Fibrin but Not Adsorbed Fibrinogen Supports Fibronectin Assembly by Spread Platelets

EFFECTS OF THE INTERACTION OF αIIbβ3 WITH THE C TERMINUS OF THE FIBRINOGEN γ-CHAIN

Jaehyung Cho1, Jay L. Degen2, Barry S. Coller1, and Deane F. Mosher1,11

From the 1Molecular and Cellular Pharmacology Program and Department of Medicine, University of Wisconsin-Madison School of Medicine, Madison, Wisconsin 53706, 2Children’s Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, and 4Laboratory of Blood and Vascular Biology, The Rockefeller University, New York, New York 10021

We investigated the assembly of soluble fibronectin by lysophosphatidic acid-activated platelets adherent to fibrinogen or fibrin. More fibronectin was assembled by activated platelets spread on fibrin matrices than by platelets spread on adsorbed fibrinogen. The difference between platelets adherent to fibrinogen and fibrin occurred under both static and flow conditions. Similar differences were seen in binding of the 70-kDa N-terminal fragment of fibronectin that recognizes fibronectin assembly sites on adherent cells. Antibody and peptide blocking studies demonstrated that αIIbβ3 integrin mediates platelet adhesion to fibrinogen, whereas both αvβ3 and αIIbβ3 mediate platelet adhesion to fibrin. The hypothesis that engagement of the C-terminal QAGDV sequence of the fibrinogen γ-chain by αIIbβ3 inhibits the ability of the platelet to assemble fibronectin was tested by several experiments. Activated platelets adherent to adsorbed mutant fibrinogen lacking the QAGDV sequence (γΔ5FG) were assembly-competent, as were platelets adherent to adsorbed normal fibrinogen that had been pretreated with the 7E9 antibody to the C terminus of the γ-chain. Moreover, adsorbed normal fibrinogen but not γΔ5FG suppressed the ability of co-adsorbed fibronectin to direct assembly of soluble fibronectin by spread platelets. The suppressive effect was lost when a surface of co-adsorbed fibronectin and fibrinogen was pretreated with 7E9. These results support a model in which the engagement of αIIbβ3 by the C-terminal sequence of the fibrinogen γ-chain initiates signals that suppress subsequent fibronectin assembly by spread platelets. This interaction is less dominant when platelets adhere to fibrin, resulting in enhanced fibronectin assembly.

Platelets at sites of vascular and tissue injury are essential for the formation of a hemostatic plug and subsequent arrest of bleeding (1). The initial step for hemostasis and thrombosis is adhesion of platelets by interaction of platelet surface receptors with extracellular matrix components of the exposed subendothelium of injured vessels. Intravital microscopy using mice lacking various adhesive proteins demonstrates that post-adhesive events are important in stabilizing the hemostatic plug in the injured mesenteric arteriole, which has a wall shear rate of ~1300 s⁻¹ (2, 3). For instance, mesenteric arteriole thrombi detach and embolize more readily in mice lacking plasma fibronectin, suggesting that the interaction of plasma fibronectin with platelets after adhesion plays a role in the formation of stable thrombi (3).

Fibronectin is a glycoprotein dimer of 230–250-kDa subunits that is present in a soluble form in plasma and other body fluids and in an insoluble form in tissues (4, 5). In contrast to integrin-mediated adhesion of cells to the RGD-containing midpiece of insolubilized fibronectin, assembly of fibronectin into thin, aperiodic extracellular matrix fibrils is mediated by interaction of the N-terminal 70-kDa portion of fibronectin (70k) with poorly characterized cell surface molecules (6). Microscopic studies from our laboratory demonstrated that soluble fibronectin is assembled into fibrillar arrays by lysophosphatidic acid (LPA)-stimulated spread platelets by a process that, as with fibroblasts (7), is blocked by the 70-kDa fragment (8). During the final stage of fibrin assembly, which is catalyzed by cross-linking of the e-(γ-glutamyl)-lysyl bond caused by thrombin-activated coagulation factor XIII (FXIIIa) (9–11), plasma fibronectin is cross-linked to the α-chain of fibrin by FXIIIa (11). α5β1 integrin mediates platelet adhesion to adsorbed fibronectin (12), and αIIbβ3 mediates binding of fibronectin to thrombin-activated platelets in suspension (13). Fibronectin, therefore, could stabilize platelet thrombi by several possible mechanisms, which are polymerization on the surface of adherent platelets, incorporation in fibrin clots, and participation in platelet adhesion and aggregation.

Studies of adherent platelets stimulated by LPA (8) or of fibroblasts (14, 15) have revealed that platelets or fibroblasts spread on vitronectin assemble soluble fibronectin poorly, whereas activated platelets or fibroblasts spread on fibronectin assemble soluble fibronectin well. We, therefore, initiated a survey of other adhesive ligands for platelet receptors to learn which ones support assembly of soluble fibronectin. In the current studies, we were intrigued to discover that fibronectin and its 70-kDa fragment similarly deposit in fibrillar arrays on platelets spread on fibrin but not on platelets spread on adsorbed fibrinogen. The experiments described below lead us to the surprising conclusion that the inability of platelets interacting with immobilized fibronectin to assemble fibronectin results from a suppressive effect of the interaction of αIIbβ3 with the C-terminal sequence of the fibrinogen γ-chain.

MATERIALS AND METHODS

Tetramethylrhodamine B isothiocyanate-conjugated phalloidin, GRGDS, α-phenylenediamine, and fatty acid-free bovine serum albumin (BSA) were purchased from Sigma. GRGES and dodecapeptide (HHLGGAKQAGDV) were from Peninsula Laboratories (San Carlos,
Fibrin Matrix Supports Fibronectin Assembly

Human or mouse fibrinogen, fibronectin, or vitronectin, 10 µg/ml, was coated directly on coverslips or wells without coverslips. These surfaces were compared with surfaces to which fibrin was deposited. For co-coating experiments, coverslips or wells were coated with a mixture of fibrinogen, 5 µg/ml, and either normal mouse fibrinogen or γSFG, 0–10 µg/ml, overnight at 4 °C. Surfaces coated with adsorbed proteins or fibrin matrices were post-coated with 1% fatty acid-free BSA for 1 h. In some experiments, adsorbed normal fibrinogen or γSFG was further treated with thrombin, 3 units/ml, for 30 min or hamster IgG or IgE, 10 mg/ml, for 1 h. After incubation, thrombin or the IgG was removed by three washes. Platelets, 1.5–2 × 10^8 in 0.5 ml, were added and incubated with HEPES-Tyrode buffer including 2 mM CaCl_2, 0.1% fatty acid-free BSA for 20 min at 37 °C. After washing 3 times, adherent platelets were incubated with 100 nM FITC-fibronectin or the FITC-70K fragment in the same buffer with 1 µM LPA for 1 h at 37 °C.

For microscopy, platelets on coverslips were washed, fixed, permeabilized, blocked, stained with 0.1 µg/ml rhodamine-conjugated phalloidin, and examined as described previously (8). In some studies of platelets binding the FITC-70K fragment, endogenous platelet fibronectin was stained with rabbit polyclonal antibody against fibronectin and rhodamine-conjugated anti-rabbit IgG. Samples were observed on an Olympus epifluorescence microscope using a 100× objective. Care was taken to image a given fluorochrome at the same settings for all experimental permutations using SPOT RT software Version 3.4 (Diagnostic Instruments, Inc.). For immunoblotting, platelets on wells without a coverslip were washed 3 times and lysed with 100 µl of SDS lysis buffer. Equal proteins (3 µg) of platelet lysates were analyzed by SDS-PAGE (a 3.3% stacking gel and a 5% separating gel) under reduced conditions and immunoblotted with a polyclonal antibody against FITC (1:2000 dilution).

Platelet Adhesion Assay under Static Conditions—Fibronectin, vitronectin, fibrinogen, or fibrin was coated onto wells of a 96-well plate with a black opaque well wall and transparent bottom (Corning Inc.). After unadsorbed proteins or fibrin clots were removed, the wells were post-coated with heat-inactivated fetal bovine serum. In some experiments, 10 µg/mlhamster IgG or IgE were preincubated with adsorbed mouse fibrinogen or fibrin for 1 h. After 3 rinses, 1 × 10^7 platelets premixed with 10 µg/ml antibody against integrin or 200 or 500 µM peptide for 10 min were added in 100 µl of HEPE-Tyrode buffer including 1 µM LPA, 2 mM CaCl_2, and 0.1% fatty acid-free BSA and incubated for 20 min at 37 °C. After washing out non-adherent platelets, HEPE-Tyrode buffer, 100 µl, and the cell viability assay reagent, 100 µl, were added and incubated for 20 min. The reagent determines the number of viable, adherent cells based on luminescence due to ATP in cell lysates (24).

Luminescence was detected with a multifunctional GENios Pro plate reader (TECAN, Research Triangle Park, NC). Nonspecific attachment of platelets to surfaces coated with fetal bovine serum alone gave a signal of 1600 ± 250, which was subtracted from the value by the specific binding.

Deposition of FITC-Fibronectin or FITC-70K Fragment by Adherent Platelets under Flow Conditions—The parallel plate flow chamber (25, 26) was purchased from Glycotech (Rockville, MD). The flow deck and a silicone rubber gasket were placed on a 3.5-cm-diameter coverslip coated with fibrinogen or fibrin in a 6-cm-diameter Petri dish. A reservoir, 4 ml, in which washed platelets, 8 × 10^8, were prewarmed at 37 °C in a water bath and premixed with 30 nM FITC-fibronectin or FITC-70K fragment in HEPE-Tyrode buffer including 2 mM CaCl_2, 0.1% fatty acid-free BSA, and 5 µM LPA was used. A peristaltic pump connected to the inlet of the flow chamber provided constant velocity flow through the chamber. The platelet solution was recirculated through the chamber at
**Fibrin Matrix Supports Fibronectin Assembly**

a flow rate of 2 ml/min, resulting a shear rate of 1250 s^{-1}, and each perfusion was done with 4 ml of separate, fresh platelet solution. The flow chamber and tubes were maintained at 37 ± 1°C. Platelet solutions were circulated through the chamber for 30 min. For microscopy, platelets on coverslips were washed, fixed, permeabilized, blocked, stained with rhodamine-conjugated phalloidin, and examined as described above.

**Enzyme-linked Immunosorbent Assay to Quantify the Amounts of Adsorbed Fibrinogen and Fibrin and Exposure of 7E9 Epitope**—The wells of a 96-well plate were coated with fibrinogen or non-cross-linked or cross-linked fibrin. After unadsorbed fibrinogen or fibrin clots were removed, the wells were post-coated with 1% BSA. After 3 rinses, 10 μg/ml mouse IgG, mouse 2C2-G7 anti-human fibrinogen, hamster IgG, 7E9, normal rabbit serum, or rabbit serum against mouse fibrinogen (1:10,000 dilution) was incubated for 1 h followed by incubation with peroxidase-conjugated goat anti-mouse IgG (1:10,000 dilution), antihamster IgG (1:5,000 dilution), or anti-rabbit IgG (1:20,000 dilution). After washing 3 times, 100 μl of 500 μg/ml o-phenylenediamine in a substrate buffer (25 mM citric acid and 100 mM NaH₂PO₄, pH 5.0) mixed with 30% H₂O₂ stock solution (1:1000 dilution, Sigma) was added to wells and incubated for 10 min. The enzyme reaction was stopped by the addition of 50 μl of 4 M H₂SO₄. The absorbance was measured at 490 nm. Specific binding of antibody was calculated by subtracting the signal by nonspecific binding of hamster IgG or a normal rabbit serum from the signal by binding of 7E9 or rabbit antiserum against mouse fibrinogen to each substrate.

**Replication of Results and Statistical Analysis**—Microscopic results were obtained from at least three separate occasions with similar results. Experiments with quantitative endpoints were pooled. Pooled data were expressed as the mean ± S.D. and statistically analyzed by analysis of variance using GraphPad Prism (GraphPad Software, Inc.). If the analysis of variance conveyed a value of p < 0.05, Dunnett’s test was performed to further assess significance. Differences were taken to be significant at p < 0.05.

**RESULTS**

FITC-Fibronectin or FITC-70K Fragment Is Deposited in Fibrillar Arrays by Platelets Spread on Human Fibrin but Not by Platelets Spread on Human Fibrinogen—Correlative light and electron microscopy have demonstrated that FITC-fibronectin is assembled into <10-nm diameter fibrils by platelets spread on fibronectin much more than by platelets spread on vitronectin (8). As part of a survey of other ligands of platelet adhesive receptors, we investigated if platelets stimulated by LPA and spread on human fibrinogen or cross-linked fibrin assemble soluble fibronectin. On platelets adherent to cross-linked fibrin, short fibrils of FITC-fibronectin formed in association with most spread platelets (Fig. 1A). In contrast, a little FITC-fibronectin was associated with platelets spread on coverslips coated with a solution of 10 μg/ml fibrinogen (Fig. 1A). Consistent with previously published results (8), FITC-fibronectin was found to assemble at the periphery of platelets spread on fibronectin-coated coverslips but was not organized into fibrils by platelets spread on vitronectin-coated coverslips (Fig. 1A).

A variety of experiments failed to find conditions under which adsorbed fibrinogen does support fibronectin assembly by spread platelets. An enzyme-linked immunosorbent assay with a monoclonal antibody (2C2-G7) that recognizes fibrinogen and fibrin demonstrated that the amount of fibrin left behind was similar to the amount of adsorbed fibrinogen (not shown), suggesting that the inability of fibrinogen to support fibronectin assembly by spread platelets did not result from absence of enough fibrinogen. Fibronectin was not assembled by platelets adherent to the coverslips coated with solutions of 3–500 μg/ml fibrinogen (not shown), a range of coating concentrations that has been shown to result in different conformations of adsorbed fibrinogen and different morphologies of platelets adherent to the coating (27–29). Thus, lack of assembly occurs at a variety of densities or conformations of adsorbed fibrinogen. In addition, fibronectin assembly was absent of when platelets adherent to adsorbed fibrinogen were incubated with FITC-fibronectin for 15, 30, or 180 min (not shown). Therefore, the inability of fibrinogen to support fibronectin assembly by spread platelets is not due to delayed expression of sites for assembly.

In the previous studies (8), co-incubation with the 70-kDa fragment resulted in decreased deposition of FITC-fibronectin by platelets adherent to fibronectin, presumably by competing with the N-terminal region of intact fibronectin for cell surface sites that initiate fibronectin assembly. We, therefore, compared distribution of FITC-70K fragment to that of FITC-fibronectin. The FITC-70K fragment bound to fibrin matrix in the absence of platelets (not shown). Samples were observed by epifluorescence microscopy. Bar, 10 μm. B, adherent platelets in wells without coverslips were lysed with 100 μl of SDS lysis buffer. Equal proteins of lysates (3 μg) were electrophoresed and immunoblotted with antibody against FITC, C, deposition of FITC-FN or FITC-70K fragment (not shown) on platelets adherent to FG or fibrin were observed after circulation in a parallel plate flow chamber with a wall shear rate of 1250 s^{-1} as described under “Materials and Methods.” Bar, 30 μm.

![FIGURE 1. Deposition of FITC-fibronectin or FITC-70K fragment by adherent platelets.](http://www.jbc.org/)
Thus, binding of the 70-kDa fragment could not be attributed to secretion and preassembly of endogenous fibronectin. These results indicate that the nature of the adhesive event governs the display of binding sites for the N-terminal region of fibronectin. The differences in fibrillar deposition of FITC-fibronectin or FITC-70K fragment by platelets spread on fibronectin or cross-linked fibrin versus vitronectin or fibrinogen were not associated with differences in platelet spreading or organization of filamentous actin cytoskeleton that correlated with ability to assemble (Fig. 1A).

Corroborative experiments were performed in which FITC-fibronectin or the FITC-70K fragment bound to spread platelets was extracted and immunoblotted using equal amounts of platelets lysate proteins. Consistent with the microscopy results, band density of FITC-fibronectin or the FITC-70K fragment bound to platelets spread on fibronectin or cross-linked fibrin was greater than band density of FITC-fibronectin or FITC-70K fragment bound to platelets spread on vitronectin or fibrinogen (Fig. 1B). These differences were confirmed by densitometry of immunoblots of replicated experiments (not shown).

Fibrin-coated surfaces were reported to result in increased adhesion and aggregation of platelets at a wall shear rate of 1600 s⁻¹ as compared with fibronectin-coated surfaces (30). To learn if fibronectin is assembled differently by platelets adherent to adsorbed fibronectin or fibrin under flow conditions, platelets premixed with FITC-fibronectin or the FITC-70K fragment were circulated through the flow chamber at a shear rate of 1250 s⁻¹. Platelets adhered and spread but did not aggregate on fibrinogen. FITC-fibronectin or FITC-70K fragment did not deposit on platelets spread on fibrinogen (Fig. 1C and not shown). Platelets adhered and aggregated more robustly on fibrin, and FITC-fibronectin or the FITC-70K fragment was incorporated into platelet aggregates that formed on fibrin (Fig. 1C and not shown). These results indicate that, as in the static condition, adherence to fibrin but not to adsorbed fibronectin leads to binding of soluble fibronectin or its N-terminal 70-kDa fragment by adherent platelets under flow conditions.

Platelets Adhere to Fibrinogen via αIIbβ3, to Fibrin or Vitronectin Via Both αvβ3 and αIIbβ3, and to Fibronectin via α5β1, αvβ3, and αIIbβ3—Suppression of fibronectin assembly by fibroblasts adherent to vitronectin is dependent on the integrin expression patterns of adherent cells (14). We, therefore, used function-blocking antibodies or peptides to determine which integrins mediate static adhesion of LPA-stimulated platelets to human fibronectin, vitronectin, fibrinogen, or cross-linked fibrin. Antibodies were tested at a single concentration of 10 μg/ml, which is 3-fold higher than the concentration of 7E3 or LM609 that saturates binding sites on 3 × 10⁵/ml platelets (31, 32). Of the platelets added, ~2–5% adhered per well coated with proteins or cross-linked fibrin (TABLE ONE). Platelet adhesion to fibronectin, vitronectin, or fibrin was diminished significantly by anti-αvβ3 as well as by anti-β3 or anti-αIIbβ3 antibody. In contrast, only anti-β3 or anti-αIIbβ3 antibody blocked platelet adhesion to fibrinogen significantly (TABLE ONE). Anti-β1 attenuated platelet adhesion to fibrinogen but not significantly to other substrates. Treatment with both anti-αvβ3 and anti-β1 antibodies inhibited platelet adhesion to fibrinogen more than the individual antibodies, whereas the combination of two antibodies did not show increased inhibition of platelet adhesion to vitronectin, fibrinogen, or fibrin (TABLE ONE). Treatment with 200 μM GRGDS, an inhibitor of adhesion mediated by αIIbβ3, αvβ3, and α5β1 (33–35), perturbed platelet adhesion to fibrinogen and, to a greater extent, to fibronectin, vitronectin, or fibrin. GRGES, a control peptide, was inactive. A dodecapeptide, 500 μM, which contains the QAGDV sequence of the fibronectin γ-chain that is a major site for the interaction of αIIbβ3 with fibrinogen (19, 36, 37), inhibited platelet adhesion to fibrinogen more than it inhibited adhesion to fibrin (TABLE ONE). The peptide did not diminish platelet adhesion to fibronectin or vitronectin. These findings indicate, therefore, that platelets stimulated by LPA adhere to fibrinogen mainly via αIIbβ3, to fibrin or vitronectin via both αvβ3 and αIIbβ3, and to fibronectin via αvβ3, α5β1, and αIIbβ3.

Interaction of αIIbβ3 with the C-terminal QAGDV Sequence of Fibrinogen γ-Chain Negatively Regulates Fibronectin Assembly by Spread Platelets—The findings that the adhesion of platelets to fibrinogen has a greater dependence on αIIbβ3 than adhesion of platelets to fibrin and that platelets interacting with fibrinogen do not assemble soluble fibronectin or its 70-kDa fragment suggest that the interaction of αIIbβ3 with fibrinogen results in an adherent platelet that does not support fibronectin assembly. We, therefore, tested whether a surface of adsorbed γΔSFG, purified from mice that have been genetically modified to lack the C-terminal QAGDV sequence of the fibrinogen γ-chain (19), supports fibronectin assembly by LPA-stimulated spread platelets.

We began by characterizing the adhesive activities of normal mouse fibrinogen and γΔSFG for human platelets. Microscopic examination of platelets activated by LPA and added to surfaces coated with fibrinogen

| Antibody or peptide | Luminescence \( \times 10^{-3} \) (％ inhibition) |
|---------------------|--------------------------------------------|
|                     | Fibronectin | Vitronectin | Fibrinogen | Fibrin |
| Control             | 13.3 ± 1.4  | 11.1 ± 1.2  | 11.4 ± 1.6  | 5.5 ± 1.2  |
| + mouse IgG1        | 12.5 ± 1.8 (6) | 10.4 ± 1.8 (6) | 10.7 ± 1.5 (6) | 5.4 ± 1.0 (2) |
| + rat IgG2          | 12.2 ± 1.3 (8) | 10.0 ± 1.7 (10) | 10.6 ± 1.5 (7) | 5.4 ± 1.1 (2) |
| + anti-β3 (7E3)     | 7.0 ± 1.1 (47)* | 4.5 ± 1.2 (59)* | 1.1 ± 0.2 (90)* | 2.0 ± 1.0 (64)* |
| + anti-αIIbβ3 (10E5) | 7.4 ± 1.2 (45)* | 5.5 ± 1.1 (51)* | 0.6 ± 0.2 (95)* | 2.5 ± 0.9 (55)* |
| + anti-αvβ3 (LM609) | 7.2 ± 1.1 (46)* | 4.0 ± 1.8 (64)* | 8.5 ± 2.0 (25) | 1.9 ± 1.0 (65)* |
| + anti-β1 (mAb13)   | 5.8 ± 1.7 (56)* | 9.0 ± 1.4 (19) | 8.6 ± 2.0 (24) | 4.0 ± 1.8 (27) |
| + anti-αvβ3 + anti-β1 | 5.0 ± 1.8 (63)* | 4.7 ± 1.4 (58)* | 8.3 ± 1.8 (27)* | 2.2 ± 1.1 (60)* |
| + GRGDS             | 5.8 ± 1.4 (56)* | 4.5 ± 1.5 (59)* | 7.5 ± 1.2 (34)* | 2.6 ± 1.0 (53)* |
| + GRGES             | 11.3 ± 1.0 (15) | 9.5 ± 1.4 (14) | 11.0 ± 1.6 (4) | 4.8 ± 1.3 (12) |
| + dodecapeptide     | 11.0 ± 1.5 (17)* | 9.2 ± 1.6 (17)* | 2.6 ± 1.8 (77)* | 3.4 ± 1.4 (38)* |

* \( p < 0.01 \) as compared with a control group.
* \( p < 0.05 \).
* Values are from three separate duplicate experiments.

The luminescence signal was obtained as described under “Materials and Methods.” Dilutions of a standard platelet suspension were assayed to relate luminescence to the number of adherent platelets. A value of 10,000 represents about 3.2% of the 1 × 10⁵ platelets added to each well. Values represent the mean ± S.D. of 6–7 separate duplicate experiments. The numbers in parentheses show inhibitory percentages of platelet adhesion by an antibody or peptide.
or cross-linked fibrin showed that platelets adhered and spread on normal fibrinogen, γΔ5FG, or fibrin made from either fibrinogen (Fig. 2). The number of platelets adherent to γΔ5FG was about 33% fewer than the number of platelets adherent to normal fibrinogen. There was no difference between numbers of platelets adherent to the cross-linked fibrins made from normal fibrinogen or γΔ5FG (TABLE TWO). Anti-β3, anti-αIIbβ3, or anti-ανβ3 antibody, 10 μg/ml, diminished platelet adhesion to normal fibrinogen significantly, whereas anti-β3 or anti-ανβ3 but not anti-αIIbβ3 antibody perturbed platelet adhesion to γΔ5FG or cross-linked fibrin made from normal fibrinogen or γΔ5FG (TABLE TWO). Preincubation of adsorbed normal fibrinogen or fibrin with 10 μg/ml 7E9, a hamster monoclonal antibody against the C terminus of the mouse fibrinogen γ-chain (38), blocked adhesion of LPA-stimulated platelets to normal fibrinogen or cross-linked fibrin made from normal fibrinogen by 74 or 42%, respectively (TABLE TWO). Co-incubation with dodecapeptide, 500 μM, inhibited platelet adhesion to normal fibrinogen and cross-linked fibrin made from normal fibrinogen by 73 and 29%, respectively. Neither pretreatment of adsorbed γΔ5FG or cross-linked fibrin made from γΔ5FG with 7E9 nor incubation of platelets with dodecapeptide perturbed platelet adhesion to these substrates. Co-incubation with GRGDS but not GRGES, 200 μM, blocked platelet adhesion to each substrate by 50–60% (TABLE TWO). These results with LPA-stimulated platelets are consistent with previous reports that washed platelets adhere to normal mouse fibrinogen by interaction with an intact QAGDV sequence via αIIbβ3 (19) and extend these findings by showing that adhesion of LPA-activated platelets to γΔ5FG or fibrin made from γΔ5FG as well as from normal mouse fibrinogen is mainly via ανβ3.

LPA-activated platelets spread on normal mouse fibrinogen, like platelets spread on human fibrinogen, did not assemble FITC-fibronectin (Fig. 2). Remarkably, FITC-fibronectin was assembled by platelets spread on γΔ5FG (Fig. 2). Platelets spread on cross-linked fibrin made from normal mouse fibrinogen or γΔ5FG assembled FITC-fibronectin equally well. These results, taken together with the results of the adhesion studies, indicate that the interaction of αIIbβ3 with the QAGDV sequence results in an adhesive phenotype that is not supportive of fibronectin assembly by LPA-stimulated spread platelets.

To test the role of the QAGDV sequence of fibronectin by a second method, adsorbed normal mouse fibrinogen or γΔ5FG was treated with hamster IgG or the 7E9 antibody before the addition of platelets. Adsorbed normal mouse fibrinogen treated with 7E9 supported FITC-fibronectin assembly by LPA-stimulated spread platelets (Fig. 3A). Adsorbed normal mouse fibrinogen treated with hamster IgG, like untreated adsorbed mouse fibrinogen, did not support assembly. γΔ5FG treated with either hamster IgG or 7E9 supported FITC-fibronectin assembly equally (Fig. 3A). When platelet lysates were immunoblotted, more FITC-fibronectin was found in the lysates of platelets spread on 7E9-treated normal fibrinogen or hamster IgG- or 7E9-treated γΔ5FG than in lysates of platelets spread on hamster IgG-treated normal fibrinogen (Fig. 3B). As a control for possible effects of stimulation of platelet Fc receptors on fibronectin assembly, adsorbed human fibrinogen was treated with mouse anti-human fibrinogen antibody before adhesion of platelets. Platelets adherent to antibody-treated or untreated human fibrinogen assembled FITC-fibronectin equally poorly (not shown).

The results described above are compatible with the conclusion that the interaction of αIIbβ3 with the C terminus of the fibrinogen γ-chain results in signals that either negatively regulate or do not support fibronectin assembly by LPA-stimulated spread platelets. In studies of fibroblasts, vitronectin co-coated with fibronectin substrate has a dominant-negative effect on assembly of soluble fibronectin by adherent β1- or fibronectin-null fibroblasts (14, 15). To test for a similar suppressive effect of the interaction of αIIbβ3 with the C terminus of the fibrinogen γ-chain on fibronectin assembly, we performed a co-coating experiment using a mixture of fibronectin and either normal mouse fibrinogen or γΔ5FG. The presence of increasing amounts of adsorbed normal fibrinogen had a dominant-negative effect on the ability of adsorbed

![FIGURE 2. Assembly of FITC-fibronectin by platelets spread on mouse fibrinogen or fibrin. Platelets were plated onto a glass coverslip coated with normal mouse FG, γΔ5FG, or fibrin made from either FG. The microscopic experiment was performed as described for Fig. 1. Images were captured in fields in which similar numbers of platelets were present. The insets are overlapped enlargements of the areas within the dotted rectangles. FN, fibronectin. Bar, 10 μm.](http://www.jbc.org/)

| Antibody or peptide | Fibrinogen | γΔ5FG | Fibrin | γΔ5FG | Fibrin | γΔ5FG |
|--------------------|------------|--------|--------|--------|--------|--------|
| Control            | 13.7 ± 1.7 | 8.7 ± 1.5 | 7.3 ± 1.7 | 6.9 ± 1.1 |
| + mouse IgG1       | 12.7 ± 1.8 (7) | 8.9 ± 1.7 (–2) | 6.9 ± 1.1 (5) | 6.8 ± 1.3 (2) |
| + hamster IgG      | 12.4 ± 2.0 (9) | 8.2 ± 1.3 (6) | 6.7 ± 1.8 (8) | 6.9 ± 1.1 (0) |
| + anti-β3 (7E3)    | 4.0 ± 1.4 (71)* | 5.0 ± 1.4 (43)* | 3.0 ± 1.4 (59)* | 3.6 ± 1.5 (48)* |
| + anti-αIIbβ3 (10E5) | 4.4 ± 1.2 (68)* | 7.2 ± 1.9 (17) | 5.7 ± 2.0 (22) | 6.0 ± 1.7 (13) |
| + anti-ανβ3 (LM609) | 9.8 ± 1.8 (32)* | 5.5 ± 1.6 (37)* | 4.3 ± 1.4 (41)* | 4.7 ± 1.1 (32)* |
| + 7E9              | 3.6 ± 1.3 (74)* | 6.9 ± 1.3 (20) | 4.2 ± 1.7 (42)* | 6.6 ± 1.0 (5) |
| + GRGDS            | 6.6 ± 1.5 (52)* | 4.2 ± 1.4 (52)* | 3.5 ± 1.2 (53)* | 2.8 ± 1.6 (59)* |
| + GRGES            | 11.6 ± 2.0 (15) | 8.4 ± 1.2 (4) | 6.6 ± 1.6 (10) | 6.8 ± 1.0 (2) |
| + dodecapeptide    | 3.7 ± 1.5 (73)* | 8.2 ± 1.4 (6) | 5.2 ± 1.2 (29)* | 6.6 ± 1.2 (5) |

* p < 0.01 as compared with a control group.
* p < 0.05 as compared with a control group.
fibronectin to support assembly of soluble fibronectin by spread platelets (Fig. 4A). In contrast, the dominant-negative effect on fibronectin assembly was not observed when fibronectin was co-coated with γΔ5FG (Fig. 4A). In immunoblotting, the relative band density in lysates of platelets spread on the mixture with γΔ5FG was more intense than that in lysates of platelets spread on the mixture with normal fibrinogen as the concentration of fibrinogen during coating increased from 0.1 to 10 μg/ml (Fig. 4B).

The suppressive effect of vitronectin on binding of the N-terminal 70-kDa fragment of fibronectin to adherent fibroblasts was lost when the mixture of fibronectin and vitronectin was preincubated with an antibody against vitronectin (14). The same strategy was applied to the co-coat of fibronectin and normal mouse fibrinogen by treating the adsorbed proteins with the 7E9 monoclonal antibody to the γ-chain tail. 7E9, but not hamster IgG, neutralized the suppressive effect of surface fibrinogen on assembly of fibronectin by spread platelets (Fig. 4C). These results indicate that the failure of platelets interacting with IIb/IIIa via the QAGDV sequence of the fibrinogen γ-chain to assemble fibronectin is not due to a simple inability to assemble but, rather, a suppressive effect on fibronectin assembly.

Polymerization of Fibrin but Not Thrombin Treatment or FXIII-mediated Cross-linking Is Essential for Induction of Fibronectin Assembly Competency by Spread Platelets—A series of experiments was done to assess which feature of fibrin formation is required for fibrin to support fibronectin assembly by adherent platelets stimulated by LPA. When fibrinogen adsorbed at a wide range of concentrations was treated with thrombin, the thrombin-treated fibrinogen did not support fibronectin assembly by treating the adsorbed proteins with the 7E9 monoclonal antibody to the γ-chain tail. 7E9, but not hamster IgG, neutralized the suppressive effect of surface fibrinogen on assembly of fibronectin by spread platelets (Fig. 5A). This result indicates that fibrin must form a three-dimensional fibrillar network. Treatment of fibrin with D-Phe-Pro-Arg-chloromethyl ketone had no effect on the ability of fibrin fibrils to support assembly of soluble fibronectin by spread platelets (not shown), suggesting that fibronectin assembly by spread platelets is not due to the catalytic activity of thrombin bound to fibrin.

Fibrin made from human or normal mouse fibrinogen in the absence of FXIIIa, which has only trace amounts of cross-linked γ-chain dimers, was equivalent to extensively cross-linked fibrin made from either species of fibrinogen in supporting fibronectin assembly by spread platelets (Fig. 5B). These results indicate that fibrin polymerization per se rather than FXIII-mediated cross-linking of the fibrin γ-chains is responsible for the switch in the effect of fibrinogen-fibrin on fibronectin assembly by adherent platelets.

**Exposure of the 7E9 Epitope in Non-cross-linked and Cross-linked Fibrin**—The C terminus of the γ-chain is disordered in the structure of the D-D dimer of cross-linked fibrin but is close to the interface of the two γD modules (39). The C terminus is the site of FXIIIa-mediated
Fibrin Matrix Supports Fibronectin Assembly

intermolecular γ-γ cross-linking that occurs between two γ-chains in a reciprocal anti-parallel fashion (36, 40, 41). The results described above suggest that the QAGDV sequence of the γ-chain is partially sequestered from interaction with αIIbβ3 in both non-cross-linked and cross-linked fibrin. To determine the accessibility of the γ-chain C termini in non-cross-linked and cross-linked fibrin to the 7E9 antibody, which blocks the suppressive effect of adsorbed normal mouse fibrinogen on fibronectin assembly, binding of antibody to adsorbed normal mouse fibrinogen, γΔ5FG, or non-cross-linked or cross-linked fibrin made from normal fibrinogen or γΔ5FG was quantified by an enzyme-linked immunosorbent assay. 7E9 bound to normal mouse fibrinogen but not to γΔ5FG (Fig. 6A), indicating that the C-terminal QAGDV sequence is a necessary feature of the 7E9 epitope. Binding of 7E9 to non-cross-linked fibrin made from normal mouse fibrinogen was the same as normal mouse fibrinogen, whereas binding to cross-linked fibrin was 3-fold less (Fig. 6A). A rabbit antiserum bound equally well to normal fibrinogen, γΔ5FG, and fibrins made from either fibrinogen (Fig. 6B), showing that these wells were coated with similar amounts of the protein. Thus, these results indicate that ~70% of the C termini of the γ-chain are inaccessible to 7E9 in cross-linked fibrin as compared with immobilized fibrinogen or non-cross-linked fibrin. Even though the ability to down-regulate fibronectin assembly is blocked by 7E9, however, exposure of the 7E9 epitope does not correlate with the ability of fibrin to support fibronectin assembly by spread platelets. The fact that loss of the 7E9 epitope was not complete even though SDS-PAGE indicated complete cross-linking of γ-chain could be due to partial retention of the epitope in cross-linked fibrin. Alternatively, a proportion of the γ-chain may be linked by only one of the two potential reciprocal ε-(γ-glutamyl)-lysyl cross-links, leaving the C-terminal tail of one of the cross-linked γ-chains free to interact with the antibody.

DISCUSSION

The present investigations uncovered major differences in the effects of two adhesive substrates, fibrinogen and fibrin, on a post-adhesive activity of platelets, supporting the assembly of fibronectin. Previous correlative fluorescence and electron microscopic analyses demonstrated that soluble fibronectin assemblies to form <10-nm diameter fibrils on platelets adherent to fibronectin (8). We found that a substrate of fibrin but not of immobilized fibrinogen supports assembly of soluble fibronectin by LPA-stimulated spread platelets. This difference was observed under both static and flow conditions. Subsequent experiments demonstrated that the interaction of αIIbβ3 with the QAGDV sequence of the fibronectin γ-chain is responsible for the lack of fibronectin assembly by platelets spread on fibrinogen, and, furthermore, that the αIIbβ3-QAGDV interaction has a dominant-negative effect on assembly of fibronectin by platelets spread on a substrate that contains both fibronectin and fibrinogen. Finally, the results suggest that platelets spread on fibrin are induced to adopt a phenotype favorable for fibronectin assembly as a consequence of the interaction of αβ3 with fibrin.

Fibronectin assembly is more robust on platelets adherent to fibronectin than on platelets adherent to vitronectin in the absence of stimulus or in the presence of LPA, ADP, or a thrombin receptor agonist (8). Fibronectin and vitronectin are completely different proteins, whereas fibrinogen and fibrin are so alike that the term “fibrinogen” is
often used as a common descriptor for both. Conversion of fibrinogen to fibrin is initiated by thrombin-mediated cleavage of fibrinopeptide A and B from the N termini of the Aα- and Bβ-chains, respectively. The newly exposed N-terminal sequences in the E domain of fibrin function as Eα and Eβ polymerization sites. The Eα polymerization sites interact with complementary binding sites located in the γD modules of adjacent molecules (40, 42). This interaction brings the peripheral D domains of two fibrin monomers together and drives formation of double-stranded twisting fibrils. The fibrinogen-to-fibrin conversion also exposes the β15–42 sequence that interacts with vascular endothelial cadherin to favor spreading of platelets, fibroblasts, and endothelial cells on fibrin (40, 43–46).

The finding that a matrix of polymerized fibrin is a supportive substratum for assembly of soluble fibronectin by adherent platelets, whereas thrombin-treated adsorbed fibrinogen is inactive, indicates that the key difference between fibrinogen and fibrin is due to the polymerization step of the fibrinogen-to-fibrin conversion. This result also indicates that exposure of the β15–42 sequence is not responsible for the difference between fibrinogen and fibrin.

The findings that γΔFG- and 7E9-treated normal mouse fibrinogen have effects on adherent platelets similar to the effect of fibrin suggest that a change in the accessibility of the C-terminal tail of the γ-chain to αIIbβ3 accounts for the difference between fibrinogen and polymerized fibrin. The crystal structure of a complex of αIIbβ3 with epitibatide, a cyclic peptide derivative containing the Arg-Gly-Asp cell recognition sequence, demonstrates that a carboxyl group coordinates to Mg²⁺ in the metal ion-dependent adhesion site of the I-like domain of β3, and the arginine, which likely mimics Lys-406 in the flexible KQAGDV sequence at the C terminus of the fibrinogen γ-chain, interacts with an aspartate residue in β-propeller of αIIb subunit (47). Assembly into fibrils is accompanied by intramolecular γ-γ cross-linking mediated by FXIIIa (36, 40, 48). Lys-406 also participates in reciprocal γ-γ cross-linking (40). FXIIIa-mediated cross-linking of fibrin and interaction of the KQAGDV sequence of fibrinogen with αIIbβ3, therefore, are predicted to be mutually exclusive functions because cross-linking would modify the positive charge on Lys-406 as well as immobilize the sequence. Assembly of fibronectin by platelets adherent to non-cross-linked fibrin, however, indicates that the KQAGDV sequence is less available to αIIbβ3 in both cross-linked and non-cross-linked fibrin. Of note, even though both non-cross-linked and cross-linked mouse fibrin supported fibronectin assembly, 7E9 bound well to non-cross-linked fibrin but not to cross-linked fibrin. Thus, the findings indicate that fibrin formation in the absence of cross-linking alters access of the γ-chain C terminus dodecapeptide to αIIbβ3 but not to 7E9.

αIIbβ3 has been found in many studies to mediate adhesion of washed platelets to fibrinogen and fibrin under static (49–51) and flow conditions (30, 52–57). Our data with anti-β3 or anti-αvβ3 antibody indicate, however, that αvβ3 contributes to static adhesion of LPA-activated platelets to γΔFG or fibrin made from human fibrinogen, normal mouse fibrinogen, or γΔFG fibrin. This finding is remarkable because there are only 50–100 αvβ3 receptors per platelet compared with 80,000–100,000 αIIbβ3 receptors per platelet (31, 32). Human smooth muscle cells and glioma cells also adhere to fibrin via αvβ3 (58, 59), and the small number of αvβ3 receptors on platelets effectively mediates adhesion to vitronectin in the absence of αIIbβ3 (32). The α-chain of fibrin contains an RGD sequence at Aa572–574 that is recognized by endothelial cell αvβ3 integrin (60). The αvβ3-RGD interaction is significantly enhanced when α-chains are cross-linked into multimers (61). The fact that substrates of both cross-linked and non-cross-linked fibrin resulted in adherent platelets that are competent for fibronectin assembly indicates, however, that formation of α-chain multimers is not essential for the interaction of platelet αvβ3 integrin with fibrin. Studies with inhibitory peptides suggest that γ190–202 (GWTVFQKRSLG5V) and γ346–358 (GYVYQGTTYSKAS) of the γ-chain, which are adjacent on the surface of the fibrin D domain, mediate adhesion of β3-expressing Chinese hamster ovary cells to fibrinogen via αvβ3 (62). Our findings leave open the possibility that the difference between fibrinogen and fibrin is due not only to a diminished interaction of αIIbβ3 with the C-terminal sequence of the γ-chain of polymerized fibrin but also an enhanced interaction of αvβ3 with the RGD sequence in the α-chain and/or with other regions in the γD module in fibrin as compared with fibrinogen.

In co-coating experiments done with fibroblasts (14, 15), vitronectin had a dominant-negative effect on the ability of adsorbed fibronectin to support soluble fibronectin assembly. We found a similar effect when platelets were spread on a mixed coating of fibronectin and vitronectin (not shown). The integrin usage for adhesion of platelets to vitronectin was via both αvβ3 and αIIbβ3, i.e. was similar to the integrin usage for adhesion to fibrin, which supported fibronectin assembly. Thus, integrin usage during adhesion cannot by itself explain why adherent platelets do or do not assemble soluble fibronectin. Vitronectin is not known to contain a sequence similar to the flexible KQAGDV C-terminal “tail” in fibrinogen that interacts with αIIbβ3. Nevertheless, the demonstration that suppression of fibronectin assembly is a consequence of interaction of αIIbβ3 with the QAGDV sequence of fibrinogen suggests hypotheses about possible mechanisms of the suppressive effects. One possibility for suppression of fibronectin assembly is that further ligation of polymerizing fibronectin by its receptors is not possible because signaling molecules and/or cytoskeletal elements involved in receptor activation and localization are engaged by the cytoplasmic domains of the large number of αIIbβ3 receptors interacting with the fibrinogen γ-chain sequences (trans-dominant inhibition) (63). Inasmuch as we have been unable to demonstrate that β1 or β3 integrins act as receptors for assembling fibronectin or the 70-kDa fragment despite using both antibody inhibition and cross-linking approaches (results not shown), it would be necessary to postulate that these signaling molecules are important in activating or positioning one or more of the non-integrin receptors for fibronectin that have been reported (64–66). Alternatively, the interaction of the integrin per se with the adhesive ligand or the interaction of the integrin and integrin-associated proteins with the adhesive ligand may affect an adjacent receptor for fibronectin.

Recent in vivo investigations of genetically manipulated mice have indicated that plasma fibronectin is important for platelet function (2, 3). In mice lacking fibrinogen and von Willebrand factor, plasma fibronectin is deduced to support platelet thrombus formation, leading to occlusion of injured mesenteric arteries (2). Moreover, mice with a conditional knock-out of plasma fibronectin form unstable hemostatic plugs in the injured vessel (3). These results suggest, therefore, that plasma fibronectin contributes to the formation of stable thrombi by enhancing platelet–platelet and perhaps platelet-vessel wall interactions. Our results delineate a pathway whereby platelets interacting with fibrin networks deposit fibrillar arrays of soluble fibronectin that may serve to enhance platelets cohesiveness and support the recruitment of additional platelets to the plug. In addition, the finding that fibronectin assembly is controlled by whether platelets are adherent to fibrinogen or fibrin raises the possibility that other post-adhesive activities of platelets are modulated differently by fibrinogen and fibrin.

REFERENCES
1. Ruggeri, Z. M. (2002) Nat. Med. 8, 1227–1234
2. Ni, H., Denis, C. V., Subbarao, S., Degen, J. L., Sato, T. N., Hynes, R. O., and Wagner, J. A.
Fibrin Matrix Supports Fibronectin Assembly

D. D. (2000) J. Clin. Investig. 106, 385–392
3. Ni, H., Yuen, P. S., Papalia, J. M., Trevirtich, J. E., Sakai, T., Fassler, R., Hynes, R. O., and Wagner, D. D. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 2415–2419
4. Mosher, D. (1989) Fibrinocitin, Academic Press, Inc., San Diego, CA
5. Hynes, R. (1990) Fibrinocitin, Springer-Verlag, New York, NY
6. Mosher, D. F. (1995) Thromb. Haemostasis 74, 529–533
7. McKeeven-Longo, P. J., and Mosher, D. F. (1985) J. Cell Biol. 100, 364–374
8. Olorundare, O. E., Peyruchaud, O., Albrecht, R. M., and Mosher, D. F. (2001) Blood 98, 117–124
9. Barry, E. L., and Mosher, D. F. (1988) J. Biol. Chem. 263, 10464–10469
10. Barry, E. L., and Mosher, D. F. (1989) J. Biol. Chem. 264, 4179–4185
11. Mosher, D. F. (1995) J. Biol. Chem. 270, 6614–6621
12. Wayner, E. A., Carter, W. G., Postowicz, R. S., and Kunicki, T. J. (1988) J. Cell Biol. 107, 1881–1891
13. Plow, E. F., McEver, R. P., Coller, B. S., Woods, V. L., Jr., Marguerie, G. A., and Ginsberg, M. H. (1985) Blood 66, 724–727
14. Zhang, Q., Sakai, T., Nowlen, J., Hayashi, I., Fassler, R., and Mosher, D. F. (1999) J. Biol. Chem. 274, 368–375
15. Bae, E., Sakai, T., and Mosher, D. F. (2004) J. Biol. Chem. 279, 35749–35759
16. Coller, B. S., Peerschke, E. I., Scudder, L. E., and Sullivan, C. A. (1983) J. Clin. Investig. 72, 325–338
17. Coller, B. S. (1985) J. Clin. Investig. 76, 101–108
18. Lengweiler, S., Smyth, S. S., Jirouskova, M., Scudder, L. E., Park, H., Moran, T., and Coller, B. S. (1999) Biochem. Biophys. Res. Commun. 262, 167–173
19. Holmback, K., Danton, M. J., Suh, T. T., Daugherty, C. C., and Degen, J. L. (1996) EMBO J 15, 5760–5771
20. Bittorf, S. V., Williams, E. C., and Mosher, D. F. (1993) J. Biol. Chem. 268, 24838–24846
21. Mosher, D. F., and Blout, E. R. (1973) J. Biol. Chem. 248, 6896–6903
22. Corbett, S. A., and Schwazerbaur, J. E. (1999) J. Biol. Chem. 274, 20943–20948
23. Corbett, S. A., Wilson, C. L., and Schwazerbaur, J. E. (1996) Blood 88, 158–166
24. Crouch, S. P., Kozlowski, R., Slater, K. J., and Fletcher, J. (1993) J. Immunol. Methods 160, 81–88
25. Brown, D. C., and Larson, R. S. (2001) BMC Immunol. 2, 9
26. Li, F., and Palecek, S. P. (2003) Eukaryot. Cell 2, 1266–1273
27. Coller, B. S., Kutom, J. L., Scudder, L. E., Galanakis, D. K., West, S. M., Rudomen, G. S., and Springer, K. T. (1993) J. Clin. Investig. 92, 2796–2806
28. Moskowitz, K. A., Kudryk, B., and Coller, B. S. (1998) Thromb. Haemostasis 79, 824–831
29. Jirouskova, M., and Coller, B. S. (2001) Annu. N. Y. Acad. Sci. 936, 464–465
30. Jirouskova, M., Dyr, I., Suttman, J., Holada, K., and Trnka, B. (1997) Thromb. Haemostasis 78, 1125–1131
31. Coller, B. S., Cheshes, D. A., Asch, E., and Seligsohn, U. (1991) Blood 77, 75–83
32. Wagner, C. L., Maselli, M. A., Neublock, D. S., Weisman, H. F., Coller, B. S., and Jordan, R. E. (1996) Blood 88, 907–914
33. Suehiro, K., Smith, J. W., and Plow, E. F. (1996) J. Biol. Chem. 271, 10365–10371
34. Wang, X., Coons, L. R., Taylor, D. B., Stevens, S. E., Jr., and Gartniser, T. K. (1996) J. Biol. Chem. 271, 17785–17790
35. Hynes, R. O. (1992) Cell 69, 11–25
36. Mosesson, M. W. (2003) J. Thromb. Haemostasis 1, 231–238
37. Farrell, D. H. (2004) Curr. Opin. Hematol. 11, 151–155
38. Jirouskova, M., Smyth, S. S., Kudryk, B., and Coller, B. S. (2001) Thromb. Haemostasis 86, 1047–1056
39. Yang, Z., Pandi, L., and Doolittle, R. F. (2002) Biochemistry 41, 15610–15617
40. Mosesson, M. W., Siebenlist, K. R., and Melt, D. A. (2001) Ann. N. Y. Acad. Sci. 936, 11–30
41. Chen, R., and Doolittle, R. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 420–427
42. Shimizu, A., Nagel, G. M., and Doolittle, R. F. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2888–2892
43. Hamaguchi, M., Bunce, L. A., Sporn, L. A., and Francis, C. W. (1993) Blood 81, 2348–2356
44. Sporn, L. A., Bunce, L. A., and Francis, C. W. (1995) Blood 86, 1802–1810
45. Bunce, L. A., Sporn, L. A., and Francis, C. W. (1992) J. Clin. Investig. 89, 842–850
46. Bach, T. L., Barsigian, C., Yuen, C. H., and Martinez, J. (1998) J. Biol. Chem. 273, 30719–30728
47. Xiao, T., Takagi, J., Coller, B. S., Wang, J. H., and Springer, T. A. (2004) Nature 432, 59–67
48. Doolittle, R. F., Yang, Z., and Mochalkin, I. (2001) Annu. N. Y. Acad. Sci. 936, 31–43
49. Savage, B., and Ruggeri, Z. M. (1991) J. Biol. Chem. 266, 11227–11233
50. Savage, B., Shattil, S. J., and Ruggeri, Z. M. (1992) J. Biol. Chem. 267, 11300–11306
51. Wonerow, P., Pearce, A. C., Vaux, D. J., and Watson, S. P. (2003) J. Biol. Chem. 278, 37520–37529
52. Litjens, P. E., Kroner, C. L., Akkerman, J. W., and Van Willigen, G. (2003) J. Thromb. Haemostasis 1, 2014–2021
53. Endenburg, S. C., Hantgan, R. R., Sixma, J. J., de Groot, P. G., and Zwaginga, J. J. (1993) Blood Coagul. Fibri...
Access the most updated version of this article at doi: 10.1074/jbc.M506289200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2005/08/02/M506289200.DC1

This article cites 64 references, 35 of which can be accessed free at
http://www.jbc.org/content/280/42/35490.full.html#ref-list-1