# Deletion of the Platelet-Specific Alloantigen $Pl^{A1}$ from Platelets in Glanzmann's Thrombasthenia

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ABSTRACT Expression of a platelet-specific alloantigen ($Pl^{A1}$) was studied in five unrelated patients with Glanzmann's thrombasthenia using immunologic techniques based on release of $^{51}Cr$ from tagged platelets by $Pl^{A1}$-specific antibody. Less than 1% of the normal quantity of $Pl^{A1}$ could be detected on platelets of patients 1, 2, and 3; platelets from patients 4 and 5 contained 22 and 12% of normal levels, respectively. After treatment with bromelain, platelets from patients 4 and 5, but not those from patients 1, 2, and 3, released $^{51}Cr$ as well as normal $Pl^{A1}$-positive platelets when exposed to anti-$Pl^{A1}$. Platelets from each of the five patients reacted normally with drug-dependent antibodies and with autoantibodies specific for platelets.

Polyacrylamide gel electrophoresis of thrombasthenic platelets showed marked deficiencies of glycoproteins IIb$\alpha$ and III ($P < 0.0005$), confirming recent reports of others. Deficiency of the two proteins as determined by gel scanning was more pronounced in patients 1, 2, and 3 than in patients 4 and 5. Normal levels of glycoproteins IIb$\alpha$ and III were found in platelets from normal subjects negative for $Pl^{A1}$.

These observations are consistent with the possibility that the $Pl^{A1}$ antigen is located on one or both of the glycoproteins lacking in Glanzmann's thrombasthenia, although other explanations are possible. They further suggest that patients with thrombasthenia may be heterogeneous in respect to the degree to which these glycoproteins are deleted. The $Pl^{A1}$ antigen can be measured with considerable precision and may provide a marker useful for the diagnosis and study of Glanzmann's disease.

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## INTRODUCTION

Glanzmann's thrombasthenia is a congenital disorder of platelet function originally described in 1918 (1). The major diagnostic characteristics of this disease include markedly reduced or absent clot retraction, prolonged bleeding time in the presence of normal platelet levels, lack of in vitro aggregation after exposure to adenosine diphosphate, epinephrine, and collagen, and normal or slightly reduced aggregation in response to ristocetin (1–4). Although thrombasthenia is rare, it has been studied extensively in the hope that recognition of the basic effect in this disorder may lead to increased understanding of normal platelet function.

Various molecular abnormalities have been reported in thrombasthenic platelets. The earliest consistent observations indicated a subnormal content of externally absorbed and intrinsic platelet fibrinogen (5–9). A single report of a lower content of surface-bound IgM has not been confirmed (10). Observations on the level of reduced glutathione in thrombasthenic platelets are conflicting (11, 12). Deficiency of an unidentified membrane protein has been described (8).

Using newer methods for characterization of membrane glycoproteins, it has been found that thrombasthenic platelets exhibit quantitative, and possibly qualitative abnormalities of certain membrane constituents. Specifically, reduced content of a protein designated glycoprotein II and possible reduction of another glycoprotein, III, have been reported (13). Recent observations by Phillips et al. (14) and Phillips and Agin (15) strongly suggest that two glycoproteins (designated by them, IIb and III) are deficient in the thrombasthenic platelet.

These reports led us to study the expression of surface antigens on thrombasthenic platelets. We here

report observations on the reactions of normal and thrombasthenic platelets with a variety of platelet-specific antibodies. Our findings indicate that thrombasthenic platelets are markedly deficient in a platelet-specific alloantigen ($Pl^{A1}$)[1] (16) (also called $Zw^a$; [17]), but contain normal quantities of the membrane receptor(s) for drug-dependent platelet antibodies (18) and for autoantibodies obtained from patients with idiopathic (autoimmune) thrombocytopenic purpura (ITP). Quantitative measurement of the amount of $Pl^{A1}$ antigen present in the platelets of our patients and electrophoretic analysis of their membrane glycoproteins lend support to the view that the membrane abnormality in Glanzmann's disease is heterogeneous.

## METHODS

*Selection of patients.* Each of the five unrelated patients studied fulfilled the diagnostic criteria of Glanzmann's thrombasthenia in that each had a consistently normal platelet count, markedly prolonged bleeding time, lack of aggregation in response to adenosine diphosphate, epinephrine, and collagen, and virtual absence of clot retration. Platelets from each of four patients tested aggregated in response to ristocetin. The patients were studied through the courtesy of Doctors Jack Lazerson (Milwaukee, Wis.), Walter Bowie (Rochester, Minn.), David Green (Chicago, Ill.), Lilia Tallarico (Boston, Mass.), and John Penner (Ann Arbor, Mich.). Two of the cases have been reported previously (19, 20). The five patients did not differ obviously from one another in their bleeding history, but a detailed analysis of the severity of hemorrhage has not yet been made.

*Isolation of platelets.* Platelets were isolated by differential centrifugation of whole blood anticoagulated with EDTA (21). In four instances, in which blood was obtained from individuals in another city, a second sample was drawn from a normal individual at the same time; both samples were processed, transported, and handled under identical conditions. All studies were performed on the day the blood was drawn. The longest interval between blood drawing and the initiation of tests was 8 h.

*Preparation of $^{51}Cr$-labeled platelets and treatment with bromelain.* Labeling of platelet suspensions with sodium $^{51}Cr$ (Amersham-Searle Corp., Arlington Heights, Ill.) and treatment with bromelain (Sigma Chemical Co., St. Louis, Mo.) were performed as previously described (21, 22), with the following minor modification: the bromelain suspension contained 1 mg/ml bromelain and 1 mg/ml cysteine in phosphate-buffered saline (PBS). Bromelain treatment increases the sensitivity of $^{51}Cr$-tagged platelets to immune lysis four- to eightfold (12).

*The $^{51}Cr$ lysis test and the inhibition assay.* Antibody detection by release of $^{51}Cr$ from target platelets and quantitative measurement of platelet antigens by inhibition of $^{51}Cr$ release have been described in detail (22–24). Release of $^{51}Cr$ from platelets was assayed in the following system: 0.02 ml of suspension of $^{51}Cr$-labeled platelets in PBS at 100,000 per $mm^3$ were incubated for 2 h at 37°C with 0.02 ml of the specific serum, 0.02 ml of 0.1 M magnesium chloride, and 0.1 ml of fresh, ABO-compatible, platelet-poor plasma from a normal subject anticoagulated with EDTA as a source of complement. With quinidine- and quinine-dependent antibodies, 0.02 ml of 0.1 mM quinine in PBS or 0.02 ml of 1 mM quinidine in PBS were also added. After incubation, 2 ml of 0.5% EDTA in 0.145 M NaCl was added, the tubes were centrifuged at 3,000 *g* for 30 min, and the radioactivity of the supernate and the platelet button was measured. Percent immune release of $^{51}Cr$ was calculated as previously described (21). Control sera typically released 5–10% of $^{51}Cr$, whereas antibody-containing sera released 50–80% (23).

The quantity of $Pl^{A1}$ antigen and of the receptor(s) for quinidine- and quinine-dependent antibodies on platelets was measured by determining the number of unlabeled test platelets required to inhibit by 50% the release of $^{51}Cr$ from tagged platelets by specific antibody in a two-stage assay (22, 24). Inhibition of lysis of the $^{51}Cr$-labeled platelets added in the second stage provides a measure of the quantity of antigen in the test material added to antibody in the first stage.

*Selection of antibodies.* Three anti-$Pl^{A1}$ antisera were obtained from patients with post-transfusion purpura (16). The specificity of each was confirmed by its reactions against a large panel of platelets from $Pl^{A1}$-positive and $Pl^{A1}$-negative donors. The frequency of $Pl^{A1}$ antigen in the general population is 98% (16). Quinidine- and quinine-dependent antibodies were obtained from two patients who developed thrombocytopenia after ingestion of those drugs. These sera react with normal platelets in the presence of the appropriate drug, but fail to react in the absence of the drug (18, 23). Autoantibodies were obtained from two patients with ITP. Sera from about 25% of patients with ITP release significant amounts of $^{51}Cr$ from bromelain-treated normal platelets.[2] Each of the seven antisera was shown to lack detectable HLA antibodies by screening it against a panel of 60 lymphocyte donors, using the standard National Institutes of Health lymphocytotoxicity assay.

*Preparation of platelet suspensions for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE).* A platelet button was prepared by centrifugation and was washed three times at 4°C in 0.01 M Tris-HCl, 0.001 M EDTA, 0.145 M NaCl, pH 7.5. After the last wash, the button was resuspended in this buffer, and the platelet concentration was determined by phase microscopy. The ratio of erythrocytes and leukocytes to platelets was always <1:3,000. A platelet button containing $10^9$ platelets was prepared by centrifuging a portion. The supernatant buffer was discarded and as much residual buffer as possible was removed by inverting the tubes for several minutes and flushing out excess liquid with nitrogen. The total protein content of a separate portion of each platelet preparation was determined by the method of Lowry et al. (25). The platelets were then prepared for SDS-PAGE as described by Phillips and Poh Agin (15, 26). The resolving slab gel contained 7.5% acrylamide and 0.1% SDS, and was covered by a 3% acrylamide stacking gel.

*Quantitation of protein bands on SDS-polyacrylamide gels.* The identity of membrane glycoproteins was determined after electrophoresis of solubilized whole platelets (20 μl contain-

[1] *Abbreviations used in this paper*: ITP, idiopathic (autoimmune) thrombocytopenic purpura; PAGE, polyacrylamide gel electrophoresis; PAS, periodic acid-Schiff reagent; PBS, phosphate-buffered saline (0.013 M phosphate, 0.145 M NaCl, pH 7.4); $Pl^{A1}$, a platelet-specific alloantigen; SDS, sodium dodecyl sulfate.

[2] Aster, R. H., unpublished observations. Antibody activity in these sera was recovered in IgG fractions and could be totally absorbed with platelets, but not with lymphocytes, granulocytes, or erythrocytes.

ing 100 μg of protein) that had been fully reduced in a sample buffer containing 10% 2-mercaptoethanol (26). The following purified proteins (Sigma Chemical Co.) were co-electrophoresed as molecular weight markers: *Escherichia coli* β-galactosidase, 130,000 mol wt; bovine serum albumin—fraction V, 68,000 mol wt; ovalbumin, 43,000 mol wt; chymotrypsinogen A, 25,600 mol wt. Platelet myosin (mol wt 200,000) served as an internal molecular weight marker. Protein bands were stained with either Coomassie Blue R or with periodic acid-Schiff reagent (PAS) (27). The location of membrane protein bands was previously established by numerous electrophoretic analyses of isolated human platelet plasma membranes and whole platelet preparations stained with Coomassie Blue R. Glycoproteins were located by PAS staining of samples run in parallel, or by staining first with PAS reagent, marking the bands obtained with India ink, and then restaining the same gel with Coomassie Blue R (28). To adequately visualize PAS-stained bands, 40-μl platelet samples containing 200 μg of protein were electrophoresed. Molecular weights estimated for glycoproteins Ibα, IIbα, and III on the basis of their rates of migration were 145,000, 132,000, and 120,000 mol wt, respectively. Essentially the same values have been reported by Phillips and Poh Agin (26).

Gel densitometry was performed with a Gilford spectrophotometer equipped with a linear transport accessory (model 2410; Gilford Instrument Laboratories Inc., Oberlin, Ohio). Gels stained with Coomassie Blue R were scanned at 600 nm (27). The density of each protein band was determined by calculating the peak area on the recorder tracings obtained from the gel scans. Quantitative comparisons of glycoproteins based on the density of bands stained with either Coomassie Blue R or PAS reagent are made difficult by variation in the affinity of individual proteins for these stains as a result of differences in conformation and degree of glycosylation (26). For this reason the sum of the densities of three platelet proteins—platelet myosin and two as yet unnamed nonmembrane proteins (labeled *a* and *b* in Fig. 1)—were used as a reference. The relative density of each of the membrane glycoprotein bands, Ia, Ibα, IIbα, and III (15, 26) (Fig. 1) was determined by calculating the ratio of the density of that glycoprotein band to the sum of the band densities of the three protein standards in the same gel scan. These three platelet proteins were chosen as reference standards because (*a*) the level of platelet myosin, as reflected in the level of platelet thrombosthenin, does not vary significantly from one normal platelet preparation to another (30) and the level of thrombosthenin in thrombasthenic platelets is at least 95% of normal (31); (*b*) the levels of the two other nonmembrane proteins do not vary significantly when different normal platelet preparations are compared to thrombasthenic platelet preparations. The mean ratio of protein a (Fig. 1) to myosin in normal platelets was 0.32±0.08 (SD); in thrombasthenic platelets, 0.35±0.08. The mean ratio of protein b (Fig. 1) to myosin in normal platelets was 0.52±0.05; in thrombasthenic platelets, 0.59±0.10.

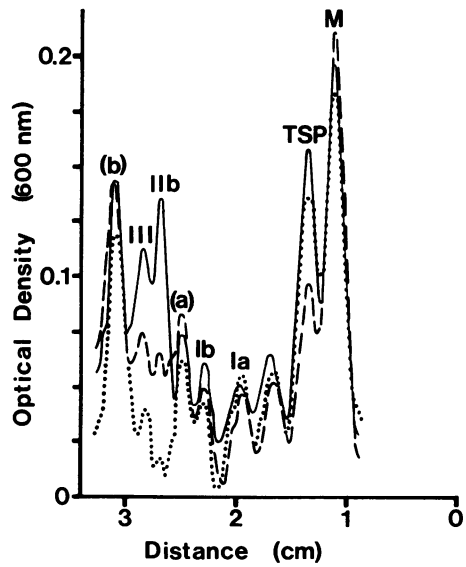


FIGURE 1 Spectrophotometric scan of gels (fully reduced samples) prepared from whole platelets solubilized with SDS and stained with Coomassie Blue R. Each sample contained ≅100 μg of protein. Ordinate denotes optical density at 600 nm. Abscissa indicates distance of individual bands from top of gel (cathodal end). Three individual gel scans are superimposed: solid line denotes scan of sample from a normal $Pl^{A1}$-positive subject; dashed line denotes scan of a sample from thrombasthenic subject 4; dotted line denotes scan of a sample from thrombasthenic subject 2. Membrane glycoproteins Ia, Ibα, IIbα, and III are indicated; (*a*) and (*b*) are two nonmembrane protein standards; M denotes platelet myosin (200,000 mol wt); TSP refers to "thrombin-sensitive protein" (mol wt 185–190,000) (29). The identity of the protein band between Ia and thrombin-sensitive protein is not known.

## RESULTS

*Reaction of $^{51}Cr$-labeled thrombasthenic platelets with anti-$Pl^{A1}$.* Platelets from each of the five patients with thrombasthenia responded subnormally to the three anti-$Pl^{A1}$ antibodies (Fig. 2). Of the nine $Pl^{A1}$-positive normal donors used as controls, six were known to be homozygous, and three heterozygous, for the $Pl^{A1}$ allele. The low reactivity of the thrombasthenic platelets cannot, therefore, be ascribed to heterozygosity for this antigen. Platelets from thrombasthenic patients 4 and 5 reacted more strongly with anti-$Pl^{A1}$ than did platelets from patients 1, 2, and 3. After bromelain treatment of platelets, the contrast between patients 1, 2, and 3 and patients 4 and 5 became accentuated (Fig. 3); platelets from patients 4 and 5 reacted normally, whereas platelets from patients 1, 2, and 3 remained poorly responsive. Identical results were obtained with each of the three anti-$Pl^{A1}$ sera.

*Sensitivity of $^{51}Cr$-labeled thrombasthenic platelets to other platelet antibodies.* Platelets from each of the five thrombasthenic patients reacted normally with quinidine- and quinine-dependent antibodies (Fig. 4) and with the two autoantibodies from patients with ITP (not shown).

*Inhibition of $^{51}Cr$ lysis.* The $Pl^{A1}$ content of platelets from patients 3, 4, and 5 was assayed by determining their ability to compete for platelet-specific antibody and thereby inhibit the lysis of $^{51}Cr$-labeled target platelets added subsequently (22, 24). As seen in Fig.

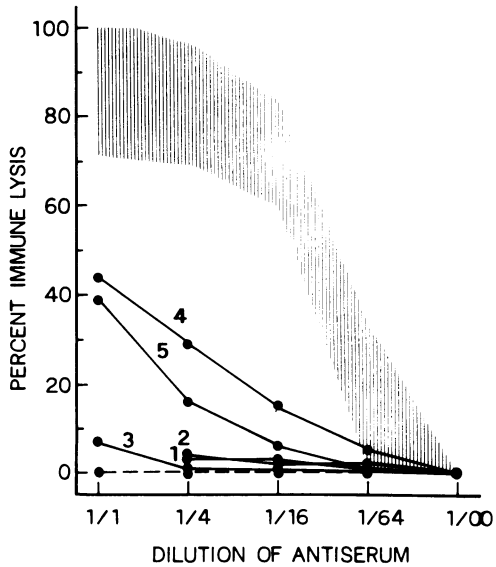


FIGURE 2 Lysis of $^{51}$Cr-labeled platelets from normal and thrombasthenic donors by anti-Pl$^{A1}$ antibody. Ordinate denotes percent immune lysis of $^{51}$Cr-tagged platelets induced by anti-Pl$^{A1}$ in dilutions shown on the abscissa. Platelets from patients 1 to 5 are identified by number. The shaded area denotes lysis (mean±2 SD) observed with platelets from nine Pl$^{A1}$-positive normal individuals. The dashed line indicates lysis observed with platelets from two Pl$^{A1}$-negative normal individuals.

5, platelets from patients 4 and 5 contained ~22 and 12% of the normal quantity of Pl$^{A1}$, respectively. Platelets from patient 3 contained no detectable Pl$^{A1}$.

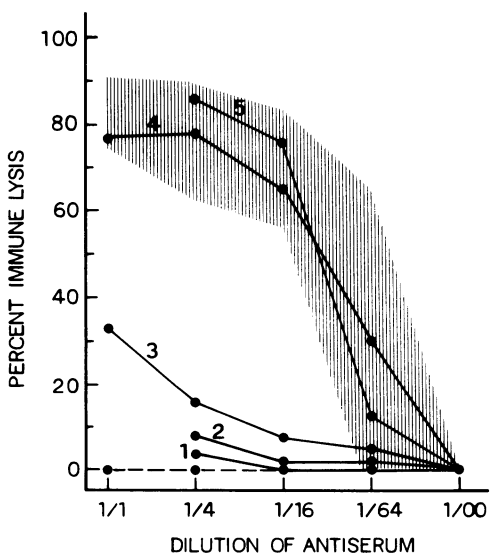


FIGURE 3 Lysis of $^{51}$Cr-labeled bromelain-treated platelets from normal and thrombasthenic donors by anti-Pl$^{A1}$ antibody. Legend as in Fig. 2 except that target platelets were treated with bromelain to increase their sensitivity to immune cytolysis.

Insufficient numbers of platelets were available from patients 1 and 2 to perform this assay.

*SDS-PAGE.* Platelets from all five thrombasthenic patients exhibited a marked reduction in glycoproteins IIb$\alpha$ and III. The decrease in IIb$\alpha$ (the larger subunit obtained upon reduction of IIb in 10% 2-mercaptoethanol) and III (26) was evident on spectrophotometric scans of gels stained with Coomassie Blue R (Fig. 1). No significant variation is apparent in levels of proteins (a) and (b) or platelet myosin. Membrane proteins Ia and Ib also show little variation. Thrombin-sensitive protein varied significantly in platelets of normal subjects; the variability shown in Fig. 1 is not peculiar to thrombasthenia. Platelets from Pl$^{A1}$-negative normal subjects contained normal amounts of glycoprotein IIb$\alpha$ and III. The relative densities of each of four membrane glycoproteins in the platelets of six normal subjects and five thrombasthenic subjects are presented in Fig. 6 which demonstrates (*a*) a significant decrease ($P < 0.0005$) in the levels of IIb and III in thrombasthenic platelets relative to normal, and (b) heterogeneity of this defect among the five thrombasthenic individuals reflected in significantly lower levels of IIb and III in patients 1, 2, and 3 than in patients 4 and 5 ($P < 0.025$).

## DISCUSSION

The Pl$^{A1}$ antigen, originally designated Zw$^{a}$, was first recognized by van Loghem and co-workers in 1959

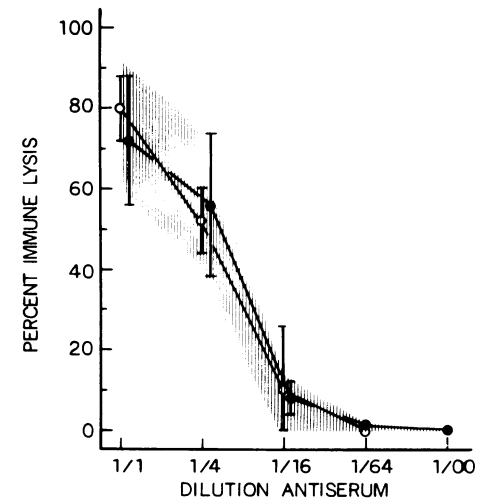


FIGURE 4 Lysis of $^{51}$Cr-labeled platelets from normal and thrombasthenic donors by quinine-dependent anti-platelet antibody. Legend as in Fig. 2 except that quinine-dependent antibody was used instead of anti-Pl$^{A1}$ and 0.02 ml of $10^{-4}$ quinine sulfate was added to reaction mixtures. (●) denotes mean lysis of platelets obtained from thrombasthenic patients 1 to 5 (±2 SD). (○) denotes mean lysis obtained with platelets from 2 Pl$^{A1}$-negative normal subjects (±2 SD). Shaded area denotes range of lysis obtained with platelets from nine normal, Pl$^{A1}$-positive subjects.

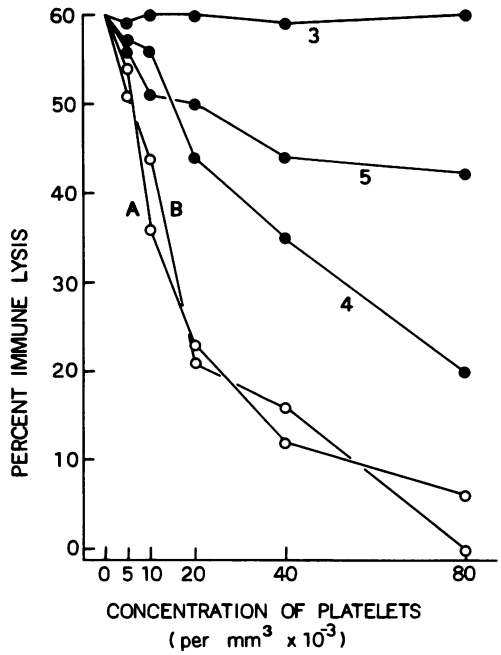


FIGURE 5 Inhibition of the lytic activity of anti-$Pl^{A1}$ antibody by nonlabeled platelets from two normal $Pl^{A1}$-positive subjects (○) and from thrombasthenic patients 3, 4, and 5 (●). 0.02 ml of nonlabeled platelets in PBS at concentrations shown on the abscissa was incubated with 0.02 ml of anti-$Pl^{A1}$ antibody for 2 h at 37°C. Complement and $^{51}Cr$-tagged, $Pl^{A1}$-positive target platelets were then added and percent immune lysis (ordinate) was determined after an additional 2-h of incubation as described in Methods. Inhibition curves shown for normal platelets are typical of those obtained with platelets from 10 normal individuals. Platelets from patient 3 failed to inhibit anti-$Pl^{A1}$ in the highest concentration used. Platelets from patients 4 and 5 produced significant but subnormal inhibition. Platelets from a normal, $Pl^{A1}$-negative donor (not shown) behaved like platelets from patient 3.

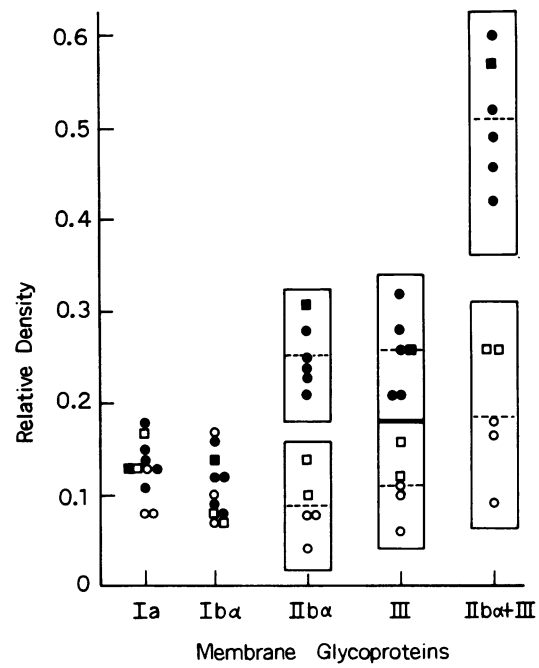


FIGURE 6 Relative amounts of four membrane glycoproteins in platelets from normal and thrombasthenic subjects. After PAGE of whole platelet preparations from normal and thrombasthenic subjects, gels were stained with Coomassie Blue R, scanned at 550 nm in a spectrophotometer and the area under each peak measured. Ordinate denotes the ratio of the area of each glycoprotein peak to the sum of the areas of peaks corresponding to three reference proteins (Fig. 1 and Methods). Values are shown for five $Pl^{A1}$-positive (●) and one $Pl^{A1}$-negative (■) normal subject and for thrombasthenic patients 1, 2, and 3 (○) and 4 and 5 (□). Dashed horizontal lines indicate mean values for normal and thrombasthenic subjects and boxes indicate ±2 SD from the mean. Differences between the means are significant in the three columns on the right ($P < 0.0005$).

(17), with an agglutinin present in the serum of a patient who developed severe thrombocytopenia after receiving a blood transfusion. Shulman et al. (16) subsequently detected antibodies of the same specificity in two additional patients with post-transfusion thrombocytopenia and established post-transfusion purpura as a specific disorder associated in nearly all cases with an alloantibody specific for the $Pl^{A1}$ antigen. Utilizing complement fixation, they found the gene frequency of the allele coding for $Pl^{A1}$ to be 0.87 and the frequency of $Pl^{A1}$ in the general population to be 98%. The antigen appears to be restricted to platelets. Van der Weerdt et al. (32) have described an antibody that appears to react with an antigen allelic to $Pl^{A1}$.

Our observations demonstrate a relationship between thrombasthenia and expression of the $Pl^{A1}$ antigen. That thrombasthenic platelets are not simply negative for $Pl^{A1}$ on a genetic basis is indicated by: (*a*) contrasting reactions of these platelets and those of $Pl^{A1}$-negative normal individuals (Figs. 1–3); and (*b*) the statistical unlikelihood ($P < 10^{-9}$) of choosing five successive $Pl^{A1}$-negative persons at random (the frequency of the $Pl^{A1}$-negative phenotype in the normal population is 0.02).[3] The normal responsiveness of platelets from patients with thrombasthenia to quinidine- and quinine-dependent antibodies and to autoantibodies from the patients with ITP rules out the possibility that their platelets are unable to undergo immune cytolysis. The inhibition studies performed with platelets from patients 3, 4, and 5 (Fig. 5) confirm their reduction in $Pl^{A1}$ content.

The correlation observed between the degree to which $Pl^{A1}$ is expressed (Figs. 2, 3, and 5) and platelet

[3] Patients 4 and 5 would have been typed as "$Pl^{A1}$-positive" by $^{51}Cr$ release if a qualitative test had been performed using undiluted antibody and bromelain-treated target platelets, but as "negative" with untreated platelets and a weaker anti-$Pl^{A1}$ typing serum.

content of membrane glycoproteins IIb and III among the thrombasthenic patients (Figs. 1 and 6) is consistent with the possibility that either IIb or III, or both, carry the $Pl^{A1}$ antigenic determinant. Alternative possibilities are that $Pl^{A1}$ resides on a third protein, not yet characterized, which is also deficient in Glanzmann's disease or that deficiency of IIb and III may somehow affect the expression of $Pl^{A1}$ on the platelet surface. Regardless of the mechanism responsible for deletion of $Pl^{A1}$, our data suggest that thrombasthenia can be likened to several other disorders in which a membrane alloantigen is subnormally expressed. These are the En(a-) condition, in which erythrocytes of En(a-) individuals lack a major membrane glycoprotein (PAS-1 and part of PAS-2) which appears to carry the M (33) and possibly the Wright a and b alloantigens (34); chronic granulomatous disease in which granulocytes and erythrocytes lack Kx, a precursor in the biosynthetic pathway of the Kell blood group antigen system (35); and (probably) the Rh null anomaly of erythrocytes which is associated with mild hemolytic anemia (36). It is to be expected that thrombanthenic platelets will also be found to lack the antigen $Pl^{A2}$ ($Zw^b$) allelic to $Pl^{A1}$ (32, 37), but we did not have anti-$Pl^{A2}$ available to us to confirm this. Degos et al. (38) have described an antibody found in a patient with thrombasthenia which reacts by complement fixation with a platelet membrane glycoprotein of mol wt = 125,000. This antibody reacted equally well with platelets from $Pl^{A1}$-positive and $Pl^{A1}$-negative normal subjects, but failed to react with platelets from eight patients with thrombasthenia. It is likely that their antibody identifies a second marker, distinct from $Pl^{A1}$, which is present on one or both of the glycoproteins deleted in thrombasthenic platelets.

Our findings suggest that patients with "thrombasthenia" vary in respect to the degree to which $Pl^{A1}$ and proteins IIb and III are deleted. The observations summarized in Figs. 2, 3, and 5 indicate that in patients 1, 2, and 3, $Pl^{A1}$ is almost totally absent (<1% of normal), whereas platelets of patients 4 and 5 contain ~22 and 12% of the normal quantity of $Pl^{A1}$, respectively. The difference in $Pl^{A1}$ expression on platelets of the two groups of patients was accentuated when bromelain-treated platelets were used as targets in the cytolytic assay (Fig. 3). Further studies are required to determine whether the normal behavior of bromelain-treated platelets from patients 4 and 5 in this assay is merely a consequence of their increased sensitivity to complement-mediated cytolysis or reflects "unmasking" of additional quantities of $Pl^{A1}$ antigen by enzymatic treatment.

That thrombasthenia may be a heterogeneous condition has been suggested previously. A decreased content of ATP and decreased activity of pyruvate kinase and glyceraldehyde-3-phosphate dehydrogenase have been found in platelets of some patients studied (39), but not in others (3, 5, 9). Variability in the response of platelets from four thrombasthenic subjects to ristocetin has been found (40), and apparent variation in the ability of thrombasthenic platelets to take up latex or Thorotrast (Fellows Manuf. Co., Inc., Detroit, Mich.) has been described (41). In the latter study, it was concluded that thrombasthenia might be classified into two varieties on the basis of abnormalities in cellular metabolism and/or membrane function. However, Degos et al. (38) failed to observe heterogeneity in eight thrombasthenic patients using their complement-fixing antibody and Phillips and Poh Agin (15), using gel electrophoresis, found the membrane protein abnormality to be uniform in thrombasthenic platelets from two families. Quantitation of membrane glycoproteins by scanning of stained gels has serious limitations, due to large measure to incomplete resolution of individuals bands and the variable degree to which individual proteins are glycosylated. Assay of the $Pl^{A1}$ alloantigen by inhibition of $^{51}Cr$ release is precise (SE±10%), and the information obtained in this way, coupled with our cytolytic and electrophoretic studies, is consistent with the possibility that Glanzmann's thrombasthenia is a heterogeneous disorder. Family studies and sequential studies in individual patients are now being undertaken to determine whether expression of $Pl^{A1}$ is constant in individual patients and to analyze its genetic control. It will also be of interest to study patients with the Bernard-Soulier abnormality whose platelets are deficient in membrane glycoprotein I (42).

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