A Peptide Model of Basement Membrane Collagen α1(IV) 531–543 Binds the α3β1 Integrin*

(Received for publication, September 25, 1995)

Andrew J. Miles†‡§, Jennifer R. Knutson‡, Amy P. N. Skubitz‡§, Leo T. Furcht‡§, James B. McCarthy‡§, and Gregg B. Fields‡§**

From the Departments of Laboratory Medicine and Pathology and Biochemistry, and the Biomedical Engineering Center, University of Minnesota, Minneapolis, Minnesota 55455

Tumor cell adhesion to the triple-helical domain of basement membrane (type IV) collagen occurs at several different regions. Cellular recognition of the sequence spanning α1(IV)531–543 has been proposed to be independent of triple-helical conformation (Miles, A. J., Skubitz, A. P. N., Furcht, L. T., and Fields, G. B. (1994) J. Biol. Chem. 269, 30939–30945). In the present study, integrin interactions with a peptide analog of the α1(IV)-531–543 sequence have been analyzed. Tumor cell adhesion (melanoma, ovarian carcinoma) to the α1(IV)531-543 chemically synthesized peptide was inhibited by a monoclonal antibody against the α3 integrin subunit, and to a lesser extent by monoclonal antibodies against the β1 and α5 integrin subunits. An anti-α5 monoclonal antibody and normal mouse IgG were ineffective as inhibitors of tumor cell adhesion to the peptide. Two cell surface proteins of 120 and 150 kDa bound to an α1(IV)531–543 peptide affinity column and were eluted with 20 mM EDTA. When the eluted proteins were incubated with monoclonal antibodies against either the α3 or β1 integrin subunit, proteins corresponding in molecular weight to α3 and β1 integrin subunits were precipitated. No proteins were immunoprecipitated with monoclonal antibodies against the α2 or α5 integrin subunits. Thus, the α3β1 integrin from two tumor cell types has been shown to bind directly to the α1(IV)531–543 peptide. The α1(IV)531–543 peptide is the first collagen-like sequence that has been shown to bind the α3β1 integrin.

Basement membranes are specialized extracellular matrices that delineate tissue boundaries. The metastatic process involves tumor cell interaction with and invasion through the basement membrane. Basement membrane (type IV) collagen can promote the adhesion and migration of diverse cell types including melanoma, corneal epithelial, endothelial, and neural crest cells (Herbst et al., 1988; Chelberg et al., 1989; Etch et al., 1993; Olivo and Furcht, 1993; Perris et al., 1993). Normal and tumorigenic cellular interactions with type IV collagen can be mediated by integrins and/or cell surface proteoglycans (reviewed in Faassen et al., 1992). The αβ2 integrin can bind directly to type IV collagen (Staatz et al., 1989; Vandenberg et al., 1991; Kern et al., 1993; Eble et al., 1993; Carmeliet et al., 1994), while the αβ2 integrin has been implicated in cell migration on type IV collagen (Yoshinaga et al., 1993; Vink et al., 1994; Melchiori et al., 1995). Identification of specific adhesion sites within type IV collagen for the individual integrins has proven difficult, most likely due to the conformational dependence of integrin binding to the collagen triple-helix (Kühn and Eble, 1994). Type IV collagen cyanogen bromide fragment 3 (CB3(IV)), which includes α1(IV) residues 388–551 and α2(IV) residues 407–570, has been reported to contain the individual binding sites for the αβ2 and αβ2 integrins (Vandenberg et al., 1991; Kern et al., 1993). The N-terminal region of a trypsin-derived fragment of CB3(IV), incorporating residues α1(IV)414–452 and α1(IV)432–469, is believed to be a high affinity binding site for the αβ2 integrin from fibrosarcoma cells (Eble et al., 1993). The αβ2 integrin from human fibroblasts and human platelets adheres to a peptide model of α1(IV)430–442 in a Mg2+–dependent fashion (Staatz et al., 1990, 1991). This peptide inhibits platelet and breast adenocarcinoma cell adhesion to type I collagen (Staatz et al., 1991) but does not inhibit αβ2–mediated chondrosarcoma cell adhesion to type II collagen (Tuckwell et al., 1994).

Other collagen-derived sequences may also function as integrin binding sites. Peptide models of the α1(IV)1263–1277 region promote the adhesion, spreading, and migration of highly metastatic tumor cells (Chelberg et al., 1990; Mayo et al., 1991; Fields et al., 1993). A peptide incorporating the α1(IV)531–543 (Gly-Glu-Phe-Tyr-Phe-Asp-Leu-Arg-Leu-Lys-Gly-Asp-Lys-Tyr) sequence promotes keratinocyte, corneal epithelial, melanoma, ovarian carcinoma, and Jurkat cell adhesion (Wilke and Furcht, 1990; Cameron et al., 1991; Miles et al., 1994) and migration of corneal epithelial cells and keratinoctyes (Cameron et al., 1991; Kim et al., 1994). Cellular recognition of the α1(IV)531–543 peptide is, in general, independent of substrate conformation and configuration (chirality) (Miles et al., 1994). Competition studies suggested that the L- and D-peptides incorporating α1(IV)531–543 are bound by the same receptor (Miles et al., 1994). Preliminary results indicated that the β1 integrin subunit was involved in mediating cell adhesion to this sequence (Miles et al., 1994). We have presently examined integrin binding to the α1(IV)531–543 sequence. Since an αβ1 integrin binding site in type IV collagen has already been identified (see above), we have focused on the αβ1 integrins as possible receptors for α1(IV)531–543. In addition, the αβ1 integrin has been considered as a potential receptor for α1(IV)531–543 in this study, as it can mediate cell adhesion to denatured collagen (Gullberg et al., 1992; Tuckwell et al., 1994). We have utilized a number of assays including inhibition of cell adhesion and affinity chromatography of solubilized cells to characterize the integrin(s) involved in cellular recognition of the α1(IV)531–543 sequence. Two different tumor cell lines

* This work was supported in part by National Institutes of Health Grants K02 44494 and AR 01929 (to G. B. F.), CA 21463, CA 29995, and EY 09065 (to L. T. F.), CA 60638 (to A. P. N. S.), and CA 63671 (to J. B. M. and G. B. F.) and by the American Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Allen-Pardue Professor.

‡§ Recipient of a National Institutes of Health research career development award. To whom correspondence should be addressed: Department of Laboratory Medicine and Pathology, Box 107, 420 Delaware St. S.E., University of Minnesota, Minneapolis, MN 55455. Tel.: 612-626-2446; Fax: 612-625-1121.

** Recipient of a National Institutes of Health research career development award. To whom correspondence should be addressed: Department of Laboratory Medicine and Pathology, Box 107, 420 Delaware St. S.E., University of Minnesota, Minneapolis, MN 55455. Tel.: 612-626-2446; Fax: 612-625-1121.

The abbreviations used are: α1(IV)531–543 peptide, Gly-Glu-Phe-Tyr-Phe-Asp-Leu-Arg-Leu-Lys-Gly-Asp-Lys-Tyr; mAb, monoclonal antibody.
have been utilized, melanoma and ovarian carcinoma, since both of these cell types adhere well to type IV collagen and the α1(IV)531–543 peptide (Miles et al., 1994).

**EXPERIMENTAL PROCEDURES**

Materials—All standard peptide synthesis chemicals were analytical reagent grade or better and purchased from Applied Biosystems, Inc. (Foster City, CA) or Fisher. The synthesis, purification, and characterization of the peptide incorporating the α1(IV)531–543 sequence has been described (Miles et al., 1994). Monoclonal antibody (mAb) P502 was prepared against the β1 integrin subunit using methods described previously (Wayner and Carter, 1987). mAbs prepared against the integrin subunits α2 (P1E6), α3 (P1B5), and α5 (P1D6) were purchased from Chemicon International (Temecula, CA). The anti-α3 mAb was obtained as ascites fluid (~2.5 mg of IgG/ml of ascites); all other mAbs were purified IgGs. Purified normal mouse IgG was purchased from Organon Teknika Corp. (Durham, NC).

Cells—A375SM melanoma cells were originally obtained from Dr. I. J. Fidler, M. D. Anderson Cancer Center, Houston, TX, and propagated as described previously (Miles et al., 1994). Briefly, melanoma cells were cultured in Eagle’s minimum essential medium supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 0.1 mg/ml gentamicin (Boehringer Mannheim), 50 units/ml penicillin, 0.05 mg/ml streptomycin, and minimum essential medium vitamin solution. SKOV3 ovarian carcinoma cells were obtained from Dr. Robert C. Bast, Jr., M. D. Anderson Hospital, Houston, TX. Ovarian carcinoma cells were cultured in modified McCoy’s 5A medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 units/ml penicillin, and 0.05 mg/ml streptomycin.

Cells were passaged for 4–5 weeks and then replaced from frozen stocks of early passage cells to minimize phenotypic drift. All cells were maintained at 37°C in a humidified incubator containing 5% CO2. All media reagents were purchased from Sigma except where noted.

Cell Adhesion—Melanoma and ovarian carcinoma cell adhesion and inhibition of cell adhesion assays were performed as described previously (Chelberg et al., 1990; Wilke and Furcht, 1990; Miles et al., 1994) using the α1(IV)531–543 peptide at a substrate concentration of 5.7 μM. For inhibition assays, cells were preincubated for 1 h at 37°C with various concentrations (0.005–5 μM) of the anti-integrin subunit mAb; then the cells (50,000/ml), in the continued presence of the mAb, were added to the Immulon plate wells and allowed to adhere for another 1 h at 37°C.

Affinity Chromatography—The α1(IV)531–543 peptide was coupled to activated CH-Sepharose according to the manufacturer’s instructions (Pharmacia Biotech Inc.). In addition, a mock-coupled column was made without peptide for use as a control. Briefly, 30 mg of high purity liquid chromatography-purified peptide was dissolved in 200 μl of Me2SO and diluted to 5 ml with coupling buffer (15 mM sodium carbonate and 35 mM sodium bicarbonate, pH 8.6). The peptide solution was added to 3 ml of preswollen beads and mixed overnight at 4°C. Unbound peptide was removed by washing the beads with coupling buffer, and the remaining reactive groups were hydrolyzed at pH 8.0 with 0.1 M Tris-HCl for 2 h. Cells were surface labeled with [35S]methionine as described (Gehlsen et al., 1992), extracted in buffer (50 mM Tris-HCl, pH 7.4, 50 mM 2-mercaptoethanol (10%) was added to some samples (see “Results”), followed by heating at 100°C for 5 min to reduce disulfide bonds. Elution fractions were electrophoresed by 7.5% SDS-polyacrylamide gel electrophoresis and analyzed by autoradiography (Gehlsen et al., 1992). Molecular mass standards (Sigma) were rabbit muscle myosin (205 kDa), Escherichia coli β-galactosidase (116 kDa), rabbit muscle phosphorylase b (97.4 kDa), bovine albumin (66 kDa), and chicken egg albumin (43 kDa).

**RESULTS**

We had shown previously that 40–60% maximum cell adhesion is achieved with 5–10 μM amounts of a peptide analog of the α1(IV)531–543 sequence (Miles et al., 1994). Inhibition of cell adhesion studies were thus performed at a peptide concentration of 5.7 μM. Ovarian carcinoma cell adhesion to the peptide could be inhibited in a dose-dependent manner by mAbs against β1, α2, or α3 integrin subunits, with maximum inhibition occurring at the highest mAb concentration tested (5 μg/ml) (Fig. 1A). The mAb against the α3 subunit was the most effective, producing ~60% inhibition of adhesion at a mAb concentration as low as 2.5 μg/ml (data not shown). A similar level of inhibition (~50%) was achieved by the anti-β1 subunit mAb at a concentration of 5 μg/ml (Fig. 1A). The anti-α2 subunit mAb was less effective as an inhibitor, causing only ~35% inhibition at 5 μg/ml (Fig. 1A). A mAb against the α3 integrin subunit and normal mouse IgG did not give dose-dependent inhibition of cell adhesion, even up to a concentration of 5 μg/ml (Fig. 1A). In a similar fashion, melanoma cell adhesion to the peptide could be inhibited in a dose-dependent manner by mAbs against the β1, α2, or α3 integrin subunits, with maximum inhibition occurring at a mAb concentration of 5 μg/ml (Fig. 1B). The anti-α3 subunit mAb was the most effective inhibitor, causing ~60% inhibition. A mAb against the α3 integrin subunit and normal mouse IgG did not give dose-dependent inhibition of melanoma cell adhesion up to a concentration of 5 μg/ml (Fig. 1B).

The α1(IV)531–543 peptide was immobilized to CH-Sepharose, and affinity chromatography was performed with an 125I-labeled extract of the ovarian carcinoma cells. Following application of cells, the column was first washed with extraction buffer, then eluted by 20 ml EDTA. Eluants were incubated with 5 μg/ml mAbs against α2, α3, α5, or β3 integrin subunits. Precipitated proteins were then analyzed by 7.5% SDS-polyacrylamide gel electrophoresis with detection by autoradiography. Immunoprecipitation of the EDTA eluant with the anti-β1 integrin subunit mAb resulted in detection of a 135-kDa protein under reducing conditions (Fig. 2). Immunoprecipitation of the same eluant with the anti-α3 integrin subunit mAb, followed by reduction with 2-mercaptoethanol, resulted in detec-
tion of a 135-kDa protein (Fig. 2). In other studies, the $\beta_1$ and $\alpha_3$ integrin subunits have been shown to have similar apparent molecular weights under reducing conditions (Gehlsen et al., 1992; Sonnenberg, 1993). No proteins were seen following incubation of the EDTA eluant with anti-$\alpha_2$ integrin subunit mAb or normal mouse IgG (Fig. 2). When the immunoprecipitants were not reduced, two proteins of 150 and 120 kDa were immunoprecipitated from the EDTA eluant by the anti-$\beta_1$ integrin subunit mAb (Fig. 3). The molecular weights correspond to the $\alpha_3$ and $\beta_1$ integrin subunits, respectively (Sonnenberg, 1993). In similar fashion, two proteins of 150 and 120 kDa were immunoprecipitated from the EDTA eluant by the anti-$\alpha_3$ integrin subunit mAb (Fig. 3). No proteins were seen using normal mouse IgG or mAbs against the $\alpha_2$ or $\alpha_3$ integrin subunits (Fig. 3).

Affinity chromatography and immunoprecipitation experiments were repeated using $^{125}$I-labeled melanoma cells. Incubation of the EDTA eluant with the anti-$\alpha_3$ integrin subunit mAb resulted in immunoprecipitation of a 135-kDa protein under reducing conditions (data not shown). Similarly, incubation of the eluant with the anti-$\beta_1$ integrin subunit mAb resulted in immunoprecipitation of a 135-kDa protein under reducing conditions (data not shown). Under non-reducing conditions, two proteins of 150 and 120 kDa were immunoprecipitated from the EDTA eluant by the anti-$\alpha_3$ integrin subunit mAb (Fig. 4). The apparent molecular weights correspond to the $\alpha_3$ and $\beta_1$ integrin subunits, respectively (Sonnenberg, 1993). No proteins were seen using normal mouse IgG or mAbs against the $\alpha_2$ or $\alpha_3$ integrin subunits (data not shown).

**DISCUSSION**

In an attempt to further refine our knowledge of cellular receptors for type IV collagen, melanoma and ovarian carcinoma cell adhesion to the $\alpha 1(IV)531-543$ peptide was examined in the presence of anti-integrin subunit mAbs. For both cell types, adhesion to this peptide was most effectively inhibited by the anti-$\alpha_3$ integrin subunit mAb, followed by the anti-$\beta_2$ and anti-$\alpha_2$ integrin subunit mAbs. Cell surface proteins with molecular masses of 120 and 150 kDa bound to a $\alpha 1(IV)531-543$ peptide affinity column in an EDTA-dependent fashion. These proteins could be immunoprecipitated with mAbs against either the $\alpha_2$ or $\beta_1$ integrin subunit, and the protein molecular weights corresponded to the $\alpha_3$ and $\beta_1$ integrin subunits. Thus, it would appear that the $\alpha_2\beta_1$ integrin binds directly to the $\alpha 1(IV)531-543$ peptide.

The $\alpha 1(IV)531-543$ peptide is the first collagen-like sequence identified as a specific $\alpha_2\beta_1$ integrin/ligand binding site. Two different tumor cell types, melanoma and ovarian carcinoma, bind this site via the $\alpha_2\beta_1$ integrin. At present, it is unclear as to what role this integrin plays during tumor cell invasion, where extravasating cells have contact with type IV collagen. The level of $\alpha_2\beta_1$ expression varies amongst tumor cell types. Metastatic melanoma cells up-regulate the $\alpha_2\beta_1$ integrin compared with primary melanoma cells (Yoshinaga et al., 1993), while highly invasive prostate carcinoma cells have decreased expression of the $\alpha_3\beta_1$ integrin compared to the parental cell line (Dedhar et al., 1993). Transformed fibroblasts retain the same level of $\alpha_2\beta_1$ as non-transformed cells while decreasing levels of other integrins (Plantefaber and Hynes, 1989).

The level of $\alpha_2\beta_1$ integrin expression may correlate to the utility of this integrin for cell migration. mAbs to the $\alpha_3$ subunit inhibit melanocyte and melanoma cell motility (Morcelli et al., 1993; Yoshinaga et al., 1993; Melchiori et al., 1995) and dysplastic nevus cell spreading and migration (Vink et al., 1994) on type IV collagen. Tumorigenic cell types such as metastatic melanoma cells may have increased levels of $\alpha_2\beta_3$, which enhances motility on the basement membrane or basement membrane molecules. Other cell types may use different receptors for migration. For example, keratinocytes use $\alpha 2\beta_3$ integrins for migration on type IV collagen (Chen et al., 1993; Kim et al., 1994). There are suggestions that cellular interactions with the basement membrane via $\alpha_2\beta_3$ integrins may also lead to basement membrane degradation. Antibodies to $\alpha_3\beta_1$ integrin stimulate the expression of matrix metalloproteinase-9 (92 kDa type IV collagenase) (Larjava et al., 1993). Matrix metalloproteinase-9 can be induced in transformed cells (Wilhelm et al., 1989) or by direct contact with tumorigenic cells (Himmelstein et al., 1994) and has been localized to the invasion front of oral squamous cell carcinoma (Kawahara et al., 1993). Matrix metalloproteinase-9 efficiently degrades type IV collagen (Morodomi et al., 1992).

The $\alpha_2\beta_1$ integrin may have a role in cellular recognition of the $\alpha 1(IV)531-543$ sequence based on the inhibition of cell adhesion assays. $\alpha_2\beta_1$ integrin binding to the anti-$\alpha_2$ integrin
subunit mAb used in this study (P1E6) does not result in signal transduction (Kapron-Bras et al., 1993); thus, the function of the αβ3 integrin is probably not indirectly altered by α2β1 interaction with the mAbs used here. More likely, the α2β1 integrin has lower affinity for the α1(IV)531–543 peptide than αβ3. An α2β1 binding site is found within α1(IV)453–551 (Vandenbergh et al., 1991; Eble et al., 1993). The overlap between the α2β1 and the sequence examined in this study (α1(IV) residues 531–543) may be part of the α2β1 binding site. There is also a greater expression of the αβ3 integrin than the α2β1 integrin for the ovarian carcinoma and melanoma cell types studied here, possibly contributing to more αβ3 receptor-ligand interactions and a more avid binding.
A Peptide Model of Basement Membrane Collagen α 1(IV) 531- 543 Binds the α3β1 Integrin
Andrew J. Miles, Jennifer R. Knutson, Amy P. N. Skubitz, Leo T. Furcht, James B. McCarthy and Gregg B. Fields

J. Biol. Chem. 1995, 270:29047-29050.
doi: 10.1074/jbc.270.49.29047

Access the most updated version of this article at http://www.jbc.org/content/270/49/29047

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 47 references, 26 of which can be accessed free at http://www.jbc.org/content/270/49/29047.full.html#ref-list-1