Inheritance of the Human Platelet Alloantigen, Pl^{A1}, in Type I Glanzmann's Thrombasthenia

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ABSTRACT The heredity of the human platelet alloantigen, Pl^{A1}, has been studied in Glanzmann's thrombasthenia. The Pl^{A1} content of platelets from three patients, 20 kindred of these patients, including parents and siblings, and 15 unrelated normal individuals was determined using immunologic techniques based on the release of ^{51}Cr from labeled platelets. The amount of membrane glycoproteins (GP) IIb and IIIa in the platelets of these individuals was determined by quantitative crossed immunoelectrophoresis of Triton X-100 soluble proteins using a multispecific rabbit antibody raised against normal platelets.

Platelets from the three thrombasthenic patients contained neither detectable GP IIb and GP IIIa nor detectable Pl^{A1} antigen. Platelets from seven kindred with normal amounts of GP IIb and GP IIIa contained Pl^{A1} antigen levels identical to those detected in platelets of normal individuals.

Platelets from 13 kindred, including each parent studied, were shown to contain an amount of GP IIb and GP IIIa equivalent to 53% of that amount detected on normal platelets. Platelets from the same individuals expressed amounts of Pl^{A1} antigen that were either 54.0±4.1 (mean±SD) or 28.0±2.7% of that present on platelets of normal individuals homozygous for the A1 allele.

The results presented in this report provide evidence that the expression of the thrombasthenic glycoprotein abnormality and the inheritance of Pl^{A1} antigen are controlled by different genes. These results further suggest that lack of expression of the Pl^{A1} antigen on thrombasthenic platelets results from the decrease or absence of the glycoprotein carrier of the Pl^{A1} determinant, previously shown to be GP IIIa.

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INTRODUCTION

Glanzmann's thrombasthenia (GT)[1] is an inherited disorder of platelet function characterized, in vitro, by the absence of aggregate formation in the presence of the physiologic agents, ADP, collagen, epinephrine, or thrombin, as well as absent or significantly impaired clot retraction, and, in vivo, by impaired thrombus formation (1–3).

Comparisons of the protein and glycoprotein composition of normal and thrombasthenic platelets by sodium dodecyl sulfate (SDS)- polyacrylamide gel electrophoresis (PAGE) have demonstrated a significant reduction in the periodic acid-Schiff reagent stain density of two bands known as glycoprotein (GP) IIb and GP IIIa[2] in thrombasthenic platelet samples (6–8). Furthermore, decreased levels of radioactivity in these regions of acrylamide gels were observed on analysis of thrombasthenic platelets whose surface proteins were labeled with ^{125}I by the lactoperoxidase-catalyzed procedure (8, 9), and an analysis of thrombasthenic platelets by two-dimensional reduced-nonreduced SDS-PAGE confirmed an absent Coomassie Blue R (CBR)-staining in the position of GP IIb and GP IIIa (8).

Using crossed immunoelectrophoresis (CIE), Hagen et al. (10) showed that the most prominent CBR-stained immunoprecipitate of normal platelet extracts, which is also labeled with ^{125}I in samples from platelets iodinated by the lactoperoxidase method, was absent or significantly reduced in extracts from thrombasthenic platelets. SDS-PAGE of the antigens present in this

[1] *Abbreviations used in this paper*: CBR, Coomassie blue R; CIE, crossed immunoelectrophoresis; GP, glycoprotein; GT, Glanzmann's thrombasthenia; PRP, platelet-rich plasma; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TG, 0.038 M Tris, 0.10 M glycine, pH 8.7; TX, Triton X-100.

[2] The nomenclature for platelet membrane glycoproteins used in this report is adapted from Phillips and Poh Agin (4) as previously described (5).

precipitate given by samples from normal ^{125}I-labeled platelets demonstrated the presence of two ^{125}I-labeled glycoprotein bands that migrate as GP IIb and GP IIIa.

A deletion of the platelet-specific alloantigen, Pl^{A1}, on platelets of five patients with thrombasthenia was first reported by Kunicki and Aster (11). This observation was subsequently confirmed by Muller et al. (12) and van Leeuwen et al. (13) who studied 12 different thrombasthenic patients. Recently Kunicki and Aster (5) demonstrated that the Pl^{A1} determinant is either closely associated with or is an integral part of the structure of membrane GP IIIa, thus establishing a link between the glycoprotein abnormality characteristic of thrombasthenic platelets and the abnormal expression of the Pl^{A1} alloantigen.

We now report further studies on the platelets of three thrombasthenic patients and 20 members of their immediate families (kindred). With the use of CIE for the determination of levels of platelet membrane proteins or glycoproteins (10), we have been able to distinguish those kindred who are heterozygous for the membrane GP IIb + GP IIIa abnormality from those who are unaffected. These results combined with determination of the amount of Pl^{A1} on platelets of these individuals, by inhibition of ^{51}Cr platelet lysis, provide further information regarding the relationship between the inheritance of GT and the expression of Pl^{A1} alloantigen activity.

METHODS

Patients. Each of the three related patients are members of two large families, H. and W., which include descendants of the Manouches gypsy tribe. This tribe had previously migrated throughout the Germanic countries and has recently settled, in part, within the Alsace region of France. These patients (J.H., S.H., and F.W.) were previously shown by Levy et al. (14) to fulfill the diagnostic criteria of GT. A fourth patient, previously diagnosed as having thrombasthenia (14), and who was a cousin of F.W., is deceased.

Preparation of platelets. The preparation of platelet-rich plasma (PRP) from EDTA-anticoagulated whole blood, platelet aggregation studies, clot retraction assays, and human leukocyte antigen (HLA)-A, B, and C antigen typing were performed in Strasbourg. On a given day, four numbered but otherwise unidentified samples of freshly prepared PRP from any combination of thrombasthenic patients, GT kindred, or normal individuals were shipped by train on ice to Paris where CIE and inhibition of ^{51}Cr lysis assays were performed. A fifth sample of PRP from a normal individual was prepared simultaneously in Paris and stored under similar conditions. The identity of the platelet donors from Strasbourg as well as the pedigrees of families H. and W. were not divulged until the completion of all CIE and inhibition of ^{51}Cr lysis assays and the quantitative analysis of data from each individual.

Upon arrival in Paris, platelets were isolated from PRP and washed free of residual plasma, erythrocytes, and leukocytes by differential centrifugation in 0.01 M Tris-HCl, 0.001 M EDTA, 0.145 M NaCl, pH 7.4, as previously described (5).

Inhibition of ^{51}Cr release. The selection of antibodies reactive with the Pl^{A1} antigen, the choice of quinine- and quinidine-dependent antiplatelet antibodies, the techniques for ^{51}Cr-labeling of platelets, the ^{51}Cr-release assay, and the assay of antibody-reactive sites by inhibition of ^{51}Cr release have been previously described (5).

In a single experiment, washed platelets from four Strasbourg donors to be tested for antibody-reactive sites and from a normal donor known to be homozygous for the Pl^{A1} antigen were resuspended in phosphate-buffered saline and analyzed. Several dilutions of each platelet suspension to be assayed were prepared in phosphate-buffered saline to contain a final protein concentration, based upon the method of Markwell et al. (15), ranging from 100 μg to 2.0 mg/ml.

CIE. Triton X-100 (TX)-soluble platelet protein was electrophoresed against multispecific rabbit anti-human platelet antibody preparations essentially as described by Hagen et al. (10) with minor variations that are described in detail elsewhere.[3]

Antisera against whole human platelets were prepared in rabbits using the immunization scheme described by Bjerrum and Bog-Hansen (16). Sera collected during 3-mo periods were pooled and IgG was isolated by ammonium sulfate precipitation followed by DEAE-cellulose chromatography, as described by Harboe and Ingold (17). Three pools of purified IgG, obtained sequentially from the same sensitized rabbits, were used in these experiments, and were obtained 6, 9, and 12 mo, respectively, after the primary immunization.

Washed platelets from Strasbourg donors and known normal individuals were resuspended in 0.038 M Tris, 0.10 M glycine (TG) to a final platelet concentration of $4-6 \times 10^9$/ml and solubilized by addition of TX to a final concentration of 1% (vol/vol). Following centrifugation at 80,000 *g* for 1 h at 4°C, the protein concentration of the TX-soluble supernates was determined by the method of Markwell et al. (14).

15-μl samples of TX-soluble protein containing 70–130 μg of platelet protein were electrophoresed at 10 V/cm for 60 min in first dimension gels consisting of 1% agarose and 0.5% (vol/vol) TX in TG. Second dimension electrophoresis was performed at 2 V/cm for 18 h into a biphasic gel system that consisted of (*a*) an intermediate gel containing 1% agarose and 0.5% (vol/vol) TX in TG, and (*b*) an upper gel containing the same suspension plus the IgG fraction isolated from rabbit antiplatelet antisera (750–800 μg/cm^2). All electrophoretic operations were performed at 15°C using a temperature-regulated circulating water bath (Haake, Inc., Saddle Brook, N. J. model NK 22). After electrophoresis, immunplates were washed and stained with CBR (16). In a single experiment, platelet samples from four Strasbourg donors were always analyzed along with a sample from a known normal Paris donor.

After electrophoresis, an enlarged image (four to five times) of each 5 × 7-cm dried CBR-stained immunplate was traced onto drawing paper with the aid of a Leitz (E. Leitz, Inc., Rockleigh, N. J.) photographic enlarger. The area beneath selected precipitates in the subsequent tracing was determined by planimetry. In a previous study[3] two precipitates were shown to contain GP IIb + IIIa and GP IIIb, respectively (shown in Fig. 1). The calculated area beneath each of these precipitates on immunplates derived from increasing dilutions of an identical platelet extract was directly proportional to the amount of total protein applied to the respective immunplate.

Additional methods. The relationships between the three thrombasthenic patients and 20 members of their immediate

[3] Kunicki, T. J., A. T. Nurden, D. Pidard, and J. P. Caen. Different hereditary disorders of platelet function are characterized by different molecular and antigenic abnormalities as shown by crossed immunoelectrophoresis. Submitted for publication.

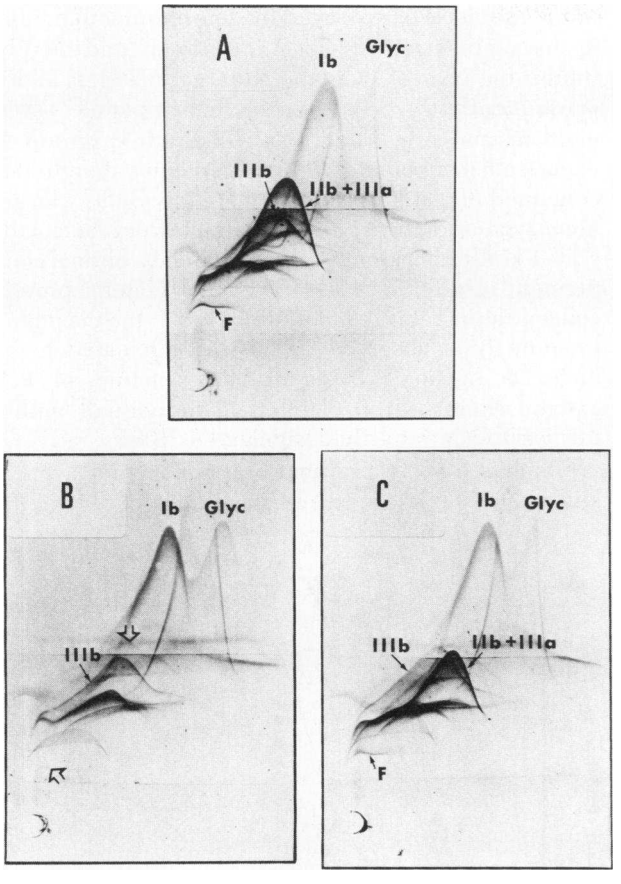

FIGURE 1 Crossed immunoelectrophoresis of TX solubilized proteins from (A) normal platelets, from (B) type I thrombasthenic platelets (F.W.), and (C) from the platelets of a sibling of thrombasthenic patient F.W. 100 μg of platelet proteins solubilized in 0.038 M Tris, 0.1 M glycine, pH 8.7 containing 1% (vol/vol) TX were electrophoresed against a multispecific rabbit anti-human platelet antibody preparation (purified IgG; 750 μm/cm^2). No antibodies were present in the intermediate gel. Electrophoresis in the first dimension (left to right) was performed at 10 V/cm for 1 h; in the second dimension (bottom to top), at 2 V/cm for 18 h. After electrophoresis, immunplates were washed, dried, and stained with CBR. The precipitates containing fibrinogen (F) and membrane glycoproteins Ib, IIb + IIIa, IIIb, and glycocalicin (Glyc) are indicated. Note the difference in area beneath the GP IIb + IIIa and the fibrinogen precipitates when comparing Figs. 1A and 1C, and the absence of these precipitates in Fig. 1B (open arrows).

families, who all live in the area of Strasbourg, was confirmed by HLA-A, -B, and -C typing by the microlymphocytotoxicity method of Terasaki and McClelland (18).

Samples of 0.5 ml of citrated PRP were used to study platelet aggregation by ADP and collagen using a turbidometric method (19). Clot retraction was measured semiquantitatively by weighing the serum expressed from non-anticoagulated blood after 3 h of incubation (19).

RESULTS

CIE analysis. A typical CIE profile of a platelet protein sample from a normal individual is shown in Fig. 1A. The platelet protein and glycoprotein antigen in 11 major precipitates have been identified.[3] These identifications were based upon the results of competitive precipitation with monospecific antibodies, and SDS-PAGE analysis of individual precipitates excised from immunplates, and were aided by comparisons of CIE profiles of normal platelet extracts and those given by platelets from patients with GT, the Bernard-Soulier syndrome, the Gray Platelet syndrome, and von Willebrand's disease. For the purposes of this report, only those precipitates containing fibrinogen, glycocalicin, and membrane GP Ib, IIb + IIIa, and IIIb are indicated. No major variations were observed in the profiles of platelet extracts from the 15 normal individuals. When soluble platelet protein from each of the three thrombasthenic subjects was analyzed (Fig. 1B), the complete absence of precipitates containing fibrinogen and GP IIb + IIIa was evident. This profile is characteristic of that previously observed with soluble platelet protein from two type I thrombasthenic patients (10). When soluble protein samples from the platelets of the GT kindred were analyzed, 7 were shown to be identical to that of normal samples (Fig. 1A), while 13 exhibited a significant and similar reduction of the GP IIb + IIIa precipitate (Fig. 1C).

The levels of precipitates containing other membrane glycoproteins that are not affected by the thrombasthenic defect, such as GP Ib, glycocalicin, and GP IIIb, appeared normal in samples from all thrombasthenic patients and their kindred studied (Figs. 1B and 1C).

Quantitative analysis of GP IIb and IIIa by CIE. Taking advantage of the fact that the area beneath a given precipitate is proportional to the quantity of antigen present (in the presence of a constant amount of antibody), an attempt was made to determine the relative amounts of GP IIb + IIIa in platelet samples from GT kindred compared with normal platelet samples.

To minimize the variability in the absolute area of precipitates on immunplates developed on different days, the area beneath the GP IIb + IIIa precipitate was determined relative to the area on the same immunplate beneath a second precipitate (GP IIIb) not affected by the thrombasthenic defect. The ratio of the area of the GP IIb + IIIa precipitate to that of the GP IIIb precipitate in a single normal platelet sample electrophoresed on five different occasions was 2.24±0.17 (mean±SD). For a second normal platelet sample, the value was calculated to be 2.23±0.21 (mean±SD, n = 5).

The area beneath the precipitates containing GP IIb + III and GP IIIb, respectively, in platelet samples from the 15 normal individuals and 20 GT kindred studied in this report was calculated (Fig. 2). The results were seen to be divided into two groups, desig-

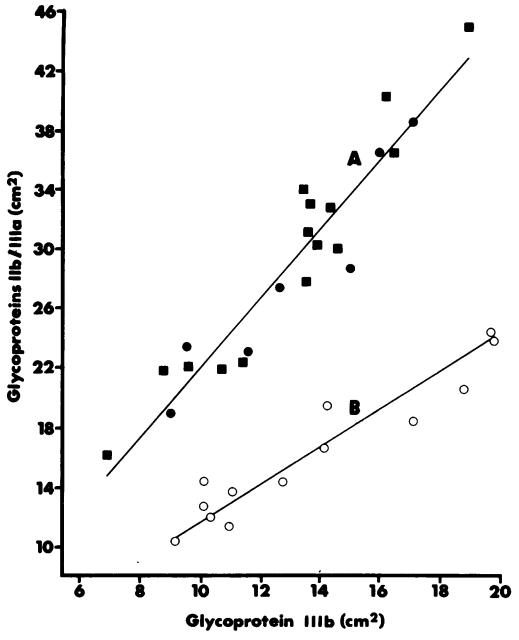

FIGURE 2 Quantitation of the platelet GP IIb and GP IIIa content. The area (square centimeters) beneath precipitates containing GP IIb + IIIa (ordinate) and GP IIIb (abscissa) for each platelet sample studied is shown. The area of the precipitates in enlarged images of CBR-stained immunplates such as those shown in Figs. 1A and 1C was determined by planimetry. TX-soluble protein from 20 kindred of the thrombasthenic patients (●; ○) and from 15 normal individuals (■) was analyzed, generating data that fell into two distinct groups designated A and B. The ratio of the area beneath the GP IIb + IIIa precipitate to that beneath the GP IIIb precipitate was calculated to be: for group A, 2.21±0.20 (mean±SD); for group B, 1.18±0.11.

nated A and B. Within a threefold range of absolute precipitate areas, a significant correlation exists between the area of the precipitate containing GP IIb + IIIa and that containing GP IIIb among samples in group A ($r = 0.9610$; $P < 0.001$) or group B ($r = 0.9574$; $P < 0.001$). In group A, the ratio of the area of the GP IIb + IIIa precipitate to that of the GP IIIb precipitate was 2.21±0.20 (mean±SD, $n = 22$); in group B, this value was 1.18±0.11 ($n = 13$). Group A included platelet protein samples from all of the normal individuals studied and samples from seven of the GT kindred. Group B consisted of samples from the remaining 13 GT kindred. Based upon the mean ratios determined for each group, it can be calculated that platelets represented in group B contain, on the average, 53% of the amount of GP IIb + IIIa present in platelets of group A. These results provide quantitative evidence to strongly suggest that 13 of the 20 GT kindred studied by CIE in this report (see Fig. 1C and Fig. 2, group B) are heterozygous for the thrombasthenic abnormality.

Expression of Pl^{A1} antigen. The Pl^{A1} content of platelets from thrombasthenic patients, kindred, and normal subjects was assayed by determining their ability to compete for Pl^{A1}-specific antibody and thereby inhibit the lysis of ^{51}Cr-labeled target platelets added subsequently (5). As shown in Fig. 3, platelets from eight normal subjects and six GT kindred (group A) expressed an amount of Pl^{A1} antigen equivalent to that contained in platelets from normal individuals who are homozygous for the A1 allele. Platelets from each of the six GT kindred in group A also contained a normal complement of GP IIb + IIIa. The final platelet protein concentration required to inhibit by 50% the maximum immune lysis observed (ID_{50}) was calculated to be 22.5±1.5 (mean±SD) μg/ml. The amount of Pl^{A1} antigen expressed on platelets of individuals within this group is arbitrarily designated as 100%.

Platelets from two normal individuals and five GT kindred (group B) expressed an average of 54% of the

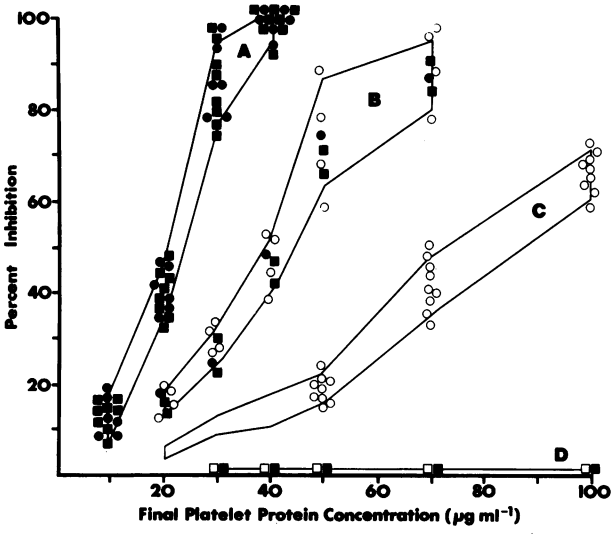

FIGURE 3 Inhibition of the lytic activity of anti-Pl^{A1} antibody by nonlabeled platelets from normal subjects, thrombasthenic patients, and kindred of thrombasthenic patients. 0.02 ml of nonlabeled platelets in 0.013 M phosphate buffer, 0.145 M NaCl, pH 7.4, was incubated with 0.02 ml of anti-Pl^{A1} antibody for 2 h at 37°C. Complement and ^{51}Cr-tagged, Pl^{A1}-positive target platelets were then added, and the percent immune lysis was determined after an additional 2 h of incubation at 37°C. At each final platelet protein concentration tested (abscissa), the percent inhibition of maximum immune lysis observed in the absence of inhibitor platelets was determined (ordinate). Those kindred who were determined to be unaffected by the thrombasthenic defect (●) are distinguished from those determined to be carriers of the defect (○) (see legend to Fig. 2). Inhibition curves fell into four groups which included platelets from: (A), eight controls (■) and six thrombasthenia kindred (●); (B), two controls (■) and five thrombasthenia kindred (●,○); (C), nine thrombasthenia kindred alone (○) and (D), one Pl^{A1}-negative control (■) and three thrombasthenic patients (□). The delineated areas represent the mean±SD for each group. The ID_{50} (micrograms per milliliter) for groups A, B, and C was calculated to be 22.5±1.5 (mean±SD), 41.8 ±3.2, and 81.1±7.8, respectively.

maximum level of Pl^{A1} antigen, an amount equivalent to that observed on platelets of normal individuals heterozygous for the A1 allele. The ID_{50} for platelets within this group was calculated to be 41.8±3.2 (mean±SD). Of the five kindred within this group, the platelets of one individual were shown to contain a normal complement of GP IIb + IIIa, while those of the remaining four individuals were shown to contain one-half the normal level of these GP.

Group C included platelets from nine GT kindred, all of which express one-half normal levels of GP IIb + IIIa. Based upon an ID_{50} value of 81.1±7.8 (mean ±SD) for this group, the platelets of these individuals express ~28% of the amount of Pl^{A1} antigen found on platelets of normal individuals homozygous for the A1 allele.

Finally, platelets from one Pl^{A1}-negative normal individual and the three thrombasthenic subjects (group D) were incapable of inhibiting lysis induced by the Pl^{A1} antibody, even as final platelet protein concentrations as high as 200 μg/ml. Because platelets from these three patients contained no detectable GP IIb + IIIa (see Fig. 1B), these individuals can be considered Pl^{A} null (11).

The specificity of the thrombasthenic abnormality was again confirmed by the fact that platelets from all of the individuals studied in this report (thrombasthenics, kindred, and normal individuals) expressed a normal amount of the receptor for quinine- and quinidine-dependent platelet-specific antibodies, a receptor previously shown to be normally expressed on the platelets of five different thrombasthenic patients (11).

Inheritance of the Pl^{A1} antigen and the thrombasthenic abnormality. The results of the quantitation of GP IIb + IIIa and of the Pl^{A1} antigen on platelets of the members of the two thrombasthenic families, H. and W., are summarized in the family pedigrees shown in Fig. 4.

In family H., subject II-2 is the thrombasthenic patient J.H. Her mother (I-1) is, as expected, a carrier of the thrombasthenic abnormality. A brother (II-3) was also identified as a carrier, whereas the remaining siblings are unaffected. The father is deceased. The thrombasthenic patient, S.H. (II-1) is a first cousin of J.H.

In family W., two thrombasthenic patients (II-2 and II-9) were identified: subject II-2 is patient F.W.; subject II-9 is deceased. Again, the mothers of both thrombasthenic patients (I-1 and I-4) are carriers, as is the only father studied (I-5). Among the remaining members of family W., eight individuals are carriers and four are unaffected.

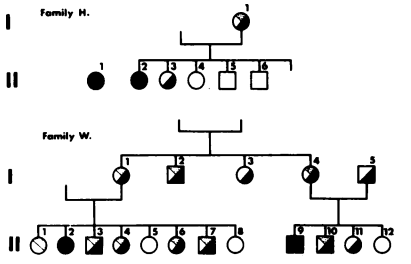

FIGURE 4 Pl^{A} and thrombasthenia genotypes of families H. and W. Roman numerals describe the generations within each family. Males are represented by square symbols; females, by circle symbols. Black symbols represent individuals homozygous for the thrombasthenia defect; half-black symbols, individuals heterozygous for this defect. White symbols represent individuals homozygous for the Pl^{A1} antigen; half-white symbols, individuals heterozygous for this antigen. The stippled symbols represent the corresponding absence of expression of the Pl^{A1} antigen. As an example of the interpretation of these pedigrees, subject I-2 in family W. is both heterozygous for the thrombasthenia defect and heterozygous for the Pl^{A1} antigen. Platelets from I-2, therefore, express 25% of the amount of Pl^{A1} antigen expressed on platelets of subject II-8 in the same family, who is homozygous for the Pl^{A1} antigen and unaffected by the thrombasthenia defect. Familial relationships were confirmed by analyses of lymphocyte HLA-A, -B, and -C antigens of each subject.

DISCUSSION

The Pl^{A} (Zw) alloantigen system was established through the combined studies of von Loghem et al. (20), Shulman et al. (21), and Van der Weerdt et al. (22), and shown to be represented by the allelic counterparts, Pl^{A1} and Pl^{A2} (also known as Zw^{a}, and Zw^{b}, respectively). Pl^{A1}, present in 98% of the general population, has been implicated in the pathogenesis of the syndrome known as post-transfusion purpura (21) and appears to be the alloantigen that most often provokes isoimmune neonatal thrombocytopenia (23, 24). Recent studies by Van Leeuwen et al. (13) have demonstrated that anti-Pl^{A1} antibodies can inhibit the in vitro aggregation of homozygous Pl^{A1}-positive platelets as induced by the physiologic agents, adenosine diphosphate, and collagen. Taken together, these observations suggest that Pl^{A} is, clinically, an important platelet alloantigen system, and that the Pl^{A1} antigenic determinant is associated with a platelet surface component of potential significance in normal platelet function.

Pl^{A1} antigenic activity was shown by Kunicki and Aster (5) to be associated with a major platelet membrane glycoprotein, IIIa, using three independent isolation procedures. The precise role of GP IIIa in platelet function remains to be elucidated, but the severe dysfunction of platelets that lack both GP IIIa and GP IIb in GT suggests that these glycoproteins are critical to platelet-platelet cohesion and, possibly, other aspects of platelet function (7).

A better understanding of the genetic basis of the thrombasthenic defect, and of its relationship to the expression of the Pl^{A1} alloantigen, has awaited improved methods for the precise quantitation of platelet membrane glycoproteins and antigens. This capability is re-

quired for the detection of individuals who are heterozygous for either or both the thrombasthenia gene and the A1 allele. Using a complement fixation assay and an antibody, IgG L., present in the serum of a polytransfused thrombasthenic patient that was shown to recognize an unidentified 120,000-mol wt antigen present on normal platelets, Degos et al. (25) were able to detect intermediate levels of the antigen on platelets of individuals presumably heterozygous for the thrombasthenic defect. No attempt was made to quantitate these intermediate levels. Subsequent studies have shown that this antigen is located on GP IIb and/or GP IIIa (10). Recently, McEver et al. (26) demonstrated that platelets from five obligate and four presumed thrombasthenia heterozygotes bound roughly 62% of that amount of a ^{125}I-labeled monoclonal anti-GP IIb + GP IIIa antibody that bound to normal platelets.

In the present study, we have used CIE of TX soluble platelet protein against a multispecific rabbit anti-human platelet antibody (Fig. 1) to quantitate the levels of GP IIb + GP IIIa in platelets from several normal individuals, three thrombasthenic patients, and 20 members of the immediate families (kindred) of these patients. Our results (Fig. 2) demonstrate that CIE, when used in the quantitative manner described in this study, is both precise and reproducible (SE ±8%). Triton extracts from the platelets of each of the three thrombasthenic patients contained no trace of GP IIb + GP IIIa. Such results are similar to those previously shown for two other type I thrombasthenic patients (10). Triton extracts from the platelets of seven kindred contained an amount of GP IIb + GP IIIa identical to that observed in normal platelets. These individuals were apparently unaffected by the thrombasthenic abnormality. Triton extracts from the platelets of the remaining 13 kindred expressed levels of GP IIb + GP IIIa that were calculated to be, on the average, 53% of those observed in extracts of normal platelets. Individuals in this group, which included all parents of the patients studied, are suggested to be carriers of the thrombasthenic abnormality.

It is probable that the glycoprotein abnormality of thrombasthenia is a direct effect of the inheritance of an altered gene or genes that normally control the expression of GP IIb + GP IIIa. The presence of 0, 50, or 100% of normal levels of GP IIb + IIIa on platelets of thrombasthenic patients or their kindred argues against a role for proteolysis in the etiology of thrombasthenia, as recently suggested by Shuman and Karpatkin (27). To our knowledge, the molecular abnormality of GT is unique among such abnormalities involving other human cell systems, in that two apparently different membrane glycoproteins, IIb and IIIa, are consistently reduced or absent. No evidence has yet been reported that the decrease in one of these glycoproteins is unaccompanied by an equivalent decrease in the other regardless of the absolute levels of both glycoproteins observed.

The expression of the Pl^{A1} alloantigen on platelets from the three patients and 20 kindred was precisely determined (SE ±6%) by inhibition of ^{51}Cr-labeled platelet lysis (Fig. 3). As expected, platelets from the three patients contained negligible amounts (<5%) of Pl^{A1} alloantigen. Platelets from those kindred that were normal, with respect to GP IIb + GP IIIa content, expressed either 100 or 50% of the maximum level of Pl^{A1} (i.e., the amount detectable on platelets of normal individuals homozygous for the A1 allele). These individuals can be classified as homozygous and heterozygous, respectively, for the A1 allele.

Platelets from those kindred previously determined to be carriers of the thrombasthenic abnormality expressed roughly 50 or 25% of the maximum level of Pl^{A1}. The logical interpretation of these results is that (*a*) these individuals are, in fact, homozygous and heterozygous, respectively, for the A1 allele, and (*b*) the relative amount of A1 antigen expressed is diminished by a factor of two as a result of the presence of one-half the normal content of GP IIIa, the carrier of the A1 determinant, on platelets of these individuals. It follows from these results that if the genes controlling the expression of Pl^{A} were the same as those controlling expression of the thrombasthenic defect, individuals who were double heterozygotes would express either 50% of maximal amounts of Pl^{A1} or no Pl^{A1} whatsoever. That individuals whose platelets contained 25% of maximum levels were consistently found strongly suggests that the genes controlling these two traits are not the same.

In early studies, Caen (3) differentiated type I thrombasthenia, in which clot retraction is absent and platelets do not contain detectable fibrinogen, from type II thrombasthenia, in which clot retraction is nearly normal and fibrinogen is detectable. Hagen et al. (10), using CIE, demonstrated that the platelets of a type II thrombasthenic patient contained 13% of the normal amount of GP IIb + IIIa. The residual GP IIb and GP IIIa in the platelets of this patient were antigenically indistinguishable from the same glycoproteins on normal platelets by crossed immunoelectrophoresis against rabbit antibody and by crossed affino immunoelectrophoresis against IgG L. (28). Using the same methods reported in that study, we observed an amount of GP IIb + IIIa in the platelets of a different type II thrombasthenic patient that was 15% of that detected in normal platelets.[3] At the same time, the levels of Pl^{A1} antigen in the platelets of the same two thrombasthenic patients were calculated to be 13 and 11%, respectively, of that detected on platelets from individuals homozygous of the A1 allele.[4]

[4] Kunicki, T. J. Unpublished observations.

At the present time, we have no explanation for the presence of residual amounts of GP IIb and IIIa and Pl^{A1} antigen in platelets of type II patients in the etiology of this variant of thrombasthenia. In light of these observations, it would be of interest to compare more precisely the molecular structure and antigenicity of GP IIb and GP IIIa isolated individually from normal platelets, to compare these with the same glycoproteins isolated from type II thrombasthenic patients, and to determine the inheritance of the membrane glycoprotein abnormality in the kindred of type II thrombasthenic patients.

In the CIE analyses of the platelets from each of three type I thrombasthenic patients studied in this report, the precipitate containing fibrinogen was not detected. A similar observation was made by Hagen et al. (10) who studied two different type I thrombasthenic patients. In contrast, platelets from two type II thrombasthenic patients (10)[3] contained elevated but apparently subnormal amounts of fibrinogen. Due to the flat shape of the fibrinogen precipitate, estimates of the amount of fibrinogen in samples from type I thrombasthenic patients (Fig. 1C) could not be made following CIE and will be the subject of a subsequent investigation.

The results reported here suggest that the gene controlling the inheritance of the allelic forms of the Pl^{A} alloantigen system is not the same gene as that (those) involved in the inheritance of the thrombasthenic defect. Type I thrombasthenic individuals can correctly be classified as Pl^{A} null since, regardless of their genotype with respect to the Pl^{A} system, these alloantigens which are normally associated with GP IIIa (5) cannot be expressed.

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