invasive and metastatic ability of CS-1 melanoma tumors in vivo since these CS-1 melanoma cells lacked β3 and β5 integrin subunits and thus fail to attach or migrate on vitronectin (17). These melanoma cells were transfected with a cDNA encoding the β5 integrin subunit, enabling them to attach to vitronectin (26). These αvβ5-expressing CS-1 cells (β5CS-1) were then tested for their ability to migrate towards the extracellular matrix proteins vitronectin or laminin in the presence or absence of insulin or IGF-1. As shown in Fig. 2 *A*, CS-1 cell migration toward vitronectin required both αvβ5 expression and exposure of cells to insulin or IGF-1 (data not shown). In contrast, CS-1 cell migration toward laminin was independent of either expression of αvβ5 or cytokine stimulation. Furthermore, cytokine stimulation did not alter the surface expression of integrin αvβ5 or induce the expression of αvβ3 as determined by flow cytometric analysis (data not shown). In fact, CS-1 cell migration on vitronectin was blocked with monoclonal antibodies directed to αvβ5 or vitronectin but not to αvβ3 (Fig. 2 *B*). These find-

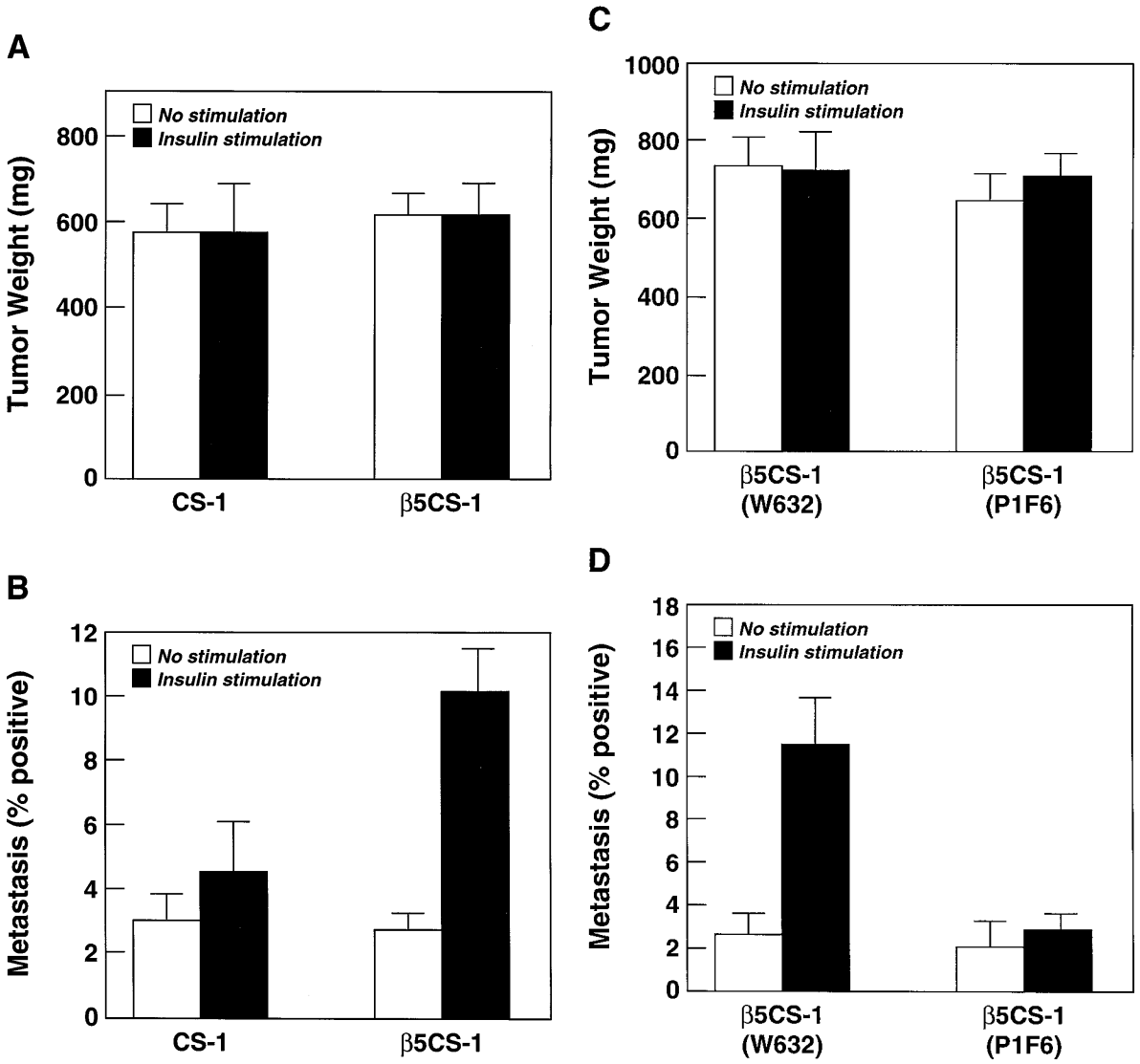

Figure 5. Cytokine stimulation of CS-1 melanoma cell metastasis in vivo. Control CS-1 and β5CS-1 cells were incubated in the presence or absence of either insulin (10 μg/ml) or IGF-1 (20 ng/ml) and inoculated onto the CAM of 10-d-old chick embryos. Tumors were allowed to grow for 7 d. (*A* and *C*) Open bars represent the mean tumor weights in the absence of cytokine, black bars in the presence of cytokine. (*B* and *D*) Open bars indicate mean percentage of tumor cells in the chick lungs in the absence of cytokine, black bars in the presence of cytokine. (*C* and *D*) Tumorgenicity and metastasis in the presence of systemically administered monoclonal antibodies P1F6 (anti–αvβ5) or W6/32 (anti–MHC). Metastasis assays were performed two to four times with five to ten embryos per condition.

ings demonstrate that insulin or IGF-1 can specifically stimulate an αvβ5-dependent motility response.

Ligation of IGF-1 receptor potentiates αvβ5-dependent CS-1 cell migration. Experiments were designed to determine whether ligation of the IGF-1 receptor is required for αvβ5-dependent cell motility. CS-1 cell migration was analyzed in the presence or absence of a monoclonal antibody directed to the IGF-1 receptor. As shown in Fig. 3 *A*, IGF-1 induced β5CS-1 cell migration, which was specifically blocked by the addition anti–IGF-1 receptor antibody αIR3, whereas an isotype-matched control antibody had no effect. Furthermore, this effect was specific to migration as αIR3 had no effect on CS-1 cell adhesion to vitronectin (Fig. 3 *B*). These results support the contention that αvβ5-dependent CS-1 cell migration requires cooperation between the insulin-like growth factor receptor and integrin αvβ5.

Cytokine stimulation promotes the redistribution and colocalization of integrin αvβ5 and α-actinin. The cytoskeletal protein α-actinin, which associates with both actin and the cytoplasmic tail of integrin beta subunits, was recently implicated in cell motility since it preferentially localized to the leading edge of migrating cells (33). Therefore, to investigate the biological response of CS-1 cells to cytokine stimulation, αvβ5-expressing CS-1 cells attached to vitronectin were permeabilized and stained for the presence of the cytoskeletal protein α-actinin and integrin αvβ5. Unstimulated β5CS-1 cells at-

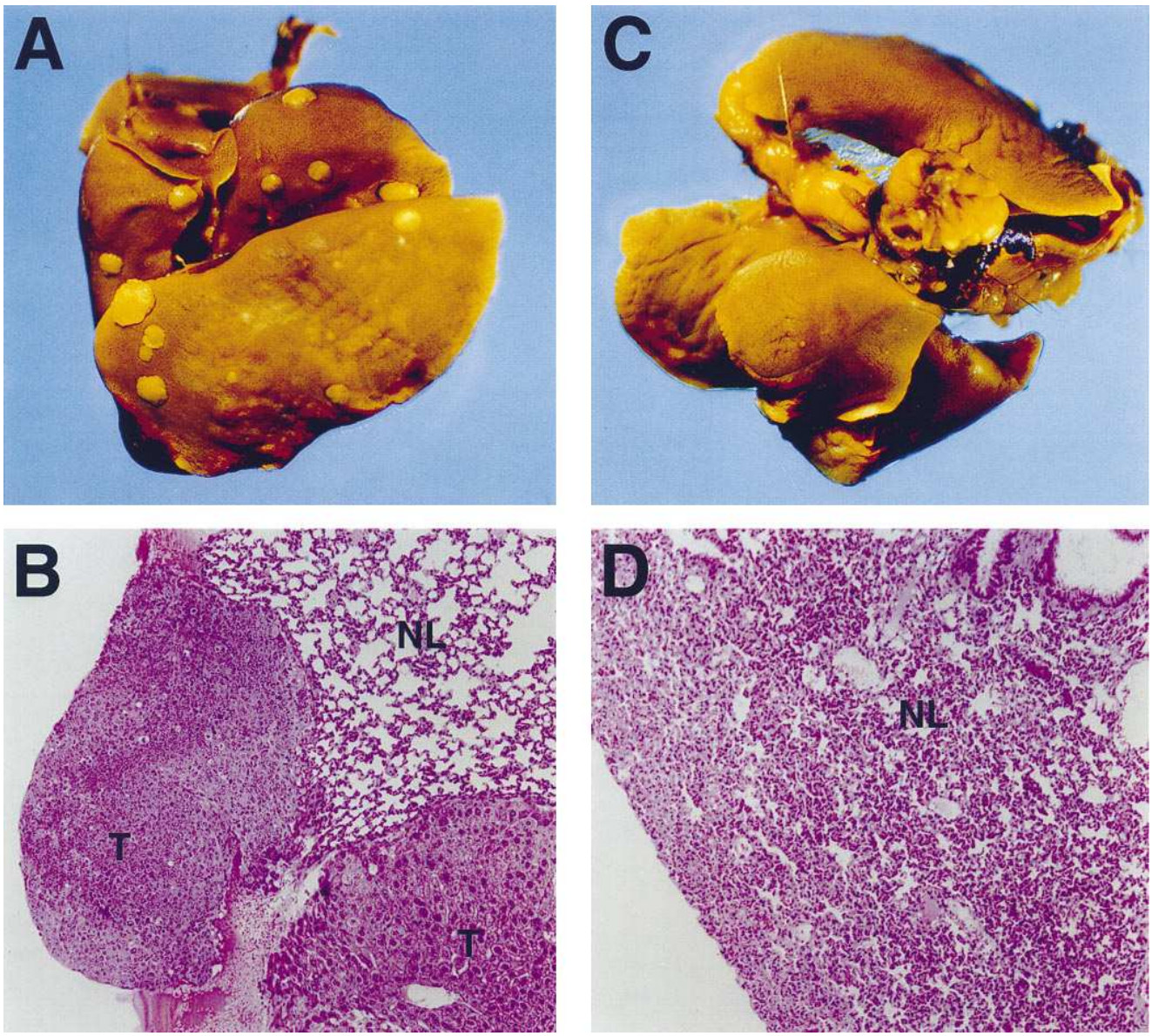

Figure 6. Spontaneous metastasis in SCID mice. β5CS-1 melanoma cells treated with or without insulin were injected intradermally into full thickness human skin transplanted on SCID mice. After 4 wk, lungs from tumor-bearing mice were resected and analyzed for pulmonary metastasis. (*A* and *B*) Representative example of lungs from animals injected with insulin-stimulated β5CS-1 melanoma cells. (*C* and *D*) Representative example of lungs from animals injected with unstimulated β5CS-1 melanoma cells. (*A* and *C*) Stereo micrographs (10×) of lungs from tumor-bearing mice. (*B* and *D*) Photomicrographs (200×) of lung tissue sections stained with hemotoxylin and eosin. NL, normal lung tissue; T, metastatic tumor lesions.

tached to vitronectin and showed a distinct compartmentalization of αvβ5 and α-actinin (Fig. 4 *A*). However, after exposure of β5CS-1 cells to either IGF-1 or insulin, these cells showed a specific redistribution and extensive colocalization (*yellow*) of αvβ5 and α-actinin at the cell substrate interface as detected by confocal image analysis (Fig. 4 *B*). Thus, cytokine stimulation of β5CS-1 cells that was sufficient to induce cell motility is associated with the colocalization of integrin αvβ5 and α-actinin in these melanoma cells. This association may provide a critical link between αvβ5 and the actin cytoskeleton necessary for migration of these cells on vitronectin.

Cytokine stimulation of CS-1 cell metastasis. To assess the biological significance of αvβ5-dependent CS-1 cell motility, we examined the growth and spontaneous metastatic properties of these CS-1 cells in the chick embryo and human skin/mouse chimeric models. Control or β5CS-1 cells pretreated with or without cytokine were placed on the chorioallantoic membrane of 10-d-old chick embryos and allowed to propagate for 7 d. Primary tumors were then resected and weighed, and the lungs were removed to identify CS-1 cells that had established pulmonary metastases as described previously (17). As shown in Fig. 5 *A*, the primary tumors that had developed were not significantly influenced by either the expression of αvβ5 ($P > 0.580$) and/or prior exposure of cells to either insulin or IGF-1 ($P > 0.748$). However, when β5CS-1 cells were exposed ex vivo to cytokine, the cells acquired the ability to spontaneously metastasize to the lungs of chick embryos (Fig. 5 *B*). Importantly, neither αvβ5 expression alone or cytokine stimulation of αvβ5-negative CS-1 cells (CS-1) promoted tumor cell metastasis. To establish whether αvβ5 directly contributed to the metastatic properties of these cells, tumor-bearing animals were injected intravenously with monoclonal antibody directed to integrin αvβ5. This antibody specifically blocked spontaneous pulmonary metastasis of β5CS-1 cells ($P < 0.002$), yet had negligible effects on primary tumor growth ($P > 0.210$) (Fig. 5, *C* and *D*). Together, these findings provide evidence for a functional cooperation between ligation of a cytokine receptor and integrin αvβ5 during spontaneous pulmonary metastasis in vivo.

CS-1 cell metastasis in the SCID mouse/human chimeric model. Primary melanomas develop in the skin and, once they invade vertically into the dermis, become highly metastatic. Therefore, we directly injected these β5CS-1 melanoma cells into the dermis of human skin transplanted on the flanks of SCID mice. β5CS-1 cells treated with or without insulin or IGF-1 were injected intradermally within the human skin as previously described (27). The tumors were allowed to grow for 4 wk, at which time the mice were killed and the resulting primary tumors were resected and weighed. The lungs were removed to determine the extent of pulmonary metastasis. As shown in Fig. 6 *A*, cytokine-treated β5CS-1 cells formed numerous large, well defined metastatic lung lesions that could also be readily detected by histological analysis (Fig. 6 *B*). In contrast, lungs from mice injected with unstimulated β5CS-1 cells (Fig. 6, *C* and *D*) or αvβ5-negative CS-1 cells (data not shown) treated with either insulin or IGF-1 showed few if any metastatic lung lesions. This metastatic behavior was not due to an increase in tumorgenicity since exposure to cytokine did not significantly influence the tumor size (Fig. 7 *A*); however, it did cause a fivefold increase in metastatic lung lesions (Fig. 7 *B*). These results confirm our previous findings in the chick embryo and provide a second physiologically relevant example of a functional cooperation between ligation of a cytokine receptor and integrin αvβ5 during spontaneous tumor cell metastasis in vivo.

Discussion

Cell adhesion molecules and cytokines have been suggested to contribute to the metastatic spread of tumor cells (1–5, 34, 35). Numerous studies have suggested possible mechanisms by which these distinct families of molecules could independently contribute to the metastatic cascade (3, 6, 22, 36, 37). However, the possibility that cell adhesion molecules and cytokines func-

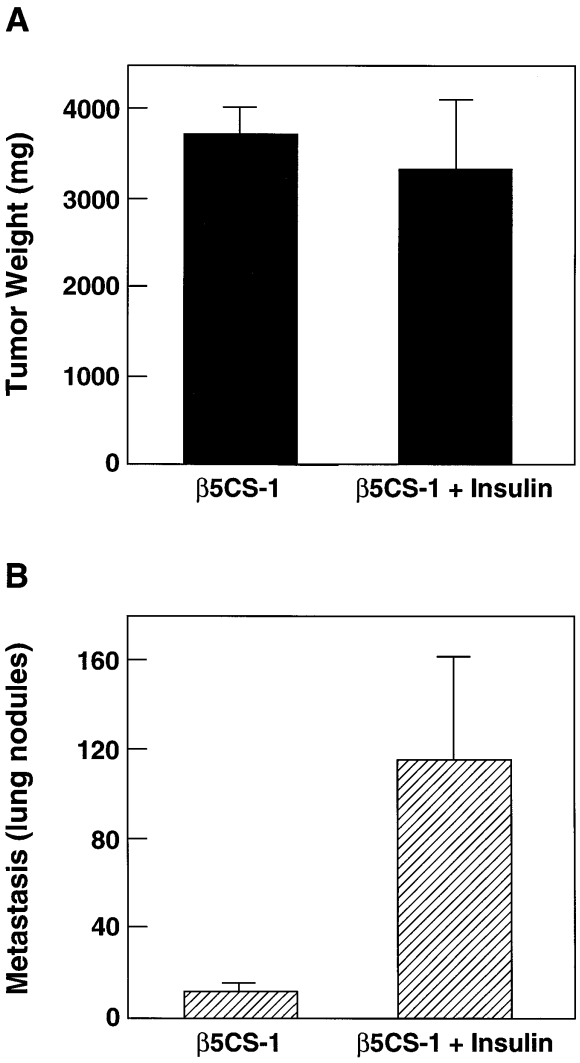

Figure 7. Quantification of β5CS-1 tumorgenicity and metastasis in the SCID mouse/human chimeric model. Tumor growth and metastasis of β5CS-1 melanoma cells untreated or pretreated with insulin, were evaluated in the SCID mouse/human chimeric model. (*A*) Black bars indicate the mean±SEM of tumor weights of β5CS-1 melanoma tumors grown in the microenvironment of human skin. (*B*) Hatched bars indicate the mean±SEM numbers of metastatic lung surface colonies from mice injected with either untreated or insulin-treated β5CS-1 melanoma cells. Experiments were performed twice with five to eight mice per condition.

tion cooperatively to potentiate a complex process like metastasis has not been fully appreciated.

Recent studies have implicated the vitronectin receptor integrin αvβ3 in angiogenesis and melanoma cell invasion and metastasis (6–8, 31, 38–40). In fact, we have recently shown that expression of integrin αvβ3 in CS-1 melanoma cells leads to vitronectin-dependent migration and pulmonary metastasis without the apparent need for exogenous cytokine activation (17). However, integrin αvβ3 has a relatively limited tissue distribution, and many tumor cells lacking this integrin readily metastasize, suggesting a requirement of other molecules (39, 40). In contrast, αvβ5, the most widely expressed vitronectin receptor, mediates cell adhesion but is unable to initiate cell motility (16). However, treatment with agonists of tyrosine kinase receptors or activators of protein kinase C facilitated αvβ5-dependent cell spreading and migration on vitronectin (16, 41). Thus, it appears that integrin αvβ5 may be structurally designed to respond to specific cytokine-derived signaling events that enable it to engage the cell's motility machinery. Since the vitronectin receptor αvβ5 is widely expressed by many malignant tumor cells (42), combined with the fact that many cytokines such as IGF-1 are secreted by tumors (43, 44), we investigated the possible cooperative role of this cytokine and αvβ5 in spontaneous tumor cell metastasis in vivo.

In this report and previous studies, evidence is provided that stimulation of αvβ5-expressing CS-1, FG, and MCF-7PB cells with either insulin or IGF-1 promoted vitronectin-dependent motility. In fact, we provide evidence that the β5CS-1 migratory response was dependent on both expression of αvβ5 and cytokine activation since neither αvβ5-negative CS-1 cells stimulated with cytokine nor β5CS-1 cells in the absence of cytokine migrated toward vitronectin. Interestingly, cytokine stimulation of β5CS-1 cells attached to vitronectin caused a specific redistribution and colocalization of α-actinin with integrin αvβ5. α-Actinin has been implicated in cell motility and is known to associate either directly or indirectly with both the actin cytoskeleton and integrin receptors (45–47). Therefore, this association between α-actinin and αvβ5 after cytokine activation may allow αvβ5 to engage the cell's motility machinery and promote migration toward vitronectin.

Since tumor cell motility contributes to the invasion and/or dissemination of malignant tumor cells, we investigated the biological relevance of cytokine-activated αvβ5-dependent tumor cell motility in two independent in vivo models. We demonstrate that β5CS-1, FG, and MCF-7PB tumor cells readily metastasize to the lungs of either chick embryos and/or SCID mice, after stimulation with either insulin or IGF-1. These results have clinical relevance, since primary melanoma tumors that invade vertically into human dermis are often highly metastatic, whereas horizontal growing melanomas in the epidermis are typically benign (48). The SCID mouse/human chimeric model provided the unique ability to study the invasive and metastatic properties of melanoma cells in the microenviroment of the human skin. In addition, many invasive human carcinoma tumors lack expression of integrin αvβ3, but readily express αvβ5, IGF-1, and IGF-I receptors that may cooperate to promote metastasis in vivo.

While it has been shown that cytokine stimulation of tumor cells can potentiate tumor growth, little if any changes were observed in tumorgenicity of the cell types tested after stimulation with either insulin or IGF-1, suggesting that cytokine stimulation can selectively influence the invasive and metastatic properties of tumor cells. In fact, it has been reported that ligation of growth factor receptors can activate ras-dependent signaling pathways leading to tumor cell metastasis (49, 50). Our findings are consistent with these results, but suggest the additional involvement of the integrin αvβ5 in metastatic behavior. Furthermore, this invasive cellular response depended on both expression of integrin αvβ5 and exposure to cytokine, since αvβ3-negative FG human pancreatic carcinoma, MCF-7PB breast carcinoma, CS-1 melanoma cells, or αvβ5-positive β5CS-1 cells in the absence of cytokine exhibited little if any metastatic ability. Moreover, both FG and β5CS-1 tumor cell metastasis could be blocked by systemic administration of monoclonal antibody directed to integrin αvβ5, thus demonstrating the importance of αvβ5 in this process.

These findings have significant consequences for many solid tumors since αvβ5 is among the most widely expressed members of the integrin family and has been detected on most normal and transformed adherent cell lines examined (42). In contrast, the vitronectin receptor αvβ3, which promotes cell motility without cytokine stimulation (17), has a relatively limited cellular distribution compared with that of αvβ5 (42, 48, 51). These results, combined with the fact that cytokines such as IGF-1 can be secreted by many tumors (20–23), suggest a novel mechanism of tumor progression in which αvβ5-dependent metastasis occurs.

Finally, results presented here support the notion that adhesion and growth factor receptors influence tumor cell behavior in vivo. Evidence is provided that the spontaneous metastasis of a variety of distinct tumor types can be specifically regulated by the functional cooperation of integrin αvβ5 and cytokine-dependent signals. These studies suggest that antagonists of integrin αvβ5 and/or IGF-1 may provide novel strategies for the treatment of metastatic disease.

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