Plasmin cleaves fibrin at or near sites involved in platelet recognition and may modulate platelet adhesion and spreading. Using an in vitro system, we have characterized the effects of limited plasmic degradation of polymerized fibrin on platelet adhesion and spreading. As shown by scanning electron microscopy, exposure to plasmin changed the tight fibrillar fibrin surface to a less dense structure with irregular and broken fibers. There was a gradient of proteolytic degradation through the fibrin clot as shown by sodium dodecyl sulfate polyacrylamide gel electrophoresis with the most extensive degradation at the surface. Plasmic degradation resulted in a rapid and progressive decrease in platelet adhesion. Plasmin exposure for 5 minutes resulted in only 6% solubilization of the fibrin but a 56% decrease in platelet adhesion. After 30 minutes of plasmin exposure, spreading of adherent platelets on fibrin also decreased sharply to a minimum of 35% of baseline. Inhibition experiments with specific monoclonal antibodies (MoAbs) indicated that platelet adhesion to undegraded fibrin involved residues within the sequence 566 through 580 of the α chain (including the RGDS site), the carboxyl terminal dodecapeptide of the γ chain, and the amino terminus of the β chain. MoAb 7E3, reactive with αmβ3, inhibited platelet adhesion to fibrinogen by 90% ± 5%, and to desA fibrin, prepared with Reptilase (American Diagnostica, Greenwich, CT), by 94% ± 6%, whereas inhibition of adhesion to undegraded desAB fibrin was significantly less (48% ± 8%, P < 0.01). The addition of 7E3 to MoAb T2G1, reactive with β15-21, significantly increased inhibition to desAB fibrin to 69% ± 6% (P < 0.05), suggesting that the newly exposed amino terminus of the β chain contributes to platelet adhesion. The results show that plasmin exposure of fibrin markedly decreases platelet adhesion and spreading, suggesting that plasmin degradation may play a role in modulating cellular responses to fibrin.

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The structural changes that accompany the conversion of fibrinogen to fibrin may form the basis of the fibrin-specific platelet responses. This conversion is initiated by thrombin, which cleaves fibrinopeptide A (FPA) and fibrinopeptide B (FPB), exposing polymerization sites involving the new amino termini of the α and β chains. FPB cleavage also exposes a site involving the amino terminus of the β chain that stimulates vWF release from Weibel-Palade bodies and interacts with a nonintegrm endothelial cell protein that may be a receptor. Spreading of endothelial cells and platelets on fibrin is also mediated by this site.

The fibrinolytic system is activated in response to fibrin formation, and plasmin action may also play a role in platelet interactions with fibrinogen and fibrin by cleaving or altering sites involved in platelet responses. In the present study, we have developed a system for examining the effects of plasmic degradation on platelet adhesion and spreading of fibrin. Monoclonal antibodies (MoAbs) reactive with specific adhesive sites on the fibrin molecule have been used in conjunction with characterization of plasmin-induced polypeptide chain cleavages to identify fibrin structural modifications associated with alterations in platelet response.

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MATERIALS AND METHODS

Fibrinogen and fibrinogen-cleaving enzymes. Human fibrinogen (grade L) was purchased from Kabi Vitrum (Franklin, OH), and as described previously.23 Factor XIII inhibition was confirmed by showing the absence of cross-linked y chains after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the reduced polypeptide chains of fibrin that were prepared after clotting. Rabbit polyclonal IgG antihuman fibrinectin (Organon Teknika, Durham, NC) was bound. The fibronectin concentration of depleted rabbit polyclonal IgG antihuman fibronectin (Organon Teknika, Durham, NC) was bound. The fibronectin concentration of depleted fibrin was measured by enzyme-linked immunosorbent assay (American Bioproducts, Parsippany, NJ) and represented less than 0.02% of total protein. Fibrinogen was radiolabeled with 114 Cu as described previously.23 Human thrombin (3.25 U/mg), was obtained from Calbiochem-Behring Corp (La Jolla, CA), and reptilase snake venom was from American Diagnostica (Greenwich, CT).

Platelet preparation. After obtaining informed consent, blood was obtained by venipuncture from volunteers who had not taken any medications in the previous two weeks. Platelets were prepared from freshly drawn blood anticoagulated with a 0.1 volume of 0.13 mol/L sodium citrate, pH 5.0. After centrifugation at 160g for 15 minutes, the platelet-rich plasma (PRP) was removed, and the pellet was gently resuspended in HEPES-Tyrode’s-EDTA buffer (0.13 mol/L NaCl, 2.6 mmol/L KCl, 12 mmol/L NaHCO3, 0.42 mmol/L Na2HP04, 0.55 mmol/L dextrose, 5 mmol/L HEPES, 2 mmol/L Na2EDTA, 0.35% [wt/vol] bovine serum albumin [BSA], pH 6.8) and washed 3 times. Washed platelets were then resuspended in HEPES-Tyrode’s-sodium-albumin buffer (0.13 mol/L NaCl, 2.6 mmol/L KCl, 12 mmol/L NaHCO3, 0.42 mmol/L Na2HP04, 0.55 mmol/L dextrose, 5 mmol/L HEPES, 1% [wt/vol] BSA, 2 mmol/L CaCl2, pH 7.35) and used within 2 hours of preparation. Washed platelets showed irreversible aggregation when stimulated with 20 μmol/L ADP. After initial preparation, the platelet pellet was resuspended in 2 mL of 50 mmol/L Tris, 0.15 mol/L NaCl, pH 7.4 (TBS) supplemented with 5 mmol/L dextrose and was radiolabeled with 51Cr by incubation for at least 30 minutes at 25°C with 100 μCi Na2Cr2O7 (Amersham, Arlington Heights, IL). The platelet suspension was then washed 3 times with TBS with 5 mmol/L dextrose and resuspended in TBS containing 5 mmol/L dextrose, 2 mmol/L CaCl2, 2 mmol/L MgCl2, and 1% (wt/vol) BSA. The platelet count was adjusted to 10^6/mL before use in the adhesion assay. Washed 51Cr-radiolabeled platelets showed irreversible aggregation when stimulated with 20 μmol/L ADP. Previous studies in our laboratory24 showed release of only 6% ± 3% of 51Cr in 3-5 hours in the supernatants from 51Cr-labeled platelets prepared with this method.

Platelet adhesion assay. The platelet adhesion assay used for these experiments is similar to that described previously.22 Factor XIII-inactivated and fibronectin-depleted fibrinogen was dissolved in McCoy’s 5A Medium (TCN/Flow Laboratories, McLean, VA). Fibrinogen was prepared by incubation at 25°C of 0.4 mL of 3 mg/mL fibrinogen with 2.5 U/mL thrombin in the presence of trace amounts of fibrinogen for 30 minutes in 1-mL wells of 24-well cell culture plates (Corning Glass Works, Corning, NY), resulting in 2-mm-thick fibrin clots. The concentration of 3 mg/mL of fibrinogen was chosen because it is similar to the normal plasma concentration. Plasmin (provided at 10.2 Committee on Thrombolytic Agents [CTA] U/mL by Dr D. Aaronson, Bureau of Biologics Standards, Bethesda, Maryland) was added to a final concentration of 0.5 U/mL in McCoy’s buffer and incubated at 37°C for 5 to 30 minutes. The reaction was terminated by addition of the enzyme inhibitor D-phenylalananyl-L-prolyl-L-arginyl-chloromethyl ketone (PPACK; Bachem, Torrance, CA) at a final concentration of 10 μg/mL. The radioactivity of the supernatant was measured, and the percent degradation of total fibrin was determined. After plasmin digestion and PPACK incubation, an aliquot of 0.4 mL of radiolabeled platelets was added to each well and incubated for 1 to 2 hours at 37°C without shaking. The unbonded platelets were removed by aspiration followed by 3 washings. Adherent platelets were then removed by trypsin-EDTA, and the percentage of adherent platelets was calculated. After correcting for the nonspecific adhesion on 60 mg/mL BSA, the percentage of baseline-specific adhesion was determined.

Platelet spreading assay. The spreading assay is also similar to that described previously.22 Surfaces coated with fibrin were prepared using LabTek chamber 8-well slides (Nunc, Naperville, IL). Briefly, fibrin coating was performed by adding 0.25 mL of 3 mg/mL fibrinogen in buffer and 0.5 U/mL thrombin at 25°C, followed by aspiration of excess solution before solid clot formation, and incubation for 1 hour leaving a residual fibrin coating on the slides. Slides were then incubated with 0.25 mL of 0.5 CTA U/mL plasmin in McCoy’s buffer for 5 to 30 minutes at 37°C, followed by 0.25 mL of 10 μg/mL PPACK to inactivate residual enzymes and then washed 3 times in McCoy’s buffer. Aliquots of 0.25 mL of washed platelet suspension were then added to the wells and incubated for 1 to 2 hours at 37°C. The nonadherent platelets were removed by aspiration, and adherent platelets were fixed for 20 minutes in 3.7% formaldehyde in phosphate-buffered saline (PBS), permeabilized for 20 minutes in 0.5% Triton X-100 in PBS, and stained with goat antiplatelet αIIbβ3 polyclonal antibody (kindly provided by Dr Mark Ginsberg, Scripps Clinical and Research Foundation, La Jolla, CA), followed by rhodamine-labeled rabbit anti-goat antibody (Cappel Research Products, Durham, NC) as described.26 Coverslips were then mounted onto glass slides, and spreading was evaluated by fluorescence microscopy. A minimum of 100 platelets was examined by an observer blinded to the plasmogen digestion time, and platelets were scored as spread if they showed a flattened morphology, if they had greater than two distinct pseudopods, or if they showed both features. Spheroid or oval-shaped platelets with no pseudopods and those with only one or two pseudopods were classified as unspread. Spreading was quantitated as the percentage of the spread platelets on duplicate slides.

Studies with MoAbs. MoAbs T2G1, reactive with residues β15-21,27 was kindly provided by B. Kudryk (The New York Blood Center, New York, NY). MoAbs LJ134B29 (IGG; reactive with residues Aα566-580), LJ155B16 (reactive with residues Aα87-100; IGG), and LIZ69-8 (reactive with γ400-411; IGM) were kindly provided by Dr Z.M. Ruggeri (Research Institute of Scripps Clinic, La Jolla, CA).28 MoAbs 7E3, reactive with platelet αIIbβ3 was kindly provided by Dr B.S. Coller (State University of New York, Stony Brook, NY). For inhibition experiments, MoAbs (50 μg/mL) were incubated with fibrin for 30 minutes at 37°C before radiolabeled platelets were added. MoAbs 7E3 (50 μg/mL) was incubated with radiolabeled platelets before addition to the fibrin. MoAbs were 125I-radiolabeled using the lactoperoxidase method, and unbound was separated by gel filtration. Radiolabeled MoAbs were diluted to desired concentrations with 50 mmol/L Tris, 0.15 mol/L NaCl, 5 mmol/L EDTA buffer (TNE), pH 7.4, containing 1 mg/mL BSA and were incubated with fibrin for 1 hour to allow binding. After washing 5 times with TNE buffer containing 0.05% (vol/vol) Tween 20, bound antibody was removed by incubation with trypsin-EDTA. Bound antibody was calculated after subtracting nonspecific binding of 7E3 to fibrin clots.
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Gel electrophoresis. The residual plasmin-digested fibrin clot was sliced serially from top to bottom resulting in layers of 200 μ. Each was dissolved in an SDS-containing diluent and electrophoresed under reducing conditions using 7% polyacrylamide gels as described. The gels were stained with Coomassie Blue.

Scanning electron microscopy. The fibrin sample was fixed in 1% glutaraldehyde and Sorenson’s buffer for 1 hour, rinsed with buffer, then postfixed in 1% osmium tetroxide for 1 hour, and rinsed again. They were then dehydrated in a gradient ethanol series, critical-point–dried, coated with gold, and observed with a JEOL T330A scanning electron microscope (JEOL, Tokyo, Japan).

Statistical analysis. Comparison of means was performed using the two-tailed Student’s t-test.

RESULTS

Before plasmin exposure, the fibrin surface had a uniform appearance by scanning electron microscopy with a fibrillar structure evident in some areas. Adherent platelets were mostly spread, showing extension of pseudopods and varying degrees of flattening (Fig 1A). Exposure to plasmin caused a marked change in the appearance of the fibrin surface. Overall, the surface was less dense, with open spaces of varying size between fibers. There appeared to be breaks in fibers with numerous fiber ends visible. The plasmin-digested surface viewed at higher magnification showed that the width of individual fibers was variable, and they displayed an irregular appearance (not shown). Many fewer platelets were visible, and those that were present had a round appearance with no flattening and few surface projections (Fig 1B).

After plasmin exposure, the residual, partly degraded fibrin was sectioned in sequential layers from top to bottom, and the polypeptide chain composition was characterized by SDS-PAGE (Fig 2). In comparison with fibrinogen (Fig 2, lane 1) the undegraded fibrin (Fig 2, lane 2) showed increased migration of the α and β chains, indicative of cleavage of FPA and FPB. Sections taken from the top, middle, and bottom of the fibrin clot after 30 minutes of exposure to plasmin showed varying degrees of polypeptide chain degradation. Fibrin removed from the top surface, which was exposed to plasmin (Fig 2, lane 3), showed extensive degradation of the α chains with none of the highest molecular weight forms present. Low molecular weight peptides migrating near the bottom of the gel (Fig 2, arrow) represent degradation products of the α chain. A prominent β chain was present, but small amounts of the β-chain derivative with a molecular weight of 43 kDa could be observed. Sections taken from the middle (Fig 2, lane 4) and bottom (Fig 2, lane 5) showed the same changes but a smaller proportion of total chains were affected. α Chains at the bottom of the fibrin clot showed evidence of degradation in comparison with the intact fibrin (Fig 2, lane 2), but some of the largest forms were still present. The section taken from the middle portion of the fibrin showed degradation of the α and β chains, intermediate in extent, between the top and bottom sections. These findings indicate that plasmin degradation was not uniform throughout the clot but was greatest at the top, with progressively less proteolysis in deeper areas.

For experiments with platelet interaction, we selected a low concentration of plasmin that left most of the fibrin intact even after 30 minutes (Fig 3). After 5 minutes plasmin digestion, 6.4% ± 0.3% of the fibrin had been solubilized, and this increased progressively to 17.9% ± 2.0% after 30 minutes. The adhesion of platelets to undegraded fibrin was 13.0% ± 2.2%, which represents 100% baseline adhesion in Fig 3. Exposure of the fibrin surface to plasmin resulted in a dramatic decrease in platelet adhesion. Platelet adhesion after 5 minutes was decreased by 56% (P < .025), whereas there was only 6.4% solubilization of the fibrin. Adhesion reached a minimum of 16% of baseline on fibrin that had been exposed to plasmin for 20 minutes, at which time there was 13% fibrinolysis.

Spreading of platelets adherent to fibrin also showed a similar decrement after plasmin exposure (Fig 4). The baseline spreading on undegraded fibrin was 79% ± 7%. Exposure of the fibrin surface to plasmin for 5 minutes resulted in a 28% decrease in spreading (P < .005), with further, progressive decreases to 35% of baseline after 30 minutes plasmin exposure.

MoAbs were used to determine the role of specific sites on the fibrin molecule in mediating adhesion before and after exposure of fibrin to plasmin (Table 1). A control antibody (12E4) reactive with factor VIII resulted in no decrement in adhesion. Similarly, MoAb LJ155B16, reactive with residues Aα87-100 including the RGDF site, did not significantly inhibit platelet adhesion to either undegraded or plasmin-treated fibrin. The greatest decrease in adhesion by fibrin antibodies was caused by LJ134B29 reactive with an epitope within residues 566-580 of the Aα chain and including the RGDS site. After incubation with undegraded fibrin, this MoAb reduced adhesion by 43% (P < .005), and it also decreased adhesion to plasmin-treated fibrin by 28% (P < .025). An antibody reactive with the γ chain carboxy terminal dodcapeptide (LJZ69-8) inhibited adhesion to undegraded fibrin by 25% (P < .05), and a similar response was observed using T2G1, reactive with the amino terminus of the β chain, which reduced adhesion by 20% (P < .05). Both LJZ69-8 and T2G1 inhibited adhesion to plasmin-degraded fibrin slightly, but this was not statistically significant. The MoAbs that inhibited adhesion were radiolabeled and incubated with fibrin before and after plasmin exposure to determine their degree of binding. At a concentration of 50 μg/mL, there was binding of 12.4% ± 1.5% of LJ134B29, of 7.1% ± 1.1% of LJZ69-8, and of 9.6% ± 1.7% of T2G1 to undegraded fibrin (Fig 5), indicating that sufficient antibody was present to saturate available binding sites on the exposed fibrin surface. After exposure to plasmin, binding of LJ134B29 decreased significantly by 46%, and binding of LJZ69-8 decreased by 51%, consistent with loss of these epitopes on the exposed plasmin-degraded surface. T2G1 binding also decreased by 27%, but this was not statistically significant.

Platelets were incubated with MoAb 7E3 before exposure to the fibrin surface to determine the role of the integrin α₉β₃ in platelet adhesion to the fibrin surface. 7E3 inhibited adhesion to a fibrinogen-coated surface by 90% ± 5% and to undegraded desA fibrin, prepared with reptilase to cleave
fibrinopeptide A only, by 94% ± 6% (Fig 6). Inhibition of adhesion to desAB fibrin, prepared with thrombin, was significantly less (48% ± 8%, \( P < .01 \)). Addition of T2G1 significantly increased inhibition of adhesion to desAB fibrin to 69% ± 6% \( (P < .025) \), suggesting that the amino terminus of the \( \beta \) chain contributes to platelet adhesion.

DISCUSSION

Physiologically, platelets may be exposed to fibrin in several forms including soluble fibrin in plasma, polymerizing protofibrils, and fully polymerized fibrin. We have focused on the latter, examining platelet adhesion and spreading on a surface of preformed, polymerized fibrin and on fibrin after limited plasmic degradation. The results show that the exposure of fibrin to plasmin results in a substantial and rapid decrease in platelet adhesion and spreading. Electron microscopic observation shows a change from a homogeneous surface to a more irregular and open structure after plasmin treatment, which is consistent with dissolution of exposed fibrin fibers leaving a surface with a lower fibrin density. The evident breakage of fibers after plasmin exposure is further evidence of proteolytic action on individual fibrinopeptide A only, by 94% ± 6% (Fig 6). Inhibition of adhesion to desAB fibrin, prepared with thrombin, was significantly less (48% ± 8%, \( P < .01 \)). Addition of T2G1 significantly increased inhibition of adhesion to desAB fibrin to 69% ± 6% \( (P < .025) \), suggesting that the amino terminus of the \( \beta \) chain contributes to platelet adhesion.

DISCUSSION

Physiologically, platelets may be exposed to fibrin in several forms including soluble fibrin in plasma, polymerizing protofibrils, and fully polymerized fibrin. We have focused on the latter, examining platelet adhesion and spreading on a surface of preformed, polymerized fibrin and on fibrin after limited plasmic degradation. The results show that the exposure of fibrin to plasmin results in a substantial and rapid decrease in platelet adhesion and spreading. Electron microscopic observation shows a change from a homogeneous surface to a more irregular and open structure after plasmin treatment, which is consistent with dissolution of exposed fibrin fibers leaving a surface with a lower fibrin density. The evident breakage of fibers after plasmin exposure is further evidence of proteolytic action on individual
FIBRIN DEGRADATION AND PLATELET ADHESION

Fig 2. SDS-PAGE of fibrinogen and fibrin before and after plasmin exposure. Samples of 10 μg were electrophoresed on SDS 7% polyacrylamide gels after disulfide bond reduction and were stained with Coomassie Blue. The location of polypeptide chains is indicated. Lane 1, fibrinogen; lane 2, fibrin showing increased migration of the α and β chains resulting from cleavage of FPA and FPB; lane 3, the top layer (approximately 0.2 mm) sliced from the fibrin after 30 minutes of plasmin digestion showed nearly complete loss of the α chains and the presence of smaller peptides and β/chain; lane 4, fibrin in a slice approximately half-way from the top surface to the bottom showed a small amount of intact α chain and less prominent β/chain and α-chain derivatives; and lane 5, a section from the bottom of the fibrin clot had the least degradation with some intact α chain.

Similar irregularities have been found by Braaten et al who examined plasmin degradation of PRP clots formed in vitro. Our findings also show a gradient of plasmin action within the fibrin with the greatest degradation at the fibrin surface and with less in deeper layers as shown by SDS-PAGE. This is compatible with previous findings that used another in vitro model of plasmin degradation which showed “outside in” solubilization. As shown by scanning electron microscopy, the interaction of platelet with the fibrin was limited to the surface, with no evidence of platelet migration into the fibrin network. These findings suggest that the basis for the change in platelet response to fibrin after plasmin exposure depends on the availability of adhesive sites on the exposed surface.

Because we could not directly determine the structure of the surface-exposed fibrin, MoAbs were used to identify specific sites involved in platelet-fibrin interactions. The greatest inhibition of adhesion to undegraded fibrin was observed with MoAb LJ134B29, which is reactive with an epitope within the sequence Aα566-580 including the RGDS sequence at Aα 572-575, a site which binds within residues 109-171 of β3 subunit of αthβ3. The importance of the Aα-chain RGDS site in mediating platelet adhesion is compatible with the report of Savage and Ruggeri who examined adhesion of unstimulated platelets to immobilized plasmin-degraded fibrinogen. Adhesion to fragment X, which lacks the carboxyl terminal Aα chain and the RGDS site, was decreased compared with adhesion to intact fibrinogen. The relative importance of specific fibrin-adhesive sites in the platelet-fibrin interaction was also investigated by Hantgan et al using synthetic peptides in a whole blood perfusion system. They found greater inhibition of platelet adhesion to fibrin with an RGDS peptide than with the γ dodecapeptide, although inhibition was only partial with either. We found that MoAb LJZ69-8, which is reactive with γ400-411, inhibited adhesion to undegraded fibrin significantly but to a lesser degree than did the antibody to RGDS, suggesting that the γ-chain carboxyl terminus plays a secondary role in supporting platelet adhesion to fibrin. This is also consistent with a previous report showing a low level of adhesion supported by fibrinogen fragment D, which contains γ400-411 but lacks RGDS.

Fig 3. Platelet adhesion to intact and plasmin exposed fibrin. The fibrin clot was exposed to 0.5 U/mL plasmin. The percentage of remaining fibrin was calculated from the solubilization of radiolabeled fibrin after plasmin exposure. The baseline platelet adhesion to undegraded fibrin (time 0) was 13.0% ± 2.2% and is represented as 100%. Values represent the mean of 3 to 4 determinations ± SE. The reduction in adhesion by 5 minutes was statistically significant (P < .025). For each determination, the adhesion to BSA, representing nonspecific adhesion, was subtracted. The mean adhesion to BSA was 3.6% ± 0.5%.
Fig 4. Platelet spreading on fibrin before and after plasmin exposure. Spreading was quantitated as the percentage of adherent platelets that showed more than two distinct pseudopods, a flattened morphology, or both features after staining with a polyclonal antibody and examination by fluorescence microscopy. Time represents minutes of exposure of the fibrin surface to plasmin. The baseline platelet spreading before plasmin exposure was 79% ± 7%. The degree of spreading was significantly reduced at 5 minutes and at later times (P < .005).

The secondary role of 7400-41 in mediating platelet adhesion to fibrin is in contrast to its importance in binding of soluble fibrinogen to platelets and supporting aggregation. Evidence with recombinant human fibrinogen5 and purified rat plasma fibrinogen variants6 indicates that the y dodecapeptide is essential for platelet aggregation, with RGDS playing a secondary role. The molecular basis for this difference in the role of these two sites is not clear but may be because of alteration in affinity of the receptor for the soluble as compared with insoluble ligand or because of a change in the fibrin(ogen) conformation resulting from surface immobilization or polymerization. The polymerization site that mediates binding of the central and lateral domains during fibrin formation is in proximity to the y-chain dodecapeptide,17 providing a possible structural basis for its altered conformation or availability in polymerized fibrin as compared with fibrinogen.

Table 1. Inhibition of Adhesion of Platelets to Intact and Plasmin-Degraded Fibrin by MoAbs

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Adhesion to Fibrin*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Undegraded</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>100 ± 4</td>
</tr>
<tr>
<td>LJ134B29</td>
<td>α566-580 (RGDS)</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>LJZ69-8</td>
<td>γ400–411</td>
<td>75 ± 65</td>
</tr>
<tr>
<td>T2G1</td>
<td>β15–21</td>
<td>80 ± 55</td>
</tr>
<tr>
<td>LJ155B16</td>
<td>α87–100 (RGDF)</td>
<td>90 ± 8</td>
</tr>
<tr>
<td>7E3</td>
<td>α87β13</td>
<td>48 ± 8</td>
</tr>
<tr>
<td>12E4</td>
<td>Factor VIII</td>
<td>100 ± 12</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.
* Mean ± SEM in relation to 100% baseline adhesion with no antibody and undegraded fibrin.
† Five minutes of plasmin exposure.
‡ P < .005 compared with no antibody.
§ P < .05 compared with no antibody.

Fig 5. Binding of MoAbs to fibrin before and after plasmin exposure. The MoAbs LJ134B29 (anti-α566-580), LJZ69-8 (anti-γ400-411) and T2G1 (anti-β15-42) were incubated at a concentration of 50 μg/mL in 400 μL with either undegraded fibrin (0) or fibrin that had been exposed to plasmin for either 5 minutes or 30 minutes. After 50 minutes unbound MoAb was removed; the surface was washed 5 times with TNE buffer containing 0.05% Tween 20; and the percentage of antibody was determined by counting of supernatant and washes. Statistical significance is indicated with an asterisk (*), P < .025 in comparison with time 0.

Fig 6. Inhibition of platelet adhesion to fibrin. Radiolabeled platelets (0.4 mL at 10^7/mL) were incubated with surfaces coated with fibrinogen, desAB fibrin (prepared with thrombin), or desA fibrin (prepared with reptilase). The percentage of inhibition of adhesion is shown by the addition of MoAb 7E3 or 7E3 plus T2G1 compared with the absence of antibody. Inhibition of adhesion to desAB fibrin was significantly less than to fibrinogen or desA fibrin (P < .01). The addition of MoAb T2G1 to 7E3 significantly increased the inhibition of adhesion (P < .025).
by MAb T2G1, reactive with β15-21 at the amino terminus of the β chain, suggests that it may play a previously unidentified role in mediating platelet adhesion. We have shown that the amino terminus of the β chain, including residues β15-42, is required for spreading of both platelets and endothelial cells on fibrin. A surface receptor for β15-42 has been identified on endothelial cells, but no information is available regarding its presence on platelets. The β15-42 site may either contribute to the initial platelet-fibrin attachment or reinforce attachment by promoting spreading, thereby strengthening primary adhesion so that detachment during washing is reduced. The data do not provide a basis for choosing between these possibilities. However, the experiments were performed in a static system with low shear forces, and this suggests that the effect of β15-42 involves initial attachment. The possible role in adhesion of β15-42 and other sites not interacting with the integrin αIbβ3 is further suggested by results with MoAb 7E3, reactive with αIbβ3. Whereas this antibody inhibited adhesion to fibrinogen by over 90%, attachment to fibrin was blocked by only 52%. Our results also confirm the lack of involvement in platelet adhesion of the RGDF site located at Aa95-98.

Exposure of fibrin to plasmin resulted in a marked decrease in platelet attachment and also in the inhibitory effects of MoAbs. The antibody to RGDS inhibited adhesion less on degraded than on undegraded fibrin (Table 1). There was no significant inhibition by antibodies either to the γ-chain dodecapeptide or to β15-42 on plasmin-degraded fibrin. These changes after plasmin exposure are compatible with the expected proteolytic modifications which have been characterized in greatest detail for fibrinogen. Plasmin initially cleaves the carboxyl terminal portion of the Aa chain and the β42-43 bond, and the carboxyl terminus of the γ chain also has several sites susceptible to plasmin cleavage. The sequence of degradation of noncross-linked fibrin, which was used in this study, is the same and has the effect of removing the carboxyl terminus of the α chain containing RGDS, of removing the amino terminus of the β chain, and also of removing the carboxyl terminus of the γ chain. This pattern is consistent with the changes observed using SDS-PAGE (Fig 2). The decrease in spreading of adherent cells (Fig 4) is also consistent with this pattern, because the amino terminus of the β chain, needed to support spreading, is lost early during degradation.

The opposing effects of thrombin and plasmin on the cellular response to fibrin are an additional element contributing to control of hemostasis by the balance of the coagulation and fibrinolytic systems. Through cleavage of FPB from fibrinogen, thrombin exposes a new amino terminus of the β chain which stimulates endothelial cell release of Weibel-Palade body contents, supports endothelial cell spreading, and also contributes to platelet adhesion. In addition to its effects on fibrinogen, thrombin exerts direct effects on vascular cells affecting proliferation, synthesis, and migration. The effects of plasmin are different. Plasmin can affect platelet function directly by cleavage of glycoprotein Ib and by alteration of arachidonic acid metabolism. Strikingly, the initial sites of plasmin action on fibrin involve those that support cell interaction, and plasmin has effects on fibrin that oppose those of thrombin with regard to platelet responses. By initially cleaving the carboxyl terminus of the Aa chain and the amino terminus of the β chain, plasmin cleaves both the RGDS site that is primarily involved in adhesion and also the β15-42 site needed for spreading, and this may limit further cellular response to the fibrin deposit.

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Plasmic degradation of fibrin rapidly decreases platelet adhesion and spreading

M Hamaguchi, LA Bunce, LA Sporn and CW Francis