Sr	extsuperscript{a}, a Private Platelet Antigen on Glycoprotein IIIa Associated With Neonatal Alloimmune Thrombocytopenia

By Hartmut Kroll, Volker Kiefel, Sentot Santoso, and Christian Mueller-Eckhardt

A new platelet alloantigen, Sr	extsuperscript{a}, is described that was defined by an alloantibody detected in the serum of a healthy mother who delivered a child with typical clinical signs of neonatal alloimmune thrombocytopenia (NAIT). The antibody reacted strongly with the child’s and father’s platelets, but not with platelets of the mother or with those of a highly selected panel representing all known platelet alloantigens. Platelets from 300 unselected normal blood donors also tested negative, suggesting a phenotype frequency in the German population of less than 0.01. The antigen was present in 9 of 20 members within three generations of the paternal family, indicating autosomal codominant inheritance. By immunochemical analysis using a glycoprotein (GP)-specific immunoassay and a variety of GP IIb/IIIa-specific monoclonal antibodies for antigen immobilization (MAIPA assay), radioimmunoassay, and Western blotting, we could show that the antigen resides on a 68-Kd proteolytic fragment of GP IIa. Immunogenetic data and gene dosage studies revealed that the Sr	extsuperscript{a} antigen is not related to any of the other known platelet alloantigens. In accordance with established criteria, the Sr	extsuperscript{a} antigen represents the first example of a “private” platelet alloantigen that bears significance in rare instances of NAIT.

© 1990 by The American Society of Hematology.
for 30 minutes at 37°C, washed and solubilized in 0.01 mol/L Tris, 0.5% Nonidet P-40, and 0.154 mol/L NaCl. The lysates were centrifuged (13,000 g for 30 minutes at 4°C) and the supernatants transferred to a microtiter tray coated with a goat antihuman platelet polyclonal antibody (Dianova, Hamburg, West Germany). After incubation for 90 minutes at 4°C the microtiter tray was washed four times with 0.01 mol/L Tris, 0.5% Nonidet P-40, 0.05% Tween 20, 0.5 mmol/L CaCl₂, and 0.154 mol/L NaCl (washing buffer). During this step, the GPs recognized by the mouse MoAb were fixed by washing of the wells and addition of p-nitrophenyl phosphate substrate solution (Sigma, Munich, West Germany). Optical density was measured in a Titertek Multiscan photometer.

During this step, the GPs recognized by the mouse MoAb were fixed by washing of the wells and addition of p-nitrophenyl phosphate substrate solution (Sigma, Munich, West Germany). Optical density was measured in a Titertek Multiscan photometer.

The immunoblotting procedure was modified from Towbin et al.²⁴ Either fresh or stored platelets were washed and solubilized in 0.01 mol/L Tris, 1% leupeptin, and 1% Nonidet P-40, and sonicated for 3 minutes. The platelet proteins were separated on 7.5% SDS-PAGE under nonreducing conditions and electrophoretically transferred to nitrocellulose sheets by the semidry technique of Kyhse-Anderson.²⁵ After blocking with phosphate-buffered saline with 3% bovine serum albumin (3% BSA/PBS) for 60 minutes at 37°C, the nitrocellulose strips were washed three times with 3 mL PBS containing 0.1% Tween 20 (washing buffer) and incubated overnight with 100 to 200 µL of eluates of the human sera diluted in 3 mL of 1% BSA/PBS. After three washings, 3 mL 17-I-labelled sheep antihuman IgG (100,000 cpm; Amersham Buchler, Braunschweig, West Germany) per strip were added for 60 minutes. The nitrocellulose strips were washed three times again, dried, and autoradiographed.

The preparation of eluates for immunoblotting was adapted from the acid elution technique described by Hotchkiss et al.²⁶ Aliquots of 3 x 10⁸ platelets were incubated with 60 to 300 µL human serum for 30 minutes at 37°C, washed three times in isotonic saline, and acidified to a pH of 2.8 by addition of 150 mmol/L acetic acid in 0.1 mol/L NaCl containing 1.5% BSA for 10 minutes. After centrifugation, the pH of the supernatant was adjusted to 7.2 with 2.5 mol/L Tris base.

For removal of blood group A antibodies, 1 mL of serum containing anti-Sr⁺ was absorbed three times (for 60 minutes at 37°C) with 2 mL of packed red blood cells (RBCs) from a blood group A donor.

RESULTS

The serum containing anti-Sr⁺ did not react with a panel of nine donor cells in the LCTT, indicating the absence of HLA-specific lymphocytotoxic antibodies. When tested in the PAIFT using a platelet panel of five typed donors, the reactions were negative; strong fluorescence was observed with paternal platelets. This reactivity was still discernible when serum Sr was exhaustively preabsorbed with blood group A, RBCs. In the MAIPA assay, serum Sr was strongly positive with paternal platelets using the GP Ib/IIIa-specific MoAb Gi 5 for antigen immobilization, whereas the platelets of 28 typed panel donors, including those of a

---

**Fig 1.** Reactivity of anti-Sr⁺ (●) tested in MAIPA assay with five panel platelets and paternal platelets using MoAb Gi 5 for immobilization of the GP Ib/IIIa complex. Antigens of the Zw and Bak system of panel platelets are indicated. Paternal platelets tested without antibody (□) as negative and Zw(a + ) platelets with a strong anti-Zw⁺ (■) as positive control. Anti-Sr⁺ reacted with paternal platelets but not with panel platelets carrying all known alloantigens of the Zw or Bak system on the GP Ib/IIIa complex. OD, optical density.
Yuk(a+b+) individual (kindly made available to us by Dr Shibata, Tokyo) did not react with anti-Sr*. The results of a representative experiment are illustrated in Fig 1. Identical results with serum Sr were obtained when other MoAbs directed against the GP IIb/IIIa complex (MoAbs P2, SZ21, and SZ22) were used for immobilization of this GP complex. Serum Sr failed to react if MoAbs specific for GPs Ia/IIa, 1c/IIa, 1c'/IIa, lb/IX, and HLA class I were used (data not shown). These data indicate that the antibody in serum Sr has specificity for an epitope on the GP IIb/IIIa complex, which is different from all other known platelet alloantigens on this complex.

To further localize the antigen, serum Sr was investigated by immunoprecipitation and immunoblotting. Figure 2 shows the results of immunoprecipitation by anti-Sr* and anti-Zw* in the presence of 3 and 5 mmol/L EDTA. Anti-Sr* precipitated predominantly GP IIIa with a molecular mass of 89-Kd (lanes 3 and 5), much like anti-Zw* (lanes 2 and 4). The slight coprecipitation of GP IIb was abrogated in the presence of 5 mmol/L EDTA (lanes 4 and 5). In immunoblotting experiments using Sr(a+), Zw(a+), and Bak(a+) platelets and antibody eluates (Fig 3), anti-Zw* and anti-Sr* reacted with GP IIIa (lanes 2 and 3), clearly distinct from anti-Bak*, which bound to GP IIb (lane 4). Under reducing conditions both the Zw* antigen and the Sr* antigen were no longer discernible.

When immunoprecipitation was performed after proteolytic digestion of platelets with chymotrypsin and trypsin (Fig 4), anti-Sr* and anti-Zw* precipitated fragments with a molecular weight of 68 and 74 Kd after cleavage with chymotrypsin (lanes 2 and 3) or trypsin treatment (lanes 4 and 5). No fragments were precipitable from the platelet supernatants after enzymatic degradation.

The inheritance pattern of the Sr* antigen in family Sr is presented in Fig 5. Table 1 compiles immunogenetic data of

---

**Fig 3.** Immunoblot under nonreducing conditions of solubilized Sr(a+), Zw(a+), Bak(a+) platelets with eluates from AB-serum as control (lane 1), anti-Zw* (lane 2), anti-Sr* (lane 3), and anti-Bak* (lane 4). Anti-Zw* and anti-Sr* recognized GP IIIa, whereas anti-Bak* reacted with GP IIb. Platelet-reactive antibodies were detected with 125I-labeled sheep antihuman Ig antibody.

**Fig 2.** A 7.5% SDS-PAGE analysis in a nonreduced system of 125I-labeled GP of Sr(a+), Zw(a+) platelets. GPs were immunoprecipitated with anti-Zw* (lanes 2 and 4) and anti-Sr* (lanes 3 and 5). Lanes 2 and 3 were precipitated in the presence of 3 mmol/L EDTA. The slight coprecipitation of GP IIb could be avoided in the presence of 5 mmol/L EDTA (lanes 4 and 5). Both anti-Zw* and anti-Sr* precipitated GP IIIa (89 Kd). Lane 1: 14C molecular weight standard.
all family members tested with regard to ABO and Rhesus blood groups, platelet antigens, and HLA-A, B, C, and DR phenotypes and haplotypes. It is evident that the Sr\(^a\) antigen segregates as an autosomal-codominant trait. Because the offspring of three Sr\((a^+)^\) fathers within two generations were Sr\((a^+)^\) as well as Sr\((a^-)^\), all Sr\((a^+)^\) individuals must be heterozygous for this character. Because both Sr\(^a\) and Zw\(^a\) are apparently localized on the same proteolytic fragment of GP III\(a\) (Figs 3 and 4) and all family members were Zw\((a+b^-)^\) (Table 1), the possibility had to be precluded that Sr\(^a\) represents a third rare allele of the Zw antigen system. For this purpose, we used the known gene dose effect for the Zw\(^a\) alloantigen, which had already been described by Shulman et al.\(^1\). They showed that anti-Zw\(^a\) reacts more strongly with platelets from Zw\((a+b^-)^\) than with Zw\((a^-)^\) homozygous controls. In contrast, platelets from Zw\((a+b^-)^\) unrelated donors bound only half the amount of Zw\(^a\) antibodies.

The frequency of the Sr\(^a\) antigen in the German population was estimated by typing of platelets from 300 unrelated blood donors with anti-Sr\(^a\) in the MAIPA assay. No positive individual was found suggesting an antigen frequency of less than 0.01.

**DISCUSSION**

We here report a new, rare platelet alloantigen. The corresponding antibody was found in the serum of a healthy white mother who gave birth to her first child. Although the neonate presented with the typical clinical features of NAIT, to our surprise no platelet antibodies were detectable on first investigation in maternal serum using a selected platelet panel possessing all known platelet antigens. The observation that the child responded well to IgG therapy further favored the clinical diagnosis. Only on retrospective analysis using the original maternal serum and paternal platelets (cross-match) was it possible to verify the NAIT serologically. This finding clearly emphasizes the necessity to perform cross-matches. Whereas this is common practice in red cell serology, it is seldom applied with platelets. This is particularly true because most reference laboratories practice one or another modification of the immunofluorescence assay for screening, which requires fresh platelets not attainable from...
referred samples. Hence, in patients with clinically suspected
NAIT where none of the already established alloantibody
specificities can be found with a well-typed platelet panel,
techniques must be used that reliably function with stored,
refrigerated platelets, ie, the MAIPA assay or similarly
adapted binding tests.

Antibody Sr reacted with platelets of the affected infant,
his father, and seven additional members of the paternal
family (Fig 5) indicating autosomal-codominant inheritance.
Because none out of more than 300 unselected blood donors
had this antigen, the phenotype frequency of Sr* is less than
0.01 and the chance to detect an Sr* homozygous individual
is extremely low. Incidentally, all members of family Sr
typed Zw(a+b-). Theoretically, the possibility existed that
Sr* would constitute a rare, as yet unknown third allele of the
Zw system. This possibility was precluded by determination
of the Zw* gene dosage of Zw(a+b-) individuals of family
Sr who were either positive or negative for the Sr* determi-
nant as compared with unrelated Sr(a-), but homozygous or
heterozygous Zw* individuals. As illustrated in Fig 6, there
was no significant difference of anti-Zw* binding between
Sr(a+) and Sr(a-) family members, with relative binding

![Graph](image-url)

**Fig 6.** Reactivity of Sr(a+), Sr(a-), Zw(a+b-), and of individuals with anti-Zw* as assessed in
MAIPA assay using the GP IIb/IIIa-specific MoAb
Gi 5 for antigen immobilization. Sr(a+) family
members (□) and Sr(a-) family members (△)
showed the same strength of reaction as Zw*Zw* homozygous
controls (○), whereas Zw*Zw* heterozygous controls (△)
showed only half the reac-
tivity. This reaction pattern indicates that Sr(a+) platelets are Zw*Zw* homozygous and that the Sr* antigen is independent of the Zw antigen system.
(□), Zw(a-) control. OD, optical density.
values equivalent to unrelated homozygous Zw\(^w\)Zw\(^w\) individu-
als, indicating that all family members are Zw\(^w\)Zw\(^w\) homozy-
gous.

Immunochemical studies provided clear evidence that the Sr\(^s\) antigen resides on GP IIIa. The antigen could only be
immobilized in the MAIPA assay by MoAbs directed against different epitopes of the GP IIIb/IIIa complex, eg, MoAb Gi 5 (Fig 1). In radioimmuno precipitation, anti-Sr\(^s\) produced a band of 89-Kd, similar to anti-Zw\(^w\), consistent with GP IIIa in the presence of high EDTA concentrations (Fig 2). After treatment of Sr\(^s\)(a+), Zw\(^w\)(a+) platelets with chymotrypsin or trypsin, the Sr\(^s\) and the Zw\(^w\) antigens were still detectable on a 68- and a 74-Kd fragment of GP IIIa, respectively. The 68-Kd fragment after chymotrypsin treatment probably corresponds to the 66-Kd fragment described by Kornecki et al\(^{32}\) or the 55- to 65-Kd band of Dancis et al\(^{27}\) whereas the 74-Kd band after tryptic cleavage confirms findings of Newman et al.\(^{27}\) The smaller fragments of 17- and 23-Kd masses reported by Newman et al\(^{27}\) and van der Schoot et al\(^{30}\) were not reliably traced. This was most likely due to the enzymatic treatment of purified GPs by the former authors instead of the use of whole platelets, as in our study. A similar discordance has already been noted by Dancis et al.\(^{27}\) In the nonreduced immunoblot, anti-Zw\(^w\) labeled the same GP IIIa band as anti-Sr\(^s\), which under reducing condi-
tions disappeared, again in agreement with previous observa-
tions of Kunicki et al\(^{32}\) with regard to the Zw\(^w\) antigen.

Taken together, our immunogenetic and immunochemo-
cal data indicate that the Sr\(^s\) antigen is a rare (private) antigen on GP IIIa that is not included in any other known platelet alloantigen system on the GP IIb/IIIa complex (ie, Zw, Yuk, or Bak).

Of interest was our observation that the immunized mother Sr carries the HLA antigen DR3, which is known to be found in 70% to 90% of individuals immunized against Zw.\(^{33}\) Although only a single observation, our case strength-
ens the view that HLA-DR3 plays a role as an immune response marker for immunization against platelet alloanti-
gens on the GP IIb/IIIa complex.

ACKNOWLEDGMENT

We thank Marianne Weisheit and Micaela Zickert for technical assistance. We appreciate the assistance of the Platelet Group of the Central Laboratory of The Netherlands Red Cross Blood Transfu-
sion Service in Amsterdam (Dr Albert von dem Borne) for con-
firming the platelet-specific antibody in serum Sr by assessing platelets of six members of family Sr (three Sr\(^s\)[a+], three Sr\(^s\)[a−]) with anti-Sr\(^s\) in the MAIPA assay. Typing results of both laborato-
ries were identical. Serum and platelet samples are available for reference laboratories upon request.

REFERENCES

1. Mueller-Eckhardt C, Kienzlen E: Alloimmune thrombocytope-
3. Shulman NR, Marder VJ, Hiller MC, Collier EM: Platelet and leucocyte iso-antigens and their antibodies: Serologic, physio-
logic and clinical studies, in Moore CV, Brown EB (eds): Progress in Neu-
roimmunology, no. 4. Philadelphia, PA, Grune & Stratton, 1964, p 222
6. Borne AEG Kr von dem, Riesz E von, Verheugt FWA, Cate JW ten, Koppe JG, Engelbert CP, Nijenhuis LE: Bak\(^{a}\), a new platelet-specific antigen involved in neonatal allo-immune thrombocyto-
7. McGrath K, Minchinton R, Cunningham I, Ayberk H: Plate-
et anti-Bak\(^{a}\) antibody associated with neonatal alloimmune thrombocyto-
penia. Vox Sang 57:182, 1989
9. Grenet P, Daussel J, Dugas M, Petit D, Boudoul J, Tanguy Y: Purpurea thrombopénique neonatal avec isoimmunisation foeto-
maternelle anti-Ko\(^{a}\). Arch Fr Pediatr 22:1165, 1965
11. Kiefel V, Santosho S, Glöckner WM, Katsmannz B, Mayr W, Mueller-Eckhardt C: Posttransfusion purpura associated with anti-
Bak\(^{a}\). Vox Sang 56:93, 1980
15. Schneider W, Schraut M: The platelet adhesion immunofluo-
rescence test: A modification of the platelet suspension immunofluo-
17. Santosho S, Lohmeyer J, Renich H, Clemetson KJ, Mueller-
20. Santosho S, Kiefel V, Mueller-Eckhardt C: Immunochemo-
cal characterization of the new platelet alloantigen system Br\(^{a}/Br\(^{b}\): Br J Haematol 72:191, 1989
21. Hemler EM, Ware CF, Strominger JL: Characterization of a novel differentiation antigen complex recognized by a monoclonal


Sra, a private platelet antigen on glycoprotein IIIa associated with neonatal alloimmune thrombocytopenia

H Kroll, V Kiefel, S Santoso and C Mueller-Eckhardt