Adhesive Protein Expression on Thrombin-Stimulated Platelets: Time-Dependent Modulation of Anti-Fibrinogen, -Fibronectin, and -von Willebrand Factor Antibody Binding

By Ellinor I.B. Peerschke

Platelets contain a pool of endogenous adhesive proteins that can be released and may bind to surface membrane receptors under appropriate conditions. Because the binding of exogenous fibrinogen to platelets was shown previously to be accompanied by a time-dependent decrease in fibrinogen accessibility to antibody and enzymes, studies were performed to evaluate changes in the expression of endogenous fibrinogen released from thrombin-stimulated platelets using monospecific polyclonal and monoclonal antibody F(ab')2 fragments. Parallel studies were performed to compare the expression of released fibrinectin and von Willebrand factor (vWF). Binding of polyclonal antibody F(ab')2 fragments directed against individual adhesive proteins was inhibited by EDTA or the 10E5 monoclonal antibody, suggesting that fibrinogen, fibronectin, and vWF expression was mediated, in large part, by divalent cation-dependent interactions with the glycoprotein IIb-IIIa complex. Interestingly, when polyclonal antibody F(ab')2 fragments were added to platelet suspensions at discrete times after thrombin stimulation, antifibrinogen F(ab')2 binding decreased by 72% ± 15% (mean ± SD, n = 22) over a 60-minute time course, whereas antifibrinogen and anti-vWF antibody F(ab')2 fragment binding changed minimally (6% ± 23%, n = 22 and 3% ± 26%, n = 14, respectively). Similar observations were made with monoclonal antibodies. Parallel experiments using 125I-labeled fibrinogen as a marker indicated that the observed decrease in antifibrinogen F(ab')2 binding was not accompanied by fibrinogen dissociation. Moreover, antibody accessibility to platelet-bound fibrinogen could be restored after Triton X-100 platelet lysis. The data suggest that fibrinogen, fibronectin, and vWF are not coordinately expressed on thrombin-stimulated platelets. Rather, fibrinogen expression appears transient compared with the expression of fibronectin and vWF. The ability of platelets to secrete and organize adhesive proteins on their surface is likely to have important implications for hemostasis and thrombosis.

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Antibody Binding

A FAMILY OF PROTEINS involved in platelet adhesive functions has been identified. This family includes such macromolecules as fibrinogen, fibronectin, and von Willebrand factor (vWF), all of which can bind to the platelet membrane glycoprotein (GP) IIb-IIIa complex under appropriate conditions. Although additional platelet membrane receptors exist for these ligands, the GP IIb-IIIa complex is apparently the most abundant. Binding of adhesive proteins to GPIIb-IIIa requires platelet activation, and is mediated in part by the amino acid sequence Arg-Gly-Asp (RGD), which functions as a basic recognition unit for a variety of cell-cell and cell-substrate interactions.

The interaction of fibrinogen with the platelet membrane GPIIb-IIIa receptor is a prerequisite for platelet aggregation. Direct correlations between fibrinogen binding and platelet aggregation have been established. When platelets are stimulated in the presence of exogenous fibrinogen, but are not aggregated, however, they lose their ability to aggregate upon subsequent stirring or restimulation with the same agonist. These findings are not associated with concomitant quantitative changes in fibrinogen binding, but are accompanied by time-dependent decreases in bound fibrinogen accessibility to polyclonal antibodies. Thus, qualitative changes in fibrinogen binding appear to correlate with the observed progressive decreases in platelet aggregation.

The release of endogenous fibrinogen also supports platelet aggregation. In addition to fibrinogen, platelets possess endogenous pools of fibrinogen and vWF. The release of these adhesive glycoproteins and their subsequent expression on the activated platelet membrane is likely to mediate a variety of cell surface reactions involved in hemostasis and thrombosis. The present study was designed to compare changes in released adhesive protein expression on the surface of thrombin-stimulated but unaggregated platelets over a 60-minute time course using monospecific polyclonal and monoclonal antibody (MoAb) F(ab')2 fragments.

MATERIALS AND METHODS

Proteins. Band I fibrinogen was purified as previously described and labeled with 125I using iodobeads (Pierce Chemical Co, Rockford, IL) according to the manufacturer's instructions. Human fibronectin was purchased from BM Biochemica (Indianapolis, IN; lot 61261700).

Antibodies. A rabbit polyclonal antihuman fibrinogen antibody (AXL203, lot 046B) was obtained from Accurate Chemical and Scientific Corp (Westbury, NY). Monoclonal antiplatelet antibodies were the kind gift of Dr B. Kudryk (NY Blood Center, New York, NY). Rabbit antiplatelet vWF was obtained from Accurate Chemical and Scientific Corp and as a generous gift from Dr I. Sussman (Montefiore Hospital/Medical Center, Bronx, NY). An MoAb against vWF (lot 05) was purchased from AMAC (Westbrook ME). Polyclonal antifibrinectin antibody was purchased from Cappel Worthington Biochemicals (Malvern, PA). A monoclonal antifibrinectin antibody (lot 072987) was obtained from Chemicon International, Inc (Temecula, CA).

Antibody F(ab')2 fragments were prepared by digestion with pepsin. Fc fragments were removed by incubating the digests with Staphylococcus aureus Protein A (10% crude cell suspension;
Platelet preparation. Blood was collected from volunteers after obtaining informed consent. Blood was anticoagulated with 0.32% sodium citrate. Platelet-rich plasma was prepared by centrifugation (280g for 15 minutes). It was acidified to pH 6.5 with 1 mol/L citric acid, and centrifuged (1,000g for 20 minutes). The resulting platelet pellet was resuspended in 0.01 mol/L HEPES-buffered modified Tyrode’s solution, pH 7.5 (HBMT) containing 0.1 µmol/L prostaglandin E1 (PGE1, Sigma Chemical Co), and passed over a column of Sepharose 2B equilibrated with HBMT.

Surface expression of adhesive proteins on stimulated platelets. Gel-filtered platelets were stimulated for 5 minutes with 0.20 U/mL human thrombin (a kind gift from Dr John Fenton, New York State Department of Health, Albany, NY) at 37°C to facilitate α-granule release. Thrombin was neutralized with hirudin (10 U/mL; Sigma Chemical Co), and the samples incubated at 22°C without stirring. The incubation temperature was chosen to reproduce conditions previously found to permit modulation of fibrinogen expression on ADP-treated platelets. At various times (10 to 60 minutes) during the incubation, I125-labeled antifibrinogen (50 µg/mL), antifibronectin (50 µg/mL), or anti-vWF (50 µg/mL) antibody F(ab)2 fragments were added to separate platelet aliquots. In some experiments, platelets were stimulated in the presence of 0.5 mmol/L gly-pro-arg (Peninsula Laboratories, Inc, Belmont, CA). Samples were placed on ice for 30 minutes to prevent further modulation of adhesive protein expression. F(ab)2 binding was assessed by counting radioactivity associated with platelet pellets after centrifugation through silicone oil (d 1.040).

Antibody specificity was assessed by examining the activity of a 10-fold molar excess of unlabelled F(ab)2 fragments or competitor adhesive proteins on labeled F(ab)2 binding to thrombin-stimulated platelets. Labeled F(ab)2 fragments were preincubated (60 minutes at 4°C) with various adhesive proteins before exposure to stimulated platelets. F(ab)2 binding to unstimulated platelets or platelets stimulated in the presence of 10 mmol/L EDTA or RGDS (80 µmol/L; Peninsula Laboratories, Inc) was monitored to evaluate nonspecific F(ab)2 binding, and/or formation of immune complex precipitates that could contribute to radioactivity recovered with platelet pellets. GPIIb-IIIa–mediated expression of adhesive proteins was assessed by preincubating platelets with the 10E5 monoclonal anti-GPIIb-IIIa antibody9 (20 µg/mL) (a generous gift of Dr Barry Coller, SUNY at Stony Brook). 10E5 was previously shown to inhibit fibrinogen, fibronectin, and vWF binding to thrombin-stimulated platelets. An anti-GPIIb MoAb (6D1),19 also from Dr B. Coller, served as a control Ig.

To confirm that observed changes in antifibrinogen F(ab)2 binding to platelets reflect qualitative changes in fibrinogen expression on thrombin-stimulated platelets rather than increased competition between released and platelet-bound fibrinogen for antifibrinogen F(ab)2 fragments, platelets were stimulated with 0.20 U/mL thrombin for 5 minutes at 37°C. Thrombin was neutralized with hirudin (10 U/mL) and platelets incubated at 22°C for 10 and 60 minutes. At each time, an aliquot of the stimulated platelet suspension was centrifuged and the resulting supernatant added to fresh, thrombin-treated platelets (1 part fresh platelets [109/µL]; 4 parts supernatant). After 10 minutes (22°C), I125–antifibrinogen F(ab)2 fragments were added, and binding was quantified as described above.

Exogenous fibrinogen binding and immunoprecipitation. I125-labeled fibrinogen binding was quantified as described previously.8 Nonspecific binding was assessed in the presence of 10 mmol/L EDTA, or 20 µg/mL 10E5. Immunoprecipitation of bound fibrinogen was accomplished after separating bound from free fibrinogen by centrifuging aliquots of stimulated platelet suspensions through silicone oil (d 1.040). The resulting platelet pellets were solubilized in 1% Triton X-100 (Eastman Kodak Co, Rochester, NY), 0.5 mg/mL leupeptin (Sigma Chemical Co), 0.15 mol/L NaCl, 0.01 mol/L Tris/HCl, pH 7.2. Samples were incubated (60 minutes at 22°C) with rabbit antifibrinogen antibody (Accurate Chemical Corp) or goat antihuman albumin (ATAB, Scarborough, ME) adsorbed to Protein A-Sepharose (Pierce Chemical Co).

RESULTS

Thrombin stimulation of gel-filtered platelets resulted in the surface expression of fibrinogen, fibronectin, and vWF, as assessed by antifibrinogen, antifibronectin, and anti-vWF antibody F(ab)2 fragment binding (Fig 1). Expression of endogenous fibrinogen occurred over a range of thrombin concentrations from 0.02 to 0.25 U/mL, whereas...
fibronectin and vWF expression required at least 0.2 U/mL thrombin.

The specificity of polyclonal antifibrinogen, antifibronectin, and anti-vWF antibody F(ab')2 fragment binding is summarized in Table 1. Binding of each F(ab')2 fragment was inhibited only in the presence of excess unlabelled, specific antibody or after antibody preincubation with specific antigen. Binding of all three F(ab')2 fragments was inhibited by greater than 70% in the presence of EDTA or if platelets were preincubated with the anti–GPIIb-IIIa, monoclonal, 10E5 antibody (Fig 2), suggesting the divalent cation-dependent interaction of released adhesive proteins with the GPIIb-IIIa receptor.

When antibody F(ab')2 fragments were added to platelets at 60 minutes compared with 10 minutes after stimulation, marked decreases (72% ± 14%, n = 22; mean ± SD) in antifibrinogen F(ab')2 binding were observed (Fig 3). Decreases in antifibronectin and anti-vWF F(ab')2 binding (6% ± 22%, n = 22; and 3% ± 26%, n = 14) were considerably smaller, and increased binding was observed in 7 and 3 of 14 experiments, respectively. Similar observations were made using MoAbs (Fig 4 and Table 2). Moreover, decreases in antifibrinogen F(ab')2 binding were also noted in the presence of the gly-pro-arg peptide, ruling out confounding effects due to fibrin formation on the platelet surface (Fig 5).

To rule out the possibility that increases in released platelet fibrinogen were responsible for the observed decrease in antifibrinogen antibody F(ab')2 binding, supernatants, derived from platelets 10 and 60 minutes after stimulation with thrombin, were added to fresh, thrombin-treated platelets, and antifibrinogen antibody F(ab')2 binding was assessed. The data are summarized in Table 3. No statistically significant differences in antibody F(ab')2 fragment binding were noted in the presence of supernatants taken from platelets 10 or 60 minutes after stimulation with thrombin. These supernatants also contained similar amounts of released fibrinogen: between 10 and 20 μg/mL fibrinogen were measured by immunoassay.

Additional studies were performed to examine the accessibility of platelet-associated fibrinogen to polyclonal anti- bodies after lysis in Triton X-100. Exogenously added 125I-fibrinogen served as a marker. Similar amounts of platelet-associated fibrinogen (84% ± 12% and 87% ± 13%, n = 4) were immunoprecipitated whether platelets were lysed 10 or 60 minutes after stimulation, respectively.

### Table 1. Specificity of Antifibrinogen, Antifibronectin, and Anti-vWF Antibody F(ab')2, Binding to Thrombin-Stimulated Platelets

<table>
<thead>
<tr>
<th>Competitor*</th>
<th>Antigen</th>
<th>Antibody F(ab')2 Binding (% of control)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antifibrinogen</td>
<td>Antifibronectin</td>
</tr>
<tr>
<td>BSA</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>15 ± 5</td>
<td>93 ± 8</td>
</tr>
<tr>
<td>Fibrinectin</td>
<td>98 ± 6</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Antifibrinogen</td>
<td>10 ± 4</td>
<td>94 ± 9</td>
</tr>
<tr>
<td>Antifibronectin</td>
<td>97 ± 9</td>
<td>8 ± 13</td>
</tr>
<tr>
<td>Anti-vWF</td>
<td>102 ± 12</td>
<td>96 ± 14</td>
</tr>
</tbody>
</table>

Abbreviation: BSA, bovine serum albumin.

*Radiolabeled antibody F(ab')2 fragments were preincubated (60 minutes at 4°C) with the respective competitors. The antigen-antibody mixture was added to platelets 10 minutes after stimulation with 0.2 U/mL thrombin. F(ab')2 binding was assessed after 30 minutes of incubation on ice. Purified vWF was not available for testing.

†Values represent mean ± SD, n = 4 relative to 125I-F(ab')2 binding in the absence of specific competitors.

**DISCUSSION**

The ability of platelets to secrete and organize adhesive proteins on their surface may have important implications for hemostasis and thrombosis. Fibrinogen, for example,
plays a central role in platelet aggregation. The observation that stimulated platelets, which do not aggregate immediately after fibrinogen binding, exhibit a diminished response to subsequent aggregation is well established. Because this phenomenon is not associated with quantitative changes in fibrinogen binding, one explanation for the loss of platelet aggregability may involve qualitative changes in fibrinogen expression at the platelet surface. Indeed, it has been shown that platelet-associated fibrinogen becomes progressively less accessible to antibodies and plasmin. Recently, Gralnick et al also reported decreases in fibrinogen expression on thrombin-stimulated platelets using an MoAb recognizing epitopes in the D domain of fibrinogen.

Because a number of adhesive proteins can interact with the platelet membrane GPIIb-IIIa, the present study was designed to investigate the surface expression of released fibrinogen, fibronectin, and vWF on thrombin-stimulated platelets at 22°C over a 60-minute time course. Greater than 70% of adhesive protein binding could be prevented by preincubating platelets with the anti-GPIIb-IIIa, 10E5 MoAb. Release of bound adhesive proteins via α-granule membrane GPIIb-IIIa, as well as expression of bound vWF from the surface canalicular system, are likely to have contributed to the inability of 10E5 to completely inhibit released adhesive protein expression on the platelet membrane.

Table 2. Modulation of Monoclonal Antifibrinogen Antibody F(ab'), Fragment Binding to Platelets at 60 Minutes Compared With 10 Minutes After Thrombin Stimulation

<table>
<thead>
<tr>
<th>Antibody*</th>
<th>Reactivity With</th>
<th>Antibody Binding (%)†</th>
</tr>
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<tbody>
<tr>
<td>DD-8D6</td>
<td>D, DD</td>
<td>30 ± 15</td>
</tr>
<tr>
<td>2N3H10</td>
<td>N, DSK, E</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>ID4-1</td>
<td>Azu 241-476</td>
<td>38 ± 17</td>
</tr>
<tr>
<td>1-8C6</td>
<td>Fibrinogen B8 1-42</td>
<td>35 ± 12</td>
</tr>
<tr>
<td>Fd(7B3)</td>
<td>Fibrinogen γ-chain, D</td>
<td>27 ± 14</td>
</tr>
</tbody>
</table>

Abbreviations: D, fragment D from fibrinogen; DD, fragment D from cross-linked fibrin; E, fragment E from fibrinogen; N-DSK, amino terminal disulfide knot of fibrinogen.

*MoAbs against fibrinogen were a gift from Dr B. Kudryk (New York Blood Center, New York, NY).

†Values represent mean ± SD (N = 6), and reflect F(ab')2 binding at 60 minutes compared with 10 minutes after platelet stimulation with 0.2 U/mL thrombin. Binding after 10 minutes was taken as 100%.

Table 3. Effect of Platelet Releasates on Antifibrinogen F(ab')2 Binding to Fresh, Thrombin-Stimulated Platelets

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Antifibrinogen F(ab')2 Binding (molecules/106 platelets)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>26,200 ± 3,742</td>
</tr>
<tr>
<td>60 min</td>
<td>25,824 ± 3,420</td>
</tr>
<tr>
<td>Releasate†</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td>25,284 ± 3,420</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
</tr>
<tr>
<td></td>
<td>27,155 ± 3,858</td>
</tr>
</tbody>
</table>

*F(ab')2 fragments were added to platelets at 10 and 60 minutes after stimulation with 0.2 U/mL thrombin. F(ab')2 binding was assessed after 30 minutes of incubation on ice. Values reflect mean ± SD, n = 4.

†F(ab')2 fragments were added to fresh, thrombin-treated platelets at 10 minutes after exposure to releasates from separate aliquots of platelets, or 10 or 60 minutes after stimulation with thrombin. F(ab')2 antifibrinogen F(ab')2 fragment binding was assessed after 30 minutes of sample incubation on ice.
course were noted in approximately 20% to 30% of experiments.

Trivial explanations for the transient expression of released fibrinogen by thrombin-stimulated platelets compared with fibronectin and vWF expression were examined. Interference of fluid phase antigen (eg, released fibrinogen) was ruled out based on the lack of effect of platelet releasates on antifibrinogen F(ab')₂, binding to freshly stimulated platelets. Moreover, similar amounts of released fibrinogen were detected 10 and 60 minutes after platelet stimulation. These observations are consistent with rapid thrombin-induced α-granule release, previously suggested by near maximal GMP140 expression 2 minutes after platelet stimulation. Moreover, similar modulation of fibrinogen expression was described after platelet stimulation with ADP in the presence of exogenous fibrinogen. Moreover, similar modulation of fibrinogen expression was described after platelet stimulation with ADP in the presence of exogenous fibrinogen.¹³

Using antifibrinogen F(ab')₂, fragments, differences in F(ab')₂ binding to thrombin-stimulated platelets in the presence or absence of gly-pro-arg were minimal. These data do not necessarily suggest the absence of fibrinogen binding or fibrin association with the platelet surface, but merely the inability of F(ab')₂ to detect increases in fibrin(ogen) associated due to fibrin formation on the platelet surface. The amount of F(ab')₂ bound to thrombin-stimulated platelets in this study is in agreement with reports by Courtois et al,¹⁴ who report the binding of approximately 50,000 antifibrinogen F(ab')₂, fragments to thrombin-stimulated platelets. Other investigators using somewhat different experimental conditions have reported the binding of nearly 250,000 antifibrinogen F(ab')₂, fragments per platelet.¹⁵

As α-granules are relatively enriched in fibrinogen¹⁶ as compared with fibronectin or vWF, released fibrinogen binding to thrombin-stimulated platelets is likely to be greater than either released fibronectin or vWF binding. Thus, the possibility of receptor clustering induced by the degree of ligand binding warrants consideration as an explanation for the observed, progressive decrease in bound fibrinogen accessibility to antibody F(ab')₂, fragments. Because fibrinogen, fibronectin, and vWF are all expressed on the surface of thrombin-stimulated platelets, coaggregation of occupied GPIIb-IIIa receptors might be expected, unless fibrinogen association with GPIIb-IIIa results in selective ligand-receptor processing. The latter would be consistent with the hypothesis that the expression of various adhesive proteins on the platelet surface via the GPIIb-IIIa receptor is discretely regulated.

Because the amount of released fibrinogen is likely to be greater than released fibronectin or vWF, the competition between released and bound ligand for their respective F(ab')₂, fragments may be greatest in the case of antifibrinogen F(ab')₂, fragments. The nonspecific fixation of antigen-antibody complexes to the platelet surface, however, was prevented by the use of F(ab')₂, fragments, lacking the Fc region, responsible for immune complex interaction with platelet Fc receptors. Thrombin stimulation of antigen-antibody lattices on the platelet surface due to the bivalency of polyclonal antibody F(ab')₂, fragments may occur, however, and makes direct correlations between F(ab')₂, binding and adhesive protein binding unreliable. Such lattice formation is unlikely to explain the time-dependent decrease in bound fibrinogen accessibility to antibody F(ab')₂, fragments, however, as F(ab')₂, fragments were not present during the incubation of stimulated platelets at 22°C, and receptor clustering induced by large antigen-antibody complexes is unlikely to occur at 4°C. Moreover, similar observations were made with a variety of MoAbs that are unlikely to support extensive lattice formation.

The interpretation of present data relies, in part, on the polyspecificity of epitopes recognized by the polyclonal antifibrinogen, anti-vWF, and antifibronectin antibodies. Labeled antifibrinogen F(ab')₂, fragments reacted with immunoblots of intact fibrinogen as well as with plasmin degradation products, fragments X, Y, D, and E.²⁻¹⁻¹² Anti-vWF F(ab')₂, fragments reacted with four major plasmin cleavage products of fibronectin (molecular weights: 170 Kd, 29 Kd, 23 Kd, and 6 Kd).³⁻¹⁻¹⁸ Digestion of cryoprecipitate with plasmin⁹ resulted in the loss of the 225-Kd native vWF subunit and the appearance of major immunoreactive bands at molecular weights 130 Kd and 90 Kd, with minor bands at 150 Kd and 45 Kd (data not shown). Artifacts due to experimental conditions favoring selection of one immunodominant epitope on adhesive proteins are also unlikely, as similar observations were made with both polyclonal antibodies and randomly selected MoAbs.

Thus, the transient expression of fibrinogen relative to fibronectin and vWF on thrombin-treated platelets may reflect the reorganization of bound fibrinogen or its "clearance" from the platelet surface. This hypothesis is consistent with the observed restoration of fibrin(ogen)-antifibrinogen antibody interactions after platelet lysis in nonionic detergents, and studies indicating that bound fibrinogen failed to undergo similar modulation on fixed platelets.¹³

The data are also consistent with recent evidence supporting the internalization of platelet-associated fibrinogen.³⁻¹⁻¹³ Fibrinogen internalization under present experimental conditions is unlikely, however, based on previous data showing virtually complete degradation of bound fibrinogen by plasmin in the absence of detectable platelet lysis. Sequestration of bound fibrin(ogen) in the surface canalicular system, which may not be readily accessible to antibody fragments but more so to proteolytic enzymes, may be an alternate explanation. Indeed, the movement of fibrinogen-gold from the cell surface to deep channels of the open canalicular system within 20 minutes after platelet stimulation with 1 U/mL thrombin was recently reported.²⁻¹ Although fibrinogen adsorbed onto gold particles represents a multimeric ligand complex that may be processed differently from native fibrinogen, Isenberg et al²⁻¹ also observed rapid surface reorganization of GPIIb-IIIa receptor occupancy by fibrinogen using immunocytochemistry.

The regulation of adhesive protein and receptor expres-
ADHESIVE PROTEIN EXPRESSION

Thrombin stimulation of human blood platelets, for example, induces both rapid exposure of fibrinogen binding sites on GPIIb-IIIa and the sequestration on stimulated platelets is likely to have important implications for understanding the molecular basis of dependent and dependent pathways. Blood 66:26, 1985


and platelets. Thromb Haemost 59:1, 1988


Hematol 22:241, 1985


Peerschke EIB: Ca$^{2+}$ mobilization and fibrinogen binding to platelets refractory to adenosine diphosphate stimulation. J Lab Clin Med 106:111, 1985


transient expression of released fibrinogen relative to fibrinogen binding and vWF. This may serve as a control mechanism to limit thrombus growth at sites of vessel injury.

ACKNOWLEDGMENT

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REFERENCES

18. Coller BS, Peerschke EI, Scudder LE, Sullivan CA: A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds to glycoproteins Ib and/or GPIIIa. J Clin Invest 72:325, 1983
25. Laudano AP, Doilittle RF: Synthetic peptide derivatives that bind to fibrinogen and prevent the polymerization of fibrin monomers. Proc Natl Acad Sci USA 75:3805, 1978
Adhesive protein expression on thrombin-stimulated platelets: time-dependent modulation of anti-fibrinogen, -fibronectin, and -von Willebrand factor antibody binding

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