Activation of Human Platelets by a Stimulatory Monoclonal Antibody

(Received for publication, October 6, 1989)

Elizabeth Kornecki, Bogdan Walkowiak, Ulhas P. Naik, and Yigal H. Ehrlich

From the Department of Anatomy and Cell Biology, State University of New York Health Science Center, Brooklyn, New York 11203, the CSI/IBR Center for Developmental Neuroscience, College of Staten Island, City University of New York, and the New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10301

The clinical significance of the interaction of antibodies with circulating platelets is well documented, but the mechanisms underlying these interactions are not fully known. Here we describe the characterization of anti-human platelet membrane protein monoclonal antibody (mAb) termed F11. Interaction of mAb F11 with human platelets resulted in dose-dependent granular secretion, measured by [3H]serotonin and ATP release, fibrinogen binding and aggregation. Analysis of the specific binding of mAb F11 to platelets revealed a high affinity site with 8,067 ± 1,307 sites per platelet with a dissociation constant (K_d) of 2.7 ± 0.9 × 10^{-11} M.

Two membrane proteins of 32,000 and 35,000 daltons, identified by Western blotting, were recognized by mAb F11. Incubation of [%]F1-plateleted platelets with mAb F11 resulted in rapid phosphorylation of intracellular 40,000- and 20,000-dalton proteins, followed by dephosphorylation of these proteins. Monovalent Fab fragments or Fc fragments of mAb F11 IgG did not induce platelet aggregation or secretion; however, Fab fragments of mAb F11 IgG blocked mAb F11-induced platelet aggregation and the binding of [125I]-mAb F11 to platelets. The addition of an anti-GPIIIa monoclonal antibody (mAb G10), which inhibits [125I]-fibrinogen binding and platelet aggregation, completely blocked mAb F11-induced [3H]serotonin secretion and aggregation but not the binding of [125I]-mAb F11 to platelets. mAb G10 also inhibited the increase in the phosphorylation of the 40,000- and 20,000-dalton proteins induced by mAb F11. These results implicate the involvement of the GPIIIa molecule in the chain of biochemical events involved in the induction of granular secretion.

Platelets and platelet membrane glycoproteins play a significant role in immunologic reactions. Early studies have suggested that allantibodies developed in patients following multiple transfusions activate platelets in vivo resulting in thrombocytopenia (1, 2). Specific anti-platelet autoantibodies and allantibodies to membrane glycoproteins (GP) such as GPIb, GPIIb, GPIIIa, and GPV now have been identified in patients with clinical disorders of drug-dependent thrombocytopenia purpura, posttransfusion purpura, neonatal isoimmune thrombocytopenia, chronic immune thrombocytopenia purpura, and septicemia (3-16). AIDS patients with acute thrombocytopenia purpura were shown to have anti-platelet antibodies (17, 18). The study of the interaction of immunoglobulins with platelets has been enhanced by the development of monoclonal antibodies which induce platelet aggregation (19-26). The study of such monoclonal antibodies enables the identification of specific platelet membrane antigens involved in platelet activation by immunoglobulins in vivo, and in the elucidation of the molecular mechanisms resulting in this activation process.

In this paper we describe the properties and mechanism of action of a novel monoclonal antibody which acts as a potent inducer of aggregation and secretion in human platelets. This monoclonal antibody recognizes a unique receptor on the platelet surface which is involved in platelet activation, and we present data showing the association of the fibrinogen receptor in platelet secretion.

**EXPERIMENTAL PROCEDURES**

**Collection of Blood and Plasma**—Blood was obtained from normal individuals who were free of any medication for at least 1 week prior to experimentation. All volunteers signed an informed consent form approved by the State University of New York, Health Sciences Center, Brooklyn, New York Committee on Human Research.

**Preparation and Washing of Platelets**—Platelets were washed and isolated from blood freshly collected in acid citrate dextrose (27). Platelets were washed by differential centrifugation and resuspended in a Tyrode-albumin (0.35%) solution buffered with 11.9 mM sodium bicarbonate (pH 7.4) containing heparin (2 units/ml) and 0.5 mg/ml potato amyrase (28). Platelets were finally suspended in a final Tyrode-albumin solution containing 2 mM CaCl_2, 1 mM MgCl_2, 11.9 mM NaHCO_3, NaH_2PO_4 (0.36 mM), glucose (0.1%), and bovine serum albumin (0.3%). Gel-filtered platelets were prepared as described previously (30, 31). Platelets were counted microscopically using a phase contrast microscope and a hemocytometer.

**Platelet Aggregation**—The experiments were carried out in a Chronolog Lumi-Aggregometer (Chronolog Corp., Havertown, PA). Platelet aggregation was initiated by the addition of monoclonal antibodies (10 μl) at various concentrations to 0.45 ml of a platelet suspension containing 2.4 × 10^9 platelets/ml. The extent of platelet aggregation was expressed in light transmission units and the initial velocity of aggregation was measured from the slope of platelet aggregation tracings (light transmission units/min). The aggregometer was calibrated with 0.45-mI suspensions of washed platelets for 10% light transmission.

**Platelet ATP Release**—The experiments were carried out in a Lumi-Aggregometer using the Chronolog luciferin/luciferase reagent. Platelet Serotonin Release—Serotonin (side chain-2-[14C]hydroxytryptamine) cystine sulfate, 56 μCi/mmol) (Amersham Corp.) was added to washed platelets or platelet-rich plasma and incubated for 30 min at 22°C. Imipramine (2 μM) was added to prevent reincorporation of serotonin and incubation continued for 5 min. Formaldehyde (135 mM) was added to stop the release reaction and platelet suspensions were centrifuged for 1 min at 11,000 × g (32). The extent of release was calculated as the percentage of thrombin-releasable [14C]DOPAC.
Platelet Activation by a Monoclonal Antibody

5HT appearing in the supernatant fraction following stimulation as described previously (33). Iodination of Antibodies and Antibody Binding to Platelets—Purified monoclonal antibodies were radiolabeled by using the Iodo-Gen method (34) or by use of Iodo-Beads (Pierce Chemical Co.). The specific activities were approximately 7.4 x 10^6 cpm/μg when 1 mg/ml of monoclonal antibodies were radiolabeled by the Iodo-Gen method and 2 x 10^6 cpm/μg when 50 μg/ml of monoclonal antibodies were radiolabeled by the Iodo-Bead method. Binding of radiolabeled antibody to platelet-rich plasma or to washed platelets was performed over 15 μl of silicon oil or 20% sucrose (35). The incubation mixture consisted of platelet aliquots (2-5 x 10^10/ml) and radiolabeled monoclonal antibodies in total volumes of 220 or 300 μl. Values for specific binding were analyzed according to the method of Scatchard (36).

Platelet Aggregation and Secretion by mAb F11:—Washed platelets (10^8/ml), resuspended in phosphate-free Tyrode's buffer containing albumin (pH 7.4), were incubated with 1 mCi/ml 125I for 30 min at 37°C. The platelets were washed and resuspended in phosphate-containing bovine serum albumin-free Tyrode's solution. Aliquots (40 μl) of the platelet suspension were incubated with agonists at 22°C under nonstirring conditions. The reactions were stopped by the addition of SDS Laemmli solution containing 2% β-mercaptoethanol and processed for SDS-gel electrophoresis followed by autoradiography.

Immunization and Hybridoma Production—Balb/c mice were injected with human platelet membranes emulsified with incomplete Freund's adjuvant containing 4 mg/ml M. butyricum. Following weekly injections, mouse spleens were removed and fused with SP2/0-Ag14 myeloma cells. Supernatants were tested for effects on platelet function. Selected hybridomas were cloned at least twice. Antibodies were characterized by enzyme-linked immunosorbent assay with the use of subclass-specific goat or rabbit anti-mouse antibodies. Monoclonal antibody F11 is an IgG2a isotype.

Preparation of IgG and Fab Fragments—IgG was obtained from hybridoma supernatants by initial precipitation with 50% saturated ammonium sulfate followed by two washings with 40% ammonium sulfate (37). Following dialysis, the samples were chromatographed on a DEAE-cellulose column in 17 mM phosphate buffer (pH 7.0). The Fab fragments were prepared by papain digestion followed by carboxymethylcellulose column chromatography (38). IgG was also prepared from mouse ascites fluid by protein A-Sepharose column chromatography (39).

SDS-Polyacrylamide Gel Electrophoresis and Western Blotting—SDS-polyacrylamide gel electrophoresis was performed in 4% stacking gels and in 7.5 or 10% separation polyacrylamide slab gels or 7-14% polyacrylamide exponential gradient gels (40). The gels were stained for proteins with Coomassie Brilliant Blue, destained in 10% acetic acid, 50% methanol, dried in a vacuum, and exposed to Kodak X-Omat developer. Reduced samples (reduced with 2% β-mercaptoethanol) or nonreduced samples were applied to SDS-polyacrylamide slab gels for electrophoresis. Molecular weight determinations were made by comparison to Bio-Rad reduced samples of myosin (200,000), Escherichia coli β-patulolysin (116,000), phosphorylase b (97,400), bovine serum albumin (66,200), ovalbumin (42,700), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400). The completed nonstained gels were transferred also to nitrocellulose paper for staining with specific antibodies using the method of Towbin et al. (41).

RESULTS

Induction of Platelet Aggregation and Secretion by mAb F11:—Involvement of the Platelet F11 Antigen in Platelet Activation—Fig. 1 shows the effects of addition of mAb F11 to intact, washed platelet suspensions. Following the addition of mAb F11, there was an initial lag period followed by ATP release and platelet aggregation. We also measured serotonin release induced by mAb F11. At concentrations of mAb F11 as low as 0.25 μg/ml, [14C]serotonin release was calculated to be 70.04 ± 8.2% (mean ± S.D.) of total uptake in seven separate experiments performed in triplicate. In comparison, thrombin (10 units/ml)-induced [14C]5HT release was consistently lower, and thrombin induced 48.5 ± 13.1% (mean ±

![Fig. 1. Effect of mAb F11 on the aggregation and secretion of platelets.](http://www.jbc.org/)

As shown in Fig. 1, mAb F11 induced platelet aggregation (top panel) and ATP release (bottom panel) were not immediate events but were initiated after a latency period which was dependent on the concentration of mAb F11. The latency observed for platelet activation following the addition of mAb F11 was shortened with increasing concentrations of mAb F11. Approximately 5.8 μg/ml of mAb F11-induced platelet activation within 3 min, whereas with higher concentrations of mAb F11 the latency period decreased to less than a minute.

Binding of 125I-mAb F11 to Platelets—The binding of 125I-mAb F11 to platelets increased rapidly with time and reached equilibrium within 15 min. The binding of mAb F11 to platelets was dependent on the concentration of radiolabeled ligand. Fig. 2 shows the results obtained in 11 separate experiments using blood obtained from 11 different donors. This figure displays the exasperating but often present variability that can be observed in binding studies with human platelets from separate donors. In several experiments, the maximal amount of mAb F11 bound at saturation was approximately 0.35 μg/10^10 platelets, whereas in other experiments the binding of mAb F11 at saturation was approximately 0.1 μg/10^10 platelets. The two experiments which represent the maximal and minimal binding of mAb F11 to platelets, in our studies to date are highlighted in this figure. Scatchard analysis of
the data from all 11 experiments indicated a single class of binding sites with 8067 ± 1307 binding sites/platelet with a dissociation constant of 2.7 ± 0.9 × 10⁻⁸ M. These values are the weighted means (X ± S.E.) which were calculated by using the correlation coefficient as a weighting factor.

**Stimulation of Protein Phosphorylation in Platelets by mAb FII**—The effect of mAb FII on the phosphorylation of intracellular platelet proteins is shown in Fig. 3. Following incubation with mAb FII, we found a selective and time-dependent increase in the phosphorylation of proteins with apparent molecular weights of 40,000 and 20,000. Such changes in phosphorylation pattern were found in intact platelets and in chymotrypsin-pretreated platelets. The phosphorylation of the 40,000- and 20,000-dalton proteins increased significantly within seconds following the addition of mAb FII. In intact platelets, the maximal increase in the phosphorylation of these proteins occurred following 5 min of incubation with mAb FII. After longer incubations there was a decrease in the phosphorylation state of both the 40,000- and 20,000-dalton proteins. The changes in the phosphorylation state of the 40,000- and 20,000-dalton proteins induced by mAb FII in platelets pretreated with chymotrypsin followed essentially the same time course shown for intact platelets in Fig. 3.

**Platelet Proteins Recognized by mAb FII**—The platelet proteins recognized by mAb FII in a Western immunoblotting procedure are shown in Fig. 4. mAb FII recognized epitopes on two platelet-membrane proteins with molecular masses of 32 and 35 kDa. Both of these proteins were recognized by mAb FII in 18 separate experiments conducted to date. Another monoclonal antibody, named mAb G10, which is described below, as well as immunoglobulins obtained from Sp2/0-injected mice (Fig. 4), showed no interaction with these proteins.

**Involvement of GPIIb-IIIa in the Activation of Intact Platelets by mAb FII**—We isolated a second monoclonal antibody, termed G10, which is directed against the platelet GPIIIa molecule. Fig. 5 shows the results of Western blotting experiments using three different antibodies for comparison. mAb G10 is shown to immunoblot GPIIIa (lane A). As previously shown by us (30), glycoproteins IIb and IIIa were recognized by a polyclonal anti-human platelet membrane antibody (lane B). A polyclonal anti-human 66-kDa protein antibody (kind gift from Dr. S. Niewiarowski, Temple University, Philadelphia, PA) also immunoblotted GPIIIa (lane C). This 66-kDa protein was shown by us previously to be a proteolytically derived fragment of the GPIIIa molecule (30). An antibody prepared against this protein immunoblotted GPIIIa in undigested platelets (42). As control, immunoglobulins obtained from ascites of mice injected with Sp2/0 hybridoma cells did not immunoblot GPIIIa (lane D).
Fll Fab
upper
induced platelet aggregation. Fifty percent inhibition of plate-
in the presence of the anti-glycoprotein IIIa monoclonal an-
tibody mAb GlO. mAb G10 completely inhibited mAb Fll-
hibition of mAb Fll binding to platelets, as
described below.

The mAb Fll-induced platelet aggregation, ATP release, 
[14C]5HT secretion and protein phosphorylation were tested in the presence of the anti-glycoprotein IIIa monoclonal antibody mAb G10. mAb G10 completely inhibited mAb Fll-induced platelet aggregation. Fifty percent inhibition of plate-
et aggregation and ATP release occurred at concentrations of mAb G10 ranging from 0.35 to 0.45 μg/ml (Fig 6A). A slightly higher concentration of mAb G10 (1.8 μg/ml) inhibited 50% of the mAb Fll-induced [14C]5HT release. Fig. 6B shows that the stimulation by mAb Fll of the phosphorylation of the intracellular 40,000- and 20,000-dalton proteins by mAb F-11 was completely inhibited by mAb G10. Fig. 6B also shows that the mAb Fll-induced increase in phosphorylation starts before aggregation (compare time points marked d in upper and lower panels of Fig. 6B). These results were not due to blockade by mAb G10 of mAb Fll binding to platelets, as described below.

Inhibition of mAb Fll-induced Platelet Aggregation by mAb
Fll Fab Fragments—Fab fragments were prepared from pu-
rified IgG of mAb Fll. Neither these monovalent molecules nor Fc fragments induced granular secretion or platelet ag-
gregation. The effects of monovalent Fab fragments on mAb Fll-induced platelet aggregation are shown in Fig. 7A. The addition of increasing concentrations of Fab fragments pro-
longed the latency of mAb Fll-induced platelet aggregation from 2 min to 1 h and longer. The mechanism responsible was found to be the inhibition of the Fab fragments of mAb Fll binding, as shown in Fig. 7B. The IC50 of Fab fragment inhibition of mAb Fll binding is approximately 5 μg/ml. On the other hand, Fc fragments prepared from mAb Fll-IgG had no effect on mAb Fll binding to platelets, and did not inhibit mAb Fll-induced platelet aggregation even at a concentra-
tion as high as 435 μg/ml. In five separate experiments, mAb G10-IgG did not inhibit the binding of 125I-mAb Fll to platelets. The binding data was similar to that seen in Fig. 7B using mAb Fll Fc fragments which did not inhibit mAb Fll binding.

Comparison of Fibrinogen-induced Platelet Aggregation and
mAb Fll-induced Aggregation in Chymotrypsin-potentreated Platelets—Fig. 8, A and B, shows the spontaneous aggregation of chymotrypsin-treated platelets upon the addition of fibrin-
ogen in the presence and absence of PGE
. The result indicates that the elevation of cyclic AMP does not interfere with this type of aggregation. In contrast, the mAb Fll-induced aggregation of chymotrypsin-treated platelets is completely inhibited by PGE, as shown in Fig. 8 C, even though the fibrinogen-induced aggregation still occurs as shown in Fig. 8 D. This result indicates that mAb Fll-induced platelet aggregation is sensitive to raised levels of intracellular cyclic AMP.}

Inhibition of mAb Fll-induced Secretion by ATP—mAb
Fll-induced platelet aggregation can be inhibited by apyrase,
Platelet Activation by a Monoclonal Antibody

Platelet Activation by a Monoclonal Antibody

A *rn

FIG. 7. Inhibition of mAb F11-induced platelet aggregation and 125I-mAb F11 IgG binding by mAb F11 Fab fragments. A, inhibition of platelet aggregation. mAb F11 (5 &g/ml)-induced platelet aggregation was performed in the presence of mAb F11 Fc fragments (90 &g/ml) or mAb F11 Fab fragments (90 &g/ml) as shown above. mAb F11 Fab and Fc fragments were incubated with platelets for 1 min at 37 °C prior to the addition of mAb F11. mAb F11 Fc fragments did not inhibit mAb F11-induced platelet aggregation. B, dose-dependent inhibition of 125I-mAb F11 binding to platelets by mAb F11 Fab fragments. mAb F11 Fab (circles) or mAb F11 Fc fragments (triangles) were incubated with platelets for 1 min prior to the addition of 125I-mAb F11. The binding of 125I-mAb F11 IgG to platelets was performed as described under “Experimental Procedures.” Each point is the mean of at least two separate experiments. mAb G10 IgG gave results in at least five separate experiments which were similar to those observed with mAb F11 Fc fragments (triangles).

ATP, and ATP analogues. Table I shows the IC50 values for such inhibition by ATP, 5'-p-fluorosulfonylbenzoyladenosine, and AMP-PNP. The simultaneous secretion of [14C]5HT from platelet dense granules following the addition of mAb F11 was also measured (Fig. 9). We found that although ATP inhibited [14C]5HT secretion induced by mAb F11 (Fig. 9), maximal inhibition of secretion, even at high ATP concentrations, was never greater than 70%. Thirty percent of the [14C]5HT release induced by mAb F11 could not be inhibited by ATP. Similar results of inhibition of [14C]5HT were found with apyrase. A high concentration of apyrase (0.9 mg/ml) inhibited 70% of [14C]5HT secretion induced by mAb F11, and 29.3 ± 2.6% of the secretion was not inhibited by apyrase. This would indicate that mAb F11 acts directly on the platelet surface to induce 30% granular secretion. Is the ADP receptor involved in this initial action? To test this possibility, platelets were made refractory to ADP by adding nanomolar concentrations of ADP as shown in the bottom panel of Fig. 10. Platelets which were made refractory to ADP still responded to mAb F11 with a shortened latency, even though maximal concentrations of ADP could not induce aggregation. This result indicates that mAb F11 does not interact directly with the ADP receptor site.

DISCUSSION

We report here the characteristics and mechanisms of action of a monoclonal antibody named mAb F11, a potent platelet agonist. mAb F11 directly stimulates platelet secretion, measured as ATP and serotonin release, and fibrinogen-dependent platelet aggregation. By interacting with a unique receptor, termed F11, mAb F11 induces rapid intracellular phosphorylation of two major proteins: a 40,000-dalton protein which is a known substrate for protein kinase C, and a 20,000-dalton protein, the light chain of myosin and the substrate for myosin light chain kinase, a Ca2+-dependent enzyme. Thus, the cascade of intracellular biochemical events triggered by mAb F11 involves stimulation of protein kinase C and elevation of free calcium ion levels, in all likelihood through activation of the phosphoinositide cycle (43).

We have found that mAb F11 recognizes platelet surface membrane proteins of approximately 32,000 and 35,000 daltons, as determined by Western blotting and by analysis of the bound material eluted from a mAb F11 affinity column. By Scatchard analysis we have shown that there are approximately 8,000 high affinity F11 binding sites per platelet. The platelet F11 antigen appears to be resistant to surface proteolysis since we observed that chymotrypsin- and elastase-pretreated platelets are stimulated by mAb F11 to secrete and aggregate. These proteolytically treated platelets show significant increases in intracellular phosphorylation of the 20,000-
Table I

Inhibition of mAb F11-induced platelet aggregation

| Compounds tested | ICso values (μM) |
|------------------|------------------|
| ATP              | 220              |
| AMP-PNP          | 83               |
| 5'-FSBA          | 85               |

FIG. 9. Inhibition of mAb F11-induced [3H]5HT release by ATP. Platelet-rich plasma was incubated with [3H]5HT for 30 min at 37°C as described under “Experimental Procedures.” Aliquots (0.45 ml) of platelet-rich plasma were incubated for 1 min at 37°C under stirring conditions with various concentrations of ATP. Aggregation was initiated by the addition of mAb F11 (5 μg/ml).

FIG. 10. Stimulation of ADP refractory platelets by mAb F11. Top panel, washed platelets were induced to aggregate by the addition of ADP (100 μM). The addition of mAb F11 (5 μg/ml) also induced platelet aggregation in separate aliquots of washed platelets. Bottom panel, platelets were made refractory to ADP by adding aliquots of suboptimal concentrations of ADP (0.5 μM). Finally, ADP at 100 μM (final concentration) was totally ineffective in inducing platelet aggregation. mAb F11 (5 μg/ml) was able to aggregate platelets made refractory to ADP.

and 40,000-dalton proteins following the addition of mAb F11. The platelet receptor(s) recognized by mAb F11 consists of a protein duplex of molecular mass of 32,000 and 35,000 daltons. This appears to be a unique platelet antigen(s) previously not recognized by other stimulatory antibodies. Detailed characterization of the structure of this unique receptor and its associated glycoproteins is in progress.

The fibrinogen receptor, consisting of glycoproteins Ib-IIIa, appears to play an important role in the action of mAb F11. A monoclonal antibody developed in our laboratory (named mAb G10), which blocks aggregation and 125I-fibrinogen binding to ADP-stimulated platelets, was found to be directed against GPIIIa. mAb G10 potently and completely inhibited mAb F11-stimulated platelet aggregation. Moreover, mAb G10 blocks intracellular events induced by mAb F11: these events include the increase in the phosphorylation of 40,000- and 20,000-dalton proteins and the intuation of [3H]5HT and ATP secretion. The complete inhibition by mAb G10 of intracellular protein phosphorylation events and the secretion induced by mAb F11 indicates that the GPIIIa molecule functions not only as a fibrinogen binding site required for fibrinogen-dependent platelet aggregation, but that GPIIIa also plays an important role in the transmission of signals that activate second messenger-generating systems leading to secretion. These results describe a new role for GPIIIa in platelet function. In a previous report we have described the platelets of a Friedreich's ataxia patient with unique thrombopathy (44). mAb F11 induced secretion in platelets of this patient but not aggregation, due to a defect in the exposure of fibrinogen receptors. Interestingly, also in this patient mAb G10 inhibited mAb F11-induced secretion, indicating that the role of GPIIIa in signals that lead to secretion can be separated from its role in exposure of fibrinogen binding sites.

In addition to mAb G10, the Fab fragments of mAb F11 also inhibited the activation of platelets by mAb F11. This inhibition was found to be due to direct interference of the Fab fragments with the binding of the mAb F11 IgG molecule to the platelet surface. Such interference is consistent with the possibility that mAb F11-induced platelet activation involves receptor dimerization and micellization (45) or the platelet FC receptor (46). Agents which increase the level of cyclic AMP also inhibit mAb F11-induced platelet aggregation, and this may be due to the inhibition of fibrinogen receptor exposure (47). The involvement of released ADP in mAb F11-induced platelet aggregation is indicated by the finding that ATP and ATP analogues, which block the ADP receptor, and apyrase, which degrades the released ADP, completely inhibit aggregation. However, significant granular secretion (30% of uptake) still occurs in response to mAb F11 even in the presence of either ATP or apyrase, indicating that the direct interaction of mAb F11 with its receptor results, in part, in granular release.

In conclusion, mAb F11 interacts with specific protein components (32 and 35 kDa) at the platelet surface. This interaction leads to platelet granular secretion and aggregation. The biochemical pathways of platelet activation by mAb F11 involve stimulation of the activity of protein kinase C and of the Ca++/calmodulin-dependent myosin light chain kinase, and is inhibited by elevating intracellular cyclic AMP. The mAb F11-induced aggregation of platelets appears to be secondary to ADP release. The mAb F11-induced secretion appears to involve action of glycoprotein IIIa. At least 30% of the granular secretion induced by mAb F11 is not mediated by ADP but by a specific F11 receptor. Detailed characterization of this unique receptor will provide novel information on the process of platelet activation.

Acknowledgments—We wish to thank Dr. Czeslaw Cierniewski for stimulating discussions and Monika Lange, Laurie DiCesare, Mark Fleming, David Harwick, Dan De Mars, and Nancy Harber for excellent technical assistance.

REFERENCES
1. De Gaetano, G., Vernynen, J., and Verstraete, M. (1970) Thromb. Diath. Haemorrh. 24, 419-431
2. Tobelem, G., Levy-Toledano, S., Nurden, A. T., Degas, L., Caen,
Platelet Activation by a Monoclonal Antibody

J. P., Malmsten, C., and Kindahl, H. (1979) Br. J. Haematol. 41, 427-436

Kunicki, T. J., Christie, D. J., and Aster, R. H. (1983) Blood Cells 9, 293-301

Woods, V. L., Jr., Kurata, Y., Montgomery, R. R., Tani, P., Mason, D., Oh, E. H., and McMillan, R. (1984) Blood 64, 156-160

Woods, V. L., Jr., Oh, E. H., Mason, D., and McMillan, K. (1984) Blood 63, 368-375

Kunicki, T. J., and Aster, R. H. (1978) J. Clin. Invest. 61, 1225-1231

van Leeuwen, E. F., van der Ven, J. Th. M., Engelfriet, C. P., and van dem Borne, A. F. G. K. (1982) Blood 59, 23-26

Varon, D., and Karpatkin, S. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6992-6995

Beadnell, D. J. S., Spiegel, J. E., and Jacobs, M. M. (1984) J. Clin. Invest. 73, 1701-1707

Vander Lelie, J., Van der Plas-Van Dalen, C. M., and Van dem Borne, A. E. G. K. (1988) Thromb. Haemostasis 60, 68-74

Kornecki, E., Ehrlich, Y. H., and Lenox, R. H. (1984) Science 226, 1454-1456

Kornecki, E., Tusuznics, G. P., and Niewiarowski, S. (1983) J. Biol. Chem. 258, 9349-9356

Kornecki, E., Ehrlich, Y. H., Egbring, R., Gramse, M., Seitz, R., Eckardt, A., Lukasiewicz, H., and Niewiarowski, S. (1985) Am. J. Physiol. 255, H651-H658

Akermann, J. W. N., Gotter, G., and Kloprogge, E. (1982) Thromb. Res. 27, 59-64

Kornecki, E., and Feinberg, H. (1980) Am. J. Physiol. 238, 54-60

Tusuznics, G. P., Knight, L. C., Kornecki, E., and Srivastava, S. (1983) Annu. Rev. Immunol. 1, 635-660

Feinberg, H., Michal, F., and Born, G. V. R. (1974) J. Lab. Clin. Med. 84, 926-934

Seathead, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672

Jonak, Z. L. (1980) in Monoclonal Antibodies (Kennett, R. H., ed.) pp. 405-416, Plenum Publishing Corp., New York

Porter, R. R. (1959) Biochem. J. 73, 119-126

Eyr, P. L., Prowse, S. J., and Jenkins, C. R. (1978) Immunchemistry 15, 429-439

Ehrlich, Y. H., Devis, T., Gardfield, M., Bock, E., Kornecki, E., and Lenox, R. H. (1986) Nature 320, 67-69

Towbin, H., Staehelin, T., and Gordon, J. (1978) Proc. Natl. Acad. Sci. U. S. A. 76, 4350-4354

Niewiarowski, S., Norton, K. J., Eckardt, A., Lukasiewicz, H., Holt, J. C., and Kornecki, E. (1989) Biochim. Biophys. Acta 983, 91-96

Lapetina, E. G. (1987) in Advances in Hematology and Oncology (Bechtol, K. B., ed.) pp. 323-324, Plenum Publishing Corp., New York

Kornecki, E., Cooper, B. A., and Ehrlich, Y. H. (1988) J. Lab. Clin. Med. 111, 618-626

Hollenberg, M. D., and Goren, H. J. (1985) in Mechanisms of Receptor Regulation (Pogue, G., and Crooke, S. T., eds) pp. 323-373, Plenum Publishing Corp., New York

Anderson, C. L. (1989) Clin. Immunol. Immunopathol. 53, 563-571

Kornecki, E., Ehrlich, Y. H., Hardwick, D., and Lenox, R. H. (1988) Am. J. Physiol. 254, H550-H557

Morel, M.-C., Lecompte, T., Champeix, P., Favier, R., Potevin, F., Samama, M., Salmon, C., and Kaplan, C. (1989) Br. J. Haematol. 71, 57-63

Mustard, J. F., Perry, D. W., Ardlie, N. G., and Packman, M. A. (1972) Br. J. Haematol. 22, 193-204

Molinari, J., and Larand, L. (1981) Arch. Biochem. Biophys. 203, 199-203
Activation of human platelets by a stimulatory monoclonal antibody.
E Kornecki, B Walkowiak, U P Naik and Y H Ehrlich

J. Biol. Chem. 1990, 265:10042-10048.

Access the most updated version of this article at http://www.jbc.org/content/265/17/10042

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

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/17/10042.full.html#ref-list-1