Distinct Biological Consequences of Integrin αvβ3-mediated Melanoma Cell Adhesion to Fibrinogen and Its Plasmic Fragments*

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Brunhilde Felding-Habermann‡, Zaverio M. Ruggeri§, and David A. Cheresh‡
From the Departments of Immunology and Molecular and Experimental Medicine and Committee on Vascular Biology, The Scripps Research Institute, La Jolla, California 92037

Fibrinogen/fibrin and its proteolytic fragments serve as potential adhesive substrates during thrombosis, wound healing, and cancer. In this report we examined the biological response of human melanoma cells exposed to fibrinogen and its naturally occurring plasmic breakdown products that are known constituents of the tumor stroma. Plasmin treatment of fibrinogen first results in fragment X, which is characterized by removal of the COOH-terminal portion of the α chain including an RGD sequence (Aα 572–575). Further digestion leads to fragment D comprising primarily an intact COOH-terminal stretch of the γ chain containing the platelet adhesion sequence HHLGGAKQAGDV. In a sensitive adhesion assay M21 human melanoma cells utilized integrin αvβ3 to attach to all three of these ligands. However, only intact fibrinogen promoted significant cell spreading, while fragment X produced minimal spreading and fragment D promoted only adhesion. These results indicate that fibrinogen contains at least two αvβ3-dependent adhesive sites and these promote distinct biological responses of human melanoma cells. The differential functional properties of these ligands directly correlate to their relative binding affinity for purified αvβ3 as measured in a solid-phase receptor binding assay. These results provide evidence that a single integrin can promote distinct biological signals depending on the molecular nature of the ligand binding event.

EXPERIMENTAL PROCEDURES

Materials—Sepharose CL-4B, MonoQ, and Superose 12 HPLC1 columns and PD-10 gel filtration columns were from Pharmacia LKB Biotechnology Inc. Plasmin from human plasma was purchased from Boehringer Mannheim. Aprotinin, Nonidet P-40, digitonin, and phenylmethylsulfonyl fluoride were from Sigma. Fibronectin and collagen I were from Collaborative Research, and vitronectin was prepared by the method of Yatohgo et al. (26). Octyl-β-D-glucopyranoside was from Calbiochem. RGD containing and random peptides (GRGDSPK and SPGDRGK) were from E. Merck, Germany. The dodecapeptide HHLGGAKQAGDV was from Multiple Peptide Systems (San Diego, CA). The Na151 and 51Cr were obtained from Amersham.

Monoclonal Antibodies—Anti-fibrinogen monoclonal antibodies (mAbs) are as follows. mAb 11A against distinct sequences on human fibrinogen were used to characterize the generated plasmic fibrinogen fragments and to monitor their purity. mAb LI-134B29 recognizes a COOH-terminal epitope on the αa chain within Aα 566–580, including

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† Supported by E. Merck, Darmstadt, Germany. Present address: E. Merck, Preclinical Research, PhaFo IMP, P. O. Box 4119, Frankfurter Str. 289, D-6100 Darmstadt, Germany.

1 The abbreviations used are: HPLC, high-performance liquid chromatography; mAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EGTA, (ethylenebis(oxyethylenenitrito))tetra-acetic acid; BCA, bicinchoninic acid; CAFS, (cyclohexylamino)propanesulfonic acid; PBS, phosphate-buffered saline.
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ing residues RGDS at 572-575 (21). mAb LJ-155B39 is directed against an epitope within the NH2-terminal sequence Aα 87-100, including residues RGDF at 98-99 (21). mAb LJ-Z-69/8 is specific for the COOH-terminal region on the γ chain encompassing residues γ400-411, known as γ chain dodecapeptide (27). Anti-integrin antibodies D710 and D70 were raised against specific monoclonal antibody, mAb LM609 (20), and P3G2 (anti-ovβ5) (28) were used as purified immunoglobulins. mAbs P4C10 (anti-β1), P1H5 (anti-α2), P1D6 (anti-α5), and P1B5 (anti-α3) were gifts of Dr. E. A. Wayner (University of Minnesota) and were used as ascites at a final dilution of 1:500.

Cells—M21 human melanoma cells were used as a tumor cell model characterized by high ovβ3 expression. As a control cell line for integrin ovβ3 function M21-L cells were selected from the parental M21 cell line based on their lack ovβ3 expression due to nonexpression of the ov gene (22). Both cell variants exhibit identical expression and binding of a2 and a5 integrins as assessed by lectin panel integrin αlβ3, as shown by FACS analysis and immunoprecipitation tests using anti αlβ3 mAb CP8 which in control experiments reacted positively with M21-L cells that were transfected with the ovβ3 gene. The cells were cultured in 10% fetal calf serum containing RPMI medium (GIBCO) at 37 °C in a 5% CO2 atmosphere. For adhesion assays the cells were harvested by EDTA (0.02%) treatment from subconfluent cultures in the logarithmic growth phase.

Fibrinogen Purification and Plasmic Fragmentation—Fibrinogen was purified from human blood freshly collected into 0.011 M trisodium citrate; 0.1 M L-α-aminoacetic acid, and 500 KIU/ml aprotinin (final concentration). Fibrinogen purification was carried out using the method described by Kazal et al. (29). High molecular weight contaminants were removed by gel permeation chromatography using Sephacrose CL-4B. The resulting fibrinogen preparation did not contain von Willebrand Factor or fibronectin as determined by ELISA analysis using monoclonal antibodies directed to these human plasma proteins. Fibrinogen fragments were prepared by limited plasmin digestion of the purified protein in 20 mM Hepes/150 mM NaCl, pH 7.4. Reactions were stopped by adding 500 KIU aprotinin/U of plasmin. Cleavage conditions were optimized after monitoring the fragments by reduced SDS-PAGE analysis. Each well was then washed once with the same buffer. Radiolabeled ligands were added in the absence of inhibitor and incubated for 2 h at 30 °C. Following three washes with binding buffer, bound material was solubilized with boiling 5 N NaOH and radioactivity measured by γ counting. Data points were Maken intriplicates, with standard deviations typically less than 5%. M21 Cell Adhesion Assay—Cell adhesion assays were performed as previously described (36). The specific activities of labeled ligand proteins were typically in the range of 10,000 cpm/ng. Ligand binding to immobilized receptor protein was done as detailed earlier (37) using the following modifications. Receptor-coated polysyntene plates were blocked with blocking/blocking buffer (50 mM Tris, pH 7.4, 100 mM NaCl, 2 mM CaCl2, 2 mM MgCl2, 1% BSA) for 2 h at 30 °C and washed once with the same buffer. Fibrinogen fragments were analyzed using anti-αlβ3 mAb CP8 which in control experiments reacted positively with M21-L cells that were transfected with the ovβ3 gene.
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Fibrinogen

Fragment X

Fragment D

Fragment E

FIG. 1. Schematic diagram of the fibrinogen molecule and its plasmic fragments X and D. Model of intact fibrinogen with putative adhesive sites identified for β3 integrins. These include RGDS at the COOH terminus of the α chain Aα 572–575, RGDF at the NH2-terminus of the α chain Aα 95–98, and the dodecapeptide (ddp) sequence, HHLGGAKQAGDV, at the COOH terminus of the γ chain (γ400–411). The latter is present in fragment D100 but missing in fragment D80. The cleavage site for plasmin in the absence of Ca2+ leading to FD80 is indicated by an arrow.

To demonstrate the homogeneity of these fragments each was separated by SDS-PAGE under reducing conditions (Fig. 2A). The approximate molecular masses for their α, β, and γ chains are indicated in Table I. These fragments were further purified by ion-exchange and gel-permeation chromatography. The schematic composition of the fibrinogen fragments obtained and the positions of the putative β3 integrin binding sites are depicted in Fig. 1. mAbs specific for these sequences (see below) were used to confirm the absence of material containing a sequence relevant for cell attachment in any fragment that lacks this site.

To assess the potential adhesive role of the γ chain COOH-terminal dodecapeptide sequence a variant of fragment D was prepared that lacks this site. This fragment, termed fragment D80, was generated by plasmin digestion in the absence of calcium and did not react with either antibody used indicating that it lacks any of the adhesive sequences depicted in Fig. 1.

FIG. 2. Characterization of fibrinolytic fragments. A, SDS-PAGE analysis of intact fibrinogen and fragments X, D100, D80, and E. The fragments were obtained from plasmic digests in the presence (fragments X, D100, and E) or in the absence of Ca2+ (fragment D80) and were isolated by ion-exchange and gel-filtration chromatography as detailed under “Experimental Procedures.” The samples were separated on a 10–20% gradient polyacrylamide gel under reducing conditions. Protein bands were visualized by Coomassie Blue staining. Molecular weight markers are indicated at the left (kDa). B, ELISA analysis of fibrinogen (Fg) and the fibrinolytic fragments, for the detection of potential recognition sites for β3 integrins. The proteins

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**Table I**

Molecular mass ranges of the fibrinogen fragments and their subunits

Fibrinogen was subjected to plasmin cleavage, and the resulting fragments were isolated as detailed under "Experimental Procedures." The fibrinolytic fragments were analyzed by SDS-PAGE on a 10–20% gradient gel under reducing conditions. The molecular masses of the fragment subunits were determined by comparison to molecular mass standards and are given in kDa.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>α chain</th>
<th>β chain</th>
<th>γ chain</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FX</td>
<td>36</td>
<td>53</td>
<td>45</td>
<td>270</td>
</tr>
<tr>
<td>FDm₀</td>
<td>14</td>
<td>37</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>FDw</td>
<td>11</td>
<td>14</td>
<td>7</td>
<td>60</td>
</tr>
</tbody>
</table>

**Table II**

NH₂-terminal sequences of fragment Dm₀ and D₀ subunits

In order to identify the 100- and 80-kDa fibrinogen fragments as members of the fragment D family the NH₂-terminal amino acid sequences of their subunits were determined by automated Edman degradation as detailed under "Experimental Procedures."

<table>
<thead>
<tr>
<th>Fragment subunit</th>
<th>N-terminal sequence</th>
<th>N-terminal amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDm₀ α</td>
<td>DNENVVRV</td>
<td>α 105</td>
</tr>
<tr>
<td>FDm₀ β</td>
<td>VSEDLR</td>
<td>α 111</td>
</tr>
<tr>
<td>FDm₀ γ</td>
<td>DNENVV</td>
<td>β 134</td>
</tr>
<tr>
<td>FDm₀ β</td>
<td>DNENVV</td>
<td>β 134</td>
</tr>
<tr>
<td>FDm₀ γ</td>
<td>AQLNTY</td>
<td>γ 63</td>
</tr>
<tr>
<td>FDm₀ γ</td>
<td>MLEEIM</td>
<td>γ 89</td>
</tr>
</tbody>
</table>

In order to establish its identity as a member of the fragment D family, the NH₂-terminal amino acid sequences of its α, β, and γ chains were determined and compared to the results obtained for fragment Dm₀ (Table II). The α chain NH₂ termini of FDm₀ and FDw differ by 6 amino acid residues (DNTYVR). This difference accounts for the molecular mass difference of the FDm₀ and FDw α chains (approximately 1 kDa) and indicates that the COOH termini of the two α chains are identical. NH₂-terminal sequences and molecular weights for the β chains of both D fragments were also identical. The NH₂-terminal sequences of the γ chains in FDm₀ and FDw were found to be 26 amino acid residues apart, accounting for an approximate molecular mass difference of 3 kDa. According to the total molecular masses of the fragment D γ chains, a 12-kDa stretch is missing at the γ COOH terminus in FDm₀. This is in accordance with the loss of the COOH-terminal dodecapeptide sequence as indicated by results from ELISA (Fig. 2B).

**Fig. 3.** M21 melanoma cells bind to fibrinogen and its fragments X and D₁₀₀ via integrin αvβ₃. The adhesive proteins were immobilized on 48-well plates and ⁵¹Cr-labeled cells were allowed to attach as detailed under "Experimental Procedures." A, ligand dose dependency of M21 melanoma cell adhesion documented after a 90-min incubation; B, time dependency of adhesion. The cells were allowed to attach for various times to ligand-coated wells in the absence (open circles) or presence of 50 µg/ml antibody. Cell adhesion was specifically inhibited by function blocking mAb LM609 directed to the integrin αvβ₃ complex (closed circles) but not with control IgG (mAb W632 directed to human MHC class I). Other controls consisted of cell pretreated with mAbs P3G2 (anti-αvβ₃), P4C10 (anti-β₁ integrins), P1H5 (anti-α₂), P1D6 (anti-α₅), and P1B5 (anti-α₃). These were identical to that observed with mAb W632. Each point represents the mean of triplicates.
of fibrinogen (see “Experimental Procedures”). When the COOH-terminal portion of the γ chain of fragment D was cleaved to yield fragment Dm (see Fig. 1), cell adhesion was abolished indicating that this region of the molecule contains an adhesive sequence for M21 cells. In addition, fragment E, which contains the α chain NH2-terminal RGD sequence of fibrinogen (Aα 95–97) failed to support adhesion even though the plasmin cleavage site involved in the generation of this fragment is located only 5 residues away from this RGD sequence (Fig. 1).

To investigate the mechanisms of cell adhesion to these ligands M21 cells were allowed to attach to immobilized fibrinogen, fragments X and D, for various times in the presence of several function blocking anti-integrin antibodies including mAb LM609 (αβ3), mAb P5H9 (αβ5), and mAb P4C10 (various β1 integrins). As shown in Fig. 3B, cell adhesion to all of these ligands was completely blocked by mAb LM609 directed to integrin αβ3. In contrast, all other anti-integrin antibodies were without effect (not shown) even though they blocked M21 cell adhesion to their respective ligands (28). Cell adhesion to fibrinogen or fragments X and D100 was also blocked with an RGD peptide (GRGDSPK) at similar concentrations of this inhibitor (IC50 = 0.1–1.0 μM) (Fig. 4) known to inhibit αβ3-mediated cell adhesion (22) while a random peptide (SPKDGPR) had no effect on attachment to any of these adhesive ligands (data not shown). It is of interest that the γ chain peptide HLGGAKQAGDV was incapable of inhibiting cell attachment to these ligands (Fig. 4). These results provide further evidence for a role of αβ3 in these adhesion events and indicate that the same or mutually exclusive sites on the receptor likely potentiate adhesion to each of these ligands.

To characterize potential binding sites for integrin αβ3 within fibrinogen that are shared by fragments X and D100, M21 cells were allowed to attach to these ligands in the presence of a monoclonal antibody directed to the dodecapeptide sequence HLGGAKQAGDV at the COOH terminus of the γ chain (27). This site on fibrinogen has been shown to be a major adhesive sequence for platelet integrin αIIbβ3 (10–12, 15, 16, 18, 19). In this case mAb LJ-Z-69/8 inhibited M21 cell adhesion to the fragments X and D to 40 and 70%, respectively (Fig. 5). However, this antibody had no significant effect on the adhesion of M21 cells to intact fibrinogen, a finding which is consistent with the presence of the RGD site at position 572–575 of the fibrinogen α chain that was previously shown to be a major adhesive sequence for integrin αβ3 (21). These results are not likely due to simple steric hindrance of antibody binding since mAb LJ155B39 directed to the α-chain NH2-terminal RGD sequence has absolutely no effect on M21 cell adhesion to fragment X (not shown). Taken together these results suggest that integrin αβ3 is capable of promoting M21 cell adhesion to the COOH-terminal region of the fibrinogen γ chain and that this accounts for much of the adhesion to fragments X and D100.

Fibrinogen and Its Adhesive Fragments Promote Distinct Biological Signals through Integrin αβ3—To examine the biological consequences of M21 cell adhesion to fibrinogen or its fragments, these cells were allowed to attach to each of these ligands and then monitored for changes in cellular morphology (i.e. spreading). As shown in Fig. 6, A and B, M21 cells readily spread on fibrinogen in a time-dependent manner while little or no spreading could be observed on fragments X and D100, respectively. Specifically, 70% of the attached cells spread within 90–120 min on fibrinogen, whereas <20 and <5% of the cells spread on fragments X and D within this period of time. In fact, additional spreading was not observed on these ligands when monitored for longer periods (4 h). The localization of αβ3 in focal contacts and an organized actin-cytoskeleton was associated with the spread appearance of M21 cells on fibrinogen as determined by staining with rhodamine-labeled phalloidin (not shown) and as previously observed in M21 cells attached to vitronectin (22, 28). It is important to point out that this difference in spreading on each of these ligands was not due to variations in coating efficiency since radiolabeled fibrinogen, fragments D or X, were found to coat with equimolar efficiency (see “Experimental Procedures”). In addition, mAb LJ134B29 directed to the α-chain RGD sequence 572–575 specifically inhibited melanoma cell spreading on intact fibrinogen (not shown) resulting in comparable adhesion and spreading on fragment X as observed in Fig. 6.

Integrin αβ3 Binds to Fibrinogen, Fragment X, and D100 with Variable Affinity—To account for the capacity of M21 cells to adhere to but respond differently to intact fibrinogen

![Figure 4](image-url) An RGD peptide inhibits adhesion of M21 melanoma cells to fibrinogen and fragments X and D100. 65Cr-Labeled cells were plated on 48-well plates coated with 10 μg/ml of fibrinogen (open circles), fragment X (closed circles), or fragment Dm (open triangles) in the presence of increasing concentrations of soluble peptide GRGDSPK. The cells were incubated for 60 min. Controls consisted of 50 μg/ml of the fibrinogen γ chain-derived dodecapeptide HLGGAKQAGDV (inverted triangles, squares, and diamonds, indicated for cell attachment to fibrinogen, fragment X and D, respectively) and had no significant effect on cell adhesion to either of the substrates. Each point represents the mean of triplicates.

![Figure 5](image-url) mAb LJ-Z-69/8 directed to the COOH-terminal fibrinogen γ chain sequence HLGGAKQAGDV inhibits M21 cell attachment to fragments X and D100 but not to fibrinogen. Cells were allowed to attach to plates coated with 10 μg/ml of the substrates in the absence (open bars) or presence (hatched bars) of 50 μg/ml of monoclonal antibody LJ-Z-69/8 directed to the γ chain COOH-terminal dodecapeptide sequence of fibrinogen. Control IgM had no effect. Each bar represents the mean of triplicates ± S.D.
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A

30 min

90 min

Fg

Fx

FD100

B

80

Percent Cells Spread

60

40

20

0

0

30

60

90

120

150

Adhesion Time (min)

Fig. 6. Morphology of M21 cells attached to fibrinogen or fragments X and D100. M21 cells were plated on 48-well plates coated with 10 μg/ml of the substrates. After various times unbound cells were aspirated and the wells were washed twice. A, for documentation of the morphology of adherent cells, random fields were chosen from quadruplicate wells after 30 and 90 min of incubation and photographed using a Nikon inverted microscope at 100 x magnification. B, for the quantitation of cell spreading, spread cells and total attached cells were counted in four random areas from each of two wells per time point. The data presented are means ± S.D. of these counts.

versus fragments X and D100, we tested each of these ligands for their ability to interact with purified αvβ3 in a solid-phase receptor binding assay. As shown in Fig. 7A, all three ligands bound to this receptor in a specific (RGD-dependent) dose-dependent and saturable manner. However, the concentration required for half-maximal binding varied considerably, demonstrating values of 2 × 10^{-9}, 1.5 × 10^{-8}, and 1.5 × 10^{-7} M for fibrinogen, fragment X, and D100, respectively. These results indicate that the relative affinity of αvβ3 for fibrinogen is about 10-fold higher than that for fragment X and 100-fold higher than for fragment D100. While these ligands clearly bound to αvβ3 with different relative affinities it was not possible to determine an actual dissociation rate constant since integrin αvβ3 bound fibrinogen or fragments X and D100 in a nondissociable manner. This is documented in Fig. 7B and was previously demonstrated for other ligands capable of binding to this receptor (36). Specifically, once allowed to bind αvβ3, these ligands could not be dissociated by up to 100-fold excess soluble ligand (Fig. 7B). However, the relative affinities of these ligands for αvβ3, as measured by their half-maximal binding concentration, directly correlate with their ability to promote spreading of attached M21 cells. Thus, these cells readily attach and spread on fibrinogen which serves as the highest affinity ligand for αvβ3 tested. Fragment X, which has moderate affinity, promotes partial spreading, while fragment D100, having the lowest affinity, fails to promote any significant spreading of these cells. In support of these results are data demonstrating that vitronectin, which binds to αvβ3 with a higher affinity than that observed with
fibrinogen in this assay (38), promotes the most complete spreading of M21 cells (28). Taken together these results demonstrate that a given integrin can promote distinct cell biological signals which correlates with the relative affinity of the integrin-ligand binding event.

**Discussion**

The interaction of tumor cells with fibrinogen and its naturally occurring breakdown products is of importance during tumor development and metastasis. Solid tumors are characterized by the presence of stroma that accounts for up to 80% of the tumor mass (9). This stromal material is primarily comprised of fibrin that is generated by extravascular clotting which in turn, is triggered by the release of potent tumor-associated vascular permeability factors (39, 40). Initial stages of metastasis likely depend on the adherence and eventual degradation of fibrinogen/fibrin within the tumor stroma by tumor cell-derived plasminogen activators (24). The resulting fibrin/fibrinogen fragments may then serve as further adhesive substrates for tumor cell migration and/or invasion. Therefore, this study was designed to characterize the molecular basis of tumor cell adhesion to intact fibrinogen and its natural plasmonic proteolytic fragments.

Fibrinogen is an adhesive molecule for various cell types including platelets (10-19), endothelial cells (20, 21), as well as tumor cells (22). Cell adhesion studies have revealed at least three putative adhesive sequences within the fibrinogen molecule (Fig. 1). These include two RGD sequences at the NH2 and COOH termini of the α chain and a site at the COOH terminus of the γ chain (10-21). By cleaving fibrinogen into fragment X one selectively eliminates the α chain COOH-terminal RGD sequence (Aα 572-575). Previous studies demonstrated that this cleavage resulted in a significant reduction in endothelial cell adhesion mediated by integrin αvβ3 (21). We now provide evidence that although M21 human melanoma cells attach to fragment X they spread rather poorly. In contrast these cells attached and fully spread on intact fibrinogen (this study) or vitronectin (28) in a manner dependent on integrin αvβ3.

The cell adhesion assay used in this case was very sensitive and thus was able to measure cell adhesion events that did not depend on cell spreading. Specifically, cells were allowed to attach to relatively large (10 mm) wells thereby minimizing the shear forces observed in smaller microtiter wells during subsequent washing steps. This assay also allowed us to measure specific adhesion to fibrinogen fragment D100 which contains neither RGD sequence but maintains an intact COOH-terminal γ chain. Two lines of evidence were provided which indicate that M21 cells attach to the COOH terminus of the γ chain of fragment D50. First, a mAb directed to this sequence significantly inhibited M21 cell attachment to fragment D but had no effect on cell attachment to intact fibrinogen. Second, further cleavage of fragment D to D50 resulted in a loss of the γ chain COOH terminus eliminated all detectable M21 cell adhesion. While a soluble RGD peptide could block adhesion of M21 cells to each of these ligands the soluble γ chain peptide HHLGGAKQAGDV had no effect. This is likely due to the comparatively low affinity of the soluble form of this peptide for αvβ3 as reported previously (37).

That αvβ3 mediates adhesion of M21 cells to each of these ligands but promotes different biological responses indicates that this integrin is capable of inducing distinct cell signals depending on the structural features of the ligand. These findings could not be explained by a difference in coating efficiency of these ligands. In addition, regardless of the number of fragment D100 molecules bound per well, we were never able to observe measurable cell spreading even after several hours.

To account for the distinct biological responses of this αvβ3-mediated adhesion event, purified αvβ3 was allowed to bind each of these ligands in a solid-phase receptor assay. Results from these studies revealed that all three ligands bound αvβ3 in a dose-dependent and saturable manner but with varying affinity. Fibrinogen bound with highest relative affinity followed by fragment X, and D100, respectively. These results directly correlated with the ability of these substrates to promote αvβ3-mediated spreading of M21 cells. Therefore, it appears that fibrinogen contains at least two sites that promote M21 cell adhesion to fibrinogen through integrin αvβ3, and these sites appear to promote distinct biological responsiveness. This finding provides the first evidence that a given integrin can promote distinct cell signals. Moreover, these results suggest that integrin-mediated signalling events may depend on the relative affinity of the integrin-ligand interaction. It is important to note that while each of these ligands bound specifically to αvβ3 they failed to achieve a measurable dissociation from this receptor. This result is consistent with previous findings from our laboratory demonstrating that integrin αvβ3 binds various ligands in a non-dissociable manner (36) thereby making it impossible to determine the dissociation rate constant (Kd). It is conceivable that some of the adhesive differences between intact fibrinogen and fragment X may be due the ability of plasmin to cleave β3 chain 1-42 since this region peptide from fibrin has been shown to be adhesive for endothelial cells and platelets (42, 43). In fact, such a cleavage event may explain, in part, why endothelial cells appear less adhesive to fragment X (21) than melanoma cells as reported in this report.

While M21 cells attached to fibrinogen fragments X and D they failed to recognize fragment E. This fragment is characterized by the presence of the NH2-terminal RGD sequence on the α chain (Aα 95-98) but lacks the COOH-terminal RGD and the γ chain COOH terminus. In fact, the α-chain cleavage site of this fragment E preparation was within 5 amino acid residues of the RGD sequence yet this fragment was still unable to promote cell adhesion. These data are consistent with our previous results indicating that an antibody to the α-chain NH2-terminal RGD sequence could not affect cell adhesion to fibrinogen (21). This was of interest since a synthetic peptide of 17 residues derived from this domain of fibrinogen could support cell adhesion. Therefore, this sequence, within the macromolecule, may be conformationally incompatible to be recognized by integrin αvβ3, an event which is likely due to flanking sequences NH2-terminal to the RGD site. In fact, structural studies have revealed that this region of fibrinogen is α-helical (41) indicating that an RGD sequence within such a secondary structure is incapable of supporting cell adhesion.

The fact that fibrinogen promotes M21 cell adhesion and spreading, while its proteolytic fragments X and D support adhesion of these cells without spreading, suggests that during fibrinolysis cells may acquire distinct signals depending on the local availability and concentration of these fragments. In addition, a tumor cell that can effectively attach to the fibrin stroma and interact with its resulting proteolytic fragments may have the potential to escape the primary tumor site and to metastasize. Thus, integrin αvβ3 may indeed be an important tumor cell surface receptor. In fact, recent studies reveal that this integrin is preferentially expressed on invasive melanoma tumor tissue since it is not found on noninvasive primary melanoma, nevi, or normal melanocytes.
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(44). This indicates that the expression of avß3 correlates with the most malignant phenotype of human melanoma in situ. That this receptor can interact with fibrinogen and its natural breakdown products suggests a potentially significant cell adhesion mechanism that may contribute toward the metastatic phenotype of human melanoma cells.

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