Human Keratinocytes Adhere to Two Distinct Heparin-Binding Synthetic Peptides Derived from Fibronectin

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Fibronectin is present at the dermal-epidermal junction in normal skin and is increased in skin tissues in inflammatory diseases, skin cancers, and wound repair. The present studies focused on further characterizing the interaction between fibronectin and keratinocytes, specifically addressing whether human keratinocytes utilize multiple adhesion promoting sequences within fibronectin. Initially, direct cell-binding assays were utilized in which keratinocyte adhesion to plastic substrata coated with fibronectin or proteolytic fragments of fibronectin was quantified. Intact fibronectin, a 75-kD proteolytic fragment containing the RGD sequence, and 33/66-kD cell adhesion/heparin binding fragments lacking the RGD sequence derived from the A and B chains of fibronectin, all promoted keratinocyte adhesion in a concentration-dependent manner. To further define putative cell-binding domains within the 33/66-kD fibronectin fragments, we studied three chemically synthesized peptides derived from the amino acid sequence of the 33-kD fragment of the fibronectin A chain: FN-C/H-I (YEKPGSPPREVVPRPRPGV), FN-C/H-II (KNNQKSEPLIGRKKT), and CS1 (DELPQLVTLPHPNLHGPEILDVPST). Substrata coated with either FN-C/H-I or FN-C/H-II promoted keratinocyte adhesion in a concentration-dependent and saturable manner, whereas peptide CS1 promoted no significant keratinocyte adhesion. In solution, both exogenous FN-C/H-I and FN-C/H-II partially inhibited keratinocyte adhesion to the 33/66-kD fibronectin fragments. Furthermore, antibodies prepared against these peptides also inhibited keratinocyte adhesion to the 33/66-kD fibronectin fragments. These data indicate that keratinocyte adhesion to fibronectin is mediated by multiple distinct amino acid sequences, at least two of which are localized to the carboxy-terminal heparin binding domain of fibronectin. *J Invest Dermatol 97:573–579, 1991*

The precise function of fibronectin in human skin remains to be elucidated. However, in normal human skin, fibronectin is visualized discretely along the dermal-epidermal junction by immunofluorescent studies [1,2] and at the basal cell plasma membrane and lamina lucida areas of the epidermal basement membrane by immunoelectron microscopy [3]. Fibronectin has been shown to be increased in skin tissues in inflammatory diseases such as psoriasis [4], lichen planus [5], discoid lupus [6], and dermatitis herpetiformis [7]. Basal cell carcinomas [8] and squamous cell carcinomas [9] of the skin have also been shown to display increased quantities of fibronectin. Finally, in wound healing, keratinocytes migrate over and through a provisional fibronectin matrix [10,11].

Plasma fibronectin is a dimer composed of two similar, but not identical, disulfide bonded subunits of approximately 220 kD each [12]. Several functionally and chemically distinct regions of fibronectin (Fig 1), which can be isolated by proteolytic digestion and purified with various antibodies or other ligands, have been defined by numerous investigators (for reviews see [13–16]) and include from the amino terminus: i) a weak heparin and fibrin-binding region, ii) a collagen-binding region, iii) a large segment with the ability to enhance cell adhesion and migration, iv) a cell-binding peptide region with the arginine-glycine-aspargine-serine (RGDS) sequence, v) a strong carboxy terminal heparin and cell-binding domain, and vi) a free sulfhydryl-containing carboxy-terminal region that possesses interchain disulfide bonds.

Cell adhesion to fibronectin is a complex process involving multiple cell-binding domains. For example, the well-characterized RGDS tetrapeptide has been shown to promote the attachment of various normal and transformed cells including keratinocytes [17–20]. The carboxy-terminal cell and heparin-binding region of fibronectin also has been shown to promote the adhesion of a variety of cells in an RGD-independent manner [19,21,22]. Studies previously done have identified three synthetic peptide sequences

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Abbreviations:

DEAE: diethyl-aminoethyl
EDC: 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride
EDTA: ethylenediaminetetraacetic acid
EHS: Engelbreth-Holm-Swarm
ELISA: enzyme linked immunosorbent assay
HEPES: hydroxyethylpiperazine-N′-2-ethanesulfonic acid
IgG: immunoglobulin G
KLH: keyhole limpet hemocyanin
PAGE: polyacrylamide gel electrophoresis
PBS: phosphate-buffered saline
SDS: sodium dodecyl sulfate

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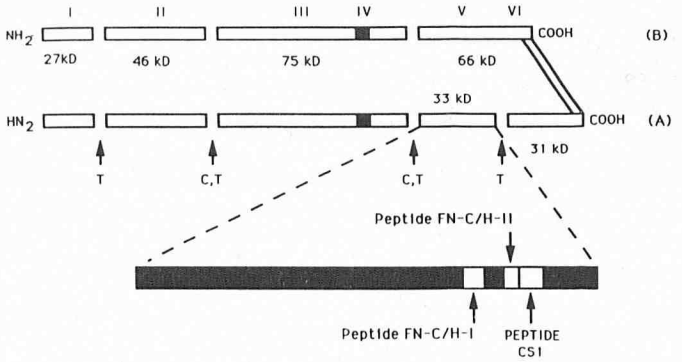


Figure 1. Diagramatic model of human plasma fibronectin. The molecule is disulfide bonded at the carboxyl-terminus and is composed of two similar, but not identical chains termed the A and B chains. Selected biologically active domains are indicated by *roman numerals* and defined in the text. The apparent molecular weights shown in the diagram refer to specific trypsin (T) or cathepsin D/trypsin (C,T) generated fragments. The 33-kD fragment of the A chain has been expanded to depict the location of synthetic peptides FN-C/H-I, FN-C/H-II, and CS1.

within this region that have cell attachment activity, including CS1 [23–25], FN-C/H-I [24,26], and FN-C/H-II [25,26]. FN-C/H-I and FN-C/H-II differ from CS1 in their ability to bind heparin [26], whereas FN-C/H-II differs from FN-C/H-I in that it is contiguous with CS1.

In this study, we focused on determining whether keratinocytes utilize cell adhesion–promoting sequences other than RGD within fibronectin. To further pursue this possibility, various proteolytic fragments of fibronectin, representing specific biologically active domains, were examined for their ability to promote keratinocyte adhesion. In addition, synthetic peptides derived from the known amino acid sequence of fibronectin A chains were tested in similar assays. The results indicate that keratinocytes are capable of adhering to fibronectin through multiple distinct cell-binding sequences, two of which are localized to the carboxy-terminal heparin-binding domain of fibronectin.

MATERIALS AND METHODS

Cell Culture Normal human keratinocyte cultures were initiated from neonatal foreskins within 1 to 2 h after routine circumcision of newborn male infants as previously described [27]. Tissue was obtained from a local nursery with the approval of the Human Studies Committee at the University of Minnesota. Primary and passaged cultures were grown in complete keratinocyte growth medium in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Complete keratinocyte growth medium consists of nutrient keratinocyte basal medium (Clonetics, San Diego, CA) supplemented with 5×10^{-7} M hydrocortisone, 5 μg/ml insulin, 10 ng/ml epidermal growth factor, and 140 μg/ml protein of bovine pituitary extract. Using the above-described conditions, keratinocyte cultures are easily initiated and serially passaged forming monolayers of undifferentiated cell colonies. However, because cultured neonatal keratinocytes undergo senescence at 50 to 60 population doublings [28], only early passage cultures (up to passage 3) were used in experiments.

Protein and Fragment Isolation Human plasma fibronectin was purified as a byproduct of factor VIII production by sequential ion-exchange and gelatin affinity chromatography as previously described [19]. The 33/66-kD fragments (Fig 1) were prepared by limited trypsin-cathespin D digestion of intact fibronectin followed by sequential gelatin- and heparin-affinity chromatography as reported earlier [19]. The 75-kD fragment (Fig 1) was prepared by extensive trypsinization followed by purification over sequential monoclonal antibody 180-8 affinity and Spherogel TSK 3000 columns as previously described [19]. The purity of the intact protein and fragments was verified by SDS-PAGE and Coomassie brilliant blue staining.

Peptide Synthesis, Purification, and Coupling to Ovalbumin Peptides derived from the A chain of fibronectin were synthesized by the Merrifield solid-phase method as described previously [29]. Briefly, deprotection and release of peptides was achieved using hydrofluoric acid containing 10% anisole for 1 h at 4°C. Peptides were then extracted with ether, dissolved in 10% acetic acid, filtered, and lyophilized. Lyophilized crude polypeptides were purified by high-pressure liquid chromatography on a reverse-phase C-18 column using an elution gradient of 0 to 60% acetonitrile with 0.1% trifluoroacetic acid in water. Purified synthetic peptides were characterized by amino acid analysis using a Beckman System amino acid analyzer. Finally, sequence determination was carried out by sequential Edman degradation from the amino terminus on a gas-phase sequenator, by the methodology of Hewick and others [30].

The synthetic peptides were chemically conjugated to ovalbumin using 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, Sigma Chemical Co., St. Louis, MO) as previously described [25]. Briefly, equal amounts (by weight) of peptides and ovalbumin were solubilized in water containing a tenfold excess (by weight) of EDC. The coupling reaction was carried out overnight at 4°C on a circular rotator. The coupled peptides were then dialyzed extensively in PBS to remove the excess EDC and free peptide (10,000 dalton exclusion, Spectrum Medical Industries, Los Angeles, CA). By use of trace amounts of radiolabeled peptides, the coupling efficiency of the various peptides to ovalbumin was determined to be approximately five peptide molecules per ovalbumin molecule.

Generation and Purification of Anti-FN-C/H-I and Anti-FN-C/H-II Antibodies Polyclonal antibodies were generated against fibronectin synthetic peptides as previously described [31]. Briefly, peptides were coupled to the carrier protein keyhole limpet hemocyanin (KLH, Sigma Chemical Co.) using EDC as a coupling reagent. Immunization of New Zealand white rabbits was performed by mixing an equal volume of peptide/KLH conjugate with complete Freund's adjuvant, and injecting this mixture subcutaneously into multiple sites on the shaved backs of the rabbits. Subsequent boosts were biweekly and performed by intramuscular injection of the immunogen into the hind legs of the rabbits using incomplete Freund's adjuvant for the emulsification. Immune sera were tested by ELISA for reactivity against uncoupled peptides, fibronectin, and various other ligands. Immunoglobulin G (IgG) was purified from pooled immune sera by precipitation with ammonium sulfate followed by DEAE anion exchange column chromatography, and purity was verified by SDS-PAGE and Coomassie brilliant blue staining of the gel.

Cell Adhesion Assay Assays for cell attachment to fibronectin, proteolytic fragments of fibronectin, and chemically synthesized peptides coupled to ovalbumin were similar to those previously described [19]. Briefly, various concentrations of the above proteins and peptides in Voller's buffer were added to 96-well Immulon 1 plates and adsorbed overnight at 37°C. Three hours prior to the start of the assay, the plates were treated with PBS containing 2 mg/ml of ovalbumin to block any non-specific binding sites on the plastic surfaces. Rapidly proliferating cultures of normal human keratinocytes were incubated overnight in medium containing 4 μCi/ml of [^{3}H]thymidine (6.7 Ci/mmole, New England Nuclear, Boston, MA). The flasks were washed 3 times with 10 mM glucose,

3 mM KCl, 130 mM NaCl, 1 mM Na_2HPO_4, 3.3 μM phenol red, and 30 mM hydroxyethylpiperazine-N'-2-ethanesulfonic acid [HEPES] at pH 7.4 [14] and harvested by gentle trypsinization (5-min incubation at 37°C with 0.025% trypsin and 0.01% ethylenediaminetetraacetic acid [EDTA]). Trypsinization was terminated by washing the flasks with bovine pituitary extract free keratinocyte growth medium to which 0.15 mg/ml soybean trypsin inhibitor, 2 mg/ml ovalbumin, and 0.02 M HEPES had been added. The cells were pelleted, resuspended in the same medium without soybean trypsin inhibitor, and passed through a nylon mesh to remove any cell aggregates. The blocking buffer was aspirated from the precoated wells and 100 μl of the cell suspension at 5×10^4 cells/ml was then added to each well and incubated at 37°C. After 30 min, the non-adherent cells were aspirated off and the wells were rinsed 3 times with the medium used to plate the cells. Adherent cells were solubilized with 0.5 N NaOH containing 1% SDS, and quantified in a scintillation counter.

Inhibition of Cell Adhesion by Synthetic Peptides In these assays, the coating of plates with various ligands and harvesting of cells was similar to that described above. However, prior to transferring the cells to the coated plates, the cells were incubated with the test inhibitors at various concentrations (50 to 250 μg/ml) for 30 min at 37°C. After this preincubation, cells in the continued presence of peptide inhibitors were transferred to plates previously coated with various proteins and cell binding was quantified as described above.

Competition Studies using Anti-Synthetic Peptide Antibodies In these assays, the coating of plates with various ligands and harvesting of cells was similar to that described above. However, prior to transferring the cells to the coated plates, various concentrations of purified anti-FN-C/H-I, anti-FN-C/H-II, or normal rabbit IgG were incubated in the wells for 1 h at 37°C. Cells were then added to the wells and allowed to attach for 30 min and cell binding was quantified as described above.

RESULTS

Keratinocyte Adhesion to Fibronectin, the 75-kD Fragment, and the 33/66-kD Fragments The first series of experiments were carried out to further delineate putative cell-binding domains within fibronectin utilizing proteolytic fragments of fibronectin. Substrata were coated with various concentrations (1 to 200 μg/ml) of test proteins and keratinocytes were allowed to attach for 30 min (Fig 2). Substrata coated with fibronectin, the 75-kD fragment, or the 33/66-kD fragments promoted keratinocyte adhesion in a concentration-dependent manner. In contrast, substrata coated with ovalbumin displayed no significant keratinocyte-adhesion–promoting activity. The observation that the 33/66-kD fragment promotes greater keratinocyte adhesion at lower concentrations than intact fibronectin is not surprising because the fragment and intact molecule were not coated in molar equivalents. Therefore, substrata coated with the smaller 33/66-kD fragment in μg concentrations equivalent to fibronectin, display more active cell binding sites per unit area of plastic.

Keratinocyte Adhesion To Peptides Synthesized from the 33-kD Fragment The above data indicate that keratinocytes are capable of not only binding to the 75-kD fragment, which contains the well-described RGD peptide sequence, but also the 33/66-kD heparin binding fragments. Because it has previously been described that keratinocytes are capable of binding to fibronectin via the RGD sequence [20], we chose to focus our studies on defining novel cell-binding domains in the 33/66-kD fragments. In this regard, three previously described cell-adhesion–promoting synthetic peptides from within the 33-kD fragment of the fibronectin A chain [23–26] were examined for their ability to promote keratinocyte adhesion. The amino acid sequence, chain of origin, residue number, net charge, hydropathy index, and location in the intact fibronectin molecule are shown for each of these peptides in Table I and Fig 1.

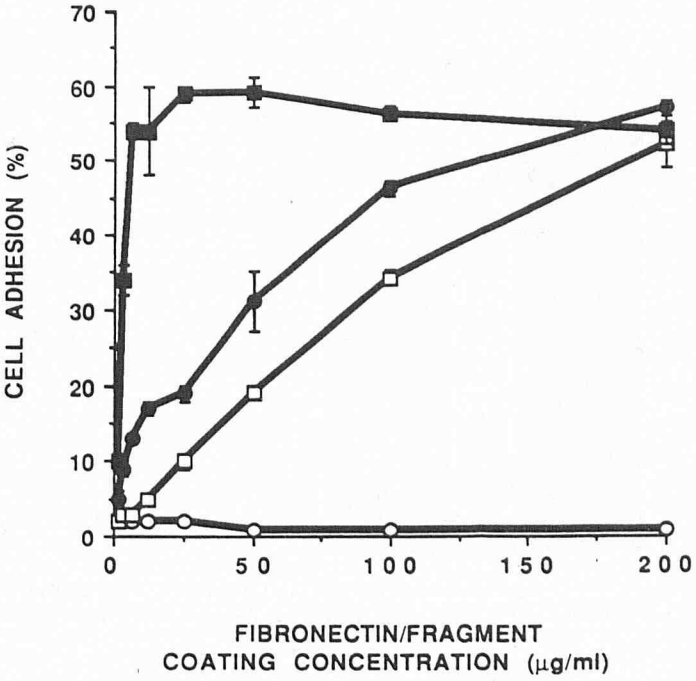


Figure 2. Keratinocyte adherence to fibronectin, the 75-kD fragment, the 33/66-kD fragments, and ovalbumin. Microtiter wells were coated with increasing concentrations of soluble fibronectin (●), the 75-kD fragment (□), the 33/66-kD fragments (■), or ovalbumin (○). Radiolabeled keratinocytes were added to each well and allowed to attach for 30 min, after which time the unattached cells were washed away, and the remaining radiolabeled cells were solubilized and quantified by liquid scintillation counting. Cell adhesion is expressed as a percentage relative to the total number of cells added. Determinations were done in triplicate. *Bars,* SEM.

Within 30 min, substrata coated with ovalbumin-conjugated peptides FN-C/H-I and FN-C/H-II promoted keratinocyte adhesion in a concentration-dependent and saturable manner, whereas ovalbumin-conjugated CS1 displayed no significant keratinocyte adhesion promoting activity in comparison to control substrata (ovalbumin-conjugated to ovalbumin), Fig 3. The inability of CS1 to promote keratinocyte adhesion was not due to ineffective substrata coating, because using similar protocols our laboratory has previously shown that substrata coated with CS1 promote the adhesion of melanoma cell and neural cell lines [25,26].

Peptide FN-C/H-I and FN-C/H-II Competition for Keratinocyte Adhesion to the 33/66-kD Fragments In order to determine whether the above fibronectin peptides represent functionally active regions within the 33/66-kD fragments, keratinocytes were preincubated with exogenous solutions of the synthetic peptides to test their ability to inhibit cell binding to substrata coated with the 33/66-kD fragments. When keratinocytes were preincubated with peptide FN-C/H-I or FN-C/H-II, there was up to 40% inhibition of keratinocyte adhesion to substrata coated with the 33/66-kD fragments (Fig 4). Pre-incubating keratinocytes with FN-C/H-I and FN-C/H-II together resulted in only a moderate additive effect and did not completely inhibit keratinocyte binding to the 33/66-kD fragments (data not shown). This suggests that additional putative cell binding sites exist within the 33/66-kD fragments. Pre-incubation of keratinocytes with peptides FN-C/H-I or FN-C/H-II had no significant effect on keratinocyte adhesion to substrata coated with fibronectin (Fig 4), presumably because cells could use the RGD sequence within fibronectin. Also, as expected, pre-incubation of keratinocytes with peptides FN-C/H-I or FN-C/H-II

Table I. Peptides Synthesized from the 33-kD Fragment of Fibronectin

Peptide Name	Sequence[a] (residue number)	Hydropathy Index[b]	Net Charge[c]
FN-C/H-I	YEKPGSPPREVVPRPRPGV (1906-1924)	−24.3	+2
FN-C/H-II	KNNQKSEPLIGRKKT (1946–1960)	−29.3	+4
CS1	DELPQLVTLPHPNLHGPEILDVPST (1961–1985)	−9.9	−4

[a] The sequences shown use the single letter amino acid code (K = lysine, R = arginine, H = histidine, E = glutamic acid, D = aspartic acid, Q = glutamine, N = asparagine, P = proline, G = glycine, S = serine, T = threonine, V = valine, I = isoleucine, L = leucine, Y = tyrosine).

[b] The hydropathy values were calculated by the method of Kyte and Doolittle [42]. According to this method, the more hydrophobic peptides correspond to the more positive numerical values.

[c] 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.

had no significant effect on keratinocyte adhesion to the 75-kD RGD-containing fragment (data not shown).

Anti-FN-C/H-I and Anti-FN-C/H-II Antibody Competition for Keratinocyte Adhesion to the 33/66-kD Fragments As another means of showing the biologic relevance of the synthetic peptides, antibodies known to react with peptides FN-C/H-I or FN-C/H-II [24,25] were utilized. In these assays, substrata were coated with the 33/66-kD fragments, then pre-incubated with purified IgG prior to the addition of the cells. Both anti-FN-C/H-I and anti-FN-C/H-II IgG partially inhibited the attachment of keratinocytes to substrata coated with 33/66-kD fragments (Fig 5). At the highest concentration of IgG tested (500 μg/ml), anti-FN-C/H-I or anti-FN-C/H-II IgG inhibited keratinocyte adhesion to substrata coated with the 33/66-kD fragments by 23% or 35%, respectively. Combining anti-FN-C/H-I IgG and anti-FN-C/H-II IgG had only a moderate additive effect (data not shown) that correlates with the inhibition of keratinocyte binding to the 33/66-kD fragments when keratinocytes are pre-incubated with combinations of peptides FN-C/H-I and FN-C/H-II. Normal rabbit IgG at similar concentrations caused no significant inhibition of cell attachment (Fig 5).

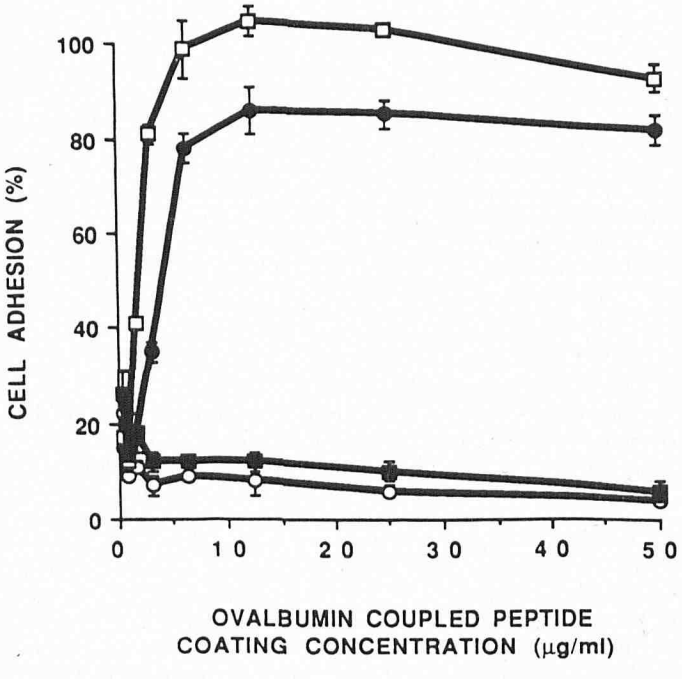


Figure 3. Keratinocyte adherence to fibronectin synthetic peptides FN-C/H-I, FN-C/H-II, and CS1. Microtiter wells were coated with soluble ovalbumin-coupled peptide FN-C/H-I (●), ovalbumin-coupled peptide FN-C/H-II (□), ovalbumin-coupled CS1 (■), or ovalbumin-coupled ovalbumin (○). The coating concentration represents the total amount of protein present (i.e., ovalbumin plus peptide) as determined by a BCA protein assay (Pierce Chemical Co.). Keratinocytes were allowed to attach for 30 min, after which time unattached cells were washed away and the attached cells were quantified as described in Fig 2. Determinations were done in triplicate. *Bars,* SEM.

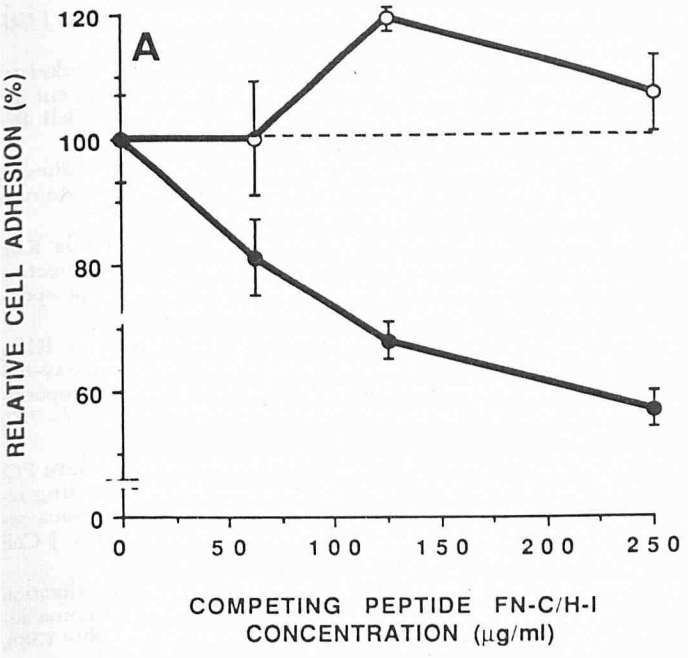
DISCUSSION

It is becoming increasingly clear that cell adhesion to fibronectin has a complex molecular basis that may involve multiple distinct domains within the molecule [19,23–26,32]. The results of the present study support this view and demonstrate that at least three, and likely more, independent sites on fibronectin mediate keratinocyte adhesion.

Previous studies have identified two proteolytic fragments of fibronectin that actively promote neuronal, melanoma, and lymphoid cell adhesion [19,22,25]. These fragments include a 75-kD tryptic fragment of fibronectin, which contains the RGDS sequence, and a 33-kD tryptic/catheptic heparin-binding fragment of fibronectin that contains the FN-C/H-I, FN-C/H-II, and CS1 cell-binding domains [23–26]. In this study, substrata coated with either the 75-kD or the 33/66-kD fragments promoted keratinocyte adhesion. This indicates that keratinocytes recognize multiple distinct cell-binding domains within fibronectin, because the 75-kD fragment does not overlap with the 33/66-kD fragments (Fig 1).

One approach to further defining putative cell-binding domains has been the use of chemically synthesized peptides. In this regard, previous reports have demonstrated that synthetic peptides containing the RGDS sequence are capable of inhibiting keratinocyte adhesion to an RGDS-containing proteolytic fragment of fibronectin [33] or to intact fibronectin [20,34]. These earlier reports suggest that keratinocyte adhesion to the 75-kD fragment of fibronectin is mediated solely via the RGD sequence present in this fragment. However, this does not define the cell-binding amino acid sequences utilized by keratinocytes adhering to the 33/66-kD fragments, because the RGD sequence is not present in these fragments [26].

Several distinct cell-binding sites within the 33/66-kD fragments of the fibronectin A and B chains have been elucidated to date. These include FN-C/H-I and FN-C/H-II, which are both present in all fibronectin isoforms, and CS1, which is only present in the fibronectin A chains [23–26]. In this study we report that synthetic peptides FN-C/H-I and FN-C/H-II promote keratinocyte adhesion, whereas peptide CS1 did not mediate keratinocyte



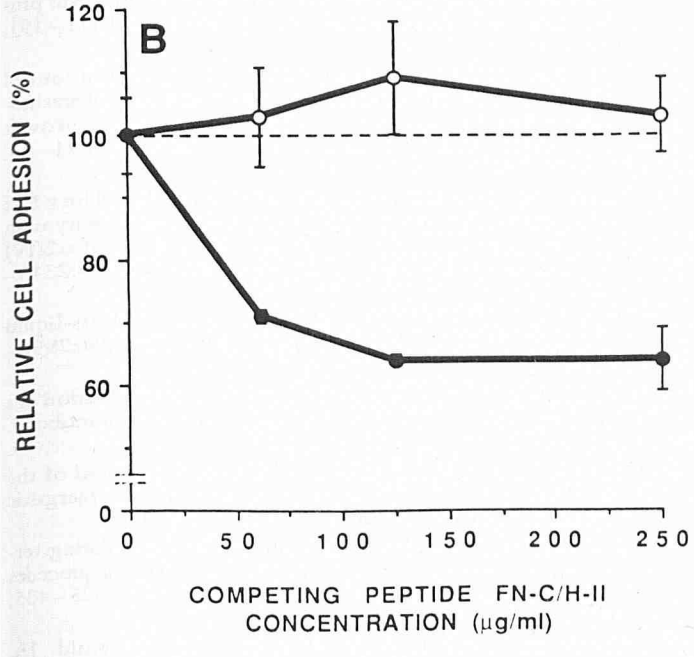


Figure 4. Effect of soluble fibronectin peptides FN-C/H-I or FN-C/H-II on competition for keratinocyte adhesion to the 33/66-kD fragments or fibronectin. Microtiter wells were coated with the 33/66-kD fragments at 2 μg/ml (●) or fibronectin at 6 μg/ml (○). Adherence of keratinocytes was then determined in the presence of increasing concentrations of soluble competing peptides FN-C/H-I *(A)* or FN-C/H-II *(B)*. Data represent the mean percentage of cells adhering, relative to control adherence in the absence of competing peptide. Determinations were done in triplicate. *Bars,* SEM.

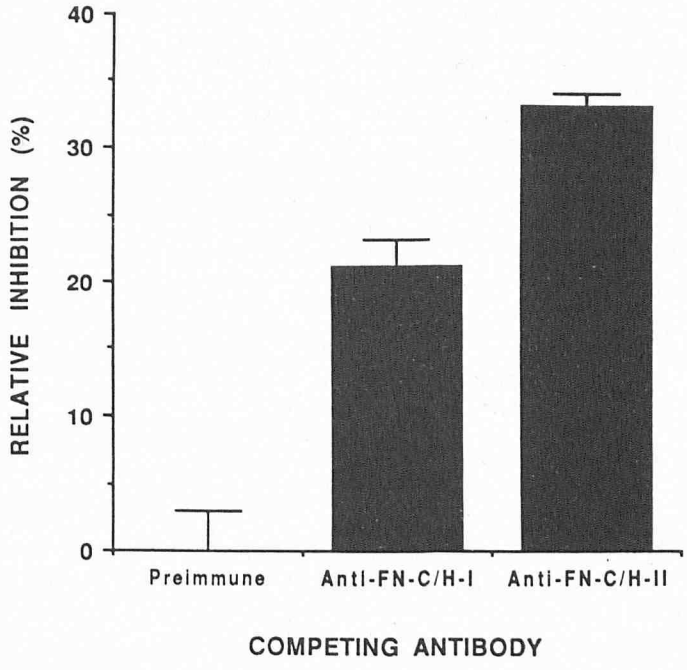


Figure 5. Effect of anti-FN-C/H-I or anti-FN-C/H-II antibodies on 33/66-kD fragment-mediated attachment of keratinocytes. Before the addition of cells, microtiter wells coated with 3 μg/ml of the 33/66-kD fragments were incubated with 500 μg/ml of pre-immune rabbit IgG, anti-FN-C/H-I IgG, or anti-FN-C/H-II IgG. Keratinocytes were allowed to attach for 30 min in continued presence of IgG, after which time unattached cells were washed away and the attached cells were quantified as described in Fig 2. Data represent the mean inhibition of cell adhesion relative to that observed in the presence of preimmune IgG. Determinations were done in triplicate. *Bars,* SEM.

adhesion. Because FN-C/H-I, FN-C/H-II, and perhaps other active sites are present in the 33/66-kD fragments, it was expected that exogenous solutions of these synthetic peptides only partially compete for cell adhesion to these fragments. Because the RGD sequence has significant keratinocyte-adhesion–promoting activity [20,33], it was also expected that exogenous solutions of synthetic peptides FN-C/H-I or FN-C/H-II would not be able to compete for cell adhesion to the intact fibronectin molecule because the RGD sequence or other sites could be utilized. Polyclonal antibodies specific for FN-C/H-I or FN-C/H-II also partially inhibited cell adhesion to the 33/66-kD fragments, further supporting the contention that these sequences are available and biologically active when presented in the context of the larger fragment.

Although it has previously been shown that synthetic peptide CS1 promotes the adhesion of a variety of cell types [23,25], substrata coated with peptide CS1 did not promote keratinocyte adherence. This indicates that certain cell-binding domains within fibronectin are cell-type specific, consistent with the results of others [23]. Furthermore, because the $\alpha 4\beta 1$ integrin has been shown to play an important role in mediating cell adhesion to peptide CS1 [34–36], keratinocytes may lack this receptor on their cell surface, or alternatively it may be present but expressed in an inactive form.

By elucidating specific keratinocyte-adhesion–promoting sequences within fibronectin, molecular mechanisms by which keratinocytes adhere to fibronectin can be further characterized. For example, monoclonal antibodies directed against the $\alpha 5$ or $\beta 1$ subunits of the $\alpha 5\beta 1$ integrin receptor, which recognizes the RGDS sequence, are capable of inhibiting keratinocyte adhesion to fibronectin [33]. With respect to keratinocyte adhesion to FN-C/H-I or FN-C/H-II, a cell-surface proteoglycan may be implicated, because both of these peptides bind the model glycosaminoglycan, heparin [26]. There is an increasing body of literature that implicates cell-surface proteoglycans in mediating cell adhesion to fibronectin as well as other extracellular matrix proteins [37–41]. Studies are currently underway to determine what role cell-surface proteogly-

cans might play in keratinocyte adhesion to fibronectin, and, in particular, to synthetic peptides FN-C/H-I and FN-C/H-II.

Further characterization of the complex molecular mechanisms by which keratinocytes adhere to fibronectin may aid in the understanding of the various contributory factors involved in normal and disease processes such as wound healing, inflammatory disease of the skin, and skin cancers.

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