Human Platelet-Specific Antigen, Sib*, Is Associated With the Molecular Weight Polymorphism of Glycoprotein Iba

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Platelet-specific antigen Sib* has been highly implicated in the pathogenesis of refractoriness to human leukocyte antigen (HLA)-matched platelet transfusions in Japan. We provide evidence that the Sib* antigen is located on the glycoprotein (GP) Iba and has a close association with the molecular weight (mol wt) polymorphism of GPIb. In modified antigen-capture ELISA (MACE), anti-Sib* antibody reacted only with GPIb/IX held by a murine anti-GPIb/IX monoclonal antibody (MoAb). The reactivity of anti-Sib* antibody to Sib*-positive (Sib*) platelets was abolished after they were treated with Serratia marcescens protease. Platelets from 50 healthy volunteers were semiquantitatively phenotyped for Sib* antigen by MACE and divided into three distinct groups: strongly positive, positive, positive, and negative. They were also analyzed by sodium dodeyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and periodic acid-silver staining for mol wt polymorphism of GPIb, phenotyped as A, B, C, or D. Without exception, Sib** platelets showed larger phenotypes (A or B). Removal of sialic acid from Sib** platelets did not reduce the binding of anti-Sib*. Finally, anti-Sib* antibody specifically immunoprecipitated A and B phenotypes of GPIb from Sib* platelets. Thus, Sib* antigen evidently is located in the region of glyocalcin that is present only on the A and B phenotypes of GPIb.

INCOMPATIBILITY in platelet alloantigen systems leads to various clinical disorders, such as posttransfusion purpura (PTP), neonatal alloimmune thrombocytopenic purpura (NATP), and platelet transfusion refractoriness (PTR). Among the platelet alloantigens defined so far, Sib* antigen is most implicated in the pathogenesis of PTR in Japan. Phenotypic frequencies of platelet alloantigens differ considerably among various races. Although PI4 alloantigen is most implicated in the pathogenesis of PTR and NATP among whites, no PI6 heterogeneity has been reported among native Japanese. Another platelet alloantigen system, Pen6 or Yuk, also located on glycoprotein (GP)IIIa, where PI6 antigens reside, is the most frequent cause of NATP in Japan.

GPIb functions as a receptor for both von Willebrand factor (vWF) and thrombin and plays an essential role in platelet adhesion to subendothelium and thus in hemostasis. A unique aspect of GPIb is that it is structurally heterogeneous and categorized into four phenotypes according to their apparent molecular weights (mol wt). Each phenotype of GPIb functions normally despite significant difference in mol wt up to 15,000. Finch et al recently reported another GPIb polymorphism demonstrated only by intragenic restriction fragment length polymorphism (RFLP) of the GPIb gene for an endonuclease, Taq I. The precise nature of this polymorphism remains unknown.

Immunologic aspects of GPIb are also of practical importance. Among platelet-specific antigens, PI1 and Ko'/Ko are present on GPIb1,15 GPIb also plays an important role as a frequent target antigen of antiplatelet autoantibodies in autoimmune thrombocytopenic purpura (AITP). In this report, we provide evidence that the Sib* antigen is located on the a-chain of GPIb and is closely associated with the mol wt polymorphism of GPIb.

MATERIALS AND METHODS

Antibodies. The antiserum containing anti-Sib* antibody used in this study was from the proband reported by Saji et al.3 Antisera specific for Br* and PI6 alloantigen were gifts from Dr C. Mueller-Eckhardt (GieBen, Germany) and Dr T.J. Kunicki (Milwaukee, WI), respectively.

Murine monoclonal antibodies (MoAbs) SZ1 (specific for GPIb/IX complex) and SZ2 (specific for GPIbαβ) were purchased from Immunotech (Marseille, France). TP 80 (specific for GPIb), 4B4 (specific for GPIIa), and OKMs (specific for GPIV) were obtained from Nichirei (Tokyo, Japan), Coulter (Hialeah, FL), and Ortho Diagnostic System (Beere, Belgium), respectively. AP1 (specific for GPIbαβ) was also a gift from Dr T.J. Kunicki.

Platelet isolation. Venous blood was drawn from healthy volunteers into acid-citrate-dextrose [ACD, National Institutes of Health (NIH) formula A] and centrifuged at 250g for 10 minutes at ambient temperature. The obtained platelet-rich plasma (PRP) was washed three times in Ringer's citrate dextrose buffer (RCD pH 6.5) containing 20 ng/mL PGE2, and 10 mmol/L NaCl, and finally resuspended in any desired buffer.

Modified antigen-capture ELISA (MACE). Fifty microliters of a murine antiplatelet MoAb in carbonate buffer (pH 9.6) at a concentration of 3 μg/mL was added to each well of a microtiter plate (ImmuModule, Nunc, Roskilde, Denmark) and incubated overnight at 4°C. The wells were washed once with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20 (PBS/Tween) and blocked with the same buffer for 30 minutes at ambient temperature.

Washed platelets were resuspended in PBS/10 mmol/L NaCl, EDTA (PBS/EDTA), and the count was adjusted to 107/mL. One hundred microliters of each platelet suspension was mixed with 5 to 20 μL sera containing antiplatelet alloantibodies and incubated for 60 minutes at ambient temperature. The sensitized platelets were washed twice in PBS/EDTA, resuspended in 50 μL PBS/EDTA and solubilized by addition of 10 μL 10% Triton X-100 (Nacalai Tesque, Kyoto, Japan). The lysate was kept on ice for 15 minutes with occasional vortexing and then centrifuged at 11,750g for 15 minutes to pellet insoluble materials. The resultant supernatant was added to each well of the microtiter plates in duplicate.
and incubated for 60 minutes. After five subsequent washes, the F(ab')2 fraction of biotinylated goat anti-human IgG (Tago, Burlingame, CA) diluted 1:3,000 in PBS/Tween was added and the plate was incubated for 60 minutes. Each well was washed five times and further incubated with alkaline phosphatase-streptavidin (Zymed, South San Francisco, CA) diluted 1:2,500 in PBS/Tween for 30 minutes. After six additional washes, water was added and the plate was allowed to develop color for 30 minutes at ambient temperature. Absorbance at 405 nm was recorded using a microplate reader (Bio-Rad Novapath Microplate Reader, Bio-Rad, Richmond, CA).

Background absorbance varied depending on the capturing MoAbs used in each assay; mean optical density of negative controls was 0.324; 0.176; 0.103; and 0.022 for 4B4, TP80, OKM5, and OKM1, respectively. The results of each assay were indicated for clarification by controls was SZ1, 0.324; 4B4, 0.176; TP80, 0.103; and OKM5, 0.022. The OD ratio of negative controls was calculated by the following formula: OD ratio = (OD of a sample – mean OD of negative controls)/mean OD of negative controls.

Acid treatment. Platelets were acid treated by the method of Kurata et al.\textsuperscript{22} to remove antigenicity of human leukocyte antigen (HLA) class I antigens expressed on platelets. Washed platelets were resuspended in acid solution [112 mmol/L citric acid, 67 mmol/L Na,HPO\textsubscript{4}, 0.1% bovine serum albumin (BSA), pH 3.0] for 10 minutes on ice and then immediately neutralized with an excess of citrate buffer, pH 6.5.

Flow cytometry. Acid-treated platelets were resuspended in PBS/EDTA at a concentration of 10\(^6\)/mL. One hundred microliters of this suspension was incubated with appropriately diluted antiplatelet antibodies or murine MoAbs for 1 hour at ambient temperature. After washing with PBS/EDTA, the platelets were further incubated with 100 \(\mu\)L appropriately diluted FITC-conjugated goat F(ab')2, anti-human IgG (Tago) or anti-mouse IgG (Becton Dickinson, San Jose, CA) for 30 minutes in the dark. After washing, samples were analyzed on an EPICS V analyzer (Coulter). Optimal conditions for each antiplatelet antibody to yield maximum fluorescence intensity were determined individually. In subsequent studies, the effect of protease from \textit{Serratia marcescens} on the binding of anti-Sib\textsuperscript{a} antibody was investigated using flow cytometry. Sib\textsuperscript{a} platelets were treated with \textit{S. marcescens} protease (a gift from Takeda Pharmaceuticals, Tokyo, Japan) at a final concentration of 24.9 U/mL for 5 minutes at 37°C. The platelets were washed in PBS/EDTA to stop the enzyme cleavage and then treated. Flow cytometric analyses were made as described above.

\textit{Gel electrophoresis and periodic acid-silver staining.} Washed platelets were resuspended at 5 \times 10\(^9\)/mL in PBS/EDTA containing 1 mmol/L phenylmethylsulfonlfuryl fluoride (Sigma) and mixed with 20% vol 10% Triton X-100 in PBS on ice. After occasional vortexing, the lysate was centrifuged at 11,750 g for 15 minutes. The supernatant was mixed with an equal volume of sample buffer [4% sodium dodecyl sulfate (SDS) in 50 mmol/L Tris-HCl (pH 6.5)] containing 100 mmol/L NaCl and 5 mmol/L MgCl\textsubscript{2}.

Immunoprecipitation and identification of GPIb. Washed intact platelets (3 \times 10\(^9\)) were suspended in 300 \(\mu\)L PBS/EDTA and incubated with antiplatelet antibodies for 120 minutes at ambient temperature. After two washes with PBS/EDTA, the sensitized platelets were solubilized in 1% Triton X-100 for 15 minutes at 4°C. Each lysate was centrifuged at 11,750 g for 15 minutes, and the supernatant was incubated with a slurry of Protein A-Sepharose CL-4B (PAS, Sigma) for 90 minutes with continuous gentle shaking to isolate immune complexes. The PAS bead were washed five times in PBS/Tween, resuspended in 50 \(\mu\)L SDS-PAGE sample buffer, and incubated at 56°C for 30 minutes. The samples were separated by SDS-PAGE and transferred to PVDF membrane. Immunoprecipitated GPIb fixed on the membrane was detected by immunoblotting with SZ2 as the probe.

\textit{Neuraminidase treatment of washed platelets.} Washed platelets were treated with neuraminidase according to the method described by Take et al.\textsuperscript{22} Platelets were suspended in 0.5 mol/L citrate buffer (pH 6.2) and mixed with neuraminidase (Nacalai Tesque) at a final concentration of 0.5 U/mL at 37°C for 3 hours. The digestion was stopped by washing the platelets twice with PBS/EDTA. Neuraminidase-treated platelets were immunoblotted to examine the effect of the digestion on the mol wt polymorphism of GPIb. Binding of anti-Sib\textsuperscript{a} antibody to the neuraminidase-treated GPIb was also investigated with MACE.

\textbf{RESULTS}

\textbf{MACE.} With MACE, target platelet GPs to which platelet allotypoids bound could be easily identified. As shown in Fig 1, anti-Sib\textsuperscript{a} IgG bound specifically to the GPs held by SZ1, which was specific for GPIb/IX complex. On the other hand, anti-B\textsuperscript{1} and anti-PI\textsuperscript{a} IgG reacted only with the GPIb/IXa complex bound by 4B4 and the GPIb/IIIa complex bound by TP80, respectively. Anti-Sib\textsuperscript{a} antibody did not react with GPs bound by 4B4, TP80, or OKM5 (specific for GPIV). The data shown in Fig 1 represent typical results from three independent experiments. With this assay, SZ1, platelets from 50 unrelated healthy Japanese volunteers were phenotyped for Sib\textsuperscript{a}. Fifteen of the volunteers (30%) were positive for Sib\textsuperscript{a} antigen (Sib\textsuperscript{a}+). Among those, platelets from three individuals bound high
levels of anti-Sib\(^a\) IgG. Judging from these data, we divided the 50 screened donors into three groups; group 1 (Sib\(^a\) strongly positive, 3 donors), group 2 (Sib\(^b\)-moderately positive, 12 donors), and group 3 (Sib\(^c\), 35 donors). The mean OD ratios ± SD for each group measured by MACE were group 1, 4.63 ± 0.94; group 2, 2.95 ± 0.27; and group 3, 0.18 ± 0.15 (\(P < .005\)). Subsequent studies showed that the donor, whose platelets were phenotyped as shown in Fig 1, belonged to group 2.

Flow cytometry. Preliminary experiments showed that incubation of anti-Sib\(^a\) antibody at a dilution of 1:20 with Sib\(^b\) strongly positive (group 1) platelets resulted in maximum fluorescence intensity in flow cytometry (data not shown). Therefore, these conditions were used for all three groups. Platelets were always acid treated to eliminate the interaction between HLA class I antigens on platelets and high-titered anti-HLA antibodies contained in the antisera. Without the acid treatment, Sib\(^b\) platelets showed strong reactivity with the anti-Sib\(^a\) antisera but no reactivity after the acid treatment, indicating that the antigenicity of HLA antigens was eliminated sufficiently for subsequent analyses (data not shown). Group 1 platelets bound larger amounts of anti-Sib\(^a\) antibody detected in flow cytometry than did group 2 platelets (Fig 2A). An anti-GPIb MoAb, SZ2, bound equally to the platelets from all three groups, however, indicating that an equal amount of GPIb was expressed on all platelets (Fig 2B). Apparent differences in the amount of bound IgG to the group 1 platelets between anti-Sib\(^a\) (Fig 2A) and SZ2 (Fig 2B) could be attributed to the difference in the molar FITC/protein ratio of the second antibody used in each assay.

Subsequently, the effect of \(S\) marcescens protease on binding of various antiplatelet antibodies to group 2 platelets was investigated in flow cytometry (Fig 3). Both anti-Sib\(^a\) and SZ2 binding was abolished. On the other hand, reactivity of the protease-treated platelets with anti-PIA\(^a\), TP80, and SZ1 was unchanged.

**Mol wt polymorphism of GPIb.** Platelets phenotyped for Sib\(^b\) were next analyzed by SDS-PAGE and periodic acid-silver staining for the mol wt polymorphism described by Moroi et al.\(^a\) One or two darkly stained band(s) with apparent mol wt ranging from 163,000 to 152,000 were observed in each platelet preparation under nonreducing conditions (Fig 4A). Platelets from any single donor did not concurrently show three or more darkly stained bands with apparent mol wt in this range. That these bands were GPIb was verified by immunoblotting with SZ2 as the probe (Fig 4B). All four phenotypes of GPIb designated by Moroi et al\(^a\) were identified in platelets from the 50 individuals. The apparent mol wt of each GPIb phenotype under nonreducing conditions was about 163,000, 160,000, 155,000, and 152,000 for A, B, C, and D, respectively. The frequency of each GPIb phenotype among the 50 individuals is summarized in Table 1.

**Association of Sib\(^a\) antigen with the mol wt polymorphism of GPIb.** The relationship in each platelet preparation between the reactivity with the anti-Sib\(^a\) antibody (represented by groups 1 through 3) and the mol wt phenotype of GPIb is summarized in Table 1. All group 1 platelets had A-phenotype of GPIb exclusively (A/A), whereas all group 2 platelets had a combination of two, A or B plus C or D. Each Sib\(^b\) (group 3) platelet preparation had not larger (A or B), but instead a combination of smaller, GPIb phenotypes (C/C, C/D or D/D). All platelets showing A or B
GPIb phenotypes were positive for Sib" according to MACE. These observations indicated that the Sib* antigen is closely associated with the mol wt polymorphism of GPIb.

**Immunoprecipitation of unlabeled GPIb.** To confirm that only A and B phenotypes of GPIb carry the Sib* epitope, 3H-labeled platelet GPs were immunoprecipitated with antiplatelet antibodies. Although anti-PIA1 and SZ2 immunoprecipitated 3H-labeled corresponding antigens, anti-Sib* antibody immunoprecipitated only HLA class I molecules (data not shown). Loss of Sib" antigenicity during 3H-labeling procedure by periodate/borohydride was suspected, and an immunoprecipitation procedure with unlabeled platelets as the source of antigens was designed. AP1 immunoprecipitated all GPIb phenotypes (Fig 5, lanes 1, 3, 6, and 9). On the other hand, the anti-Sib* antibody specifically immunoprecipitated the higher mol wt forms of GPIb, A and B (Fig 5, lanes 4, 7, and 10).

**Neuraminidase treatment of platelets.** Because GPIb is very rich in carbohydrate, involvement of the carbohydrate moiety in both expression of Sib* antigen and the mol wt polymorphism was suspected. Platelets were treated with neuraminidase to investigate this possibility. The apparent mol wt of all GPIb phenotypes, determined by immunoblotting with SZ2 as the probe, decreased significantly (Fig 6). The extent of this reduction was larger in smaller GPIb phenotypes; approximately 5,000 daltons in C and D phenotypes v 2,000 daltons in A and B phenotypes under reducing conditions. Of note are the results from MACE indicating that the binding of anti-Sib* antibody to Sib" (group 2) platelets was significantly augmented by neuraminidase treatment of the target platelets; the OD ratios (mean ± SD) of untreated platelets v treated platelets were 2.72 ± 0.44 and 3.70 ± 1.03 (n = 6, P < .05), respectively.

**Family studies on inheritance of Sib* antigen and mol wt polymorphism of GPIb.** Three families were analyzed regarding the inheritance of Sib* antigen and the mol wt polymorphism of GPIb. Figure 7 shows pedigrees of two of the three families. These studies confirmed that the inheritance pattern of both Sib* antigen and the mol wt polymorphism showed autosomal (co) dominant traits and that the expression of Sib* antigen was always linked to the A
phenotype of GPIb. It is unfortunate that no families including members with B phenotype were available for this study.

**DISCUSSION**

Platelet Sib' antigen, first described by Saji et al., was defined by an antibody detected in the serum of a patient who had become refractory to both random and HLA-matched platelet transfusions. In this report, we provide several lines of evidence indicating that the Sib' antigenic determinant is located on the α-chain of GPIb and that it is closely associated with the mol wt polymorphism of GPIb.

Localization of Sib' antigen to GPIbα was demonstrated by two lines of evidence. First, with MACE, in which murine MoAbs specific for platelet membrane GPs (including SZ1, TP80, 4B4, and OKM5) were used, anti-Sib' specifically reacted with the GPIb/IX complex bound to SZ1. Anti-Sib' did not react with the GPIb/IIa complex bound to TP80, GPIa/IIa (or GPIc/IIa) complex bound to 4B4, or with GPIV bound to OKM5. In other experiments in which SZ1 was replaced by other anti-GPIb MoAbs, such as SZ2 and AP1, anti-Sib' also reacted with the bound GPs (data not shown). Second, reactivity of anti-Sib' and SZ2 to Sib' platelets assayed by flow cytometry was abolished after treatment of the platelets with S marcescens protease, whereas binding of anti-PI'-, TP80, and SZ1 remained unchanged. Platelets treated with S marcescens protease lost their reactivity with a murine MoAb specific for the peptide tail region of GPIb, SZ2, but not with another murine MoAb specific for the membrane-associated region of GPIb/IX, SZ1. In addition, release of most of the glycoprotein on the surface of digested platelets to the supernatant under these conditions was confirmed by immunoblotting (data not shown). These observations are in accord with previous reports that S marcescens protease specifically cleaves GPIb to release glycoprotein and therefore strongly indicate that anti-Sib' is bound to a determinant on the S marcescens protease-sensitive region of GPIb, glycoprotein.

Kuijpers et al. reported that Ko'/Koh alloantigens were located on the N-terminal elastase-sensitive fragment of GPIb based on the data obtained by monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay and immunofluorescent test in combination with elastase digestion. Serologic tests indicate that anti-Sib' is similar to anti-Ko' (H. Saji and A.E.G.Kr. von dem Borne, personal communication, July 1989). Another platelet antigen, PI', was localized to glycoprotein by Furihata et al. Because the proband who had developed anti-PI' antibody might have had Bernard-Soulier syndrome, this antigen could be an isoantigen instead of an alloantigen. The precise relationship between Sib' and these antigens requires further study.

From our data, we calculated the phenotypic frequency of Sib' antigen among Japanese to be 30%, which was slightly higher than the value Saji et al. reported (25.4%). Platelets from 50 donors were divided into three groups (1, 2, and 3) according to their reactivity with anti-Sib' deter-

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<th>Table 1. Relationship Between Sib' Phenotype and Molecular Weight Polymorphism of GPIb</th>
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Abbreviation: GPIb, glycoprotein Ib.
Fig 5. Immunoprecipitation of unlabeled platelet GPs and sequential immunoblot assay under reducing conditions to identify GPlb using SZ2. Molecular weight phenotypes of the target platelet preparations were C/D (lanes 1 and 2), A/A (lanes 3 through 5), A/C (lanes 6 through 8), and B/D (lanes 9 through 11). Antibodies were a murine MoAb anti-GPlba, AP1 (lanes 1, 3, and 9), anti-Sib* (lanes 2, 4, 7, and 10), and negative control sera (lanes 5, 6, and 11). AP1 immunoprecipitated all GPlb phenotypes. In contrast, anti-Sib’ antibody specifically immunoprecipitated A and B phenotypes of GPlb from Sib’ platelets (lanes 4, 7, and 10). Negative control sera did not immunoprecipitate any type of GPlb.

Fig 6. Effect of neuraminidase treatment on the mol wt polymorphism of GPlb. Neuraminidase-treated platelets (odd lanes) and untreated platelets (even lanes) were immunoblotted under reducing conditions with SZ2 as the probe. Lanes 1 and 2 are platelets with the C/D phenotype of GPlb, lanes 3 and 4 are platelets with the B/D phenotype, and lanes 5 and 6 are platelets with the A/C phenotype. The reduction in size was greater in the smaller phenotypes C and D than in the larger phenotypes A and B.

Fig 7. Pedigrees of two families indicating genetic linkage of Sib* antigen with the A phenotype of GPlb. Capital letters below each circle and rectangle represent the mol wt phenotype. Sib* phenotypes are indicated (right).
platelet-specific alloantigen systems, these results indicate group 1 would represent a Sib heterozygote and group 2 would represent a Sib homozygote.

Although a mol wt polymorphism of GPIb was noted before in two patients with a bleeding diathesis, Moroi et al reported the existence of the genetic polymorphism of GPIb among healthy Japanese individuals. According to its apparent mol wt, each GPIb phenotype was designated A, B, C, and D (A is the largest). No individuals with any combination of GPIb phenotype showed bleeding or thrombotic diathesis. In an aggregation study, platelets with any phenotype without exception, whereas group 2 would represent a Sib heterozygote. The identity of GPIb stained by periodic acid-silver staining was verified by immunoblotting with SZ2 as the probe. Phenotypic frequencies of the mol wt polymorphism of GPIb obtained from our study significantly differed from those in previous reports. We noted three individuals with A/A phenotype in 50 unrelated volunteers whereas no other previous reports documented their existence. These discrepancies could be attributed to differences in ethnic and regional backgrounds. The expression of Ko antigen, serologically similar to Sib antigen, among whites is significantly lower than that of Sib in Japan (14.3% vs 25.4%).

Analysis of the relationship between the Sib phenotype and the mol wt phenotype of GPIb showed a perfect association of Sib antigen with the higher mol wt phenotypes of GPIb, A and B. Group 1 platelets showed the A/A phenotype without exception, whereas group 2 platelets showed A or B plus C or D. All Sib+ platelets were of C/C, C/D, or D/D phenotypes. Individuals with an A/B or B/B phenotype, which would also be Sib strongly positive, have not yet been found.

The association of Sib antigen with the mol wt polymorphism was then directly demonstrated by immunoprecipitation. An MoAb specific for GPIba, AP1, immunoprecipitated all GPIb phenotypes. In contrast, anti-Sib antibody specifically immunoprecipitated the higher mol wt phenotypes of GPIb, A and B, from the lysate of unlabeled platelets. Immunoblotting was also performed to confirm further the results of immunoprecipitation, but anti-Sib antibody failed to react with SDS-denatured GPIb.

These observations indicated that Sib antigen was located solely on the A and B phenotypes of GPIb and that the 5-Kd fragment accounting for the difference in size between B and C phenotypes might contribute to Sib antigenicity.

In a previous study, neuraminidase treatment of platelets appeared to have no effect on the mol wt polymorphism of GPIb. In our experiments in which platelets were treated with a higher concentration of neuraminidase, the apparent mol wt of all GPIb phenotypes significantly decreased. The reduction in size was greater in the smaller phenotypes, C and D, than in the larger A and B; 5,000 daltons in C and D vs 2,000 daltons in A and B. Satisfactory removal of sialic acid was also confirmed by enhancement of the reactivity of Ricinus communis agglutinin (RCA I lectin with neuraminidase-treated GPIb demonstrated by lectin-blot assay (data not shown). These data led us to consider that not only primary structures but also carbohydrate moieties were responsible for the difference in mol wt of GPIb. This hypothesis was also supported by recent report of Meyer and Schellenberg that the C-terminal region of glycoaclacin (macroglycopeptide), rich in carbohydrate, contributed to these mol wt polymorphisms of GPIb. Binding of anti-Sib antibody to Sib+ platelets determined by MACE was augmented by neuraminidase treatment of the platelets. These results suggested that expression of Sib antigen did not depend directly on the presence of sialic acid and that the Sib+ antigen would become more accessible by conformational and/or an electric change induced by removal of sialic acid.

The nature of the Sib+ antigen is different from that of the Pt and Bak alloantigen systems in that the 5-Kd fragment of GPIba, which accounts for the difference in mol wt between type B and type C, would be responsible for the expression of Sib+, whereas only a single base change (resulting in a single amino acid change) differentiates each allele in the Pt and Bak systems. One newly defined platelet antigen, Nak+, is localized to GPIV and has recently been proven an isoantigen because Nak+ platelets appeared to lack GPIX. Sib+ platelets have the normal number of fully functioning GPIb molecules. This type of alloantigen has not been reported in the field of platelet immunology and would serve as an ideal model to investigate the evolution of an essential platelet GP, GPIb.

ACKNOWLEDGMENT

We thank Professor Seiichi Furuta (The Second Department of Internal Medicine, Shinshu University School of Medicine) and Dr Hiroyuki Ogata (Transfusion Service, Shinshu University Hospital) for excellent advice, Susumu Itoh and Sayuri Hayashi (The Blood Center of Southeastern Wisconsin, WI) and C. Mueller-Eckhardt (Justus Liebig University, Germany) for their gift of antibodies.

REFERENCES

ASSOCIATION OF SII, MOL WT POLYMORPHISM OF GPIB

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involved in the refractoriness of HLA-matched platelet transfusion. Vox Sang 57:213, 1989
15. Kuipers RWAM, Modderman PW, Bleeker PMM, Ouwenthal WH, von den Borne AEGK: Localization of the platelet specific Ko system antigens Ko(a)/Ko(b) on GP Ib/IX. Blood 74:226a, 1989 (abstr)
23. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage \(\lambda\). Nature 227:680, 1970
32. Newman PJ, Derbes RS, Aster RH: Human platelet alloantigens, PI\(^a\) and PI\(^b\), are associated with a leucine/\(\gamma\)/proline/\(\alpha\) amino acid polymorphism in membrane glycoprotein IIIa, and are distinguishable by DNA typing. J Clin Invest 83:1778, 1988
Human platelet-specific antigen, Siba, is associated with the molecular weight polymorphism of glycoprotein Ib alpha

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