Synthetic Peptides from the Carboxy-terminal Globular Domain of the A Chain of Laminin: Their Ability to Promote Cell Adhesion and Neurite Outgrowth, and Interact with Heparin and the β1 Integrin Subunit

Amy P. N. Skubitz,*‡ Paul C. Letourneau,§ Elizabeth Wayner,*‡ and Leo T. Furcht*‡

Departments of *Laboratory Medicine and Pathology, ‡Biomedical Engineering Center, and §Cell Biology and Neuroanatomy, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Abstract. The large carboxy-terminal globular domain (G domain; residues 2,110–3,060) of the A chain of murine-derived laminin has been shown to promote heparin binding, cell adhesion, and neurite outgrowth. This study was conducted to define the potential sequence(s) originating from the G domain of laminin with any of these functional activities. A series of peptides were synthesized from the G domain, termed GD peptides, each ~20 amino acids long and containing multiple positively charged amino acids. In direct ^{3}H-heparin binding assays, peptides GD-1 and GD-2 bound high levels of ^{3}H-heparin, while peptides GD-3 and GD-4 bound lower levels of ^{3}H-heparin, and GD-5 bound essentially no ^{3}H-heparin. The binding of ^{3}H-heparin to peptides GD-1 and GD-2 appeared to be of high affinity, since significant binding of ^{3}H-heparin to these two peptides was still observed even when the NaCl concentration was raised to 1.0 M. Four of the peptides, GD-1, GD-2, GD-3, and GD-4, directly promoted the adhesion and spreading of HT-1080 human fibrosarcoma cells as well as the outgrowth of neurites from chick spinal cord and dorsal root ganglia neurons. In addition, solutions of these peptides or antibodies generated against these peptides inhibited laminin-mediated HT-1080 cell adhesion. Antibodies against the β1 integrin subunit inhibited HT-1080 cell adhesion and neurite outgrowth on surfaces adsorbed with peptides GD-3 and GD-4. Therefore, laminin appears to have multiple, independent sequences in the G domain that serve a similar cell adhesion promoting function for different cell types. Furthermore, these results suggest that the sequences comprising peptides GD-3 and GD-4 use an integrin as a receptor, of which the β1 integrin subunit is a component for these various cell types.

THE basement membrane glycoprotein laminin isolated from the murine Engelbreth-Holm-Swarm (EHS) tumor consists of one 400-kD A chain and two B chains, each of ~200 kD. Laminin promotes the adhesion and spreading of a variety of cells and binds many different types of molecules, such as glycosaminoglycans or proteoglycans. Studies using enzymatic digests of laminin or monoclonal antibodies raised against laminin have defined some of the biologically active regions of the 400-kD A chain of murine laminin. The large carboxy-terminal globular domain (G domain) on the long arm of the A chain of laminin is reported to be the major heparin binding domain of laminin. In early studies, enzymatic digests of laminin were passed over heparin-affinity columns and proteolytic fragments containing the G domain were found to adhere (Ott et al., 1982). Later studies using monoclonal antibodies in combination with heparin-affinity chromatography and a solid-phase radioligand binding assay showed that the G domain, also termed the Hep-1 domain, in addition to two domains from other regions of laminin, termed Hep-2 and Hep-3, was important for heparin binding (Skubitz et al., 1988). More recently, electron microscopic examination of laminin–heparin interactions have provided further evidence for the binding of heparin to the G domain of laminin (Kouzi-Koliakos et al., 1989).

Other biological activities attributed to the A chain of laminin include promotion of neurite outgrowth from neurons and cell adhesion, which is localized to part of the G domain and the region directly above the G domain, termed fragment E8 (Edgar et al., 1984, 1988; Engvall et al., 1986; Goodman et al., 1987; Deutzmann et al., 1990). In addition, the amino-terminal globular domain on the "short" arm at the top of the molecule is involved in laminin–laminin self-assembly (Yurchenco et al., 1985; Schittny and Yurchenco, 1990) and the adhesion of hepatocytes (Timpl et al., 1983).

Over the past few years, the amino acid sequences of the B1, B2, and A chains of laminin have been determined (Barlow et al., 1984; Sasaki et al., 1987, 1988; Sasaki and Yamada, 1987; Pikkarainen et al., 1987, 1988; Hartl et al.,

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1988); this has permitted the synthesis of various peptides from domains of laminin that could then be screened for specific functional activities present within the intact molecule. Four peptides from the B1 chain of laminin have been reported to promote cell adhesion: YIGSR, located near the intersection of the cross (Graf et al., 1987*a*,*b*; Clément et al., 1990); peptide F-9 (RYVVLPRPVCFEKGMNYTVR) located on the inner globule of the lateral short arm (Charonis et al., 1988) which contains the active 7′ mer RYVVLPR (Skubitz et al., 1990); peptide AC15 (RIQNLLKITNLRIKFVK) derived from the outer globule of the lateral short arm (Kouzi-Koliakos et al., 1989); and peptide PDSGR near the cross region (Kleinman et al., 1989). Only one synthetic peptide has been reported from the B2 chain of laminin to have functional activity (Liesi et al., 1989). This peptide, P20 (RNIAEIIKDI), stimulates neurite outgrowth of both central and peripheral neurons (Liesi et al., 1989).

Since the A chain was the most recent chain of laminin for which the entire amino acid sequence was predicted, only a few functional activities have been ascribed to synthetic peptides. Synthetic peptide PA 21 (residues 1,115–1,129; CQAGTFALRGDNPQG), which contains the active sequence RGD, promotes cell attachment (Clément et al., 1990; Tashiro et al., 1991) and induces the attachment of human endothelial cells through an integrin receptor (Grant et al., 1989). Peptide PA22-2 (residues 2,091–2,108; SRARKQAASIKVAVSADR), which has been reported to contain the active sequence IKVAV (Tashiro et al., 1989) promotes hepatocyte attachment (Clément et al., 1990), neuronal process extension (Tashiro et al., 1989; Sephel et al., 1989), and increases experimental metastasis of the lungs by increasing collagenase IV activity when murine melanoma cells are preincubated with the peptide (Kanemoto et al., 1990).

To our knowledge, however, there have been no studies that have reported on the biological activity of synthetic peptides or expressed fragments from the G domain of laminin, which is the major heparin binding site on laminin that also promotes cell adhesion. In this study, we have synthesized peptides, each of ~20 amino acid residues in length, from the published amino acid sequence of the G domain of the A chain of murine EHS tumor-derived laminin (Sasaki et al., 1988). Sequences from this domain were selected for synthesis based on their relatively low hydropathy indexes (indicative of hydrophilic regions) and relatively high density of positively charged amino acid residues (arginines and lysines). In addition, the peptides, termed GD-1 through GD-5, were selected from regions at the ends of the five loops of the A chain with the thought that these domains would potentially be more accessible for the binding of cells, proteoglycans, and/or glycosaminoglycans such as heparin-like molecules. The synthetic peptides were screened for their ability to bind ^{3}H-heparin and/or promote the adhesion and/or spreading of HT-1080 human fibrosarcoma cells. The peptides and antibodies raised against the peptides were also assayed for their ability to inhibit laminin-mediated HT-1080 cell adhesion. Furthermore, these peptides were tested for their ability to promote the outgrowth of neurites from chick spinal cord (SC)[1] neurons as well as dorsal root ganglia (DRG) neurons. Antibodies against the β1 integrin subunit were then used to inhibit the adhesion of HT-1080 cells and the outgrowth of neurites from DRG neurons on surfaces adsorbed with the peptides.

1. *Abbreviations used in this paper*: DRG, dorsal root ganglia; OVA, ovalbumin; RLBA, radioligand binding assay; SC, spinal cord.

Materials and Methods

Proteins and Synthetic Peptides

Laminin was isolated from the EHS tumor as described previously (Palm and Furcht, 1983) and fibronectin was isolated from human plasma as described previously (McCarthy et al., 1986). The purity of both proteins was verified by SDS-PAGE and ELISA. BSA (grade V, fatty acid free) and ovalbumin (OVA) were purchased from Sigma Chemical Co., St. Louis, MO.

The five peptides listed in Table I, GD-1 to GD-5, were derived from the G domain (residues 2,110–3,060) of the A chain of EHS laminin. The amino acid residues of the four "active" peptides, GD-1 to GD-4, were randomly scrambled (Table I) in order to ensure that the primary amino acid sequences were essential for the functional activity of these peptides and that biological activity was not merely due to the peptides' net charge or amino acid composition. Peptides were synthesized by Dr. Bianca Conti-Tronconi (University of Minnesota, St. Paul, Minnesota) or the Microchemical Facility at the University of Minnesota, then purified as previously described (Charonis et al., 1988). Purity was checked by HPLC and amino acid analysis.

Quantitation of Peptides Adsorbing to Surfaces

Each of the peptides was synthesized with a tyrosine residue at the carboxy terminus and purified by HPLC. The peptides were then radiolabeled with ^{125}I as previously described (Chelberg et al., 1990). The amount of each peptide adsorbed to the wells was quantitated as previously described (Skubitz et al., 1990). Briefly, 50 μl of radiolabeled peptides diluted in PBS to concentrations ranging from 2 to 200 μg/ml were added to 96-well polystyrene Immulon 1 microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA) and incubated overnight at 29°C. The next day, wells were treated for 2 h with 2 mg/ml BSA in DME containing 20 mM Hepes, pH 7.4, then washed three times with this buffer. The radioactivity was removed and quantitated in a gamma counter.

Solid-phase Radioimmunoassays

The binding of ^{3}H-heparin to synthetic peptides and OVA was quantitated by a solid-phase radioligand binding assay (RLBA) in microtiter plates as previously described (Skubitz et al., 1988) with minor modifications. Briefly, 50 μl of the peptides at various concentrations in PBS containing 0.02% NaN_3 were added to each well and adsorbed overnight at 29°C. The next day, 200 μl of RLBA buffer (2 mg/ml OVA in 0.02 M Tris, pH 6.8, 0.15 M NaCl) was added to each well followed by a 90-min incubation at 37°C. After removal of this buffer, 50 μl of ^{3}H-heparin (100,000 dpm; 0.3 mCi/mg; Du Pont-New England Nuclear Research Products, Boston, MA) in RLBA buffer was added to each well and the plates were incubated at 37°C for 2 h. Unbound ^{3}H-heparin was removed by washing three times with wash buffer (RLBA buffer containing 0.1% CHAPS). Tritiated heparin was solubilized by incubation with 200 μl of 0.05 N NaOH and 1% SDS for 30 min at 60°C and quantitated in a scintillation counter (model LS-3801; Beckman Instruments, Inc., Palo Alto, CA). All experiments were repeated a minimum of three times and done in quadruplicate. Other experiments were similarly conducted in which various concentrations of NaCl (0.05–2.0 M) were present in the RLBA buffer.

Antibodies

Synthetic peptides were coupled to the carrier protein keyhole limpet hemocyanin (KLH) as previously described (Skubitz et al., 1990). Briefly, peptides were conjugated to KLH by use of 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (Sigma Chemical Co.), then dialyzed into PBS, and stored at −70°C for later use. Polyclonal antibodies were generated against the synthetic peptides or the synthetic peptides coupled to KLH as previously described (Skubitz et al., 1990). Briefly, rabbits were immunized with peptides or peptides/KLH, sera was tested for specificity by ELISA, then IgG was purified by ammonium sulfate precipitation and DEAE column chromatography. Purity of IgG was assessed by SDS-PAGE and ELISA.

Monoclonal antibody P4C10 was generated in mice against the human β1 integrin subunit as described (Carter et al., 1990). Monoclonal antibody

Table I. Laminin A Chain Peptides Synthesized

Peptide	Sequence*	Sequence numbers*	Net charge‡	Hydropathy index§
GD-1	KATPMLKMRTSFHGCIK	2,615-2,631	+5	−5.1
GD-2	KEGYKVRLDLNITLEFRTTSK	2,890-2,910	+2	−10.1
GD-3	KNLEISRSTFDLLRNSYGVRK	2,443-2,463	+3	−8.6
GD-4	DGKWHTVKTEYIKRKAF	2,779-2,795	+4	−17.8
GD-5	TSLRKALLHAPTGSYSDGQ	2,547-2,565	+2	−11.8
GD-1"S"	LGHMITFKKPSKTMRAC	Scrambled	+5	
GD-2"S"	LGIKTKSRKDLEFTLVNTREY	Scrambled	+2	
GD-3"S"	SRLKNVDLGRTFRSENSLIKY	Scrambled	+3	
GD-4"S"	VKTHRWFKGAEDIKTKY	Scrambled	+4	

* Based on Sasaki et al., 1988. (*G*, glycine; *A*, alanine; *V*, valine; *L*, leucine; *I*, isoleucine; *F*, phenylalanine; *Y*, tyrosine; *W*, tryptophan; *M*, methionine; *C*, cysteine; *S*, serine; *T*, threonine; *H*, histidine; *K*, lysine; *R*, arginine; *D*, aspartate; *E*, glutamate; *N*, asparagine; *Q*, glutamine; *P*, proline.)
‡ Calculated by assuming a +1 net charge for lysine (*K*) and arginine (*R*) residues and a −1 net charge for glutamic acid (*E*) and aspartic acid (*D*) at neutral pH. Histidine is assumed to be uncharged at this pH.
§ Calculated by the method of Kyte and Doolittle (1982). According to this method, more hydrophobic peptides correspond to the more positive numerical values.

WIB10, generated in mice against the chicken β1 integrin subunit, was provided as purified IgG as a generous gift from Dr. Rick Horwitz (University of Illinois, Champaign-Urbana, IL).

Cells

The HT-1080 human fibrosarcoma cell line was purchased from the American Type Culture Collection (Rockville, MD) and was maintained in MEM with nonessential amino acids (Gibco Laboratories, Grand Island, NY) containing 10% FBS. PC12 rat pheochromocytoma cells were kindly provided by Dr. Lloyd Greene (New York University, NY) and were maintained in DME containing 10% FBS and 5% horse sera. Cells were passaged for 4–5 wk 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% CO_2.

DRG from embryonic day 12 (E12) or SC from E8 White Leghorn chicks were dissected, and the cells dissociated by a 20-min incubation in 0.25% trypsin followed by gentle trituration. Cells were washed twice to inactivate the trypsin in F14 medium (Gibco Laboratories) buffered with bicarbonate, containing 10% calf sera, 5 μg/ml insulin, 5 mM phosphocreatine, 5 ng/ml sodium selenite, 0.1 mg/ml transferrin, 0.4 mg/ml sodium pyruvate, and 20 nM progesterone (all purchased from Sigma Chemical Co.), and 15 ng/ml β-NGF, a generous gift of Dr. Bernard Mirkin, University of Minnesota. The neuronal cells were then resuspended in serum-free media for use in neurite outgrowth assays described below.

Cell Adhesion Assays

The direct adhesion of HT-1080 or PC12 cells to surfaces adsorbed with peptides or proteins was performed as described previously (Skubitz et al., 1987; Charonis et al., 1988). Briefly, HT-1080 cells were radiolabeled overnight in MEM containing 10% FBS plus 3 μCi/ml of [^{3}H]thymidine (6.7 Ci/mmol, New England Nuclear), whereas PC12 cells were radiolabeled overnight in DME containing 10% FBS, 5% horse sera, and 71 μCi/ml of Tran ^{35}S-label (70% [^{35}S]-L-methionine; 1,024 Ci/mmol; ICN Biomedicals, Inc., Irvine, CA). The next day, radiolabeled HT-1080 cells were removed from the flasks by gentle trypsinization, while HBSS was used to remove the PC12 cells from the flasks as previously described (Charonis et al., 1988). Radiolabeled cells were then added to 96-well microtiter plates adsorbed with various concentrations of synthetic peptides, laminin, OVA, or poly-L-lysine. After a 2-h incubation, loosely or nonadherent cells were removed by washing the wells three times. Adherent cells were solubilized and quantitated in a scintillation counter. Experiments were done in quadruplicate and repeated three times.

Quantitation of HT-1080 Cell Spreading

Spreading of the adherent HT-1080 cells was evaluated by performing adhesion assays similar to those described above, except instead of solubilizing the adherent cells, they were fixed with 2% glutaraldehyde in PBS at 23°C for 1 h. The glutaraldehyde was then removed and the cells were stained with Coomassie brilliant blue R (Sigma Chemical Co.) as previously described (Skubitz et al., 1990). Cell spreading was quantitated by measuring the average surface area occupied by a cell using an Opto-Max Image Analyzer. In addition, the number of cells that spread on each surface was determined by visually monitoring adherent cells in each well and determining the percent of those cells that had spread. 150 cells were visualized for spreading on each of the different surfaces. Each experiment was done in quadruplicate and repeated three times.

Inhibition of Laminin-mediated HT-1080 Cell Adhesion

Inhibition of HT-1080 cell adhesion to surfaces coated with laminin by soluble synthetic peptides or specific antibodies was performed as previously described (Skubitz et al., 1990). Briefly, radiolabeled cells at 5×10^4/ml were incubated for 30 min with various concentrations of synthetic peptides in DME/Hepes containing 2 mg/ml BSA. 100 μl of the cell suspension was then added to wells precoated with 1.5 μg of laminin or 1.0 μg of fibronectin. The cells were incubated in the wells for 20 min at 37°C, then the wells were washed, and adherent cells were quantitated. Cell viability after a 1-h incubation in the presence of the peptide "inhibitors" was assessed by trypan blue dye exclusion. In all cases, the cells were >95% viable, and no toxicity of the peptides was observed at the indicated concentrations tested. All experiments were repeated at least three times in quadruplicate.

In similar assays, 100 μl of DME/Hepes containing 2 mg/ml BSA and 100 μg/ml of purified IgG from normal rabbits or from rabbits immunized with the synthetic peptides was added to wells that were precoated in quadruplicate with 0.3 μg of laminin or fibronectin. The IgG was incubated for 1 h at 37°C in the wells, then radiolabeled HT-1080 cells were added to the wells and incubated for 30 min at 37°C. Wells were then washed and cells were quantitated.

Neurite Outgrowth Promoted by Peptides

Chick neurons were resuspended in serum-free media at 40,000 cells/ml (DRG) or 60,000 cells/ml (SC). 100 μl were added to each well of a microtiter plate precoated with various concentrations of the synthetic peptides, laminin, or OVA as described above for the cell adhesion assays. Neuronal cells were allowed to extend neurites for 24 h at 37°C, in 5% CO_2. Adherent cells were fixed with 2% glutaraldehyde for 20–40 min, washed, and counted using phase-contrast microscopy with a 10× objective on a Nikon inverted microscope. Cells were counted in 1 diameter across each well, 3.5 field of views (~20% of the total area of each well). Neurons were identified according to morphology as having round, phase-bright cell bodies, and scored as having neurites if there was at least one process longer than the cell diameter. All experiments were repeated at least two times in triplicate. Photographs of neurite outgrowth were taken on a Nikon Diaphot microscope using Kodak Pan-X film.

Inhibition of Peptide-mediated Cell Adhesion by an Antibody against the β1 Integrin Subunit

Inhibition of HT-1080 cell adhesion to surfaces adsorbed with laminin or the peptides by an antibody against the β1 integrin subunit was performed essentially as described above for the inhibition assays using the peptides as inhibitors of cell adhesion. Briefly, radiolabeled human HT-1080 cells

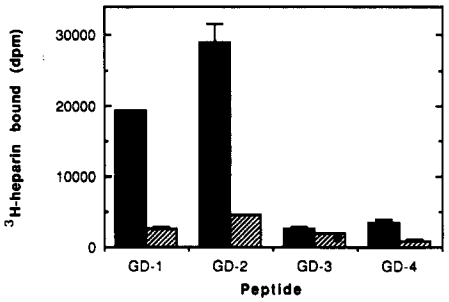

Figure 1. Binding of ^{3}H-heparin to "native" or "scrambled" peptides. ^{3}H-heparin (100,000 dpm in 20 mM Tris, pH 6.8, containing 2 mg/ml OVA and 0.15 M NaCl) was added to wells adsorbed with 3 μg of native peptides (*solid bars*) or scrambled peptides (*hatched bars*) and incubated for 2 h at 37°C. Each value represents the mean of four separate determinations ± SD. Duplicate experiments gave similar results.

were incubated for 20 min at 37°C in DME/Hepes containing 2 mg/ml BSA and various dilutions of supernatant from monoclonal antibody P4C10 against the human β1 integrin subunit or control supernatant. Cells were then added to wells preadsorbed with 0.7 μg of laminin or the peptides. All experiments were repeated at least four times in quadruplicate.

Inhibition of Peptide-mediated Neurite Outgrowth by an Antibody against the β1 Integrin Subunit

Microtiter wells were adsorbed with 1 μg of the peptides or laminin. Chick DRG neurons were cultured for 24 h in media containing 10 μg/ml of purified normal mouse IgG or mouse monoclonal antibody W1B10 IgG, which recognizes the chicken β1 integrin subunit. Neurons were then added, in the continued presence of the IgG, to the microtiter wells and cultured for 24 h. Neurite outgrowth was quantitated by counting fixed cells, as described above. All experiments were done in triplicate and repeated at least two times.

Results

Efficiency of Peptide Adsorption to Surfaces

To ensure that the peptides would adsorb to the polystyrene microtiter wells, peptides were radiolabeled with ^{125}I and the amount of each peptide bound to the wells was quantitated. Equivalent levels of adsorption were observed for all five of the peptides when they were added in the range of 0.1–10 μg per well (data not shown).

^{3}H-Heparin Binding to Laminin A Chain Peptides

The binding of ^{3}H-heparin to laminin A chain peptides was measured in a direct solid phase binding assay whereby various amounts of the peptides were adsorbed to plates and ^{3}H-heparin was added to each well. Peptides GD-1 and GD-2 bound the most ^{3}H-heparin (Fig. 1) in a concentration dependent manner (data not shown) with a maximum of ~30,000 dpm of the input ^{3}H-heparin binding to peptide GD-2 at a coating concentration of 3 μg/well. Significantly lower levels of ^{3}H-heparin bound to peptides GD-3 and GD-4; maximal levels of only ~5,000 dpm of ^{3}H-heparin bound (Fig. 1). Less than ~500 dpm of the input ^{3}H-heparin bound to peptide GD-5 even at the highest coating concentration of 5 μg peptide/well (data not shown). Interestingly, no correlation was observed between the ability of the peptide to bind ^{3}H-heparin and its net charge or hydropathy index. For example, peptides GD-2 and GD-3 both have a positive net charge (+2 and +3, respectively) and similar negative hydropathy indexes (−10.1 and −8.6, respectively; indicating relative hydrophilicity), however at a coating concentration of 3 μg/well, peptide GD-2 bound ~30,000 dpm of the input ^{3}H-heparin while peptide GD-3 bound less than ~5,000 dpm. As expected, based on the multiple heparin-binding domains on the intact laminin molecule, the binding of ^{3}H-heparin to peptides GD-1 and GD-2 was less than the binding of ^{3}H-heparin to laminin on a molar basis, i.e., 1/40th the amount of ^{3}H-heparin bound to these peptides compared to laminin on a molar basis. Also, as expected, solutions of these peptides were not able to inhibit ^{3}H-heparin binding to laminin (data not shown). This is most likely attributed to the multiplicity of heparin-binding sites on laminin (Skubitz et al., 1988), such that one peptide cannot compete for the multiple heparin-binding sites on laminin.

As further evidence that the primary sequence of amino acid residues comprising each synthetic peptide was critical for its ^{3}H-heparin binding activity, we randomly "scrambled" the amino acid residues and synthesized a series of "scrambled" peptides, GD-1 "S" through GD-4 "S" (Table I). In all cases, the "native" peptides bound ^{3}H-heparin at levels significantly higher than the respective "scrambled" peptides (Fig. 1).

Effect of Salt Concentration on ^{3}H-Heparin Binding to Peptides

To determine the relative affinity of the peptides for heparin, we performed ^{3}H-heparin binding assays in which increasing concentrations of NaCl were present in the incubation buffer. ^{3}H-heparin binding to peptides GD-1 to GD-4 decreased as the salt concentration increased. Significant binding of ^{3}H-heparin to peptides GD-1 and GD-2 above background levels was still observed in 1.0 M NaCl, suggesting that the affinity of binding is quite high relative to peptides GD-3, GD-4, and GD-5 (data not shown).

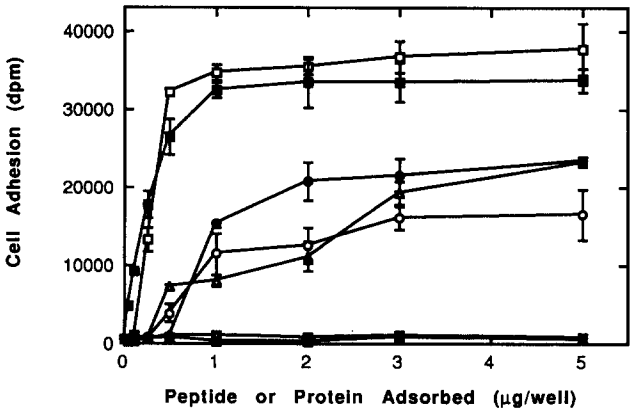

Figure 2. Adhesion of HT-1080 human fibrosarcoma cells to laminin A chain peptides. Microtiter wells were adsorbed with increasing concentrations of laminin (●), or peptides GD-1 (□), GD-2 (■), GD-3 (△), GD-4 (○), GD-5 (◇), or OVA (▲), and radiolabeled HT-1080 human fibrosarcoma cells were allowed to adhere for 2 h as described in Materials and Methods. Each value represents the mean of four separate determinations ± SD. Three separate experiments gave similar results.

Table II. Quantitation of HT-1080 Human Fibrosarcoma Cell Spreading on Surfaces Adsorbed with Laminin A Chain Peptides

Peptide or protein*	Surface area occupied by a cell	Cells spread‡
	μm^2	%
Peptide GD-1	396 ± 32	83
Peptide GD-2	388 ± 29	85
Peptide GD-3	360 ± 27	12
Peptide GD-4	372 ± 33	15
Peptide GD-5	59 ± 4	0
Laminin	610 ± 62	98
BSA	63 ± 4	0
Poly L-lysine	119 ± 32	5

* 50 μl of each peptide or protein at 50 μg/ml was adsorbed to the wells of microtiter plates, cells were incubated for 2 h, then cell spreading was quantitated as described in Materials and Methods.
‡ Cell spreading is quantitated as a percentage of the number of adherent cells that spread.

Cell Adhesion to Peptides

We were next interested in determining whether there was a relationship between the ability of peptides to bind heparin and their ability to promote cell adhesion. In this study, radiolabeled HT-1080 fibrosarcoma cells were incubated in microtiter plates adsorbed with increasing concentrations of laminin or the peptides (Fig. 2). The cells adhered to laminin and peptides GD-1, GD-2, GD-3, and GD-4 in a concentration-dependent manner. Coating concentrations as low as 0.5 μg/well of each of these four peptides promoted cell adhesion above background levels (cell adhesion on surfaces adsorbed with OVA). Maximal adhesion of the added cells (~100%) occurred at coating concentrations of 3 μg/well of peptide GD-1. Interestingly, the two peptides that bound the most ^{3}H-heparin, GD-1 and GD-2, promoted the highest levels of cell adhesion. Peptides GD-3 and GD-4 were also capable of promoting cell adhesion (albeit at a lower level, Fig. 2); these peptides had shown limited ability to bind ^{3}H-heparin (Fig. 1). Peptide GD-5, however, promoted neither cell adhesion nor bound ^{3}H-heparin. Using various coating concentrations, the following was observed: 2 μg of laminin (i.e., 2.3 pmol) promoted the adhesion of HT-1080 cells to a level comparable to 0.3 μg of peptides GD-1 or GD-2 (i.e., 150 pmol) or 5 μg of peptides GD-3 or GD-4 (i.e., 2,500 pmol). These results suggest that peptides GD-1 and GD-2 are an order of magnitude more active than peptides GD-3 and GD-4 at promoting the adhesion of HT-1080 cells under these experimental conditions. One possible explanation as to why fewer cells adhered to laminin compared to peptides GD-1 and GD-2 is that less laminin is present in the microtiter wells on a molar basis compared to the amount of peptides present in the wells. Therefore, since comparatively less laminin is present in the wells, fewer functionally active sites are available to which the cells can adhere.

Cell Spreading on Laminin Synthetic Peptides

Most of the HT-1080 fibrosarcoma cells that were incubated for 2 h on polystyrene plates adsorbed with laminin spread out and became elongated. The spreading of these cells was quantitated by measuring the mean surface area occupied by the cells. 98% of the cells that adhered to surfaces adsorbed with laminin spread out to cover an average surface area of 610 μm^2 (Table II). On surfaces adsorbed with the synthetic peptides GD-1 and GD-2, ~85% of the adherent cells spread, while on surfaces adsorbed with the synthetic peptides GD-3 and GD-4 only ~15% of the adherent cells spread. The appearance of the cells that adhered to surfaces adsorbed with the peptides was different than those cells present on surfaces adsorbed with laminin since they were more flattened and had numerous extended processes, frequently resulting in a stellate appearance (not shown). Quantitatively, those cells that spread on the synthetic peptides occupied an average area of ~380 μm^2 (Table II); ~60% of the spreading caused by intact laminin. The few fibrosarcoma cells that remained on the surfaces coated with BSA, poly-L-lysine, or peptide GD-5 were spherical and did not flatten or spread after 2 h; these cells only occupied an average area of ~60–120 μm^2 (Table II).

Inhibition of Laminin-mediated Cell Adhesion by Synthetic Peptides

To determine if the specific amino acid sequences comprising the biologically active synthetic peptides were functionally active in the intact laminin molecule, radiolabeled HT-1080 cells were preincubated with various concentrations of the synthetic peptides or OVA, then added to wells coated with laminin or fibronectin. The four peptides that directly promoted cell adhesion, peptides GD-1, GD-2, GD-3, and GD-4, were also found to inhibit laminin-mediated cell adhesion in a concentration-dependent manner when peptides were added in solution (Fig. 3). Peptide GD-1 was the most effective peptide at inhibiting cell adhesion to laminin; 100 μg/ml caused ~75% inhibition of cell adhesion (Fig. 3). Peptides GD-2 and GD-3 inhibited laminin-mediated cell adhesion to almost the same extent; this inhibition was inde-

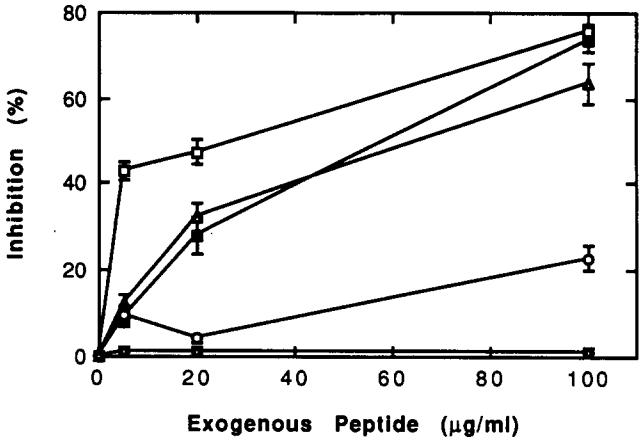

Figure 3. Inhibition of laminin-mediated cell adhesion by peptides. Radiolabeled HT-1080 human fibrosarcoma cells were preincubated for 30 min in various concentrations of peptides GD-1 (□), GD-2 (■), GD-3 (△), GD-4 (○), or GD-5 (◇), then added to wells adsorbed with 1.5 μg of laminin. After a 30-min incubation, the nonadherent cells were aspirated off and the wells were washed three times. Adherent cells were quantitated in a scintillation counter. Cell inhibition is expressed as a percent relative to the number of cells binding to laminin-coated surfaces in the absence of inhibitors. Each value represents the mean of four separate determinations ± SEM. Three separate experiments gave similar results.

Table III. Reactivity of Purified IgG Raised against Laminin A Chain Peptides

Adsorbed antigen*	Purified IgG‡			
	GD-1/KLH	GD-2/KLH	GD-3	GD-4/KLH
Peptide GD-1	1.06 ± 0.06	0	0	0.11 ± 0.06
Peptide GD-2	0	0.33 ± 0.02	0	0
Peptide GD-3	0	ND	1.14 ± 0.07	0
Peptide GD-4	0	0	0.18 ± 0.08	0.91 ± 0.11
Peptide GD-5	0	0	0	0.02 ± 0.02
Laminin	0.70 ± 0.04	0.01 ± 0.00	0.64 ± 0.01	0.10 ± 0.01
Fibronectin	0	0	0.06 ± 0.01	0
Ovalbumin	0	0	0	0.01 ± 0.01

* Wells were coated with 0.05 μg of peptide GD-1, 0.125 μg of peptide GD-2, 1 μg of peptides GD-3, GD-4, and GD-5, and 3 μg of laminin, fibronectin, or ovalbumin. *ND*, not done.
‡ Purified IgG from normal rabbits or rabbits immunized with the synthetic laminin A chain peptides was added to the wells at 100 μg/ml. The ELISA was performed as described in Materials and Methods, and absorbance readings at 490 nm were taken. The values shown are the mean of quadruplicate wells ±SD with background readings using normal rabbit IgG subtracted. Where the background reading exceeded the absorbance using IgG from the rabbits immunized with the peptides, zero has been used.

pendent of their heparin binding activity since peptide GD-2 binds high levels of ^{3}H-heparin, whereas peptide GD-3 binds low levels of ^{3}H-heparin. Peptide GD-4 inhibited cell adhesion to the least extent; 100 μg/ml inhibited cell adhesion to laminin only ~20%. Peptide GD-5, which neither promoted heparin binding nor cell adhesion, did not inhibit laminin-mediated cell adhesion. None of the peptides inhibited cell adhesion on surfaces adsorbed with fibronectin, suggesting the binding specificity of the laminin peptides.

In the next series of experiments, a checkerboard-type analysis was conducted whereby HT-1080 cells were incubated with 30 μg/ml of each of the peptides, GD-1 through GD-5, in the presence of 30 μg/ml of a second peptide in order to determine if an additive or synergistic effect of inhibition of laminin-mediated cell adhesion could be obtained. When two exogenous peptides were added concomitantly, an additive effect of inhibition was observed for peptides GD-1 through GD-4 (data not shown).

As further evidence that the precise sequence of amino acid residues comprising each synthetic peptide was critical for its cell adhesion activity, inhibition assays were performed in order to determine if peptides GD-1"S" through GD-4"S", in which the amino acid sequences were "scrambled" (Table I), could inhibit laminin-mediated HT-1080 cell adhesion. When tested at the same concentrations that caused inhibition with the "native" unscrambled peptides, the "scrambled" peptides did not cause significant inhibition (data not shown).

Inhibition of Laminin-mediated Cell Adhesion by Anti-peptide Antibodies

Polyclonal antibodies were raised in rabbits against the synthetic peptides or peptide/KLH and the specificity of the purified IgG was verified by ELISA. Each of the purified IgG's reacted specifically with their respective peptide (Table III). The IgG raised against peptides GD-1 and GD-3 were also very reactive with the intact laminin molecule, while the IgG raised against peptides GD-2 and GD-4 reacted significantly, albeit less well, with intact laminin. It is possible that the regions of laminin comprising peptides GD-2 and GD-4 are "hidden" within the globular domain, and are not as readily accessible as the sequence epitopes of peptides GD-1 and GD-3. The IgG generated against the synthetic peptides reacted at background levels, if at all, with fibronectin, OVA, or the other synthetic peptides (Table III).

As further proof that the amino acid sequences comprising peptides GD-1 through GD-4 were functionally active in the intact laminin molecule, the purified anti-peptide IgGs were tested for their ability to inhibit laminin-mediated HT-1080 cell adhesion. In all cases, the anti-peptide IgGs were able to cause partial inhibition (~30–50%) on surfaces adsorbed with laminin (Fig. 4). Since laminin is a very large molecule with many sites that promote cell adhesion, it was expected that antibodies raised against only one of these sites could not totally inhibit cell adhesion. The anti-peptide IgGs also caused complete inhibition of cell adhesion on surfaces adsorbed with their respective peptide, but had no effect on surfaces adsorbed with the other unrelated cell adhesion promoting laminin GD peptides or fibronectin (data not shown).

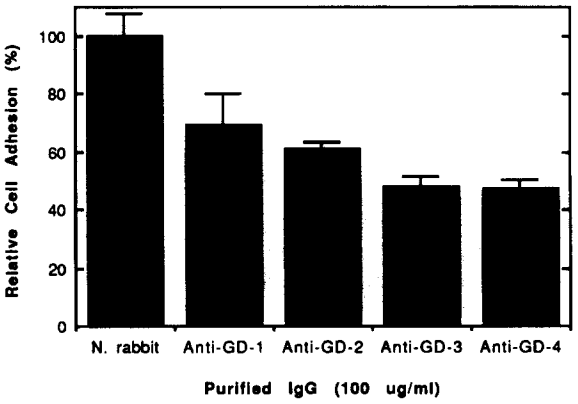

Figure 4. Inhibition of laminin-mediated HT-1080 cell adhesion with antibodies against the peptides. Microtiter wells were adsorbed with 0.3 μg of laminin, then 100 μg/ml of purified IgG from normal rabbits or rabbits immunized with the synthetic peptides GD-1/KLH, GD-2/KLH, GD-3, or GD-4/KLH were incubated in the wells for 1 h. Radiolabeled HT-1080 cells were then added to the wells and the number of cells adhering was quantitated as described in Materials and Methods. Cell adhesion is expressed as the number of cells that adhered to laminin in the presence of the IgG as a percent of the cells that adhered in the absence of the IgG. Each value represents the mean of four separate determinations ± SD. Three separate experiments gave similar results.

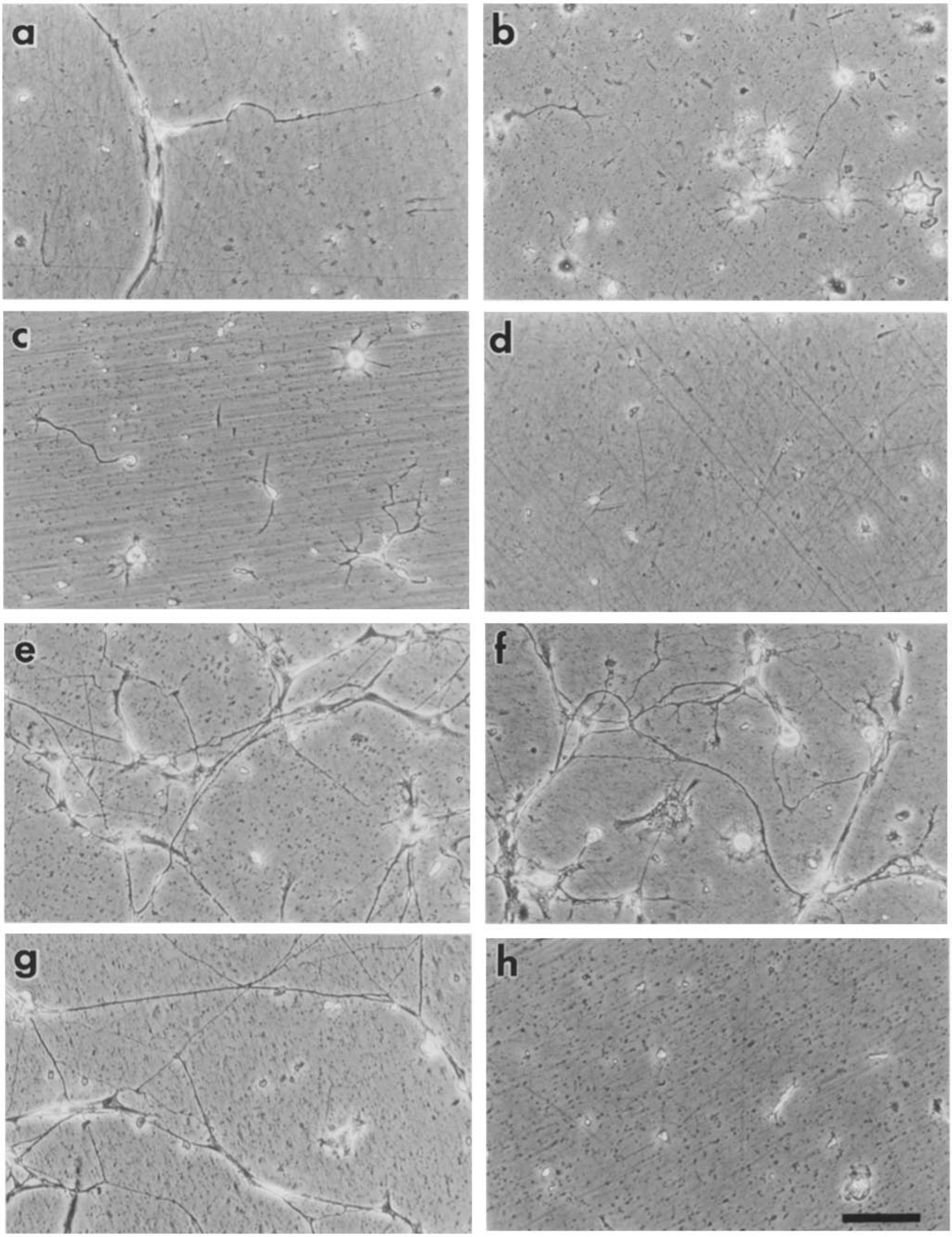

Figure 5. Morphology of chick SC neurons or DRG neurons on laminin peptides. Chick SC neurons (*a–d*) or DRG neurons (*e–h*) were incubated for 24 h in microtiter wells preadsorbed with 1 μg of laminin or the synthetic peptides, then photographed as described in Materials and Methods. SC neurons extended neurites on surfaces adsorbed with laminin (*a*), peptide GD-1 (*b*), and peptide GD-3 (*c*), but not on peptide GD-5 (*d*). DRG neurons extended neurites on surfaces adsorbed with laminin (*e*), and peptide GD-2 (*f*), peptide GD-3 (*g*), but not on peptide GD-5 (*h*). Bar, 30 μm.

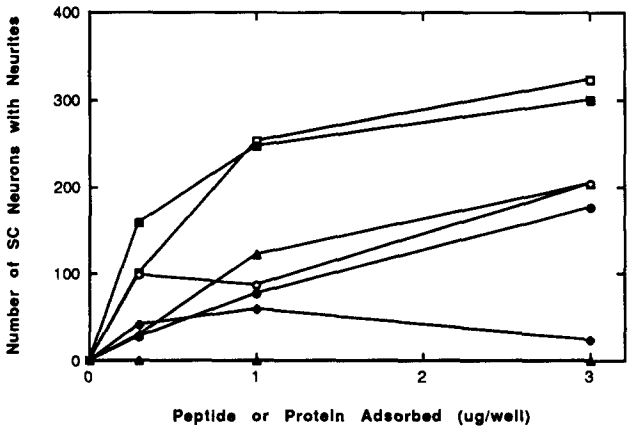

Figure 6. Laminin A chain peptides promote the outgrowth of neurites from SC neurons. Microtiter wells were adsorbed with increasing concentrations of laminin (●) or peptides GD-1 (□), GD-2 (■), GD-3 (△), GD-4 (○), GD-5 (◇), or OVA (▲), and chick SC neurons were then added. At the end of a 24-h incubation, the number of SC neurons that had extended neurites was quantitated as described in Materials and Methods. Each value represents the mean of three separate determinations and the SE was <10%. Three separate experiments gave similar results.

PC12 Cell Adhesion to Peptides

The G domain of laminin is known for its ability to promote neurite outgrowth in vitro. To ensure that the cell adhesion promoted by these laminin A chain peptides was not restricted to human fibrosarcoma cells, a neural crest derived cell line, PC12 rat pheochromocytoma cells, was examined. The PC12 cells adhered to the laminin peptides in a concentration-dependent manner similar to the HT-1080 cells (data not shown). As with the HT-1080 cells, peptides GD-1 and GD-2 promoted the highest level of cell adhesion, whereas peptides GD-3 and GD-4 promoted lower, albeit significant, levels of PC12 cell adhesion. Neither peptide GD-5 nor OVA promoted PC12 cell adhesion.

Neurite Outgrowth on Surfaces Adsorbed with Laminin or Peptides

Since neural crest derived cells were able to adhere to the laminin A chain peptides, we next tested to see if the peptides could promote neurite outgrowth of primary neuronal cells. Microtiter wells adsorbed with laminin or the peptides promoted the outgrowth of neurites from both SC and DRG neurons after a 24-h incubation. On surfaces adsorbed with laminin, SC neurons tended to extend only one long neurite per neuron and thus appeared unipolar (Fig. 5 *a*). In contrast, the majority of SC neurons appeared multipolar on surfaces adsorbed with peptides GD-1, GD-2, GD-3, or GD-4; representative examples are shown of neurons on peptides GD-1 (Fig. 5 *b*) and GD-3 (Fig. 5 *c*) where several small neuritic extensions per neuron were observed. The few SC neurons that adhered to surfaces adsorbed with peptide GD-5 (Fig. 5 *d*) or OVA (not shown), did not extend neurites.

In contrast, the morphology of DRG neurons incubated on surfaces adsorbed with laminin (Fig. 5 *e*), or peptides GD-2 (Fig. 5 *f*), GD-3 (Fig. 5 *g*), GD-1 (not shown), or GD-4 (not shown) appeared very similar. Over 95% of the DRG neurons that extended neurites on these surfaces had extended multiple neurites per neuron. No neurite outgrowth was observed from DRG neurons incubated on surfaces adsorbed with peptide GD-5 (Fig. 5 *h*) or OVA (not shown).

Neurite outgrowth was quantitated by counting the number of neurons on equivalent areas of each surface that had extended neurites. Laminin and peptides GD-1 through GD-4 promoted the outgrowth of neurites from SC neurons (Fig. 6) and DRG neurons (not shown) in a concentration-dependent manner. Peptides GD-1 and GD-2 promoted a greater number of SC neurons to extend neurites than did laminin or peptides GD-3 and GD-4. In contrast, laminin was a more potent promoter of neurite outgrowth from DRG neurons compared to any of the peptides (see below). SC and DRG neurons extended very few neurites on surfaces adsorbed with either peptide GD-5 or OVA.

Inhibition of Peptide-mediated Cell Adhesion by an Antibody against the β1 Integrin Subunit

Since integrins have been implicated as receptors that mediate the adhesion of a variety of cells to laminin, we wanted to determine if any of the laminin peptides that promote human HT-1080 fibrosarcoma cell adhesion do so by interacting with the β1 integrin subunit. To address this question, we performed HT-1080 cell adhesion assays on surfaces adsorbed with the laminin peptides in the presence of monoclonal antibody P4C10 generated against the human β1 integrin subunit. 60% fewer cells adhered to surfaces adsorbed with laminin or laminin peptides GD-3 and GD-4 when a 1:50 dilution of monoclonal antibody P4C10 was present, compared to when the antibody was absent (Fig. 7). However, monoclonal antibody P4C10, at the same 1:50 dilution, had no effect on cell adhesion to peptides GD-1 or GD-2 (Fig. 7).

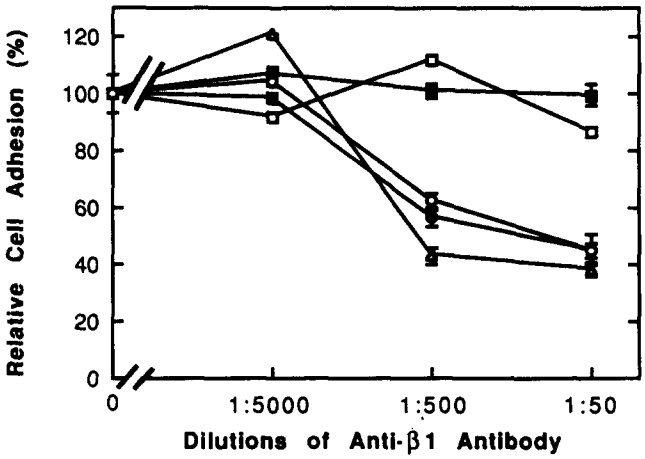

Figure 7. Inhibition of HT-1080 cell adhesion to laminin and laminin peptides by a monoclonal antibody against the β1 integrin subunit. Radiolabeled human HT-1080 fibrosarcoma cells were preincubated with various dilutions of monoclonal antibody P4C10 against the human β1 integrin subunit. Cells were then incubated for 30 min in wells preadsorbed with laminin (●) or peptides GD-1 (□), GD-2 (■), GD-3 (△), and GD-4 (○). The number of cells adhering was quantitated as described in Materials and Methods. Cell adhesion is expressed as the number of cells that adhered to the substrate in the presence of the anti-β1 integrin subunit antibody as a percent of the cells that adhered in the absence of the antibody. Each value represents the mean of four separate determinations ± SD. Triplicate experiments gave similar results.

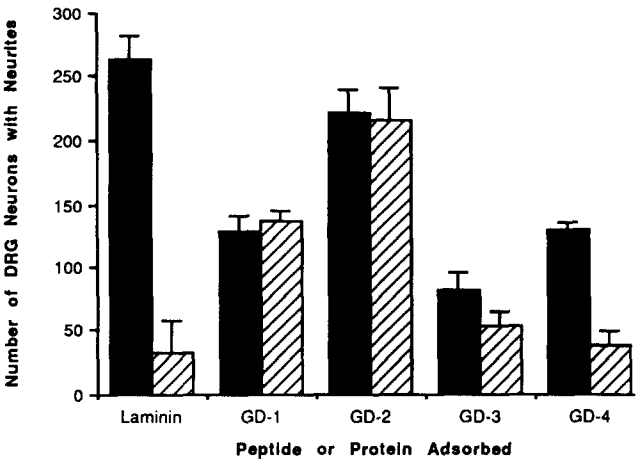

Figure 8. Inhibition of neurite outgrowth on laminin peptides by a monoclonal antibody against the β1 integrin subunit. Chick DRG neurons were preincubated with normal mouse IgG (*solid bars*) or purified IgG from the mouse monoclonal antibody WIB10 that recognizes the chicken β1 integrin subunit (*hatched bars*), then added to wells adsorbed with 1 μg of laminin or the synthetic laminin A chain peptides. After a 24-h incubation, the number of neurons that had extended neurites was quantitated as described in Materials and Methods. Each value represents the mean of three separate determinations ± SE. Duplicate experiments gave similar results.

Inhibition of Peptide-mediated Neurite Outgrowth by an Antibody against the β1 Integrin Subunit

Since expression of the β1 integrin subunit has been demonstrated on developing neurons (Bozyczko and Horwitz, 1986; Tomaselli et al., 1988*a*; Letourneau and Stattuck, 1989), we performed neurite outgrowth assays on surfaces adsorbed with laminin peptides using monoclonal antibody WIB10 against the chicken β1 integrin subunit as an inhibitor. Chick DRG neurons extended significantly fewer neurites on surfaces adsorbed with laminin, peptide GD-3, or peptide GD-4 in the presence of the anti-integrin monoclonal antibody, compared to when normal mouse IgG was present (Fig. 8). These results suggest that the sequences in laminin comprising peptides GD-3 and GD-4 use an integrin as a receptor, of which the β1 integrin subunit is a component. In contrast, the monoclonal antibody had no effect on DRG neurite outgrowth on surfaces adsorbed with peptides GD-1 or GD-2 under identical conditions.

Discussion

Based upon earlier observations indicating that the G domain of laminin binds heparin, and hence, perhaps interacts with cellular glycosaminoglycans or proteoglycans, we synthesized 20′ mer peptides from the G domain of laminin in an attempt to further define biologically active sequences from this region. Previously, this domain was shown to be the major of a number of heparin binding domains of laminin and was termed Hep-1 (Skubitz et al., 1988). The peptides synthesized were of relatively similar positive net charge and negative hydropathy. Interestingly, in direct solid-phase binding assays, two of the peptides, GD-1 and GD-2 (net charges of +5 and +2, respectively), bound heparin at very high levels while peptides GD-3 and GD-4 (net charges of +3 and +4, respectively) bound significantly less heparin, and peptide GD-5 (net change +2) did not bind heparin. This was somewhat of a surprise as one might have predicted that the more highly cationic peptides would bind heparin more effectively simply on a basis of net charge. It caused some consternation that we were unable to predict from the net charge or hydropathy index whether or not a peptide would bind heparin. The finding that two peptides from the G domain of laminin both promote high levels of heparin binding is not unexpected due to previous reports that described this domain as the major heparin binding site of laminin (Ott et al., 1982; Skubitz et al., 1988). In fact, since the G domain is predicted by computer analysis to consist of five loops (Sasaki et al., 1988), it is quite possible that regions on each of these loops may act in concert to bind heparin. Furthermore, six additional ~20′ mer peptides from the G domain of laminin also bind ^{3}H-heparin (Wilke and Skubitz, 1991; Skubitz, A. P. N., unpublished observation) and may help to account for the G domain being the major heparin binding domain of laminin.

Previous studies had shown by rotary shadowing and electron microscopy that heparin bound to laminin at the G domain in 0.2 M NaCl, while binding of heparin to other domains of laminin only occurred at lower salt concentrations (Kouzi-Koliakos et al., 1989). We were therefore interested to study the strength of the interaction between ^{3}H-heparin and the peptides by determining whether binding or dissociation of laminin A chain peptides to heparin was sensitive to increasing salt concentrations. In the present study, significant binding of ^{3}H-heparin to peptides GD-1 and GD-2 was observed even at salt concentrations as high as 1.0 M NaCl. These results indicate that there are at least two regions on the G domain of laminin that bind heparin at relatively high salt concentrations (i.e. approximately six to seven times physiological salt concentrations); this provides a potential explanation for why the G domain of laminin is the "major" heparin binding site on laminin and can be called Hep-1 in context with the other sites on laminin that appear to show lesser affinity (Skubitz et al., 1988; Kouzi-Koliakos et al., 1989).

Proteoglycans with heparin-like side chains are present on cell surfaces and in basement membranes (Lindahl and Höök, 1978; Gallagher et al., 1986; Stow and Farquhar, 1987; Ruoslahti, 1988; Klein et al., 1988). The interaction of proteoglycans with laminin by way of specific peptide sequences may serve to regulate cell adhesion and spreading. For this reason, we tested the peptides for their ability to directly promote the adhesion of HT-1080 human fibrosarcoma cells, since intact laminin significantly promotes the adhesion of HT-1080 cells. These studies showed that four of the five peptides from the G domain of laminin promoted cell adhesion. Interestingly, peptides GD-1 and GD-2, which bound heparin, promoted higher levels of cell adhesion than peptides GD-3 and GD-4, which bound less heparin. The finding that four different sequences from the G domain of laminin promote cell adhesion is not again surprising since other large extracellular matrix proteins such as fibronectin and type IV collagen are known to contain a variety of domains and specific peptide sequences involved in similar functions (Chelberg et al., 1990; McCarthy et al., 1990).

Our results also suggest that the four peptide sequences that directly promote cell adhesion play an important role in

the intact laminin molecule, since they each inhibited cell adhesion to surfaces adsorbed with laminin. The ability of any one peptide to significantly inhibit cell adhesion to an intact molecule that contains multiple cell adhesion promoting sequences suggests that there may be some common pathway or rate limiting step subsequent to cell recognition of adhesive peptide sequences. As further evidence that these four peptides play an important role in HT-1080 cell adhesion, we have shown that antibodies generated against these peptides react with laminin by ELISA and inhibit laminin-mediated HT-1080 cell adhesion.

In addition, the four peptides that promoted cell adhesion also promoted HT-1080 cell spreading. The area occupied by cells on surfaces adsorbed with any of these active peptides was ~60% of the spreading observed on intact laminin. These results indicate that the process of cell spreading is quite complex and may require multiple peptide sequences, perhaps from the other chains of laminin, for cells to acquire the characteristic phenotypic appearance of cells adherent and spreading on laminin.

Since the G domain of laminin has been shown to promote the outgrowth of neurites from neurons, we next conducted assays to determine if the GD peptides could affect the behavior of a variety of neural crest and neuronally derived cells. Peptides GD-1 through GD-4 promoted the adhesion of PC12 cells, as well as the outgrowth of neurites from both chick SC and DRG neurons. Interestingly, neurite outgrowth from DRG neurons on surfaces adsorbed with laminin appeared morphologically identical to DRG neurons on surfaces adsorbed with the peptides; whereas neurites extended in a unipolar manner from SC neurons on surfaces adsorbed with laminin and in a multipolar manner from SC neurons on surfaces adsorbed with the peptides. These results suggest that neurite outgrowth from these two different neuronal cell types may be mediated by laminin through different mechanisms. Importantly, both primary neurons and neuronally derived tumor cells are able to interact with these laminin peptides.

The amino acid residues comprising these five mouse EHS laminin A chain peptides were compared with the residues of human laminin and found to be quite similar. The murine peptides had ~70–100% identity and 93–100% similarity to the corresponding human sequences as determined using the Dayhoff matrix. When these five mouse laminin A chain peptide sequences were compared to amino acid sequences of other proteins by computer searches of the Protein Identification Resource of the National Biomedical Research Foundation, Washington, D.C., Protein Database (04/23/91), several stretches of 5 or 6 amino acid residues of identity were found. Of most potential relevance was the finding that human merosin contains the sequence G F K V G L D L L V E F E F R T T (Ehrig et al., 1990), that has ~60% identity (underlined residues) and 88% similarity to a 17 amino acid overlap in peptide GD-2. The M subunit of merosin, a molecule present in muscle fiber basal laminae, but absent from many other basal laminae (Lievo and Engvall, 1988), has been shown to be a homologue of the A chain of laminin (Ehrig et al., 1990) that also promotes the adhesion of some neurons (Sanes et al., 1990).

Another important feature of laminin is its ability to associate with cell surface receptors and consequently modify cellular phenotypes in various ways. Receptors for laminin have been isolated from a variety of normal and malignant cell lines (see reviews by von der Mark and Kühl, 1985; Martin and Timpl, 1987; Reichardt and Tomaselli, 1991). In many cases, the laminin receptor has been determined to be an integrin (Horwitz et al., 1985; Bozyczko and Horwitz, 1986; Helmer et al., 1988; Tomaselli et al., 1987, 1988*a*,*b*, 1990; Sorokin et al., 1990, and references therein). These findings suggest that different cell types adhere to specific domains of laminin based on their various cell surface receptors.

Due to the importance of integrin receptors in mediating cell adhesion to laminin, we tested the ability of monoclonal antibodies raised against the β1 integrin subunit to inhibit peptide-mediated HT-1080 cell adhesion and neurite outgrowth. On surfaces adsorbed with peptides GD-3 and GD-4, monoclonal antibodies against the β1 integrin subunit partially inhibited both HT-1080 cell adhesion and the outgrowth of neurites from chick DRG neurons. These results suggest that the sequences of laminin comprising peptides GD-3 and GD-4 (which do not bind ^{3}H-heparin) permit the interaction of laminin with cells by way of their β1 integrin subunits; whereas the sequences of laminin comprising peptides GD-1 and GD-2 (which bind ^{3}H-heparin) may be interacting with cells by way of their cell surface glycosaminoglycans/proteoglycans, though other explanations may exist.

Recently, an α3 β1 integrin isolated from osteosarcoma cells has been reported to bind to the carboxy terminus of the B1 chain of laminin (Gehlsen et al., 1989). Other groups have used monoclonal antibodies against specific integrin α or β subunits in combination with proteolytic fragments of laminin to localize regions of laminin to which integrin subunits bind. In their studies, the integrin α1 subunit has been shown to bind to the core and proximal portions of the short arms of the cross region of laminin, whereas the integrin α6 subunit and α3 β1 binds to the long arm of laminin containing the G domain (Tomaselli et al., 1990; Hall et al., 1990; Sonnenberg et al., 1988, 1990). Recently, a monoclonal antibody to VLA-6 has been reported to inhibit HT-1080 cell adhesion to laminin A chain peptide PA21, which contains the RGD sequence from the region above the intersection of the cross (Tashiro et al., 1991). To date, however, the exact amino acid sequence of the regions of laminin to which the various integrin or other receptors binds is unknown. Future studies will attempt to isolate the cell surface receptors for the biologically active A chain peptides described in this study. Of particular interest is β1,4 galactosyltransferase which has just recently been shown to serve as a cell surface receptor for the G domain of laminin (Begovac et al., 1991).

Based on the ability of these peptides to promote heparin binding and cell adhesion, the peptides fell into three categories: group I peptides (includes GD-1 and GD-2) bound high levels of heparin and were effective at promoting cell adhesion; group II peptides (includes GD-3 and GD-4) bound less heparin, but were quite active at promoting cell adhesion; and group III peptides (includes GD-5) bound neither heparin nor cells. These data indicate that multiple sequences of laminin present in the G domain may act to promote cell adhesion and/or heparin binding; potentially increasing the level of interaction due to their close proximity. These findings suggest the existence of at least two potential

classes of cell receptors which interact with the G domain of laminin: heparin-like (i.e., glycosaminoglycan/proteoglycans) and integrins.

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