

Platelet glycoprotein IIb. Chromosomal localization and tissue expression.

P F Bray, ... , R P McEver, M A Shuman

J Clin Invest. 1987;80(6):1812-1817. <https://doi.org/10.1172/JCI113277>.

Research Article

The GPIIb-IIIa complex functions as a receptor for cytoadhesive proteins on the platelet surface. Both GPIIb and GPIIIa are synthesized by a human erythroleukemia (HEL) cell line. We isolated several cDNA clones by screening a HEL cell cDNA library with an oligonucleotide derived from amino acid sequence of GPIIb. Nucleotide and amino acid sequences were determined from 703 bp of one of these clones. Amino acid sequence of purified platelet GPIIb peptides confirmed the identity of the clone. The cDNA encodes the carboxyl terminus of the large (alpha) subunit of GPIIb and all of the smaller (beta) subunit of GPIIb. By hybridizing the cDNA directly to chromosomes separated by dual laser chromosome sorting, the gene for GPIIb was mapped to chromosome 17. Northern blot analysis showed a approximately 3.4-kb GPIIb mRNA in HEL cells. We also compared the amino acid sequences determined from eight additional platelet GPIIb peptides with the derived amino acids from a published HEL cell GPIIb cDNA, and the platelet and HEL cell proteins appear to be the same. Despite previous reports that vascular endothelial cells and monocytes contain GPIIb, no GPIIb mRNA was observed in either type of cell. Thus, GPIIb appears to be specific for the platelet-megakaryocyte membrane and is distinct from the alpha subunits of the adhesion receptors in other normal tissues.

[...]

Find the latest version:

<https://jci.me/113277/pdf>



Platelet Glycoprotein IIb

Chromosomal Localization and Tissue Expression

Paul F. Bray,* Jean-Philippe Rosa,† Geoffrey I. Johnston,‡ Donny T. Shiu,* Richard G. Cook,§ Chris Lau,|| Yuet Wai Kan,*|| Rodger P. McEver,‡ and Marc A. Shuman*

*Department of Medicine, Cancer Research Institute, and ||The Howard Hughes Medical Institute Laboratory, University of California, San Francisco, California 94143; †Departments of Medicine and Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas 78284; and ‡The Howard Hughes Medical Institute Laboratory and Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030

Abstract

The GPIIb-IIIa complex functions as a receptor for cytoadhesive proteins on the platelet surface. Both GPIIb and GPIIIa are synthesized by a human erythroleukemia (HEL) cell line. We isolated several cDNA clones by screening a HEL cell cDNA library with an oligonucleotide derived from amino acid sequence of GPIIb. Nucleotide and amino acid sequences were determined from 703 bp of one of these clones. Amino acid sequence of purified platelet GPIIb peptides confirmed the identity of the clone. The cDNA encodes the carboxyl terminus of the large (α) subunit of GPIIb and all of the smaller (β) subunit of GPIIb. By hybridizing the cDNA directly to chromosomes separated by dual laser chromosome sorting, the gene for GPIIb was mapped to chromosome 17. Northern blot analysis showed a \sim 3.4-kb GPIIb mRNA in HEL cells. We also compared the amino acid sequences determined from eight additional platelet GPIIb peptides with the derived amino acids from a published HEL cell GPIIb cDNA, and the platelet and HEL cell proteins appear to be the same. Despite previous reports that vascular endothelial cells and monocytes contain GPIIb, no GPIIb mRNA was observed in either type of cell. Thus, GPIIb appears to be specific for the platelet-megakaryocyte membrane and is distinct from the α subunits of the adhesion receptors in other normal tissues.

Introduction

A critical step in maintaining normal hemostasis is the aggregation of platelets during the formation of blood clots. Failure of platelets to aggregate results in bleeding, as is seen in the hereditary disorder, Glanzmann's thrombasthenia (1). The platelets of these patients are deficient in membrane GPIIb and GPIIIa (2). GPIIb and GPIIIa mediate platelet aggregation by acting as the receptor for fibrinogen, an adhesion molecule

that binds platelets to one another in the blood clot (3). As determined by SDS-PAGE, unreduced GPIIb has an apparent M_r of 142,000 (4). Electrophoresis of the reduced protein reveals two disulfide-linked subunits; GPIIb $_{\alpha}$ (M_r of 123,000) and GPIIb $_{\beta}$ (M_r of 22,000) (4) that are synthesized as a single-chain precursor (5). GPIIIa is a single-chain glycoprotein. GPIIb and GPIIIa are associated as a noncovalent, calcium-dependent heterodimer in the platelet membrane (for review, see reference 1).

Recent evidence suggests that the GPIIb-IIIa complex belongs to a class of receptors that bind cell adhesion molecules (for review, see reference 6). These receptors share a common heterodimeric structure with α and β subunits. Platelet GPIIb and GPIIIa correspond to the α and β subunit, respectively (not to be confused with GPIIb $_{\alpha}$ and GPIIb $_{\beta}$, the subunits of reduced mature platelet GPIIb). GPIIb-IIIa is able to bind not only fibrinogen (3), but other cytoadhesive proteins, such as fibronectin (7), vitronectin (8), and von Willebrand factor (9). In contrast, Pytela et al. observed that when extracted from human placenta, the receptor for fibronectin binds only fibronectin, and that the vitronectin receptor binds only to vitronectin (8). All of these adhesion molecules contain the cell attachment sequence Arg-Gly-Asp (10), but the factors that confer ligand specificity (i.e., fibrinogen binds to GPIIb-IIIa but not to the fibronectin receptor [8]) are unknown.

Study of the genes encoding platelet proteins is particularly difficult for two reasons: (i) platelets are anucleate and synthesize little or no protein (11), and (ii) megakaryocytes, the precursor cells of platelets, comprise only 0.1% of all cells in the bone marrow and are therefore difficult to purify quantitatively. The human erythroleukemia (HEL)¹ cell line possesses some features of megakaryocytes (12, 13). Specific polyclonal antibodies raised against reduced or nonreduced GPIIb or GPIIIa have been shown to react with HEL cell proteins of comparable molecular weight on Western blots (5, 12). At least seven different monoclonal antibodies against platelet GPIIb, GPIIIa, or the GPIIb-IIIa complex react with intact HEL cells (12–15). Therefore, HEL cells represent a good model for analysis of the genes for GPIIb and GPIIIa.

We have constructed a cDNA library in the λ gt10 vector using HEL cell RNA, and have cloned and sequenced a partial cDNA for GPIIb. Poncz et al. have recently published the cDNA for the entire coding region of GPIIb, also derived from HEL cell RNA (16). We report here that the gene for GPIIb is located on chromosome 17. Also, Northern blot analysis re-

Dr. Rosa is currently at U-150 INSERM, Hopital Lariboisière, 75475 Paris Cedex 10, France, and Dr. McEver, at University of Oklahoma Health Sciences Center, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

Send reprint requests to Dr. P. F. Bray, Cancer Research Institute, Box 0128, Rm. M1282, University of California San Francisco, San Francisco, CA 94143.

Received for publication 27 May 1987 and in revised form 12 August 1987.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/87/12/1812/06 \$2.00

Volume 80, December 1987, 1812–1817

1. Abbreviations used in this paper: HEL, human erythroleukemia; HUVE, human umbilical vein endothelial.

veals the absence of GPIIb mRNA in human umbilical vein endothelial (HUVE) cells and peripheral blood monocytes, cells in which GPIIb-like proteins have been previously reported. We also provide additional platelet amino acid sequence confirming the equivalence of HEL cell and platelet GPIIb.

Methods

GPIIb amino acid sequence determination. GPIIb was isolated as described (5). Methods of amino acid determination will be described in detail in another manuscript (manuscript in preparation). Briefly, purified reduced-alkylated GPIIb was digested with trypsin and separated by reverse-phase HPLC. Amino acid sequencing from the NH₂ terminus of intact IIB_α and the resultant peptides was performed using a gas phase protein sequencer (model 470 A; Applied Biosystems, Inc., Foster, City, CA). Several attempts to sequence the NH₂ terminus of IIB_β proved unsuccessful. Therefore, IIB_β was cleaved by cyanogen bromide and subjected to sequencing.

Oligonucleotide synthesis. Using the amino acid sequence of GPIIb_β, a 48-bp oligonucleotide was designed according to Lathe (17) and synthesized by the phosphotriester method (Oligonucleotide Chemistry Systems Laboratories, Denton, TX).

Tissues. Human peripheral blood monocytes were isolated from a normal donor platelet pheresis residue bag, as described (18). After separation, Wright's stain and esterase stain showed 96% monocytes, 4% lymphocytes, and no visible platelets. HUVE cells were isolated from freshly prepared human umbilical cords by collagenase digestion and cultured with serial passages as described (19).

RNA preparation. Total RNA was extracted from various cell lines and tissues by the guanidinium thiocyanate method (20). Poly(A)⁺ RNA was selected by elution from an oligo(dT)-cellulose column (21). For construction of the cDNA library, HEL cell poly(A)⁺ RNA was fractionated on a 15–30% sucrose gradient, and then subjected to a second purification over an oligo(dT)-cellulose column. The various poly(A)⁺ RNA fractions were translated in vitro and the resulting proteins immunoprecipitated and electrophoresed to determine the fraction most enriched for GPIIb mRNA. Fractions corresponding to poly(A)⁺ RNAs of 3 kb and larger were pooled.

cDNA library construction. 4 μg of size-selected poly(A)⁺ RNA was used to synthesize the oligo(dT)-primed first strand of cDNA (22). Second strand was made using RNase H and DNA polymerase I (23). The cDNA was made blunt-ended, treated with Eco RI methylase, ligated to Eco RI linkers (Pharmacia Fine Chemicals, Piscataway, NJ) and digested with Eco RI (22). This cDNA was fractionated on a 1% agarose gel to size-select for cDNAs > 0.9 kb and to remove free linkers. cDNAs were eluted from gel slices, and then ligated into the λgt10 vector and packaged (both λgt10 vector and packaging extract from Stratagene Cloning Systems, San Diego, CA).

Screening of the λgt10 HEL cell library. The GPIIb_β 48-mer was end-labeled with γ[³²P]ATP, and 2 × 10⁶ cpm probe per milliliter hybridization solution was used to screen 700,000 recombinants. Hybridization conditions were: 5× standard saline citrate (SSC) (1× SSC is 150 mM NaCl, 15 mM sodium citrate)/5× Denhardt's solution (Denhardt's is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA)/10% formamide at 37°C for 12 h. Filters were washed in 2× SSC at 22°C, then in 0.3× SSC/0.1% SDS at 51°C. Phage DNA was prepared and digested with Eco RI. The sizes of the cDNA inserts ranged from 720 to 2,000 bp. The Eco RI insert from one of the positive clones, λH3.1, was ligated into the pBS plasmid vector (Stratagene Cloning Systems).

Nucleotide sequencing. The Eco RI insert of λH3.1 was ligated into M13mp19 and the nucleotide sequence was determined using the dideoxynucleotide chain termination method (24).

Chromosomal localization of the GPIIb gene. The chromosomal localization of the GPIIb gene was analyzed by hybridization of the cDNA directly to the DNA of chromosomes resolved by dual laser chromosome sorting (25).

cDNA probes. Northern blots were probed with the ³²P-labeled 0.7 kb Eco RI insert of λH3.1 and with a 2.2-kb Eco RI fragment from the 5' end of the cDNA for GPIIIa (manuscript in preparation). This latter cDNA was obtained from the HEL cell cDNA library described above and is 99% homologous to that obtained by Fitzgerald et al. from a HUVE cell cDNA library (26). Additional probes used as positive controls were a 404-bp Eco RI fragment of the 5' end of the von Willebrand factor cDNA (19) and the 3' 1.8 kb Eco RI fragment of the β subunit of the leukocyte adhesion proteins (27).

Northern blot analysis. Poly(A)⁺ RNA was electrophoresed on a 1% agarose 2.2 M formaldehyde gel and blotted to nitrocellulose or nylon filters. Nitrocellulose filter hybridization was performed with 2 × 10⁶ cpm/ml probe in 50% formamide/1× Denhardt's solution/3× SSC/50 mM NaPO₄ (pH 7.0)/200 μg/ml salmon sperm DNA/10% dextran sulfate at 42°C for 12 h. Filters were washed in 0.1× SSC/0.1% SDS at 55°C. Nylon filters were hybridized with 2 × 10⁶ cpm/ml probe in 7% SDS/0.5 M NaPO₄ (pH 7.2)/10% dextran sulfate at 65°C and washed in 2× SSC/1% SDS at 65°C.

Results

A cDNA library containing 2.6 × 10⁶ recombinants was constructed from HEL cell poly(A)⁺ RNA. 700,000 recombinants were screened at high plating density and 28 clones were identified that reacted with a GPIIb_β synthetic oligonucleotide. Only twelve were pursued on secondary screening, and seven of these clones were positive on secondary and tertiary screening. To confirm the authenticity of one clone, λH3.1, its nucleotide sequence was determined (Fig. 1). When translated, the open reading frame corresponds to 172 amino acids. The derived sequence was compared with 11 residues of a GPIIb_β peptide (Table I, peptide 6) obtained by tryptic digestion; all 11 matched (Fig. 1, amino acids 111–121). We also compared the derived sequence with 19 residues of a GPIIb_β peptide obtained by cleavage with cyanogen bromide (Table I, peptide βCNBr); 17 amino acids matched (Fig. 1, amino acids 154–170) and the first and last residues were ambiguous. In both discrepant residues, a minor HPLC signal was identical to the nucleotide-derived amino acid. Hiraiwa et al. (28) sequenced two GPIIb peptides, one of which matched 12 of our derived amino acids, 161–172 (Fig. 1). Because their sequence overlapped all but one of our GPIIb_β peptide residues, it was not indicated in Fig. 1. Moreover, all 15 residues of the GPIIb_β NH₂ terminus published by Charo et al. (29) were identical with a segment of our predicted sequence (Fig. 1, amino acids 36–50). We conclude that the λH3.1 cDNA includes the entire sequence for GPIIb_β. The cDNA also codes for 35 amino acids that precede the NH₂ terminus of GPIIb_β and presumably represents the COOH-terminal end of GPIIb_α.

We compared both our cDNA nucleotide sequence and platelet GPIIb peptide sequences with the data of Poncz et al. (16). All 703 nucleotides of our λH3.1 clone matched perfectly with the 3' end of their cDNA (comparison not shown). Table I lists the 10 platelet GPIIb peptides that we sequenced. Peptides 6 and "βCNBr" are contained in GPIIb_β and shown in Fig. 1. Of the remaining 8 peptides, 153 residues were assigned. When compared with the published amino acid sequence derived from the HEL cell GPIIb cDNA (16), 151 matched (comparison not shown).

The chromosomal location of the GPIIb gene was analyzed by hybridization of the ³²P-labeled λH3.1 Eco RI restriction fragment directly to the DNA of chromosomes resolved by

```

1      Val Asp Trp Gly Leu Pro Ile Pro Ser Pro Ser Pro
1      AG GTG GAC TGG GGG CTG CCC ATC CCC AGC CCC TCC CCC

13    Ile His Pro Ala His His Lys Arg Asp Arg Arg Gln Ile
39    ATT CAC CCG GCC CAT CAC AAG CGG GAT CGC AGA CAG ATC

26    Phe Leu Pro Glu Pro Glu Gln Pro Ser Arg ↓ Leu Gln Asp
78    TTC CTG CCA GAG CCC GAG CAG CCC TCG AGG CTT CAG GAT

39    Pro Val Leu Val Ser Cys Asp Ser Ala Pro Cys Thr Val
117   CCA GTT CTC GTA AGC TGC GAC TCG GCG CCC TGT ACT GTG

52    Val Gln Cys Asp Leu Gln Glu Met Ala Arg Gly Gln Arg
156   GTG CAG TGT GAC CTG CAG GAG ATG GCG CGC GGG CAG CCG

65    Ala Met Val Thr Val Leu Ala Phe Leu Trp Leu Pro Ser
195   GCC ATG GTC ACG GTG CTG GCC TTC CTG TGG CTG CCC AGC

78    Leu Tyr Gln Arg Pro Leu Asp Gln Phe Val Leu Gln Ser
234   CTC TAC CAG AGG CCT CTG GAT CAG TTT GTG CTG CAG TCG

91    His Ala Trp Phe *Asn Val Ser Ser Leu Pro Tyr Ala Val
273   CAC GCA TGG TTC AAC GTG TCC TCC CTC CCC TAT GCG GTG

104   Pro Pro Leu Ser Leu Pro Arg Gly Glu Ala Gln Val Trp
312   CCC CCG CTC AGC CTG CCC CGA GGG GAA GCT CAG GTG TGG

117   Thr Gln Leu Leu Arg Ala Leu Glu Glu Arg Ala Ile Pro
351   ACA CAG CTG CTC CGG GCC TTG GAG GAG AGG GCC ATT CCA

130   Ile Trp Trp Val Leu Val Gly Val Leu Gly Gly Leu Leu
390   ATC TGG TGG GTG CTG GTG GGT GTG CTG GGT GGC CTG CTG

143   Leu Leu Thr Ile Leu Val Leu Ala Met Trp Lys Val Gly
429   CTG CTC ACC ATC CTG GTC CTG GCC ATG TGG AAG GTC GGC

156   Phe Phe Lys Arg Asn Arg Pro Pro Leu Glu Glu Asp Asp
468   TTC TTC AAG CCG AAC CCG CCA CCC CTG GAA GAA GAT GAT

169   Glu Glu Gly Glu Stop
507   GAA GAG GGG GAG TGA TGGTGCAGCCTACACTATTCTAGCAGGAGGGTTG

556   GGCGTGCTACCTGCACCGCCCTTCTCCAACAAGTTGCCTCCAAGCTTTGGGTTGG
612   AGCTGTTCCATTGGGTCTCTTGGTGTGTTTTCCCTCCCAACAGAGCTGGGCTACC
668   CCCCCTCTGCTGCCTAATAAAGAGACTGAGCCCTGAAAAAAAAAAAAA (n)

```

Figure 1. Derived amino acid and nucleotide sequence of partial HEL cell GPIIb cDNA. The λ H3.1 clone was subcloned into M13mp19 and complete sequence was obtained in both orientations. Numbering begins at the 5' end of the λ H3.1 clone. Identical platelet peptide sequences are indicated by double-underlined regions. Underline breaks indicate ambiguities in peptide sequencing. The boxed region is the putative hydrophobic transmembrane segment (16). The single underline at nucleotide 684 represents the polyadenylation signal. The potential N-linked glycosylation site (*) and the IIB α -IIB β cleavage site (\downarrow) are indicated.

dual laser chromosome sorting (Fig. 2). The gene for GPIIb mapped uniquely to chromosome 17. Not shown are chromosomes 9-12. In a second experiment, the λ H3.1 clone did not hybridize to chromosomes 9-12, but did bind to chromosome 17.

To study tissue expression, we used the λ H3.1 cDNA and a GPIIIa cDNA fragment to probe for mRNA expression in HEL cells, HUVE cells, and normal human circulating monocytes. HEL cells contain a single ~ 3.4-kb species of GPIIb mRNA (Fig. 3, lane 1) and a ~ 6-kb species of GPIIIa RNA (Fig. 3, lane 2). The faint signal at ~ 3-kb (Fig. 3, lane 2) has been a consistent observation and may represent a spliced GPIIIa message. GPIIb message was not detected in mRNA from either HUVE cells or monocytes (Fig. 3, lanes 3 and 6, respectively). When endothelial mRNA was probed with GPIIb cDNA under less stringent conditions (same as in Methods, except hybridization performed at 37°C and washing performed in 2× SSC/0.1% SDS), no GPIIb message was detected (data not shown). However, when probed with GPIIIa cDNA, a single ~ 6-kb message was observed in mRNA from HUVE cells, but none in the monocytes (Fig. 3, lanes 4 and 7,

respectively). As positive controls, a cDNA probe for von Willebrand factor (Fig. 3, lane 5) and for the β subunit of the leukocyte adhesion proteins (Fig. 3, lane 8) identified the appropriate ~ 8.5 kb (30) and ~ 3.0 kb (27) mRNAs, respectively, in endothelial cells and monocytes. Note that when the filter used in Fig. 3, lane 3 was probed with the GPIIIa cDNA, the ~ 6-kb mRNA was seen (data not shown), and that Fig. 3, lanes 6, 7, and 8 represent the same filter hybridized in three separate experiments. Northern blot analysis indicated ~ 20-fold less GPIIb mRNA in K562 cells than in HEL cells, and absence of GPIIb mRNA in reticulocytes (data not shown).

Discussion

We have obtained a partial cDNA clone coding for the COOH-terminal portion of GPIIb. There was perfect identity between the derived amino acids of this cDNA and 43 amino acids from platelet GPIIb β . The 703 nucleotides of our cDNA clone are identical to the 3' end of the cDNA published by Poncz et al. (16). Additionally, we found 151 of 153 amino acids from 8 platelet GPIIb peptides matched the recently

Table I. Amino Acid Sequence from Platelet GPIIb Peptides

Peptide	Sequence
α NH ₂ terminus	L N L D P V Q L T F Y A G P (X) G S Q F G F S L T F H K (X) S H G (R) V A (I) V (V) G
1	N V G S Q T L Q T F K
2	I Y V (E) N D F S W D K R
3	V Y L F L Q P R
4	F (X) S A I A P L G D L D (X) D G Y N (X) I A V A A P Y G G P S G R
5	(A) L G P S Q E E T G G V F L (X) P W R
6	G E A Q V W T Q L L R
7	G P H A L G A P S L L L T G T Q L Y G R
8	(X) R P S Q V L D S P F D T G S A F G F S L R
β CNBr	(S) K V G F F K R N R P P L E E D (D) (E) (Y)

Amino acid sequences of the NH₂ termini of GPIIb _{α} , tryptic peptides of GPIIb, and the cyanogen bromide peptide (β CNBR) of GPIIb _{β} . Amino acid residues are given in the single-letter code. Tryptic peptides were identified by assigning a number to the peak on the chromatographic profile. Tentatively assigned residues are in parentheses; undetermined residues are indicated by "X". The published cDNA-derived amino acids (16) at variance with our residues are indicated above our peptides and in bold lettering.

published sequence of HEL cell GPIIb (16). When considered in light of immunologic and biochemical evidence (5, 12–15), the data strongly suggest that platelet and HEL cell GPIIb are the same protein.

Northern blot analysis of HEL cell poly(A)⁺ RNA reveals that the message for GPIIb is ~ 3.4 kb. This is somewhat smaller than the 4.1-kb reported by Poncz et al. (16). The discrepancy is likely due to the different denaturing gel systems used and/or the different molecular weight markers employed. Once the 5' untranslated region has been cloned, the true mRNA size can be determined.

Several reports have indicated that human endothelial cells synthesize a complex related to GPIIb-IIIa (31–34). When cultured HUVE cells were surface radiolabeled, solubilized, and immunoprecipitated with anti-GPIIIa antisera, an α - β heterodimeric complex was observed (31–34). Based on immunologic evidence (35) and comparisons of partial peptide sequence from GPIIIa with the sequence from an endothelial cell cDNA (26), it appears that platelet GPIIIa and the endothelial β subunit are identical. However, direct immunological evidence that the α subunit of HUVE cells is platelet GPIIb has never been obtained. Moreover, our present work shows ab-

sence of GPIIb mRNA from HUVE cells. Also, we have been unable to detect an immunologically related GPIIb molecule in HUVE cells by Western blotting using a polyclonal anti-GPIIb antibody (unpublished observation). These data indicate that once the HUVE cell adhesion receptor complex is dissociated by SDS-PAGE, the α subunit is not recognized by anti-GPIIb antisera. Our findings are in disagreement with an

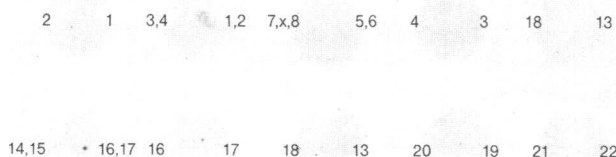


Figure 2. Autoradiogram of dot-blot filters of sorted chromosomes. ³²P-radiolabeled cDNA (0.72 kb restriction fragment) was hybridized to the DNA of chromosomes resolved by dual laser chromosome sorting and bound to nitrocellulose. Numbers refer to chromosome(s) isolated.

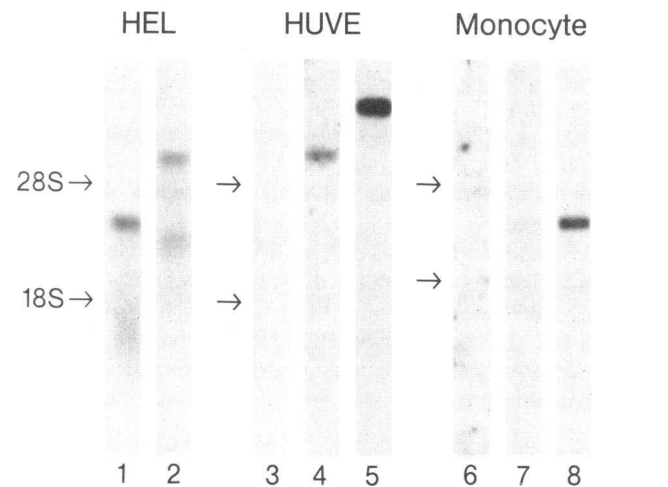


Figure 3. Identification of GPIIb and GPIIIa mRNA by Northern blot analysis Poly(A)⁺ RNA from HEL cells (10 μ g, lanes 1 and 2), HUVE cells (3 μ g, lane 3; 2 μ g, lane 4; 3 μ g, lane 5), and normal human circulating monocytes (1 μ g, lanes 6, 7, and 8) was electrophoresed and transferred to nitrocellulose. These filters were probed with ³²P-labeled cDNA for GPIIb (lanes 1, 3, and 6), GPIIIa (lanes 2, 4, and 7), von Willebrand factor (lane 5), and the β subunit of the leukocyte adhesion proteins (lane 8). Lanes 1 and 2 exposed at 22°C without intensifying screens for 12 h. Lanes 3–8 exposed at –70°C with intensifying screens for 12 h (lanes 3–5 and 8) or 4 d (lanes 6 and 7). 28S and 18S ribosomal markers are indicated by the upper and lower arrows, respectively, in each panel.

earlier report in which antisera apparently specific for GPIIb α immunoprecipitates the HUVE cell α - β heterodimeric complex (29). An explanation for this disagreement would be that the anti-GPIIb α antisera has minor reactivity with GPIIIa. Thus, in contrast to GPIIIa, our results indicate that the α subunit of the HUVE cell heterodimer is distinct from platelet GPIIb. The term "GPIIb" should not be used to describe the α subunit of HUVE cells.

The identity of the α subunit of the endothelial cell adhesion receptor is not known. When we probed endothelial mRNA with the λ H3.1 cDNA under nonstringent conditions, no homologous mRNA was identified. The adhesion receptor on endothelial cells may be analogous to the vitronectin receptor, in which the β subunit is likely to be identical to platelet GPIIIa and the α subunit different from GPIIb (36).

There has been speculation that the β subunits of other cell adhesion receptors share more homologies than the α subunits (37). Since GPIIb-IIIa binds additional ligands not bound by other adhesion receptors (7), our findings suggest that GPIIb is likely to be important in conferring receptor-ligand specificity.

Several comments regarding monocyte expression of GPIIb can be made based on the results of our Northern blot analysis. Evidence for (38-40) and against (41, 42) the presence of GPIIb and GPIIIa in monocytes has been presented. Our data reveals no GPIIb or GPIIIa mRNA in monocytes. Because we analyzed only 1 μ g of poly(A)⁺ monocyte RNA, we cannot rule out very low levels of expression. However, probing the same filter under identical hybridization conditions, we were able to detect both the Factor XIII a-chain, a cytoplasmic protein, (18) and the β subunit of the leukocyte adhesion proteins (Fig. 3, lane 8), a membrane cytoadhesion protein similar to GPIIIa. Therefore, it is unlikely that monocytes contain either GPIIb or GPIIIa.

We have mapped the gene for GPIIb to chromosome 17. We are currently conducting experiments to localize the gene for GPIIIa. These results will have implications with regard to the genetic mechanism for Glanzmann's thrombasthenia. If the gene for GPIIIa is on chromosome 17, then either a gene deletion or a defect in a regulatory element controlling transcription of both genes could explain why GPIIb and GPIIIa are absent or decreased in parallel in the platelets of patients with this disease.

The ligand binding sites on the GPIIb-IIIa complex and the structural features required for the subunit assembly have not been characterized. Cloning of the cDNA for GPIIb and GPIIIa will allow experiments in which the protein products can be selectively altered so that answers to these questions can be obtained.

Acknowledgments

The authors thank Dr. Roger Lebo and Robert Flandermeyer for providing the sorted chromosomes; Dr. J. Evan Sadler for providing HUVE cell poly(A)⁺ RNA and plasmid containing cDNA for von Willebrand factor; Dr. Timothy Springer and Takashi Kishimoto for plasmid containing cDNA for the β subunit of the leukocyte adhesion proteins; Karen Fischer for HUVE cell poly(A)⁺ RNA; Dr. Richard N. Harkins for protein sequencing; Dr. Laurie J. Weisberg for providing monocyte RNA; and Ms. Debra Singer for preparing the manuscript.

This work has been supported by grant HL-33277 from the National Institutes of Health (NIH). Dr. Bray is a recipient of Physician Scientist Award HL-01815 from the NIH, Dr. Rosa is Chargé de Re-

cherche, détaché de l'unité L'Institut National de la Santé et de la Recherche Médicale (INSERM) 150. Dr. Kan is an Investigator of the Howard Hughes Medical Institute. Dr. McEver is the recipient of Research Career Development Award HL-01733 from the NIH.

References

- George, J. N., A. T. Nurden, and D. R. Phillips. 1984. Molecular defects in interactions of platelets with the vessel wall. *N. Engl. J. Med.* 311:1084-1093.
- Nurden, A. T., and J. P. Caen. 1974. An abnormal platelet glycoprotein pattern in three cases of Glanzmann's Thrombasthenia. *Br. J. Haematol.* 28:253-260.
- Bennett, J. S. 1985. The platelet-fibrinogen interaction. In *Platelet Membrane Glycoproteins*. J. N. George, A. T. Nurden, and D. R. Phillips, editors. Plenum Press, New York. 193-214.
- Phillips, D. R., and P. P. Agin. 1977. Platelet plasma membrane glycoproteins: Evidence for the presence of nonequivalent disulfide bonds using nonreduced-reduced two-dimensional gel electrophoresis. *J. Biol. Chem.* 252:2121-2126.
- Bray, P. F., J-P. Rosa, V. R. Lingappa, Y. W. Kan, R. P. McEver, and M. A. Shuman. 1986. Biogenesis of the platelet receptor for fibrinogen: evidence for separate precursors for glycoproteins IIB and IIIa. *Proc. Natl. Acad. Sci. USA.* 83:1480-1484.
- Plow, E. F., M. H. Ginsberg, and G. A. Marguerie. 1986. Expression and function of adhesive proteins on the platelet surface. In *Biochemistry of Platelets*. D. R. Phillips and M. A. Shuman, editors. Academic Press, Inc., Orlando. 226-256.
- Plow, E. F., and M. H. Ginsberg. 1981. Specific and saturable binding of plasma fibronectin to thrombin-stimulated human platelets. *J. Biol. Chem.* 236:9477-9482.
- Pytela, R., M. D. Pierschbacher, M. H. Ginsberg, E. F. Plow, and E. Ruoslahti. 1986. Platelet membrane glycoprotein IIB/IIIa: member of a family of Arg-Gly-Asp-specific adhesion receptors. *Science (Wash. DC).* 231:1559-1562.
- Ruggeri, Z. M., L. De Marco, L. Gatti, R. Bader, and R. R. Montgomery. 1983. Platelets have more than one binding site for von Willebrand factor. *J. Clin. Invest.* 72:1-12.
- Pierschbacher, M. D., and E. Ruoslahti. 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature (Lond.)* 309:30-33.
- Booyse, F. M., T. P. Hoveke, and M. E. Rafelson. 1968. Studies on human platelets. II. Protein synthetic activity of various platelet populations. *Biochim. Biophys. Acta.* 157:660-663.
- Tabilio, A., J-P. Rosa, U. Testa, N. Kieffer, A. T. Nurden, M. C. Del Canizo, J. Breton-Gorius, and W. Vainchenker. 1984. Expression of platelet membrane glycoproteins and α -granule proteins by a human erythroleukemia (HEL) cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:453-459.
- Papayannopoulou, T., E. Raines, S. Collins, B. Nakamoto, M. Tweeddale, and R. Ross. 1987. Constitutive and inducible secretion of platelet-derived growth factor analogs by human leukemia cell lines coexpressing erythroid and megakaryocytic markers. *J. Clin. Invest.* 79:859-866.
- Rosa, J.-P., M. Cevallos, and R. P. McEver. 1986. Fibrinogen receptor assembly in human erythroleukemia (HEL) cells. *Blood.* 68:325. (Abstr.)
- Silver, S. S., M. M. McDonough, G. Vilaire, and J. S. Bennett. 1987. The *in vitro* synthesis of polypeptides for the platelet membrane glycoproteins IIB and IIIa. *Blood.* 69:1031-1037.
- Poncz, M., R. Eisman, R. Heidenreich, S. M. Silver, G. Vilaire, S. Surrey, E. Schwartz, and J. S. Bennett. 1987. Structure of the platelet membrane glycoprotein IIB: homology to the α subunits of the vitronectin and fibronectin membrane receptors. *J. Biol. Chem.* 262:8476-8482.
- Lathe, R. 1985. Synthetic oligonucleotide probes deduced from

amino acid sequence data: theoretical and practical considerations. *J. Mol. Biol.* 183:1–12.

18. Weisberg, L. J., D. T. Shiu, P. R. Conkling, and M. A. Shuman. 1987. Identification of normal human peripheral blood monocytes and liver as sites of synthesis of coagulation factor XIII a-chain. *Blood.* 70:579–582.

19. Sadler, J. E., B. B. Shelton-Inloes, J. M. Sorace, J. M. Harlan, K. Titani, and E. W. Davie. 1985. Cloning and characterization of two cDNAs coding for human von Willebrand factor. *Proc. Natl. Acad. Sci. USA.* 82:6394–6398.

20. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

21. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning. In *A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 197–198.

22. Ebina, Y., L. Ellis, K. Jarnagin, M. Edery, L. Graf, E. Clauser, J. Ou, F. Masiarz, Y. W. Kan, I. D. Goldfine, R. A. Roth, and W. J. Rutter. 1985. The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell.* 40:747–758.

23. Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. *Gene (Amst.).* 25:263–269.

24. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

25. Lebo, R., F. Gorin, R. J. Fletterick, F. T. Kao, M. C. Cheung, B. I. Bruce, and Y. W. Kan. 1984. High-resolution chromosome sorting and DNA spot-blot analysis assign McArdle's Syndrome to chromosome 11. *Science (Wash. DC).* 225:57–59.

26. Fitzgerald, L. A., B. Steiner, S. C. Rall, Jr., S-s. Lo, and D. R. Phillips. 1987. Protein sequence of endothelial glycoprotein IIIa derived from a cDNA clone. *J. Biol. Chem.* 262:3936–3939.

27. Kishimoto, T. K., K. O'Connor, A. Lee, T. M. Roberts, and T. A. Springer. 1987. Cloning of the β subunit of the leukocyte adhesion proteins: Homology to an extracellular matrix receptor defines a novel supergene family. *Cell.* 48:681–690.

28. Hiraiwa, A., A. Matsukage, H. Shiku, T. Takahashi, K. Naito, and K. Yamado. 1987. Purification and partial amino acid sequence of human platelet membrane glycoproteins IIb and IIIa. *Blood.* 69:560–564.

29. Charo, I. F., L. A. Fitzgerald, B. Steiner, S. C. Rall, Jr., L. S. Bekeart, and D. R. Phillips. 1986. Platelet glycoproteins IIb and IIIa: evidence for a family of immunologically and structurally related proteins in mammalian cells. *Proc. Natl. Acad. Sci. USA.* 83:8351–8355.

30. Shelton-Inloes, B. B., K. Titani, and J. E. Sadler. 1986. cDNA sequences for human von Willebrand factor reveal five types of re-

peated domains and five possible protein sequence polymorphisms. *Biochemistry.* 25:3164–3171.

31. Fitzgerald, L. A., I. F. Charo, and D. R. Phillips. 1985. Human and bovine endothelial cells synthesize membrane proteins similar to human platelet glycoproteins IIb and IIIa. *J. Biol. Chem.* 260:10893–10896.

32. Leeksa, O. C., J. Zandbergen-Spaargaren, J. C. Giltay, and J. A. van Mourik. 1986. Cultured human endothelial cells synthesize a plasma membrane protein complex immunologically related to the platelet glycoprotein IIb/IIIa complex. *Blood.* 67:1176–1180.

33. Newman, P. J., Y. Kawai, R. R. Montgomery, and T. J. Kunicki. 1986. Synthesis by cultured human umbilical vein endothelial cells of two proteins structurally and immunologically related to platelet membrane glycoproteins IIb and IIIa. *J. Cell. Biol.* 103:81–86.

34. Plow, E. F., J. L. Loftus, E. G. Levin, D. S. Fair, D. Dixon, J. Forsyth, and M. H. Ginsberg. 1986. Immunologic relationship between platelet membrane glycoprotein GPIIb/IIIa and cell surface molecules expressed by a variety of cells. *Proc. Natl. Acad. Sci. USA.* 83:6002–6006.

35. Thiagarajan, P., S. S. Shapiro, E. Levine, L. DeMarco, and A. Yalcin. 1985. A monoclonal antibody to human platelet glycoprotein IIIa detects a related protein in cultured endothelial cells. *J. Clin. Invest.* 75:896–901.

36. Ginsberg, M. H., J. Loftus, J-J. Ryckwaert, M. Pierschbacher, R. Pytela, E. Ruoslahti, and E. F. Plow. 1987. Immunochemical and amino-terminal sequence comparison of two cytoadhesins indicates they contain similar or identical β subunits and distinct α subunits. *J. Biol. Chem.* 262:5437–5440.

37. Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549–554.

38. Burckhardt, J. J., W. H. Kerr Anderson, J. F. Kearney, and M. D. Cooper. 1982. Human blood monocytes and platelets share a cell surface component. *Blood.* 60:767–771.

39. Gögstad, G. O., Ø. Hetland, N. O. Solum, and H. Prydz. 1983. Monocytes and platelets share the glycoproteins IIb and IIIa that are absent from both cells in Glanzmann's thrombasthenia type I. *Biochem. J.* 214:331–337.

40. Bai, Y., H. Durbin, and N. Hogg. 1984. Monoclonal antibodies specific for platelet glycoproteins react with human monocytes. *Blood.* 64:139–146.

41. Clemetson, K. J., J. L. McGregor, R. P. McEver, Y. V. Jacques, D. F. Bainton, W. Domzig, and M. Baggiolini. 1985. Absence of platelet membrane glycoproteins IIb/IIIa from monocytes. *J. Exp. Med.* 161:972–983.

42. Levene, R. B., and E. M. Rabellino. 1986. Platelet glycoproteins IIb and IIIa associated with blood monocytes are derived from platelets. *Blood.* 67:207–213.