Platelet Glycoproteins IIb and IIla Associated With Blood Monocytes Are Derived From Platelets*

By Richard B. Levene and Enrique M. Rabellino

Platelet glycoprotein IIb/IIla (GP IIb/IIla), the receptor complex for fibrinogen, has been regarded as a megakaryocyte/platelet lineage-restricted antigen. Recently, however, it has been reported that GP IIb/IIla is expressed in blood monocytes. Studies were performed to establish the origin and immunological characteristics of monocyte-associated glycoproteins IIb and IIla (GPs IIb and IIla). Preparations of monocyte preparations containing varying platelet-monocyte ratios were metabolically labeled with [35S]methionine with the expectation that any newly synthesized GPs IIb and IIla would be monocyte-derived, since platelets have only rudimentary protein synthetic apparatuses. Analyses of sodium dodecyl sulfate (SDS) gels of homogenates of cell preparations containing from 200 to 5:1 platelet-monocyte ratios revealed that unlabeled GPs IIb and IIla were readily immunoisolated using protein A-Sepharose immunobeads. However, fluorographic analyses of the same cell preparations pulse-labeled with [35S]methionine failed to demonstrate synthesis of GP IIb or IIla. Additionally, no GP IIb or IIla was detected when immunosolation was carried out in pure preparations of monocytes containing <1:100 platelet-monocyte ratios and SDS acrylamide gels were stained by the sensitive silver stain method. Furthermore, heterologous polyclonal antisera and two monoclonal antibody preparations against GPs IIb and IIla, which bound to platelets, failed to bind to monocyte membranes. Thus, evidence was presented that indicated that monocytes do not synthesize platelet GPs IIb and IIla and that detection of these molecules in blood monocyte preparations reflects platelet contamination.

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*While this manuscript was undergoing revision, a communication by Clemetson et al has reported the absence of platelet glycoproteins IIb/IIla from monocytes as analyzed by two-dimensional gel electrophoresis, immunoprecipitation, and crossed immunoelectrophoresis. (Clemetson KJ, McGregor JL, McEver RP, Jacques IV, Bainton DF, Domigz W, Baggioleini M: Absence of platelet membrane glycoproteins IIb/IIla from monocytes. J Exp Med 161:972, 1985.)

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buffered Tyrode's solution containing 3.8 mmol/L HEPES, 3.8 mmol/L NaH2PO4, 137 mmol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L MgCl2, 0.2% BSA, and 0.1% dextrose at pH 7.35, as described elsewhere.23

Antisera. Monospecific rabbit antisera against purified human platelet GPs Iib, Iib, and IIa, and polyspecific rabbit antplatelet membrane antisera were prepared and tested for specificity as described previously.21-22 Monoclonal antibody PC-1 with specific reactivity to the platelet GP Iib/IIa complex was generated and screened as described elsewhere.24 Polyspecific rabbit antithrombin Fab Ig was a generous gift from Dr Howard Gray (National Jewish Hospital, Denver). IgG fractions of all rabbit and mouse antisera were prepared by 50% saturated ammonium sulfate precipitation, DEAE-cellulose chromatography, and, subsequently, conjugated to fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC) (BBL Microbiology Systems, Walkersville, Md).7 The conjugated proteins were fractionated by column chromatography on DEAE-cellulose, using stepwise elution with sodium phosphate buffers, pH 7.4, of 0.01, 0.05, and 0.1 mol/L. In general, 0.05 mol/L sodium phosphate buffer eluted antibody preparations with a dye-protein molar ratio of 1 ± 0.3 and was found to have the highest specific staining activity. Additionally, a monoclonal antibody against platelet GP Iib was kindly provided by Dr Barry Coller (SUNY, Stonybrook, NY).24 Monoclonal antibodies B1, T3/T11, and Mol, with specific reactivities against B lymphocytes, T lymphocytes, and monocytes, respectively, were purchased from Coulter Immunology (Hialeah, Fla). Rabbit IgM antithrombin IgG used to enhance complement fixation in the cytoxicity assay was prepared by short-term immunization and subsequent precipitation and chromatography as described previously.12

Radioiodination of membrane proteins. Cell surface proteins on mononuclear blood cells and washed platelets were radioiodinated with 125I by the tetrachloro-diphenylglycoluril method (Iodo-Gen, Pierce Chemical Co, Rockford, Ill).25 Briefly, 5 x 10^6 mononuclear cells or 2 x 10^7 platelets resuspended in 200 µL phosphate-buffered saline (PBS) were incubated with 100 µCi of 125I in the presence of 250 µg 1,3,6,7-tetrachloro-3a,6a-diphenylglycoluril (Iodo-Gen) for ten minutes at 22°C. Tubes were precoated with 250 µg of Iodo-Gen dissolved in 125 µL chloroform under gentle stream of nitrogen. After iodination, unbound 125I was removed by washing cells three times with PBS, and cells were solubilized as described later.23

Metabolic labeling of mononuclear cells and platelets. Aliquots of isolated blood mononuclear cells (40 x 10^6) or platelets (1 x 10^11) resuspended in 1 mL of methionine-free medium (MEM/Eagle supplemented with 0.015 mol/L L-glutamine, 5% whole fetal calf serum (FCS), and 10% methionine-free FCS were pulse-labeled with 400 µCi [35S]methionine (1,100 Ci/mmol) at 37 °C for four hours. FCS was heat-inactivated at 56 °C for 30 minutes. Methionine-free FCS was prepared by extensive dialysis against methionine-free MEM. Unbound label was removed by washing cells, and homogenates of cell preparations were generated by solubilizing cells with 1% Triton X-100 in the presence of 2 mmol/L phenylmethylsulfonylfluoride, 5 mol/L N-ethylmaleimide, 10 mmol/L EDTA (all from Sigma Chemical Co, St Louis) and 1 µmol/L pepstatin (Protein Foundation, Osaka, Japan). Validation of endogenous incorporation of radioactive amino acids into neosynthesized protein was obtained by pulse-labeling cells in the presence of increasing concentrations of the protein synthesis inhibitor cycloheximide as well as by evaluating radioactive label incorporation into TCA-precipitable protein.

Immunoprecipitation. SDS gel electrophoresis, and fluorography. Homogenates of mononuclear cells and platelets were subjected to solid-phase immunoadsorption with protein A-Sepharose CL-4B beads (Pharmacia) coated with antibodies monospecific for platelet GPs Iib and IIa after extensive preclearing. Binding efficiency of antibody to protein A-Sepharose CL-4B beads was between 17 and 19 µg/µL beads. Binding efficiency of a radioiodinated antibody preparation was estimated by measuring radioactivity bound to washed beads over specific labeling activity expressed in cpm/µg protein. The efficiency of the immunoprecipitation system used was estimated by measuring removal of GP Iib/IIa from a radioiodinated platelet membrane preparation. Assessment of antigen precipitation was performed on platelet lysates by sequential adsorptions with antibody-coated beads. In a representative experiment, 255 µg antibody coupled to 15 µL beads removed all GP Iib/IIa derived from a lysate of 2 x 10^10 platelets detectable by autoradiography. Preclearing was performed by sequential incubation of lysates with immunoadsorbant beads and beads coated with normal rabbit or mouse IgG. After incubation of cell homogenates for four to six hours at 4 °C with rotation, immunobeads were washed three times with 20 mmol/L Tris-HCl containing 500 mmol/L NaCl and 1% ovalbumin (pH 7.8) and three times with 20 mmol/L Tris-HCl containing 150 mmol/L NaCl (pH 7.8). Subsequently, immobilized protein was eluted by incubation of beads in 20 mmol/L Tris-HCl containing 2% SDS and 6 mol/L urea (pH 6.8) for five minutes at 100 °C. Eluants were subjected to 7.5% SDS-polyacrylamide gel electrophoresis (PAGE) under reducing and nonreducing conditions.35-37 Gels were subsequently examined for immunosolated proteins after processing for fluorography, silver stain, or Coomassie blue staining with estimated levels of sensitivity of approximately 1 to 10 pg, 1 to 5 ng, and 0.5 to 1 µg protein, respectively.25-30

Immunofluorescence assays, flow cytometry, and cell sorting. For fluorescence staining of membrane-restricted components, isolated cells were incubated with 20 to 50 µL of various antibody preparations in the presence of PBS containing 0.02% sodium azide (NaN3) and 2% bovine serum albumin (BSA). After incubation at 4 °C for 30 minutes, cells were washed twice in PBS–BSA–NaN3 and examined with Nipkow illuminator and phase-contrast optics or processed by flow cytometry. To block functionality the Fc region of IgG molecules, antibody preparations were pretreated with Staphylococcus aureus protein A at wt-wt ratios of 7:1. F(ab')2 fragments of second antibodies were also used. For fluorescence staining, smears of the various types of cell preparations were fixed with pure methanol at room temperature for 15 minutes, washed with PBS three times, and incubated with the different antibody preparations in a wet chamber at 20 °C for 30 minutes. After washing twice in PBS, cells were examined by ultraviolet microscopy. Cells were double-assayed for two antigens by staining cell preparations simultaneously or sequentially with a TRITC-conjugated antibody or an FITC-conjugated specific reagent.3

Flow cytometric analysis was performed with a Coulter EPICS V (Coulter Electronics) equipped with Coherent 94-S argon ion laser. Cells in suspension were excited with laser adjusted to 1 W at 488 nm for FITC and 514 nm for TRITC at a flow rate of approximately 1,500 to 3,200 cells per second using a 76-µ flow tip. Sorting of cell subpopulations was performed using both green fluorescence and forward angle light scatter after defining appropriate electronic windows. To assess the effectiveness of the sorting procedure, positively sorted cells were reanalyzed by flow cytometry and phase-contrast microscopy for expression of Mo-I antigen and latex particle phagocytosis, respectively. Data were analyzed with a Coulter EASY data-processing computer.

Complement-mediated cytolysis. Preparations of mononuclear cells depleted of most free-floating platelets were washed twice in McCoy's 5-A medium. Aliquots of 3 x 10^6 cells were incubated with 375 µL of monospecific antisera or ammonium sulfate precipitated mouse monoclonal antibody preparations at various concentrations for 30 minutes at room temperature and, subsequently, washed twice in McCoy's 5-A medium containing 10% heat-inactivated fetal
bovine serum. Antibody-treated cells were then resuspended in 375 
\( \mu L \) undiluted oxidized normal human AB RhD+ serum that was 
prepared by iodination and used as a source of complement, and 
incubated at 37 °C for 30 minutes.\(^{10} \) To enhance complement 
fixation by monoclonal antibodies, antibody-treated cells were incu-
bated with a rabbit IgM antimouse IgG at room temperature for 30 
minutes and washed twice before complement treatment. Cell 
viability was assessed by supravital staining with trypan blue and 
observation under phase-contrast microscopy.

**RESULTS**

**Immunoisolation of platelet glycoproteins IIb and IIIa from low-density human mononuclear blood cell preparations.** The presence of platelet GPs IIb and IIIa in monocytes was investigated in preparations of low-density mononuclear cells generated after depletion of free-floating platelets. Typically, the mononuclear cell preparations contained approximately 56% monocytes and 44% lymphocytes. Cell differential analyses were performed by immunofluorescence using monoclonal antibodies Mol, B1, and T11/T3 specific for monocytes and B and T lymphocytes, respectively. Platelet-monocyte ratios, reflecting platelet contamination, were determined by examination of cell suspensions under phase-contrast microscopy and differential analyses of Wright-Giemsa–stained smears. Mononuclear cell homogenates were subjected to immunoisolation using protein A-Sepharose beads coated with rabbit antibodies monospecific for GPs IIb and IIIa. Two bands of approximately 126,000 and 116,000 daltons, corresponding to the molecular weights of reduced platelet GPs IIb and IIIa, were clearly identified in SDS gels of reduced immunoisolated material obtained from a cell preparation of 20 × 10⁶ mononuclear cells containing 11 × 10⁶ monocytes and 3.26 × 10⁸ platelets (platelet-monocyte ratio = 30) (Fig 1B). Visualization of the nonreduced monomeric GPs IIb and IIIa molecules was precluded by the heavily stained band of the antibodies’ IgG molecules (Fig 1A). Parallel controls using normal rabbit IgG are depicted in Fig 1C and D.

The presence of platelet GPs IIb and IIIa in a cell preparation of 40 × 10⁶ mononuclear cells containing 20 × 10⁶ monocytes and 2.86 × 10⁶ platelets (platelet-monocyte ratio = 0.14) was also investigated in immunoisolated material eluted from beads coated with rabbit antiplatelet GPs IIb and IIIa after radiodinating cell membrane proteins by the chloroglycoluril method. Autoradiograms of immunoisolated material electrophoresed in 7.5% acrylamide SDS gels revealed trace amounts of GPs IIb and IIIa, which comigrated with clearly identifiable GPs IIb and IIIa isolated in parallel from platelets (data not shown).

**Assessment of synthesis of platelet GPs IIb and IIIa by blood monocytes.** To assess whether platelet GPs IIb and IIIa immunoisolated from low-density blood cells were platelet- or monocyte-derived, studies were conducted to investigate the biosynthesis of these proteins. These studies were undertaken with the expectation that platelets, unlike monocytes, have only rudimentary protein synthetic apparatuses, and that any newly synthesized protein would be monocyte-derived. In a series of experiments, monocyte preparations containing varying degrees of platelet contamination were metabolically labeled with [³⁵S]methionine and analyzed for the synthesis of GPs IIb and IIIa. Material immunoisolated using antiplatelet GP IIb and IIIa antisera was electrophoresed in 7.5% SDS gels and analyzed concurrently by fluorography and Coomassie blue staining. Immunoprecipitates isolated from a preparation of 80 × 10⁶ mononuclear cells containing 40 × 10⁶ monocytes and 8 × 10⁶ platelets (platelet-monocyte ratio = 200) were analyzed under nonreducing and reducing conditions (Fig 2). Although two bands of 125,000 and 116,000 daltons were readily recognized in the reduced Coomassie blue stained gel (Fig 2B), no comparable proteins were observed in either the nonreduced or the reduced counterpart fluorograms (Fig 2A’ and B’).

In another experiment, a preparation of 80 × 10⁶ mononuclear cells containing 40 × 10⁶ monocytes and 1.48 × 10⁹ platelets (platelet-monocyte ratio = 5) was studied in a similar manner and neither GP IIb nor IIIa was detected (data not shown). Material derived from the same immunoprecipitation when analyzed in gels loaded with ninefold increased amount of protein revealed that although a trace of protein was visualized in Coomassie blue stained gels (Fig 2D), no detectable neosynthesized GP IIb or IIIa was observed (Fig 2C’ and D’). The specificity of the metabolic labeling system of monocytes was established by pulsing cells in the presence of increasing concentrations of cycloheximide (Fig 3). Cycloheximide at a concentration of 1 mmol/L fully inhibited protein synthesis of 2 × 10⁶ cells.
GPs IIb and IIIa were studied in a highly pure preparation of monocytes obtained by flow cytometric sorting. Blood monocytes depleted of free-floating and most adherent platelets were stained using a fluorescent-labeled monoclonal antibody specific for a human monocyte cell surface antigen (mol wt 90/150 kd; Mol, the CR3, or complement receptor type three) expressed on monocytes and absent in the majority of lymphoid cells (except natural killer cells) and platelets (Coulter Immunology).32 Figure 4 shows the flow cytometric distribution of the mononuclear cell preparation as a function of the light-scattering and fluorescent intensity. The boxed image corresponds to selected sort windows that includes Mol-bearing monocytes. Flow cytometry provided a preparation of 3.36 x 10⁶ monocytes, representing more than 98% nucleated cells, that contained 3.3 x 10⁴ platelets (platelet-monocyte ratio = 1:100). The presence of GPs IIb and IIIa in sorted monocyte populations was investigated by SDS-PAGE of immunoisolated material. Electrophoretic analysis revealed that no platelet GPIIb or IIIa were detected in the cell preparation despite the fact that gels were stained by the sensitive silver stain method (data not shown).

In order to exclude the synthesis of GPs IIb and IIIa by platelets, preparations of fully purified platelets were metabolically labeled and subjected to immunoadsorption in a similar manner. However, minimal specific labeling was detected corresponding to 3.9 x 10³ TCA precipitable dpm/platelet, as compared with monocytes, which incorporated 1.805 dpm/cell when pulsed under the same conditions. Furthermore, increasing concentrations of cycloheximide resulted in inhibition of [³⁵S]methionine incorporation. More important, no neosynthesis of GP IIb or IIIa was detected when platelet homogenates were subjected to immunoprecipitation.

Identification of cells bearing platelet GPs IIb and IIIa in mononuclear cell preparations. The expression of platelet GPs IIb and IIIa on the membrane and in the intracellular compartment of isolated human blood cells was investigated by immunofluorescence using heterologous and monoclonal antibody preparations. For staining of membrane-restricted
proteins, preparations of viable cells were incubated with specific antibody preparations. Staining of intracellular antigens was achieved by incubating cell preparations with antibody preparations after smearing and fixation, which rendered the membranes permeable to the antibody molecules. In mononuclear cell preparations obtained from 30 normal individuals, platelets were the only cell type stained for GPs IIb and IIIa. Monocytes were clearly not reactive with monospecific rabbit antisera against GP IIb or IIIa or two monoclonal antibodies specific for the platelet GP IIb/IIIa complex. Frequent significant fluorescence associated with mononuclear cells stained by immunofluorescence for GPs IIb and IIIa was attributable to platelets intimately associated with these cells. Figure 5 depicts mononuclear cell preparations stained for GP IIb/IIIa using monoclonal antibody preparation PC-1 (A and B) in which fluorescence staining is restricted to platelets (A' and B').

The expression of GPs IIb and IIIa by monocytes was studied further using complement-mediated cytotoxicity (Table 1). In this sensitive system, anti-Mol mediated the lysis of blood monocytes, but no monocyte cytosis was observed with polyclonal and monoclonal antibodies to platelet GPs IIb, IIIa, or Ib or a heterologous polyspecific antiplatelet membrane preparation.

**DISCUSSION**

Studies described in this communication demonstrated that GPs IIb and IIIa associated with blood monocytes are derived from platelets. Previously, GP IIb/IIIa has been regarded as a restricted antigen for the megakaryocyte/platelet lineage. However, the expression of GP IIb/IIIa by blood monocytes has been reported recently by several authors. Preparations of blood monocytes with different degrees of platelet contamination were labeled in vitro with [35S]methionine with the expectation that newly synthesized proteins would be monocyte-derived, since platelets contain only rudimentary protein synthetic systems. Analyses of immunoisolated GPs IIb and IIIa from colabeled cultures of monocytes and platelets indicated that no GP IIb or IIIa was newly synthesized. On the other hand, substantial amounts of unlabeled GPs IIb and IIIa were detected and found to vary with the level of platelet contamination. The failure to detect synthesis of GP IIb or IIIa may be attributed to the lack of synthesis by mononuclear cells. Based on the 1 to 10 pg protein level of sensitivity described for fluorography, in our system, monocytes could not have synthesized more than 0.076 to 0.76 GP IIb/IIIa molecules per monocyte. In addition, immunoprecipitated GP IIb/IIIa from mononuclear cells corresponded to about 9 to 18.5 x 10^-6 of the total TCA precipitable material. Synthesis of GP IIb/IIIa, however, was demonstrated in human marrow megakaryocytes using the same experimental approach. In 5 x 10^3 [35S]methionine-labeled megakaryocytes, immunoprecipitated GP IIb/IIIa represented only 2 x 10^-4 of all TCA precipitable counts, indicating that the detection system used was highly sensitive in detecting levels of approximately 0.01% to 0.02% of total precipitable counts. Furthermore, while synthetic rates for specific proteins have not been determined in the mononuclear cell preparations studied, synthesis of an array of proteins inhibitable by cycloheximide was observed.

Studies conducted by several groups have demonstrated that platelets contain vestigial amounts of mRNA and may be capable of minimal protein synthesis. In our studies using gel-filtered platelets and [35S]methionine with high specific activity, we have detected trace amounts of protein synthesis comparable 1/10,000 of the specific incorporation observed in other blood cell types. However, attempts to immunoisolate metabolically labeled GPs IIb and IIIa from postculture preparations of gel-filtered or washed platelets failed to demonstrate neosynthesis of these antigens.

Immunofluorescence studies detecting GP IIb and IIIa molecules in blood monocyte preparations revealed that staining was restricted to platelets. Similarly, only platelets and megakaryocytes were stained in a series of 45 immunofluorescence analyses of peripheral blood and bone marrow

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**Table 1. Detection of Cell Membrane Antigens on Blood Monocytes by Complement-Mediated Cytolysis**

<table>
<thead>
<tr>
<th>Antibody Preparation</th>
<th>Mononuclear Cells Killed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyspecific antiplatelet membrane</td>
<td>3</td>
</tr>
<tr>
<td>Heterologous antiplatelet GP IIb/IIIa</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Monoclonal antiplatelet GP IIb/IIIa (PC-1)</td>
<td>3</td>
</tr>
<tr>
<td>Heterologous antiplatelet GP Ib</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Monoclonal anti-Mol</td>
<td>39</td>
</tr>
<tr>
<td>Normal rabbit IgG</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Normal mouse IgG</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Rabbit IgM antimouse IgG</td>
<td>3</td>
</tr>
<tr>
<td>Complement</td>
<td>2</td>
</tr>
<tr>
<td>Medium</td>
<td>1</td>
</tr>
</tbody>
</table>

Cytotoxicity was performed using a two-step assay (heterologous rabbit antiserum + complement) or a three-step assay (monoclonal antibody + rabbit IgM antimouse IgG + complement). Monocytes represented approximately 40% of the low-density mononuclear blood cells (d < 1.077 g/cm³). Cell viability was assessed by trypan blue exclusion.

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Fig 5. (A through D) Mononuclear blood cells stained by immunofluorescence after fixation with monoclonal antibody PC-1 specific for platelet GP IIb/IIIa and TRITC rabbit antimouse Fab Ig. Fluorescent panels (a and b) and phase-contrast counterparts (a' and b') demonstrate restriction of immunofluorescence staining to platelets (x 595).
cell preparations using heterologous antisera monospecific for GPs IIb and IIIa as well as two monoclonal antibodies specific for the platelet GP IIb/IIIa complex. Furthermore, GPs IIb and IIIa present in mononuclear cell preparations failed to trigger complement-mediated lysis of monocytes in assays using heterologous and monoclonal antibodies specific for these antigens, indicating that these molecules were not integrally associated with monocyte plasma membranes.

Previously, Gogstad et al13 have reported the detection of an antigen immunologically indistinguishable from the platelet GP IIb/IIIa complex in blood monocytes. In crossed immunoelectrophoretic analyses of preparations of solubilized monocyte membranes using a monospecific antisera to the GP IIb/IIIa complex, Gogstad and associates demonstrated a single immunoprecipitate representing this molecular entity. However, adherent monocytes isolated by conventional techniques after full depletion of free-floating platelets typically have at least three to five platelets specifically bound to their surfaces and appear to represent the source of platelet GPs IIb and IIIa detected by Gogstad et al.13 Moreover, their observation that no monocyte-associated GP IIb/IIIa was detectable in cells in a patient homozygous for Glanzmann's thrombasthenia does not clarify its source. Detection of the platelet GP IIb/IIIa complex in mononuclear cell preparations using a monoclonal antibody has been reported by Burckhardt et al.14 However, high levels of platelet contamination are inferred from their experimental protocol. Removal of platelets adhered to monocytes requires extensive, repeated incubations with solutions containing an excess of 5 mmol/L EDTA and intervening differential centrifugations.30 Isolation of platelet GPs IIb and IIIa from blood monocytes also has been reported by Bai et al16 using two monoclonal antibody preparations against the GP IIb/IIIa complex. However, the mononuclear cell preparations were obtained from defibrinated peripheral blood, which is known to contain high concentrations of platelet microparticles.36 Serum obtained from defibrinated blood contains platelet membrane fragments generated during cell activation with quantifiable levels of platelet proteins, including platelet GPs IIb, IIa, and IIIa, as well as platelet factor 4.36 The description by Bai et al that GP Ib was readily recognized in their monocyte preparations is puzzling. Using the same monoclonal antibody (AN-51), other laboratories have not detected monocyte-associated GP Ib, suggesting that its presence may indeed represent contamination by platelet-derived proteins.37 Expression of platelet GP IIb/IIIa by an erythroleukemia-derived cell line, K-562, expressing multilinage phenotypes, has been reported by Gewirtz et al15 and Tabilio et al.7 However, using the same and different antibody preparations, other authors failed to detect GPs IIb and IIIa in K-562 cells, indicating that gene expression of these antigens in neoplastic hematopoietic cell lines may be erratic.38 HEL cell line established from a patient with Hodgkin's disease who later developed an erythroleukemia also expressed GPs IIb and IIIa.39 Two other cell lines—U-937, derived from histiocytic lymphoma cells, and HL-60, from promyelocytes—have been reported to express GP IIb/IIIa.40,41 A number of similar and shared structural properties have been recognized between platelets and endothelial cells, including the expression of factor VIII: von Willebrand factor, fibronectin, thrombin receptors, and thrombospondin.42-44 More recently, a GP IIIa-like molecule has been characterized in umbilical vein endothelial cells using a monoclonal antibody preparation.45 Intriguingly, no GP IIb was co-isolated with this GP IIIa-like molecule, suggesting that certain differences exist between this new entity and the classical GP IIb/IIIa complex, as identified on platelets and megakaryocytes.46 Structural homology between platelet GP IIIa and its endothelial analog may reflect common physiological functions or may be due to evolution from a common archetype.43 Characterization of GPs IIb and IIIa in normal hematopoietic cells by immunofluorescence have revealed that platelets and megakaryocytes are the only blood and marrow cell types expressing the GP IIb/IIIa complex.7,8,10 The clonal progenitor for megakaryocytes (CFU-Mk)—but not the progenitors for the multilineage colonies (CFU-GEMM), erythroid (BFU-E), or the myelomonocytic (CFU-GM) lineages—contain the GP IIb/IIIa complex.12 Additionally, synthesis of GPs IIb and IIIa with similar molecular characteristics as their platelet counterparts has been demonstrated in isolated human marrow megakaryocytes, thereby establishing the source of these glycoproteins in platelets.11

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