Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3844–3848, June 1985
Medical Sciences

Inhibition of platelet aggregation by a monoclonal antibody against human fibronectin

(platelet fibronectin/protease-sensitive epitope/cell-binding domain)

VISHVA M. DIXIT*†, DORIS M. HAVERSTICK‡, KAREN O'ROURKE*, SALLY W. HENNESSY*, THOMAS J. BROEKELMANN§, JOHN A. MCDONALD§, GREGORY A. GRANT*¶, SAMUEL A. SANTORO‡¶, AND WILLIAM A. FRAZIER*

Departments of *Biological Chemistry, ‡Pathology, and ¶Medicine, and Divisions of †Laboratory Medicine and §Pulmonary Medicine, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Howard A. Schneiderman, January 10, 1985

ABSTRACT **A monoclonal antibody (A3.3) has been generated against human platelet fibronectin (FN). A3.3 reacts with human plasma FN but with no other plasma proteins. A3.3 was found to inhibit thrombin- or ionophore A23187-stimulated aggregation of gel-filtered platelets in a concentration-dependent manner in both an aggregometer assay and a sensitive well plate aggregation assay. The antibody does not block secretion of serotonin. Four other anti-FN monoclonal antibodies that recognize different epitopes on FN than A3.3 does have no effect on platelet aggregation. A3.3 does not block the adhesion of CHO cells to FN-coated surfaces, indicating that it does not bind to the identified cell-binding domain of FN. A3.3 reacts with a 160/140-kDa doublet, known to contain the cell-binding domain, that is produced by digestion of FN with elastase or thermolysin. However, the antibody does not react with lower molecular weight species that also contain the cell-binding domain or with any of the other identified domains of FN. The A3.3 epitope is extremely protease sensitive and the smallest fragment found in any digest that retains reactivity with A3.3 is a 70-kDa peptide produced in low yield by mild thermolytic cleavage of FN. These data suggest that A3.3 defines a functional site present on both the platelet and plasma FN molecule that has a direct role in platelet aggregation.**

Fibronectin (FN) is a disulfide-linked dimeric glycoprotein, which is made by a variety of cells and found in extracellular matrices and fluids (1, 2). Each of the similar but not identical ≈220-kDa subunits contains several protease-resistant domains that represent functional binding sites for macromolecules such as collagen (gelatin), glycosaminoglycans, fibrin, and an as yet uncharacterized cellular receptor that functions in cell attachment to FN-containing surfaces (1, 2). The cell-binding domain of FN has been localized to a region about two-thirds distal to the NH_2 terminus of both subunits and may be a sequence of amino acids only five residues long (3, 4). Thus FN appears to play a key role in the attachment of cells to the extracellular matrix and other appropriately coated surfaces both *in vitro* and *in vivo* (1–4).

The functional role of FN in platelet aggregation remains to be clarified. Because FN was identified as one of several proteins remaining associated with collagen after collagen-induced platelet aggregation and subsequent lysis of platelets by sonication and detergent treatment, Bensusan and colleagues (5) suggested that FN serves as the collagen receptor on platelets. However, the demonstration by Santoro and Cunningham (6) that high concentrations of denatured collagen or gelatin, which has a higher affinity for FN than does native collagen, failed to inhibit either the adhesion of platelets to collagen or the aggregation of platelets induced by collagen indicated that FN was unlikely to be the collagen receptor. Subsequent investigations revealed that FN is located within the α granules of platelets (7) and is secreted after activation of platelets by collagen or thrombin (8). It is not known if this platelet FN is taken up from plasma, and hence identical to plasma FN, or synthesized by megakaryocytes during platelet maturation and hence should be considered "cellular" FN (1, 2). It is now clear that FN is not expressed on the surface of unactivated platelets (6, 8) but only becomes associated with the platelet surface after activation (8). Plow and Ginsberg (9) have demonstrated that activation by thrombin induces specific FN receptors on the platelet surface that have a much higher affinity for FN than does fibrinogen. These observations suggest that FN may in fact play a role in platelet aggregation. The recent demonstration that high concentrations of FN can inhibit platelet aggregation induced by thrombin or calcium ionophore A23187 is consistent with this concept (10).

To further investigate the role of FN in platelet aggregation and adhesion, we have raised monoclonal antibodies (MAbs) against human platelet FN. All of the MAbs obtained also recognize plasma FN. One clonal hybridoma, A3.3, produces a MAb that blocks the aggregation of thrombin-stimulated gel-filtered platelets as assayed in a sensitive well plate aggregation assay and in the aggregometer. A3.3 does not react with the identified cell-binding domain of FN in that the binding of A3.3 to FN is not inhibited by a MAb that binds to the cell-binding domain, and A3.3 does not block the attachment of CHO (Chinese hamster ovary) cells to a FN-coated surface. Evidence is presented which indicates that this MAb reacts with an epitope that defines a previously unidentified region of FN that plays an important role in platelet aggregation.

MATERIALS AND METHODS

Purification of Platelet and Plasma FN. Human platelet FN was partially purified from the supernatant of thrombin-activated platelets as described (11). After centrifugation, the supernatant was applied to a gelatin-Sepharose column equilibrated in 0.02 M Tris·HCl (Sigma), pH 7.6/0.15 M NaCl/1 mM $CaCl_2$. After washing, the column was eluted with the same buffer containing 4 M urea (Sigma). Purification of human plasma FN was exactly as described (12). Digestion of plasma FN by human leukocyte elastase (12) and purification of the various domains have been described in detail elsewhere (12, 13). Digestion of FN by thermolysin was as described (14).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FN, fibronectin; MAb, monoclonal antibody; HRP, horseradish peroxidase.

Production of MAbs Against FN. MAbs against platelet and plasma FN were prepared essentially by the method of Galfre *et al.* (15). Briefly, spleen cells from immunized 12-wk-old male BALB/c mice were fused with myeloma line SP2/AG14 and positive hybridomas were detected by solid-phase radioimmunoassay. These were subcloned into soft agar and rescreened. Positive subclones were propagated by intraperitoneal injection into pristane-primed BALB/c mice and ascites fluid was obtained. MAbs were purified from ascites fluid by precipitation with 50% saturated ammonium sulfate followed by gel filtration on Sephacryl S-200 or affinity chromatography on an affinity column containing goat antibodies to mouse gamma globulin (Cappel Laboratories, Cochranville, PA). MAb A3.3 was typed with an ELISA kit from Zymed Laboratories (South San Francisco, CA) and found to be an IgG1κ.

Solid-Phase Binding Assays. MAbs or other proteins (at 10 μg/ml) were adsorbed to polyvinyl chloride wells by incubation for 12 hr at 4°C. Wells were blocked with 1% (wt/vol) bovine serum albumin. Test solutions of the various unlabeled competitors were added at the indicated concentrations together with 10 cpm of ^{125}I-labeled MAb or FN (^{125}I-MAb or ^{125}I-FN). Plates were incubated for 4 hr at 37°C and extensively washed with the 1% bovine serum albumin solution. The radioactivity in individual wells was then measured. Purification of thrombospondin (11) and von Willebrand factor (16) was as described. Human fibrinogen was purchased from Kabi (Stockholm, Sweden) and ovalbumin and bovine serum albumin were from Sigma. α_2-Macroglobulin was a gift of P. Schlessinger and type V collagen was a gift of R. Burgeson. FN and A3.3 were iodinated with Iodo-beads (Pierce) and purified by gel filtration. The CHO cell attachment assay was performed as described (17). Plates were coated with human plasma FN, and CHO cells allowed to adhere for 2 hr at 37°C. To obtain cell count data, 20 fields (0.1 mm^2) were counted for each condition.

Platelet Preparation for Use in Aggregation Studies. Blood was drawn from healthy volunteers who had not taken aspirin for at least 10 days. Samples were mixed with 1/10 vol of the anticoagulant 38 mM citric acid/75 mM sodium citrate/135 mM glucose, pH 4.5 (ACD), and platelet-rich plasma was prepared by centrifugation at 160 × *g* for 10 min. Platelets were concentrated by centrifugation at 1300 × *g* for 10 min in the presence of an additional 1/10 vol of anticoagulant and resuspended in 5% of the original blood volume in 0.05 M Tris·HCl, pH 7.4/0.15 M NaCl/0.3% bovine serum albumin/5 mM glucose. The suspension was passed over a Sepharose 2B column (2.5 × 10 cm) equilibrated in the above Tris buffer. Platelet aggregation and [^{14}C]serotonin secretion studies were performed with a Payton (Buffalo, NY) dual channel platelet aggregometer as described (10) at a final platelet count of 2.5 × 10^8/ml.

Microtiter Assay of Platelet Aggregation. Gel-filtered human platelets (2.5 × 10^8/ml) were activated by thrombin at 0.5 unit/ml in the presence of serially diluted antibodies and 2 mM $MgCl_2$/2 mM $CaCl_2$. The microtiter plate was shaken on a Hyperion (Miami, FL) Micromix for 20 min at room temperature and then inspected under indirect lighting against a black background. Unaggregated platelets remain in suspension and appear turbid, while aggregation causes clearing of the solution. Large aggregates are readily visible as well. Serotonin release was assayed as described (10).

NaDodSO$_4$ Gels and Protein Blots. NaDodSO$_4$/PAGE was performed with the procedure of Laemmli (18). Proteins on gels (5–15% acrylamide) were electrophoretically transferred to nitrocellulose paper and the blots were blocked with 3% bovine serum albumin for 2 hr at room temperature. The blots were incubated with purified A3.3 (0.5 μg/ml) in 0.02 M Tris·HCl, pH 7.6/0.5 M NaCl/1% bovine serum albumin for 1 hr at room temperature. Transfers were rinsed twice with the above Tris-buffered saline containing 0.05% Tween 20 and then were incubated with a conjugate of horseradish peroxidase (HRP) and goat antibodies to mouse IgG in the Tris-buffered saline for 2 hr at room temperature. After several washes with the Tween-containing buffer, HRP was detected with 4-chloronaphthol and H_2O_2.

RESULTS

Specificity of MAb A3.3. This MAb was initially detected in a polyvinyl chloride plate assay with immobilized FN. To establish its specificity two approaches were used. First, A3.3 was allowed to react with a blot of purified human plasma FN and platelet-rich plasma. As seen in Fig. 1 *Left*, the antibody detects only a single antigen, which corresponds to human plasma FN. Second, A3.3 was iodinated and its binding to immobilized FN was challenged with a variety of proteins. As shown in Fig. 1 *Right*, thrombospondin, fibrinogen, von Willebrand factor, α_2-macroglobulin, type V collagen, and ovalbumin do not compete for the binding of A3.3 to FN, while FN itself effectively competes. Thus the antibody does not recognize an epitope contained on any of the other proteins in platelets or plasma.

Effect of A3.3 on Platelet Aggregation. To compare the effects of A3.3 with those of other MAbs directed against defined regions of the FN molecule, we devised a microtiter plate aggregation assay that requires smaller volumes of reagents than the aggregometer assay. As seen in Fig. 2, this clearly reveals platelet aggregation upon thrombin activation, and nonaggregated platelets appear as a turbid suspension. Further, platelets are not activated by antibody alone, thrombin is required to observe any aggregation, and platelets in buffer alone show no tendency to aggregate in the absence of thrombin. Of the total of five anti-FN MAbs tested in this assay, only one, A3.3, showed any inhibitory effect. The four other MAbs tested, all of which react exclusively with FN, had no effect on the aggregation of platelets induced by thrombin.

The results of the microtiter aggregation assay were confirmed in the platelet aggregometer at both 25°C and 37°C. At the lower temperature the aggregation reaction is slowed down compared to 37°C (Fig. 3 *Left*). MAb A3.3 shows a concentration-dependent inhibition of aggregation (Fig. 3 *Right*), with maximal inhibition at 0.2 mg/ml and partial inhibition at 0.06 mg/ml. Fig. 3 *Center* shows a negative control, MAb A2.5, which is not directed against FN. The effect of A3.3 is not due to inhibition of platelet activation by thrombin since the secretion of serotonin is the same under all conditions shown in Fig. 3. At physiological temperature, A3.3 inhibits platelet aggregation with the same concentration dependence seen at 25°C (not shown). Further, the antibody also blocks the aggregation stimulated by the ionophore A23187 with a concentration dependence very similar to that shown in Fig. 3 (not shown) at both 25°C and 37°C. The secretion of labeled serotonin was unaffected by the MAb under all of these conditions.

Mapping of the FN Epitope Recognized by A3.3. Several approaches have been taken to mapping the site on the FN molecule with which A3.3 reacts to cause the dramatic inhibition of platelet aggregation. We first determined if A3.3 had any effect on the adhesion of CHO cells to a FN-coated surface as a means of assessing its ability to react with the identified cell-binding domain of FN. In the presence of A3.3, CHO cells adhered to FN-coated plates as well as in the presence of control antibody (1751 ± 437 and 1772 ± 445 cells per mm^2, respectively). As a positive control we used antibody M3G, which reacts with the cell-binding domain (unpublished data). This anti-FN MAb abolishes the adhesion of CHO cells to the FN-coated surface (9 ± 7 cells per mm^2). This result indicates that A3.3 does not react with the

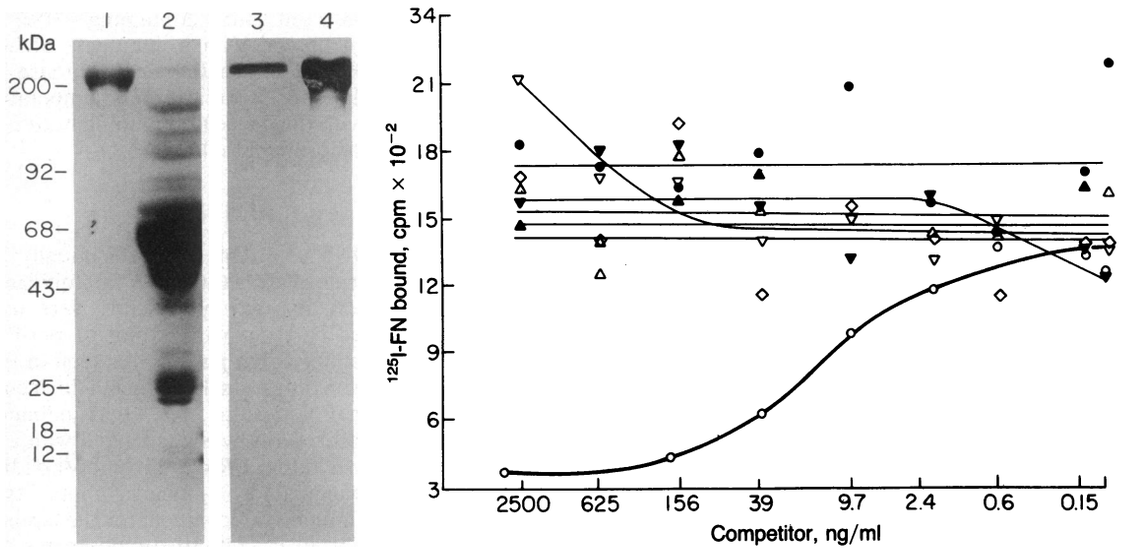

FIG. 1. Specificity of MAb A3.3 shown by protein blotting and solid-phase immunoassay. (*Left*) Purified plasma FN (lanes 1 and 4) and human platelet-rich plasma (lanes 2 and 3) were run on NaDodSO$_4$/PAGE and transferred to nitrocellulose paper. Lanes 1 and 2 were stained for protein (amido black) and lanes 3 and 4 were allowed to react with A3.3 and subsequently with an HRP-conjugated secondary antibody. The HRP was detected with 4-chloronaphthol. (*Right*) MAb A3.3 was immobilized on polyvinyl chloride wells and ^{125}I-FN was present at a constant concentration. The following competitors were tested over the indicated concentration ranges: thrombospondin (●), FN (○), human fibrinogen (▲), von Willebrand factor (△), α_2-macroglobulin (◇), type V collagen (▼), and ovalbumin (▽).

cell-binding domain of FN in any way that could compromise the interaction of the cellular receptor with this domain.

Using a solid-phase competition assay, we further investigated the relationship among A3.3 and the four anti-FN MAbs M3G, M19A, M20A, and M33. MAb A3.3 was labeled with iodine-125 and its binding to immobilized FN was examined in the presence of the four antibodies. As shown in Fig. 4, only A3.3 itself could compete for the binding of labeled A3.3 to FN. This confirms the results of the CHO adhesion experiment, which indicated that A3.3 probably does not recognize the cell-binding domain of FN, since in this competition experiment M3G shows no tendency to interfere with the binding of A3.3 to FN. Furthermore, M20A, whose epitope lies to the NH_2-terminal side of the cell-binding domain, and M33, whose epitope is COOH-terminal to the cell-binding domain (unpublished data), are both unable to compete with A3.3.

In an attempt to directly locate the epitope with which A3.3 reacts, we performed a series of experiments in which A3.3 was used to probe electrophoretic blots of previously identified fragments of FN. Fig. 5 shows that A3.3 reacts with intact FN (lanes 1 and 2) as well as the large 160/140-kDa fragments generated by digestion of FN with human leukocyte elastase (12) (lanes 3 and 4). This 160/140-kDa doublet has lost the NH_2-terminal region of FN and hence lacks the lower affinity heparin-binding domain, the gelatin-binding domain, the actin-binding domain, and the *Staphylococcus aureus*-binding domain. Further digestion of the 160/140-kDa piece with chymotrypsin yields a 105-kDa fragment that encompasses the cell-binding domain (13) and the 40/30-kDa high-affinity heparin-binding domain, which lies in the COOH-terminal region of the 160/140-kDa fragments (13). The 40-kDa piece arises from the 160-kDa fragment and the 30-kDa piece from the 140-kDa peptide (13). As seen in Fig. 5, MAb A3.3 does not react with either the 105-kDa peptide or the 40- or 30-kDa pieces. Thus it appears that the epitope for A3.3 resides in a protease-sensitive region common to both the 160- and 140-kDa fragments that is distinct from the previously identified cell-binding domain or the COOH-terminal high-affinity heparin-binding domain.

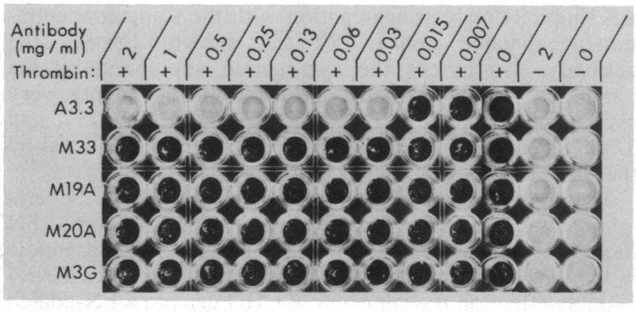

FIG. 2. Microtiter assay of platelet aggregation. Each well contained platelets at 2.5 × 10^8/ml, thrombin at 0.5 unit/ml where indicated (+), and the indicated concentration of antibody in the presence of 2 mM $CaCl_2$/2 mM $MgCl_2$. After agitation for 20 min at room temperature, the plate was photographed against a black background. Turbid wells are due to the unaggregated platelet suspension, while the platelet aggregates are visible in cleared wells. Note that only A3.3 causes inhibition of aggregation and the endpoint (last inhibitory concentration) is 0.03 mg/ml.

Because the A3.3 epitope was destroyed by elastase and chymotrypsin, several other proteases—including cathepsin D, clostripain, trypsin, pepsin, and thermolysin—were used to fragment FN. These digests were then tested in the competition assay described above to determine if they contained a fragment able to block the binding of labeled A3.3 to immobilized FN. Only the thermolytic digest (1:166, wt/wt, thermolysin to FN for 5 hr at room temperature) appeared to retain a reactive epitope. This digest was separated on NaDodSO$_4$/PAGE and a protein blot was probed with A3.3. Fig. 5, lane 9, shows the pattern of fragments obtained from FN under these conditions. The two largest correspond to the 160/140-kDa doublet found in the elastase digest (lanes 3 and 4). These retain the ability to bind A3.3 (lane 10), as do two smaller fragments of 120 and 70 kDa (lane 10). This 70-kDa fragment is a minor species (compare lanes 9 and 10), and does not contain epitopes for any of the other anti-FN MAbs employed in this study (not shown). Thus it appears that the MAb A3.3 reacts with a unique protease-sensitive epitope and defines a site on the FN molecule that plays a critical role in platelet aggregation.

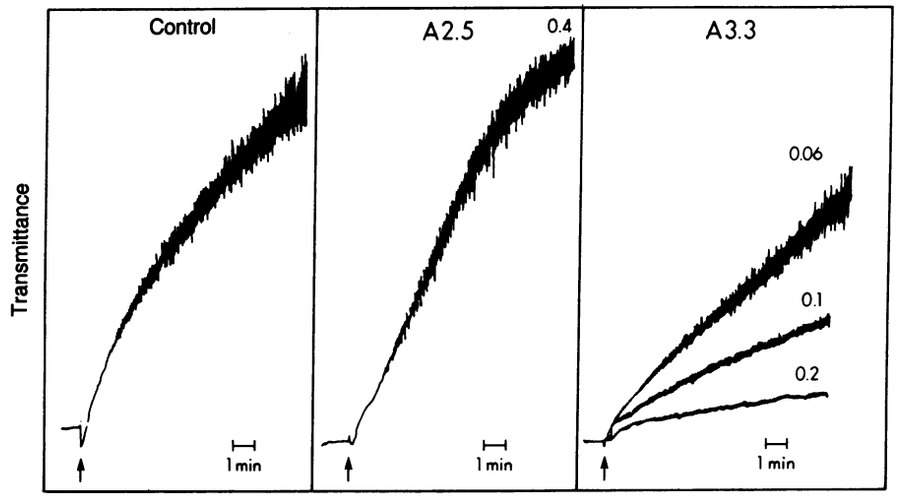

FIG. 3. Inhibition of platelet aggregation by MAb A3.3. Gel-filtered human platelets (2.5×10^8/ml) were activated by thrombin at 0.5 unit/ml (arrow) while being stirred at room temperature in the presence of 2 mM $CaCl_2$/2 mM $MgCl_2$. Aggregation of the platelets is indicated by the increase in light transmittance as monitored in a Payton aggregometer. Control, platelets in buffer alone; A2.5, platelets in the presence of a MAb (0.4 mg/ml) that does not recognize FN; A3.3, platelets in the presence of various concentrations (mg/ml) of the anti-FN MAb A3.3. Not indicated: platelet activation as monitored by [^{14}C]serotonin release after 10 min was 67 ± 4% for all the experiments shown.

DISCUSSION

The MAb designated A3.3 appears to recognize an epitope on both platelet and plasma FN that plays a critical role in the aggregation of gel-filtered platelets. This epitope is not found on any other soluble plasma proteins or platelet proteins likely to contaminate our platelet FN preparation. While our results thus far do not allow us to unambiguously locate the epitope in the linear sequence of FN, we can draw some conclusions about where it is not. Since A3.3 reacts with the 160/140-kDa doublet from both the elastase and thermolysin digests (Fig. 5), we can conclude that the epitope is not in the NH_2-terminal region of FN, which contains domains for the binding of collagen, fibrin, heparin, actin, *S. aureus*, DNA, and the transglutaminase crosslinking site (1, 2). This 160/140-kDa region is also lacking the extreme COOH terminus of both FN chains, which contains the interchain disulfides that crosslink the FN monomers into the dimer (13). This COOH-terminal domain is recovered only from cathepsin D digests (19). We have probed blots of cathepsin D digests with A3.3 and found no reaction (data not shown),

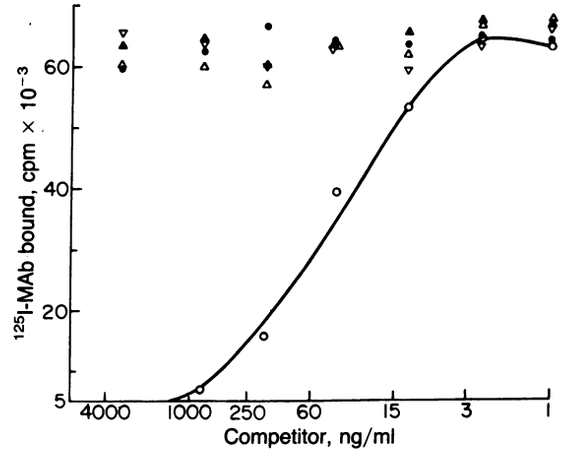

FIG. 4. Competition of MAbs for the binding of A3.3 to FN. Wells were coated with plasma FN and the ^{125}I-labeled probe is A3.3 present at a constant level in all wells. The following unlabeled competitors were tested: A3.3 (○), M3G (●), M19A (△), M20A (▲), and M33 (▽).

confirming that the epitope is not in this COOH-terminal region of the FN chains. Only two known domains have been identified within the large 160-kDa fragment: a heparin-binding domain of higher affinity than the one at the NH_2 terminus of FN (13) and a domain containing a sequence of five amino acids that binds to an as yet unidentified receptor on cells that can bind to FN-coated surfaces (1–4). We have directly tested A3.3 against the purified 105-kDa fragment containing the cell-binding domain and the 40- and 30-kDa heparin domains (Fig. 5) and found no reaction with these fragments. Ruling out these regions, which cover most of the 160-kDa fragment, one is still left with a substantial amount of protein structure as yet uncharted in terms of function. It appears as though the epitope recognized by A3.3 is located in a protease-sensitive, hence exposed, region somewhere in the remaining 10 to 20 kDa within the 160-kDa peptide. Thus, while it may be premature to refer to it as a domain, the epitope for A3.3 would seem to define a binding site on FN that has a direct role in the aggregation of platelets.

Since under normal conditions of *in situ* aggregation of platelets plasma FN is present at 300 μg/ml, it is not clear whether the relatively small amounts of FN secreted by platelets during their activation play some special role in the aggregation process or if this material simply becomes part of the available FN pool at the platelet surface. Clearly, exogenous FN is not required for aggregation to take place. The fact that other components such as fibrinogen and thrombospondin, which may be important in platelet aggregation, are all packaged together in the platelet α granule suggests that platelet FN may already exist as a complex with some of these other components. However, the finding that excess plasma FN can inhibit the aggregation of washed platelets at concentrations above 300 μg/ml indicates that soluble exogenous FN probably has access to the sites at which endogenous FN might be acting during the aggregation of washed platelets (10). Plow and Ginsberg (9) have shown that labeled plasma FN can bind to activated but not quiescent platelets. The platelet-binding site detected in those studies has a dissociation constant of 3×10^{-7} M and about 120,000 copies per platelet. Stimulation of platelets with thrombin, but not ADP or epinephrine, caused the expression of the FN-binding sites, whose presence correlated best with serotonin, and hence dense granule, release. This suggests either that the "receptor" is itself secreted by platelets or that the modification of a platelet membrane site requires the pres-

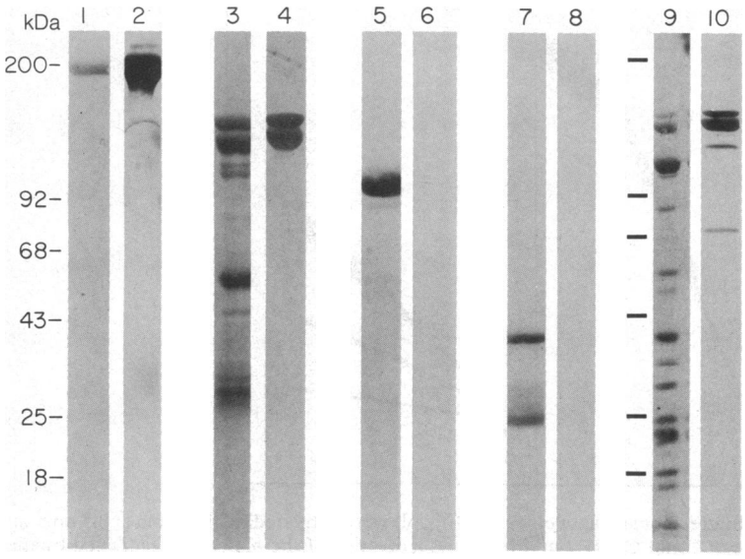

FIG. 5. Electrophoretic blots of the binding of A3.3 to FN and its fragments. The lanes contain the following: lanes 1 and 2, 10 μg of purified plasma FN; lanes 3 and 4, 40 μg of a human leukocyte elastase digest of FN; lanes 5 and 6, 20 μg of the purified 105-kDa cell-binding domain of FN; lanes 7 and 8, 20 μg of the purified 40/30-kDa heparin-binding domain; lanes 9 and 10, 40 μg of a thermolysin digest of whole FN (1:166, wt/wt, thermolysin to FN; 5 hr at room temperature). Proteins in odd-numbered lanes were stained with amido black and those in even-numbered lanes were allowed to react with A3.3 and subsequently with HRP-conjugated goat antibodies to mouse IgG, and HRP was detected with the 4-chloronaphthol reaction.

ence of a component secreted by platelets. While fibrinogen or fibrin has been suggested to be the fibronectin receptor, its affinity for FN is about two orders of magnitude lower than that found by Plow and Ginsberg (9), and FN readily dissociates from fibrinogen at 37°C. Thus it seems reasonable to search for another candidate for the FN receptor on platelets. A possibility is that activated platelets express a receptor for FN analogous to the receptor on fibroblasts that allows them to bind to FN-coated surfaces (3, 4). However, our data indicate that the cell-binding domain of FN does not react with A3.3, and further, that A3.3 does not inhibit the binding of CHO cells to FN-coated dishes. Thus the inhibition of platelet aggregation by this MAb clearly demonstrates that a determinant on FN other than the well-characterized cell-binding domain (3, 4) is important in mediating platelet aggregation.

One of the minimal models proposed for platelet aggregation is that fibrinogen itself, either from plasma or the platelet α granule, is responsible for crosslinking platelet surfaces through its ability to interact with the glycoprotein IIb/IIIa complex in the platelet membrane (20, 21). In view of the dramatic effect of A3.3, which is directed solely against FN, it is difficult to rationalize this simple model. It would seem necessary to invoke some essential role for FN in the aggregation process, but yet it is unlikely that FN binds in a stable way to fibrinogen under physiological conditions. Another component of platelet α granules secreted along with fibrinogen and FN is thrombospondin (22, 23). This large glycoprotein forms high-affinity associations with both fibrinogen (24, 25) and FN (26) and is thought to play some role in the aggregation process. However, it is unlikely that thrombospondin is the only FN receptor, since FN binds to α-granule-deficient platelets to the same extent as normal platelets (27). Thus at present the best working hypothesis would seem to be that these three proteins, fibronectin, fibrinogen, and thrombospondin (and perhaps others), form a complex matrix with themselves and with the platelet surface that must be rapidly and correctly assembled to ensure that platelets aggregate. Available data suggest that disruption of this assembly process at any one of several points is sufficient to prevent the formation of stable platelet aggregates.

We thank the staff of the Washington University Hybridoma Center, Dr. Joe Davie, Director, for their assistance with cell fusions and initial selection of hybridomas. The cooperation of the staff of the St. Louis Chapter of the American Red Cross is gratefully acknowledged. Support for this work was from the following sources: National Institutes of Health Training Grant HL-17138 to D.M.H.; National Institutes of Health Grant HL-26009 and Contract 82-22 from Mallinckrodt, Inc. (St. Louis, MO), to J.A.M.; National Institutes of Health Grant HL-29608 and a grant in aid from the American Heart Association with funds contributed in part by the Missouri Affiliate to S.A.S.; and a grant from the Monsanto Company (St. Louis, MO) to W.A.F. Also, J.A.M. and W.A.F. are Established Investigators of the American Heart Association.

1. Yamada, K. M. (1983) *Annu. Rev. Biochem.* **52,** 761–799.
2. Hynes, R. O. & Yamada, K. M. (1982) *J. Cell Biol.* **95,** 369–377.
3. Pierschbacher, M. D. & Ruoslahti, E. (1984) *Nature (London)* **309,** 30–33.
4. Yamada, K. M. & Kennedy, D. W. (1984) *J. Cell Biol.* **99,** 29–36.
5. Bensusan, H. B., Koh, T. L., Henry, K. G., Murray, B. A. & Culp, L. A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5864–5868.
6. Santoro, S. A. & Cunningham, L. W. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2644–2648.
7. Plow, E. F., Birdwell, C. & Ginsberg, M. H. (1979) *J. Clin. Invest.* **63,** 540–543.
8. Ginsberg, M. H., Painter, R. G., Forsyth, J., Birdwell, C. & Plow, E. F. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1049–1053.
9. Plow, E. F. & Ginsberg, M. H. (1981) *J. Biol. Chem.* **256,** 9477–9482.
10. Santoro, S. A. (1983) *Biochem. Biophys. Res. Commun.* **116,** 135–140.
11. Dixit, V. M., Grant, G. A., Santoro, S. A. & Frazier, W. A. (1984) *J. Biol. Chem.* **259,** 10100–10105.
12. McDonald, J. A. & Kelley, D. G. (1980) *J. Biol. Chem.* **255,** 8848–8858.
13. Ingham, K. C., Brew, S. A., Broekelmann, T. J. & McDonald, J. A. (1984) *J. Biol. Chem.* **259,** 11901–11907.
14. Sekiguchi, K. & Hakomori, S.-I. (1983) *J. Biol. Chem.* **258,** 3967–3973.
15. Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. (1977) *Nature (London)* **291,** 421–423.
16. Santoro, S. A. & Cowan, J. F. (1982) *Collagen Rel. Res.* **2,** 31–43.
17. McDonald, J. A., Broekelmann, T. J., Kelley, D. G. & Villiger, B. (1981) *J. Biol. Chem.* **256,** 5583–5587.
18. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
19. Richter, H. & Hormann, H. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363,** 351–364.
20. McEver, R. P., Bennett, E. M. & Martin, M. N. (1983) *J. Biol. Chem.* **258,** 5269–5275.
21. Berndt, M. C. & Phillips, D. R. (1981) in *Platelets in Biology and Pathology 2*, ed. Gordon, J. L. (Elsevier/North-Holland, New York), pp. 43–75.
22. Lawler, J. L., Slayter, H. S. & Coligan, J. E. (1978) *J. Biol. Chem.* **253,** 8609–8616.
23. Gartner, T. K., Williams, D. C., Minion, F. C. & Phillips, D. R. (1978) *Science* **200,** 1281–1283.
24. Dixit, V. M., Grant, G. A., Frazier, W. A. & Santoro, S. A. (1984) *Biochem. Biophys. Res. Commun.* **119,** 1075–1081.
25. Leung, L. L. K. & Nachman, R. L. (1982) *J. Clin. Invest.* **70,** 542–549.
26. Lahav, J., Schwartz, M. A. & Hynes, R. O. (1982) *Cell* **31,** 253–262.
27. Ginsberg, M. H., Wencel, J. D., White, J. G. & Plow, E. F. (1983) *J. Cell Biol.* **97,** 571–573.