The Genetic Defect in Two Well-Studied Cases of Bernard-Soulier Syndrome: A Point Mutation in the Fifth Leucine-Rich Repeat of Platelet Glycoprotein Ibα

By Chaoyang Li, S. Eric Martin, and Gerald J. Roth

Bernard-Soulier syndrome (B-Ss) is a rare congenital bleeding disorder caused by abnormal giant platelets, thrombocytopenia, and defective glycoprotein (GP) Ib-V-IX, the adhesion receptor for von Willebrand factor (vWF). This report describes the molecular defect in two related individuals with well-established B-Ss whose platelets exhibit decreased GPIba expression and normal GPV on their surfaces. The GPIb-V-IX genes of the two patients were analyzed by Southern blotting, hetero-duplex analysis, and polymerase chain reaction (PCR) amplification/sequencing. A point mutation was found in codon 129 of the GPIba gene that results in the substitution of proline for leucine in the first position of the fifth leucine-rich glycoprotein repeat of the mature gene product. The mutation (CTC: leucine, wild-type to CCC: proline, mutant) eliminates a Sac I restriction site, facilitating analysis of the mutation in the propositi (both homozygotes), unaffected family members (8 heterozygotes and 8 wild-type), and 58 normal controls (116 wild-type alleles). The status of the genomes was confirmed by the sequencing of platelet cDNA. The mutation does not affect transcription of the Ib-IX genes, as estimated by PCR and Northern blot analysis, but it does inhibit surface expression of the receptor as assessed by transient transfection of mutant and wild-type GPIba genes into mouse Ibβ-IX L cells. Many of the cells (43%) transfected with the normal gene express surface GPIba, whereas untransfected cells and those transfected with the mutant gene lack surface GPIba entirely. Patient platelets were tested both for vWF binding in the presence of ristocetin and for surface GPIb-IX expression. In these instances, despite their inability to agglutinate with ristocetin and vWF, patient platelets exhibit about 40% of normal vWF binding and 40% of normal Ib-IX surface antigens. The results suggest that the described mutation (GPIba: Leu129 → Pro) affects the conformation of the GPIb-IX receptor, alters its availability on platelet surfaces, and causes the observed Bernard-Soulier phenotype.

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We have investigated in this study two first cousins with severe B-Ss whose platelets have been the subject of earlier work by other investigators on GP Ib-V-IX, ie, observations concerning the role of the receptor in platelet adhesion and ristocetin-dependent agglutination, protein phosphorylation events due to c-AMP-dependent kinase, the binding of thrombin to platelets, the expression of platelet surface antigens, the generation of platelet coagulant activity, and platelet membrane plasticity. We report that a point mutation in the GPIba gene, causing a 129leucine → proline substitution in the fifth LRG repeat of GPIba, appears to be responsible for the phenotype of these patients. The findings suggest that the leucine-rich motif of GPIba is a critically important element in GPIb-V-IX that can influence surface expression of the receptor and that may affect the binding of ligand to the receptor.

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Submitted March 15, 1995; accepted July 19, 1995.

Supported by Grant No. HL39947 from the National Institutes of Health (G.J.R.), a Merit Review Grant from the Veterans Administration (G.J.R.), International Research Fellowship No. 93-02850 from the American Heart Association (C.L.), and a Grant-in-Aid from the Brandywine Valley Hemophilia Foundation (S.E.M.).

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0006-4971/95/8610-0014$3.00/0
MATERIALS AND METHODS

Patient Material

The two propositi (TH [III-3; male] and AJ [II-5; female]) are first cousins of maternal African-American/West Indian and paternal African-American descent. Both have suffered recurrent mucosal bleeding, and AJ has experienced excessive menstrual and postpartum hemorrhage. In both patients, bleeding responds to platelet transfusions. Platelet counts range between 7.5 x 10^9 and 2.8 x 10^11/L. The platelets are large (AJ, 12 to 26 fl; TH, 13 to 29 fl; normal 8.9 ± 1.5 fl). Bleeding times are longer than 20 minutes. The fathers of the propositi were not available for study but have no history of bleeding. Blood samples were obtained from the affected patients, their relatives, and normal individuals. Genomic DNA was prepared from white blood cells (WBCs). Leukocyte-derived, Epstein-Barr virus (EBV)-transformed cell lines from family members provided an additional source of genomic DNA. The normal population studied was composed of 58 adults, all of whom denied any history of increased bleeding. This normal population includes a variety of ethnic backgrounds, with 22 of the 58 being African-Americans. Informed consent for the studies was given by each subject as approved by the Human Subjects Committees of the Seattle VA/University of Washington and the Medical Center of Delaware.

Southern Blotting

Genomic DNA from leukocytes or EBV cell lines was digested with restriction endonucleases, electrophoresed on agarose gels, transferred to membranes, hybridized individually with labeled GPIba, IbP, V, and IX probes (random priming [32P]-dCTP, 3,000 Ci/mmol), and detected by autoradiography as described.

Polymerase Chain Reaction (PCR)

The GPIba, IbP, and IX genes were amplified 100 μL, 1 μg of genomic DNA, 200 μmol/L dNTPs, 25 pmol of each primer, 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl) in overlapping 250- to 770-bp segments using the primer pairs noted in Table 1. After preheating (5 minutes at 94°C), Taq polymerase (2 U; Perkin-Elmer, Roche Molecular Systems, Inc., Branchburg, NJ) was added and 30 cycles were performed (denaturing for 1 minute at 94°C; annealing for 1 minute at the temperature given in Table 1, and extension for 2 minutes at 72°C) with a DNA thermal cycler (Perkin-Elmer). Consistent amplification of the GPIbα gene required 10% dimethyl sulfoxide (DMSO) in the reaction mixture. Postheating was performed at 72°C for 10 minutes.

Hetero-Duplex Analysis

After the final PCR cycle, 5 mmol/L EDTA was added, and products from different PCR reactions were mixed, heated (3 minutes at 95°C), and cooled (30°C). Gel loading buffer was added, and hybrids were separated by electrophoresis (16 to 25 hours at approximately 800 V) in 1-mm Hydrolink-MDE gel (AT Biochem, Inc, Malvern, PA) as described. Products were stained with 1 μg/mL ethidium bromide and photographed under UV light.

Nucleotide Sequencing

PCR fragments were subcloned into the pCRII vector (Invitrogen, San Diego, CA) or M13 (GIBCO-BRL, Gaithersburg, MD). Plasmid DNA was prepared by adding 0.2 mol/L NaOH/2 mol/L EDTA and incubating the mixture for 15 minutes at 37°C, whereas M13 was sequenced directly. The dideoxy chain termination method was used (Sequenase 2.0; US Biochemical [USB], Cleveland, OH) with primers complementary to either the vector or the insert.

Table 1. PCR Amplification of the GPIba, GPIbP, and GPIX Genes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Location</th>
<th>Annealing Temperature</th>
<th>MgCl₂ (mmol/L)</th>
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<td>-562-539</td>
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</tr>
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<td>GPIba-2</td>
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<td>684-683</td>
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<td>1571-1546</td>
<td>55°C</td>
<td>1.5</td>
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The annealing temperature and MgCl₂ concentration refer to the pair made up of the primer in the same row and the one noted just above, except for the footnote below: Ibm-9 and -11. * Primers GPIba-1 and -2 were numbered with the transcriptional start site denoted as +1.13,18,20 whereas all the other GPIba primers (3 through 16) were numbered according to Rajagopalan and Konkle.19 * Primers GPIba-9 was used with two antisense primers, GPIba-10 and -11. The conditions for primer pair GPIba-9 and GPIba-11 were annealing temperature of 62°C and 1.5 mmol/L MgCl₂. † Primers GPIbP-1 through GPIbP-8 were numbered according to Yagi et al.23 § Primers GPIX-1 through GPIX-10 were numbered according to Hickey and Roth.26

Characterization of Platelet cDNA Obtained by Reverse Transcription-PCR (RT-PCR)

RT of buffy-coat cellular RNA was performed with random hexa-nucleotide primers (Reverse Transcription System; Promega, Madison, WI). The resultant cDNA was isolated (Wizard; Promega), and GPIbP templates were amplified (2.5 U Taq polymerase; 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1.5 minutes for 30 cycles in a Cycler; sense: nucleotides 410 → 430, biotinylated [Gene Imaging System; USB]; antisense: 806 → 786 primers) and sequenced (Sequenase; USB).

Estimation of Platelet GPIb-IX Transcripts

RNA was extracted from 7 x 10⁶ normal or homozygous mutant (II-3) platelets and subjected to Northern blot analysis for GPIb-V.
IX transcripts using described methods or reverse-transcribed as described above (Promega). Platelet cDNA was amplified as noted above for 25 cycles with identical amounts (25 pmol sense/20 pmol antisense) of primers specific for GPIIb (as above), GPIX (sense 5'-TGTTCCCTGCCTCTGGGCCCCCA/ antisense 5'-AGCTGGGGCAGTGGTCACAA), GPV (ACTCCTACTGCGGGGCGTAGCT/TTGGCAGGAAAGGAAATCGAGCT), and β actin (CTCTCTGGGCGGATGGGCACTCGTGCT/GGAGTGGTCAAA), separated on 8% agarose gels, transferred to nitrocellulose, hybridized with a 32P-labeled, 2.4-kb GPIIIa cDNA probe, and autoradiographed. Numbers indicate the size (in kilobases on the left; HindIII-digest) of λ DNA markers.

### Allele-Specific Restriction Endonuclease Analysis

PCR products (20 μL) were incubated with restriction endonuclease following the manufacturer’s instructions. The products of digestion were analyzed by electro-horesis using 4% NuSieve agarose gel (FMC, Rockland, ME).

### Assessment of Platelet Surface Antigens

Platelet-rich plasma (PRP) from normal individuals and clinically unaffected relatives was prepared from whole blood by centrifugation (300g for 5 minutes at 22°C), whereas PRP from the B-Ss patients was obtained by centrifugation at reduced speed (150g for 4 minutes at 22°C). Platelets from the two homozygotes (III-3 and III-5), one heterozygote (II-8), one related normal (II-2), and two normal unrelated individuals were analyzed for surface antigen expression using mouse IgG1 monoclonal antibodies recognizing platelet GPIbα (anti-GPIb, CD42a-FITC; Becton Dickinson, Mountain View, CA), GPIV (anti-GPIV, CD42d-FITC; CLB, Research Diagnostics, Inc. Flanders, NJ), and GPIIIa (anti-GPIIIa, CD61-FITC; unreactive with platelets lacking GPIIb-IIIa; Becton Dickinson). Mouse IgG1-FITC was used as a control. Mouse IgG1 fractions were FITC-labeled to similar extents. Platelet membrane expression of the antigens was determined by flow cytometry (FACScan; Becton Dickinson) and expressed as arbitrary mean peak channel fluorescence units, which were normalized to the signal generated with the anti-GPIIIa monoclonal antibody, which was denoted as 100%.

### Binding of vWF to B-Ss Platelets

vWF was purified from cryoprecipitate as described. The purified protein in 100 mmol/L NaPO4, pH 6.5 (100 μL, 1 mg/mL) was labeled with 1 mCi of 125I using two iododeox (Pierce, Rockford, IL), and the radiolabeled protein was isolated by chromatography on PD-10 Sephadex G-25M (Pharmacia).

Starting with 35 mL of whole blood, platelets were separated from the PRP of normal individuals and a B-Ss patient (III-5) by centrifugation (150g for 10 minutes at 22°C), suspended in 35 mL of wash buffer (10 mmol/L Tris-Cl, pH 7.0, 0.14 mol/L NaCl, 1 mmol/L EDTA), centrifuged (100g for 2 minutes at 22°C) to remove red blood cells (RBCs) and WBCs, and recentrifuged (1,000g for 10 minutes at 22°C) to provide a platelet pellet that was then resuspended in 1 mL of wash buffer.

Platelets (5 x 10^6, 50 μL) were incubated with increased concentrations of 125I-labeled vWF (1.4 to 70 μg/mL) in the presence (1.5 mg/mL) or absence of ristocetin and the presence (400 μg/mL) or absence of cold vWF. After incubation (30 minutes at 22°C), reactions were layered over 40 μL of silicone oil solution consisting of 80% Dow Corning 550 fluid (Dow Corning Corp., Midland, MI) and 20% 200 fluid (Nye Inc, New Bedford, MA). After centrifugation (10,000g for 2 minutes at 22°C), the supernatant was removed, the tube was inverted, and the pellet was clipped and assayed for radioactivity. The parallel incubations in the absence of ristocetin were performed to assess nonspecific binding.

### Surface GPIIbα Expression in Transfected Cells

**Plasmid construct.** The entire open reading frame (2 kb) of wild-type and mutant GPIIbα genes was amplified with primer pair GPIIbα-5, and GPIIbα-15 (Table 1) by PCR. The PCR fragments were subcloned into mammalian expression vector pCRII (Invitrogen) to provide wild-type (in both sense [pCR3GPIIbα-W] and antisense [pCR3GPIIbα-M] orientations) and mutant (sense [pCR3GPIIbα-WAS] orientations) and mutant (sense [pCR3GPIIbα-WAS] orientations) constructs. In this vector, transcription of the inserted gene is driven by the cytomegalovirus (CMV) immediate-early promoter. All constructs were sequenced to ensure that no additional mutagenic errors had occurred.

**Host cells.** A mouse L-cell line stably transfected with GPIIbα and GPIIbα cDNA (generous gift from Jose A. Lopez, Houston, TX) was used for transfection. Cells were grown in 5% CO2 in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL) con-
containing 10% fetal bovine serum (FBS; GIBCO), 2 mmol/L L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin (Sigma, St Louis, MO), 2.5 µg/mL amphotericin, 2.05 µg/mL sodium deoxycholate (GIBCO), 400 µg/mL G418 (GIBCO), and 50 µg/mL methotrexate (MTX; Sigma).

Transient transfection. DNA/liposome complexes prepared by gently combining the construct (10 µg) with DOTAP (50 µL; Boehringer Mannheim, Indianapolis, IN) in buffer (250 µL; 20 mmol/L HEPES, pH 7.4, 0.15 mol/L NaCl) and incubating for 10 minutes at 22°C were introduced into cells (10^6/100-mm dish; DMEM/2% FBS) over 5 hours (37°C, 5% CO2) by gently combining the construct (10 µg) with DOTAP (50 µL; 20 mmol/L HEPES, pH 7.4, 0.15 mol/L NaCl) and incubating for 10 minutes at 22°C were introduced into cells (10^6/100-mm dish; DMEM/2% FBS) over 5 hours (37°C, 5% CO2). Media was then changed to DMEM (10% FBS, 400 µg/mL G418, and 50 µg/mL MTX), and cells were grown for 48 hours. Transfection efficiency was determined by cotransfection of an SV-40-luciferase reporter gene construct, pGL2 (2 µg/dish), and luciferase activity was measured by mixing a cell lysate (20 µL) with luciferase assay reagent (100 µL; Promega) and assaying luciferase activity in a luminometer (Turner Designs, Inc, Mountain View, CA). To prepare the lysate, the cells were harvested with EDTA, washed twice in phosphate-buffered saline (PBS), incubated with 1× lysis buffer (400 µL; 100 mmol/L K2HPO4•H2O, pH 7.8, 6 mmol/L MgSO4, 1 mmol/L dithiothreitol [DTT], 0.1% Triton-100; 10 minutes at 22°C), and clarified by centrifugation.

Flow cytometry. Surface expression of GPIba was evaluated by flow cytometry using a described, affinity-purified, rabbit polyclonal antibody specific for human glycopcalicin/GPIba. Cells were harvested with EDTA 48 hours after transfection, washed twice in PBS, and incubated with rabbit antihuman platelet glycopcalicin polyclonal antibody (10 µg/mL) or normal rabbit IgG (negative control) for 30 minutes on ice. The cells were washed twice and incubated in a 1:50 (20 µg/mL) dilution of FITC-conjugated goat antirabbit IgG (Southern Biotechnology Associates, Inc, Birmingham, AL) for another 30 minutes on ice. After washing twice in PBS to remove unbound antibody, the cells were fixed in 10% formaldehyde in PBS and analyzed for surface GPIba by flow cytometry (FACSscan; Becton Dickinson). The same antibody and detection method was also applied to mutant and wild-type platelets.

RESULTS AND DISCUSSION

Identification of the Candidate Mutation in the Two Propositi

Southern blotting. To initiate the investigation of the molecular genetic defect in the two propositi, the genes of interest (GPIba, GPIb/β, GPV, and GPIX) were analyzed by means of Southern blotting using specific, labeled cDNA or PCR probes for each gene. All of the blots showed normal-sized fragments from all of the genes by single and double digestions with four different enzymes (EcoRI, HindIII, BamHI, and Xho I). The analysis of the GPIba gene is shown in Fig 1, whereas the data for the GPIb/β, GPV, and GPIX genes are not shown. The results suggest that gross deletions or rearrangements of the genes were not present and that such abnormalities did not constitute the genetic defect in the patients.

Hetero-duplex analysis. Screening for more subtle defects, such as point mutations, was performed using the hetero-duplex analysis. The technique was applied to all of the genes except those encoding GPV, based on the assumption that defects in GPV may not produce a severe bleeding diathesis, because transfected cells can express significant amounts of surface Ib-IX complex in the absence of GPV. Using a series of primers (Table 1), all of the amplified segments from the three genes (Iba, Ib/β, and IX) in the two propositi (III-3 and III-5) were found to be identical to normal using hetero-duplex analysis except for that produced by the GPIX-5 and GPIX-6 primers (nucleotides 622 to 1256; Table 1). This fragment was subcloned into the pCRII vector, and a single base substitution at codon 27 (ACG → ACA) of the GPIX gene, a silent polymorphism, was found by sequencing. Patients III-3 and III-5 proved to be heterozy-
gous and homozygous, respectively, for this polymorphism. Therefore, hetero-duplex analysis showed an apparent silent polymorphism but did not detect a mutation in the genes under study.

PCR amplification and sequencing of gene and cDNA segments. To study the genetic defect in this kindred further, the GPIba, GPIbβ, and GPIX (including promoter regions of GPIbα and GPIX) genes of the patients were amplified in overlapping segments by PCR, and the products were analyzed on 2% agarose gels, which showed normal mobility and confirmed the absence of any major deletions. Sequencing of the genes was performed after subcloning the PCR fragments into the pCRII vector. A “T” → “C” transition was found at codon 129 in GPIbα gene (Fig 2). A total of 20 different clones from III-3 (12 clones) and III-5 (8 clones) all contained the same mutation. Furthermore, with respect to the previously described normal polymorphic variation of a 13 amino acid sequence within the heavily glycosylated macroglycopeptide region of GPIbα, the two propositi (III-3 and III-5) were found to be homozygous for the two-segment, duplicated form (form C). Additional sequencing of platelet cDNA obtained by RT-PCR confirmed the CTC → CCC transition in codon 129 in both propositi (data not shown).

Distribution of the Mutant Allele in the B-Ss Kindred

Strategy of PCR amplification and restriction endonuclease digestion. A Sac I restriction endonuclease cleavage site is lost because of the mutation, and a Sac I allele-specific restriction analysis (Sac I A-SRA) was performed to determine the distribution of the mutation in the kindred (Fig 3A). A 450-bp DNA fragment was amplified from the two propositi (III-3 and III-5), available family members, and 58 normal controls (22 of them African-American) with the GPIbα-7 and GPIbα-8 primer pair (Table 1).

Results with samples from different individuals. Digestion of the 450-bp DNA fragments derived from normal controls yielded the expected 241-, 107-, and 102-bp fragments (lane 8, Fig 3B). However, digestion of the products of the two propositi (III-3 and III-5) produced only 241- and 209-bp fragments (lanes 2 and 4, Fig 3B). The daughter (IV-1) of one propositus (III-5) was found to be heterozygous for the mutation (lane 6, Fig 3B), along with 7 other family members. Sequencing of platelet cDNA from the mothers (II-3 and II-4) of the propositi indicated heterozygosity for the mutation. The mutation was absent in 8 members of the kindred and in all 58 normal controls (116 normal alleles). The results confirm that both propositi are homozygous for the mutation and are consistent with autosomal recessive inheritance of the mutant allele in the kindred (Fig 4). The results with the normal individuals indicate that the mutation does not appear to be a polymorphism (defined as present in 1% or more of normals). Site of the mutation within GPIbα. The point mutation in codon 129 of the mature gene product results in the substitution of proline (P: mutant) for leucine (L: wild-type) as the first amino acid of the fifth leucine-rich repeat of the GPIbα polypeptide (Fig 5).

The Effect of the Point Mutation on GPIbα Expression and Function

Assessment of GPIb-V-IX antigens on the platelet surface. Because diminished surface GPIb-V-IX is a hallmark of B-Ss, the extent of these antigens on the mutant platelets under study was assessed by flow cytometry. As shown in
Fig 6, the GPIb-IX surface antigens were present at a level approximately 25% to 40% of normal in both propositi as compared with both normal platelets and those from heterozygous family members. The extent of expression was determined by correcting data by means of the ratio of GPIb-IX and GPIb-IIIa on normal platelets. However, as seen in another B-Ss kindred, surface GPV was found in essentially normal amounts (93%) in the platelets of the propositi, suggesting that GPV can be expressed in a differential fashion with its expression maintained to a greater extent than that of the other members of the GPIb-V-IX system. Analysis of total GPIb-IX antigen by Western blotting using polyclonal antibodies indicated increased amounts (presumably intracellular) of GPIbα, Ibβ, and IX in the homozygous mutant platelets. Analysis of platelet RNA by Northern blotting showed increased amounts, by approximately one third, of GPIbα and IX mRNA in samples from the propositi as compared with normal samples (data not shown). Quantitative PCR showed approximately normal amounts of these same transcripts in the platelets from the two affected individuals (Fig 7). Therefore, the point mutation in codon 129 does not appear to affect transcription of the genes under study but does selectively decrease surface expression of GPIb-IX, while sparing that of GPV. The substitution of proline for leucine may alter the conformation of the Ib-IX complex in some undefined fashion, perhaps related to the fact that a proline insertion disturbs the alignment of a polypeptide chain. As a consequence of the mutation and resultant amino acid substitution, synthesis, and/or cytoplasmic transport of platelet GPIbα in the megakaryocyte appears to be affected, perhaps along with an effect on GPIbβ and GPIX; and the amount of surface GPIb-IX complex on platelets is decreased.

The effect of the mutation on ristocetin-dependent vWF binding. Earlier studies document the complete inability of the propositi' platelets to agglutinate in response to ristocetin, and we repeated the study with the same result (no ristocetin-induced agglutination; data not shown). The fact that mutant platelets possess 25% to 40% of the normal amount of surface receptor (Fig 6) makes the results with ristocetin somewhat unexpected, because one could theorize that the decreased amount of receptor might still mediate ristocetin-dependent agglutination to some extent, albeit a reduced extent. Earlier studies of the propositi report variable results in regard to the amount and activity of the surface GPIb-IX receptor of the mutant platelets. For example, about
Fig 6. Assessment of GPIb-IX antigens on the surface of platelets. Platelets from the two propositi (homozygous), heterozygous (II-8) and normal (nl) related (II-2) family members, and a normal unrelated individual were analyzed for surface antigens by flow cytometry using monoclonal antibodies to GPIb (CD42b), GPIX (CD42a), and GPllla (CD61). Fluorescence observed with an individual sample and antibody was expressed as a percentage (vertical axis) of the result with the anti-GPllla antibody (100 arbitrary fluorescence units). Nonimmune mouse IgG provided a negative control (W).

35% of normal thrombin binding and factor XI interaction was found, whereas platelet adhesion, drug-dependent antigen sites, collagen-induced coagulant activity, and GPIbβ phosphorylation were reported as absent. To pursue this type of observation, we measured the extent of 125I-vWF binding to the mutant platelets of one of the propositi and found that the extent of ligand binding paralleled that of surface antigen expression (Fig 8). When platelets were exposed to progressively increasing amounts of 125I-vWF in the presence of ristocetin, vWF binding was consistently increased in both the B-Ss sample (III-5) and the normal sample. No vWF binding was seen in the absence of ristocetin, and added cold vWF decreased the binding of labeled ligand to an appropriate degree. The extent of 125I-vWF binding to the B-Ss platelets was about 40% of that in the normal control at the saturating concentrations, and we surmise that the mutant receptor binds soluble vWF with the same stoichiometry as the normal receptor under the described conditions. The results suggest that ristocetin agglutination of platelets is not simply a function of the amount of bound vWF, because the mutant receptor in this instance appears to bind as much vWF as a wild-type receptor in the presence of ristocetin. However, such binding of vWF does not lead to agglutination. Perhaps the mutant receptor binds the ligand in a conformation that does not permit subsequent platelet-to-platelet interaction through the bound ligand and results in absent ristocetin agglutination. The same absence of Ib-vWF function, as assessed earlier in flow experiments and indicated by absent flow-dependent platelet adhesion, appears to be present.

Alternatively, the absence of ristocetin agglutination may simply reflect a reduced amount of functional receptor, and ristocetin agglutination may be a somewhat insensitive indicator of ligand binding, requiring some minimal amount of surface receptor to proceed. However, this formulation appears to be at odds with the clinical data in which the patients suffer from a severe bleeding diathesis stemming from a severe defect in vivo platelet adhesion, presumably more severely deficient in vivo than 25% to 40% of normal. The results suggest, but do not prove, that an alteration of the polypeptide chain in the LRG region of Ibα may selectively affect the chain’s interaction with vWF (absent ristocetin agglutination, severely impaired vWF-dependent platelet adhesion, and apparently normal binding of soluble vWF in...
with the mutant cDNA do not. The finding is generally consistent with the results of the flow cytometric assay of platelet surface antigens. In both instances, the point mutation appears to depress the expression of the GPIbα protein on the surface of the cells. The mutation in megakaryocytes leads to a 50% to 70% decrease in GPIbα surface expression (observed on the platelet surface), but it nearly eliminates such expression in transfected β-IX L cells. The reason for this difference may relate to differences between the two cells, ie, megakaryocytes as opposed to L cells, in their relative abilities to express surface Ib-IX. Megakaryocytes appear to express both wild-type and mutant Ib-IX to a greater extent than do L cells, and megakaryocytes may process the altered protein more successfully, thereby expressing somewhat higher levels of the mutant receptor.

The Described Point Mutation in the LRG Domain of Iba

The described point mutation in GPIbα affects surface expression of the Ib-IX complex in transfected cells in much the same manner as it appears to suppress expression of the receptor on the surface of mutant platelets. The transfection data, the finding of homozygosity for the mutant allele in the propositi, and the absence of the mutation in normals argue strongly that the described mutation is responsible for the B-Ss phenotype seen in the platelets of the affected patients. The effect of the mutation is striking. The single amino acid substitution does not influence gene transcription or intracellular protein levels, but it appears to alter the highly integrated nature of the multicomponent GPIb-IX receptor. The results imply that the multicomponent GPIb-IX receptor is highly interdependent and that its expression and perhaps function can be affected by the change of a single amino acid in a single receptor chain.

ACKNOWLEDGMENT

We thank Margaret Johnson, PhD, David C. Calverley, MD, Ma-yumi Yagi, PhD, Rachel A Hall, Sunday M. Stray, Eric G. Dobrzynshi, Eric S. Martin, and Robert F. Abel for both helpful discussions and valuable assistance.

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The genetic defect in two well-studied cases of Bernard-Soulier syndrome: a point mutation in the fifth leucine-rich repeat of platelet glycoprotein Ib alpha

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