Analysis of Platelet Aggregation Disorders Based on Flow Cytometric Analysis of Membrane Glycoprotein IIb-IIIa With Conformation-Specific Monoclonal Antibodies

By Mark H. Ginsberg, Andrew L. Frelinger, Stephen C-T. Lam, Jane Forsyth, Robert McMillan, Edward F. Plow, and Sanford J. Shattil

Normal primary platelet aggregation requires agonist-mediated activation of membrane GP1Ib-IIla, binding of fibrinogen to GP1Ib-IIla, and cellular events after ligand binding. PAC1 monoclonal antibody distinguishes between bodies preferentially recognize GP1Ib (PMI-1) or IIla (anti-platelet aggregation abnormalities. Platelets from a thrombasthenic variant, which contained near-normal amounts of GP1Ib-IIla, failed to aggregate or bind PAC1 in response to agonists. In addition, GP1b does not bind to normal GP1Ib-IIla without prior cell activation, failed to increase the binding of PMI-1 or anti-LIBS1 to the thrombasthenic platelets, suggesting a primary defect in ligand binding.

Primary platelet aggregation is essential for normal hemostasis and also plays a role in thrombosis. This process is dependent on the binding of fibrinogen to platelet membrane glycoprotein (GP) IIb-IIIa. Efficient binding of large GP1Ib-IIla ligands, such as fibrinogen or the anti–GP1Ib-IIla monoclonal antibody (MoAb) PAC1, requires platelet activation by agonists such as ADP or thrombin. In contrast, small fibrinogen-mimetic ligands, such as Arg-Gly-Asp (RGD)–containing synthetic peptides, can bind to GP1Ib-IIla in an activation-independent manner. This had led to the hypothesis that the ligand-binding pocket of GP1Ib-IIla is present on resting cells, but is of limited accessibility to large ligands such as fibrinogen and PAC1. Agonist-induced expression of the fibrinogen receptor on GP1Ib-IIla appears to involve G protein–mediated activation of phospholipase C, followed by activation of protein kinase C. Under some conditions, other metabolic pathways are also involved, but their identity remains to be determined, as do the nature of the changes in GP1Ib-IIla that render it competent to bind fibrinogen.

In addition to fibrinogen receptor exposure and ligand binding, events subsequent to fibrinogen binding appear to be necessary for maximum platelet aggregation. One such postoccupancy event involves the GP1Ib-IIla complex itself—conformational changes that can be detected by antibodies that bind preferentially to GP1Ib or IIla when the fibrinogen receptor is occupied, either by fibrinogen or by an RGD-containing peptide. One of these conformational changes is detectable with the anti-GP1Ib antibody PMI-1, and another change is detectable with the anti-GP1Ila antibody anti-LIBS1. It is not yet clear how postoccupancy events influence the extent of platelet aggregation. Of note in this regard, however, the GP1Ib-IIla complex has been implicated in stimulus-response coupling and in platelet calcium homeostasis. Moreover, the binding of fibrinogen to platelets induces conformational changes in the ligand itself, thereby possibly augmenting its adhesive function. The three processes involving GP1Ib-IIla that are necessary for platelet aggregation—activation, ligand binding, and postoccupancy events—are illustrated schematically in Fig 1.

The most profound defects in platelet aggregation occur in patients with the autosomal-recessive hereditary disorder, Glanzmann’s thrombasthenia. Although the platelets of most homozygotes with the classic form of this disease possess less than 10% GP1Ib-IIla, platelets from some functional variants possess near normal levels of GP1Ib-IIla. Accordingly, these latter individuals may have a defect involving activation, ligand binding, or postoccupancy functions of GP1Ib-IIla. It is likely that abnormalities in one or more of these three processes are also involved in the broad spectrum of acquired aggregation defects in such clinical settings as myeloproliferative disorders, drug administration, uremia, and postcardiopulmonary bypass.

Recent advances in flow cytometry have made it possible to rapidly study the quantity and function of membrane...
proteins on intact platelets. These analyses can be performed on small samples of blood or platelet-rich plasma. In the present study, we have used this approach to develop a strategy to characterize platelet aggregation abnormalities in pathophysiolgic terms as defects in either GPIIb-IIIa activation, ligand binding, or postoccupancy events. GPIIb-IIIa activation and ligand binding were assessed with the antibody PAC1, whereas postoccupancy events were assessed with the antibodies PMI-1 and anti-LIBS1. The advantages of this approach are illustrated by analysis of a functional variant of thrombasthenia, which we now report is due to a defect in ligand recognition. As a further example, we show that the platelets from an individual with myelofibrosis and an acquired aggregation disorder exhibit an agonist-specific defect in activation of GPIIb-IIIa.

**METHODS**

**Antibodies.** Preparation and characterization of the antibodies used here have been described in detail elsewhere and their properties are listed in Table 1. Tab and 4F10 were generously provided by Dr Rodger McEver, Oklahoma City, OK, and Dr Virgil Woods, UCSD Medical Center, San Diego, CA, respectively. 10E5 and 7E3 were the generous gifts of Dr Barry Coller, SUNY, Stony Brook, NY. Fluorescein isothiocyanate-conjugated antibodies were prepared as described to achieve fluorescein/protein molar ratios of 3 to 6.

**Peptides.** Peptides were synthesized in a central facility at the Research Institute of Scripps Clinic using an Applied Biosystems (Mountain View, CA) peptide synthesizer. Peptides were more than 90% homogeneous by high performance liquid chromatography, and amino acid compositions were consistent with the desired sequence. The peptides were Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP), fibrinogen (GRGDSP), fibrinogen (γc), Lys-Gly-Asp-Ser-Pro (GRGDSP), fibrinogen (γc), and Lys-Gly-Asp-Ser-Pro (GRGDSP).

Analysis of platelet surface GPIIb-IIIa by flow cytometry. Platelet rich plasma was obtained by taking venous blood from patients and controls who had not ingested medications for at least 10 days, anticoagulating with 1/10 volume of 3.8% sodium citrate, and sedimenting red and white blood cells at 180 g for 20 minutes at room temperature. Immediately thereafter, 5-µL aliquots of platelet-rich plasma were added to polypropylene tubes containing a fluorescein isothiocyanate-conjugated monoclonal antibody (10-9 to 10-12 mol/L) in Tyrode's buffer (1% bovine serum albumin, 2 mmol/L MgCl2, 137.5 mmol/L NaCl, 12 mmol/L NaHCO3, 2.6 mmol/L KCl, pH 7.4). Samples were incubated without stirring in a total volume of 50 µL for 15 minutes at room temperature with agonists and peptides as indicated. Samples were then diluted to 0.5 mL with Tyrode's buffer and analyzed on a FACStar flow cytometer (Bectin-Dickinson Immunocytometry Systems, Mountain View, CA). Light scatter and fluorescence signals were acquired at logarithmic gain, and 10,000 platelets in each sample were analyzed. Results are expressed as mean platelet fluorescence intensity in arbitrary fluorescence units or as histograms of log platelet fluorescence intensity in arbitrary units on the abscissa and platelet number on the ordinate.

Isolation and surface radioiodination of platelets. Platelets were isolated from acid-citrate dextrose anticoagulated human blood by differential centrifugation followed by gel filtration on Sepharose 2B. Lysates of surface-labeled platelets were analyzed by KYGRGDS affinity chromatography as described. Briefly, intact

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**Table 1. MoAbs Used**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Effect on Fibrinogen Binding*</th>
<th>Effect of Peptides</th>
<th>Table 1. MoAbs Used</th>
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<tbody>
<tr>
<td>PAC1</td>
<td>GPIIb-IIIa on activated platelets</td>
<td>Decrease</td>
<td>Decrease</td>
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<tr>
<td>PMI-1</td>
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<td>GPIiα</td>
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<td>Increase</td>
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<td>4F10</td>
<td>GPIiβ</td>
<td>Decrease</td>
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</tr>
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<td>10E5</td>
<td>GPIiβ</td>
<td>Decrease</td>
<td>ND</td>
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<td>7E3</td>
<td>GPIiα</td>
<td>Decrease</td>
<td>ND</td>
<td></td>
</tr>
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<td>A1/A2</td>
<td>GPIiβ</td>
<td>Decrease</td>
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<tr>
<td>TSPI-1</td>
<td>Thrombospondin</td>
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Abbreviation: ND, not determined.

*Effect of antibody on fibrinogen binding to activated platelets.

†Effect of RGD or fibrinogen γ chain, C-terminal peptides on antibody binding.
Platelets were radioiodinated by the lactoperoxidase-H₂O₂ method then resuspended to 2 × 10⁹ platelets/mL and solubilized in lysis buffer containing 10 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.5, 0.15 mol/L NaCl, 1 mmol/L CaCl₂, 1 mmol/L MgCl₂, 0.1 mmol/L leupeptin, 1 mmol/L phenylmethylsulfonylfuoride (PMSF), 10 mmol/L N-ethylmaleimide, and 50 mmol/L octylglucoside. The lysates were incubated at 4°C for 12 hours with KGYRGDS coupled to CNBr-activated Sepharose, washed, and eluted with 1 mmol/L GRGDSP. Under these conditions approximately 10% of normal GPIIb-IIIa is bound and eluted. A comparable quantity is bound on rechromatography of the pass through fraction, indicating that less than 100% binding is due to the low affinity of the interaction rather than an inactive subpopulation of GPIIb-IIIa.30

Results

Platelets were radioiodinated by the lactoperoxidase-H₂O₂ method then resuspended to 2 × 10⁹ platelets/mL and solubilized in lysis buffer containing 10 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.5, 0.15 mol/L NaCl, 1 mmol/L CaCl₂, 1 mmol/L MgCl₂, 0.1 mmol/L leupeptin, 1 mmol/L phenylmethylsulfonylfuoride (PMSF), 10 mmol/L N-ethylmaleimide, and 50 mmol/L octylglucoside. The lysates were incubated at 4°C for 12 hours with KGYRGDS coupled to CNBr-activated Sepharose, washed, and eluted with 1 mmol/L GRGDSP. Under these conditions approximately 10% of normal GPIIb-IIIa is bound and eluted. A comparable quantity is bound on rechromatography of the pass through fraction, indicating that less than 100% binding is due to the low affinity of the interaction rather than an inactive subpopulation of GPIIb-IIIa.30

The Cam variant of Glanzmann's thrombasthenia has been described in detail.17 Patients with this variant have near normal quantities of GPIIb-IIIa, undetectable fibrinogen binding to platelets, and absent platelet aggregation.

The second patient is a 58-year-old woman who noted increased bruising in 1984; studies showed a platelet count of 1,500,000/µL and a bone marrow with megakaryocytic hyperplasia and myelofibrosis. Since diagnosis, her bruising has been constant, primarily involving her extremities and anterior chest, and she notes occasional epistaxis. She has had four episodes of gastrointestinal bleeding, each requiring transfusion with 2 to 5 U of RBCs. Before the onset of bruising, she had withheld several hemostatic challenges, including tonsillectomy, hemorrhoidectomy, and coeccectomy, as well as three uncomplicated vaginal deliveries. Physical examination at present is normal except for hepatosplenomegaly and generalized edema. Laboratory findings confirmed the presence of platelet aggregation studies, performed while receiving no medications, revealed absent aggregation to ADP, 100 µmol/L epinephrine, and collagen.

RESULTS

Based on the model depicted in Fig 1, abnormal platelet aggregation could arise from a defect in the activation of platelet fibrinogen receptors, a defect in fibrinogen binding per se, or a defect in the postoccupancy events required for optimal aggregation. The potent usefulness of this model was tested by studying two patients with persistent and severe defects in platelet aggregation. To quantitate fibrinogen receptor activation and ligand binding, we used a fluorescein-labeled MoAb, PAC-1, that specifically recognizes the activated form of GPIIb-IIIa.5 Antibody binding was measured in small (5 µL) samples of platelet rich plasma by flow cytometry.

The Cam variant of thrombasthenia is due to a defect in ligand recognition. The Cam variant of Glanzmann's thrombasthenia is characterized by markedly reduced fibrinogen binding to platelets that contain near normal quantities of GPIIb-IIIa.17 The presence of GPIIb-IIIa on the Cam platelet surface was confirmed by flow cytometry, in which there was comparable binding of an anti-GPIIb MoAb (Tab) and an anti-GPIIIa MoAb (AB-15) to Cam and normal platelets. In addition, four MoAbs specific for the GPIIb-IIIa complex (A, A1, 7E3, 10E5, 4F10), all of which recognize the nonactivated form of GPIIb-IIIa at 1:1 stoichiometry22-26 and inhibit fibrinogen binding to platelets, also bound in comparable amounts to the Cam and normal platelets (Fig 2). In sharp contrast, there was a complete lack of binding of the activation-specific MoAb PAC1 to ADP-activated Cam platelets (Fig 2). This defect was not specific to ADP stimulation, because Cam platelets activated with 50 nmol/L phorbol myristate acetate (PMA) also showed no increase in PAC1 fluorescence (data not shown).

Because PAC1 may recognize the ligand binding site in GPIIb-IIIa,25-27 the absence of PAC1 binding could be due to a lack of GPIIb-IIIa activation, or it could be due to a defect in ligand binding. To distinguish these possibilities, we exploited the observation that small fibrinogen-mimetic peptides bind to GPIIb-IIIa in an activation-independent manner.

Fig 2. Flow cytometric analysis of the deficit in Cam variant of thrombasthenia. Shown are histograms with the log of fluorescence intensity on the abscissa and cell number on the ordinate. The reporting antibody is indicated in the left hand column. Cam platelets are shown in the left-hand panels and the normal control on the right. Each row of panels contains a legend indicating the added agonist, peptide, or antibody. The ADP was added at a final concentration of 100 µmol/L; the GRGDSP was at a final concentration of 200 µmol/L. The bottom row depicts results with an anti-GPIIb-IIIa complex (A, A1) and an anti-GPIIIa (AB-15). Similar data were obtained with other antibodies to GPIIb-IIIa (7E3, 10E5, 4F10) and an antibody to GPIb (Tab). Data shown are representative of determinations on two affected male siblings with identical results.
The binding of such a peptide, GRGDSP, was assayed by use of PMI-1 and anti-LIBS1 MoAbs, which preferentially recognize GPIIb-IIIa in a 1:1 stoichiometry, following the binding of either fibrinogen or small peptide ligands. Platelets from a normal individual showed a 2.5-fold increase in PMI-1 binding in the presence of 200 μmol/L GRGDSP (mean fluorescence intensity of untreated platelets = 60 arbitrary fluorescence units; GRGDSP-treated platelets = 151 fluorescence units) (Fig 2). In contrast, Cam platelets showed no increase in PMI-1 binding in response to GRGDSP (mean fluorescence intensity of untreated platelets = 102 fluorescence units; GRGDSP-treated platelets = 107 fluorescence units) (Fig 2).

It should be noted that PMI-1 binding to untreated Cam platelets was higher than normal. Therefore, the binding of this antibody could not be used here as an indicator of the binding of GRGDSP to Cam platelets. However, the anti-LIBS1 antibody was more informative in this regard. As shown in Fig 2, untreated Cam platelets bound less anti-LIBS1 than control platelets (mean fluorescence of 27 and 58 U, respectively). The fibrinogen-mimetic peptide, GRGDSP, caused a fourfold increase in anti-LIBS1 binding to normal platelets (mean fluorescence, 205 U); however, no increase in antibody binding was observed in Cam platelets (mean fluorescence, 25 U). Anti-LIBS1 binding to Cam platelets also failed to increase when platelets were treated with another fibrinogen-mimetic peptide, GRGDSP (400 μmol/L), instead of GRGDSP (control platelets, 201 fluorescence U; Cam platelets, 31 U). When anti-LIBS1 binding was examined over a range of GRGDSP peptide concentrations, control platelets showed a saturable increase in antibody binding such that half-maximal expression of the LIBS1 epitope on GPIIIa was observed at 20 μmol/L peptide. This is similar to the IC50 of this peptide for fibrinogen binding to activated platelets. In contrast, at 200 μmol/L GRGDSP, anti-LIBS1 binding to Cam platelets was less than that seen with control platelets in the presence of 1 μmol/L peptide, suggesting that the affinity of Cam GPIIb-IIIa for this peptide was at least 20-fold less than normal GPIIb-IIIa (Fig 3).

The foregoing data, by use of conformation-specific antibodies and intact cells, indicated that the Cam variant was expressed in a conformation that was different from normal. We next applied this strategy to the characterization of a patient with myelofibrosis. The work described above established the utility of conformation-specific antibodies and flow cytometry in characterizing a defect in ligand binding. We next applied this strategy to the characterization of a patient with myelofibrosis and an acquired severe defect in platelet aggregation. Platelets from this patient did not express PAC1 binding sites when stimulated with 10 μmol/L ADP (Fig 5) or 50 μmol/L epinephrine (not shown), indicating a defect either in GPIIb-IIIa activation or ligand binding. In contrast to the Cam patient, anti-LIBS1 and PMI-1 signals in the presence and absence of GRGDSP were similar to the normal control (Fig 5), indicating intact ligand binding function. Furthermore, PAC1 bound to the patient's platelets in response to PMA, which circumvents normal receptor-mediated pathways by directly activating protein kinase C. The patient's platelets also exhibited normal α-granule secretion in response to PMA, as evidenced by surface expression of the α-granule protein thrombospondin (Fig 5). The aggregation defect in the patient's platelets could not be reversed by suspending her platelets in normal plasma, and the defect could not be induced in normal platelets by incubation in the patient's plasma. Thus, the GPIIb-IIIa from this patient has the potential to bind RGD ligands, but there appears to be an intrinsic cellular defect in receptor-mediated activation of GPIIb-IIIa.

**DISCUSSION**

For two decades, studies of platelet aggregation in vitro have been routinely used in the characterization of patients with a prolonged bleeding time or a bleeding diathesis due to platelet dysfunction. The results of recent studies have
Normal Cam

Fig 4. RGD affinity chromatography of Cam versus normal platelet extract. Extracts of surface-labeled Cam and normal platelets were incubated overnight at 4°C with KYGRGDS coupled to CNBr activated Sepharose. After washing, the column was eluted with 1 mmol/L GRGDSP peptide. Starting material is shown in lane 1, column flow through in lane 2, and last wash fraction in lane 3. The GRGDSP elution began in lane 4 and continued to lane 8. Shown are autoradiograms of 7.5% SDS gels of these fractions under nonreducing conditions.

Fig 5. Flow cytometric analysis of aggregation dysfunction in a patient with myelofibrosis. Patient platelets (MPD) are shown in the left hand panels, control platelets in the right. The disclosing antibody is indicated in the left hand column. In each row, the legend indicates the added agonist or peptide. Agonist and peptide concentrations were identical to those in Fig 2. Phorbol myristate acetate (PMA) was present in a final concentration of 60 nmol/L. Anti-TSP refers to the binding of an MoAb against thrombospondin.

Table 2. Flow Cytometric Analysis of Platelet Aggregation Dysfunction

<table>
<thead>
<tr>
<th>Type of Defect</th>
<th>AGG*</th>
<th>PAC1†</th>
<th>LIBS‡</th>
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<tr>
<td>None</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Activation</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postoccupancy</td>
<td></td>
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</table>

Indicated are presence (+) or absence (−) of a given response.

*Agonist-stimulated platelet aggregation.
†Increased binding of activation-dependent ligand such as PAC1 in response to agonist.
‡Increased binding of occupancy-dependent antibody, such as anti-LIBS 1, in the presence of activation-independent ligand, such as GRGDSP.

demonstrated that normal aggregation requires the binding of fibrinogen to platelet GPIIb-IIIa. Furthermore, the aggregation process may be viewed as a series of necessary cellular events: (a) agonist-induced activation of GPIIb-IIIa, resulting in the exposure of the fibrinogen binding site; (b) fibrinogen binding; and (c) postoccupancy events that follow upon ligand binding (Fig 1). Based on the flow cytometric approach described in this report, it is possible to classify disorders of platelet aggregation in terms of this series of events. The flow cytometry method used here is rapid and can be performed on platelet-rich plasma or on whole blood. This approach has been made possible by the availability of MoAbs that can distinguish between the resting, activated, and ligand-occupied forms of GPIIb-IIIa. The three types of platelet dysfunction that can lead to reduced platelet aggregation and the predicted flow cytometry results are summarized in Table 2.

Using this approach, we have found that a variant of Glanzmann's thrombasthenia is due to a defect in the ligand-binding function of GPIIb-IIIa and that the markedly reduced aggregation in a patient with myelofibrosis is due to a defect in agonist-specific activation of GPIIb-IIIa. We have not yet identified a patient with postoccupancy dysfunction. However, such a postoccupancy defect may explain the process of agonist-induced platelet desensitization. For exam-
ple, unstimulated platelets activated for more than 10 minutes with ADP or epinephrine in the presence of fibrinogen demonstrate a marked reduction in their subsequent aggregation response when compared with the same platelets incubated without fibrinogen.10,11 This cannot be explained by a reduction in fibrinogen binding. Many patients are encountered in clinical practice with easy bruising, a prolonged bleeding time, and a decreased platelet aggregation in response to one or more agonists. After excluding aspirin ingestion and storage pool disease, the underlying cause is usually apparent. This group of individuals is often referred to as having “aspirinlike” defects, and some may have congenital or acquired platelet metabolic defects leading to an abnormality of GPIIb-IIIa activation. It seems likely that among this heterogeneous group of patients, individuals with the postoccupancy dysfunction phenotype will also be identified.

The Cam variant of thrombasthenia is due to a defect in ligand recognition by GPIIb-IIIa. This conclusion is based on (a) the failure of Cam GPIIb-IIIa to bind to an insolubilized RGD peptide and (b) a greater than 200-fold reduction in the capacity of activation-independent peptide ligands to increase the binding of the occupancy-dependent anti-LIBS1 antibody. Previous studies with proteolytic fragments of fibrinogen and fibrinogen-related peptides had implicated recognition of RGD and fibrinogen gamma chain peptide sequences in fibrinogen binding.12-15 In the present study, GPIIb-IIIa lacking the capacity to bind these peptide sequences lacked the capacity to support fibrinogen binding and platelet aggregation. In view of the apparent autosomal-recessive inheritance of the Cam variant,17 the severe functional defect in GPIIb-IIIa, and the intermediate functional defect of the parents, it is likely that the basic Cam defect is due to a point mutation in GPIIb-IIIa. Such a point mutation has recently been identified in a Cam patient.18 The observation that this single amino acid change leads to loss of binding of both the RGD and gamma chain peptide sequences favors the possibility that both peptide sequences are recognized by a common binding site.

The PAC1 antibody was used to monitor GPIIb-IIIa activation because it binds selectively and with high affinity to stimulated platelets.19 Moreover, based on the inhibition of PAC1 binding by fibrinogen20 and fibrinogen-mimetic peptides21 and the capacity of peptides derived from the hypervariable region of PAC1 to inhibit fibrinogen binding,20 it seems likely that this antibody recognizes the ligand binding site in GPIIb-IIIa. This hypothesis is strongly supported by the finding that PAC1 fails to recognize the Cam mutant GPIIb-IIIa, which lacks ligand binding function. The failure of PAC1 to interact with Cam platelets is unlikely to be due to gross denaturation of GPIIb-IIIa, because four other complex-specific anti–GPIIb-IIIa antibodies appeared to bind to the same extent as anti–GPIIb or anti–GPIIIa antibodies. Of these four antibodies, 7E3 binds more rapidly to activated cells25 and A9 is inhibited by synthetic peptides derived from the fibrinogen gamma chain.24 Because Cam GPIIb-IIIa lacks the capacity to recognize fibrinogen or the peptide ligands, it seems likely that these previous findings are due to an indirect relationship between the 7E3 and A9 epitopes and the ligand binding site of GPIIb-IIIa.

GPIIb-IIIa is a member of the integrin family of structurally related adhesion receptors.26-28 Included in this family are a platelet collagen receptor,41 leukocyte receptors involved in inflammation and defense against pyogenic infection,44 and receptors involved in lymphocyte homing.45 In the case of the leukocyte receptors, recent studies have reported that activation of these receptors induces leukocyte aggregation and endothelial cell adherence.46 Moreover, occupancy-dependent MoAbs have been prepared against an endothelial cell integrin.12 This suggests that activation-specific and occupancy-dependent antibodies might be prepared against other integrins and be used to analyze leukocyte dysfunction in a manner analogous to that described here for GPIIb-IIIa. Indeed, there is no obvious reason to preclude these strategies for the rapid analysis of binding functions of nonintegrin cell surface receptors as well.

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