International Journal of Hematology (2021) 113:945–949
https://doi.org/10.1007/s12185-021-03092-z

CASE REPORT

Down syndrome with neonatal alloimmune thrombocytopenia due to anti-HLA A31 and B61 antibodies

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Received: 15 September 2020 / Revised: 26 January 2021 / Accepted: 28 January 2021 / Published online: 11 February 2021

Abstract

Neonatal alloimmune thrombocytopenia (NAIT) arises from fetomaternal platelet incompatibility that results in transplacental passage of maternal antibodies mostly against fetal human platelet antigens (HPA), whereas NAIT due to anti-human leukocyte antigen (HLA) antibodies is extremely rare. Here, we report a case of Down syndrome (DS) with NAIT that was attributed to HLA antibodies. A boy with DS was delivered at 36 weeks' gestation. His platelet count declined to 13.0×10^9/L, suggestive of NAIT rather than other conditions, including transient abnormal myelopoiesis. Random platelet concentrates and intravenous immunoglobulin administration resolved the thrombocytopenia without clinical complications. Immunoserological investigations detected anti-HLA, but no anti-HPA antibodies in samples from the patient and the mother. HLA typing and cross-matching indicated that anti-HLA antibodies to paternal HLA A31 and B61, which had probably been induced during a prior pregnancy, led to NAIT in this case. Although it is a rare condition, healthcare providers should consider NAIT due to HLA antibodies and be vigilant for subsequent cases in DS.

Keywords Neonatal alloimmune thrombocytopenia · Down syndrome · Human platelet antigen · Human leukocyte antigen

Introduction

Neonatal alloimmune thrombocytopenia (NAIT) is among the most common causes of thrombocytopenia, manifesting around birth by marked thrombocytopenia [1, 2]. The incidence of NAIT is estimated to be 1 in 2000–3000 births [3, 4]. NAIT is caused by fetomaternal platelet incompatibility that results in transplacental passage of maternal antibodies against fetal human platelet antigens (HPA) and/or human leukocyte antigens (HLA). Anti-HLA and anti-HPA have been reported in 24.4% and 0.91% of pregnant women, respectively [5, 6]. However, much fewer reports with very small numbers of cases have described NAIT resulting from anti-HLA antibodies rather than from anti-HPA antibodies.

Down syndrome (DS) was the first described chromosomal disorder and is the most common autosomal trisomy. The incidence of thrombocytopenia in neonates with DS is higher than that in the general population [7]. Moreover, DS may present with hematological disorders such as transient abnormal myelopoiesis (TAM), occurring in approximately 10% of DS neonates [8, 9]. Thus, thrombocytopenia is among the most common hematological abnormalities associated with DS. However, cases of NAIT with DS caused by anti-HLA antibodies have barely been reported [10], although resolving the differential diagnosis of thrombocytopenia is very important to improve outcome of DS.

Here, we report a case of DS with NAIT, attributed to HLA antibodies with A31 and B61 specificity.

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Case report

The mother (gravida 9 para 5) was 38 years old with no history of blood transfusion or autoimmune disease; she was not on any medications. Her pregnancy was uneventful, with negative screening results for toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus, and vaginal group B streptococcus. Complete blood cell counts were normal throughout the pregnancy and her blood type was B Rh (D) positive. Her fetus was suspected of having DS due to brachycephaly by ultrasonography at 20 weeks' gestation. Her platelet (PLT) counts were 265×10^9/L before and 223×10^9/L after delivery.

A boy was delivered by vacuum extraction at 36 weeks' gestation due to late decelerations in fetal heart rate. He weighed 1,936 g (− 2.3 SD), and his height and head circumstance were 46.0 cm (− 0.5 SD) and 31.0 cm (− 1.2 SD), respectively. His Apgar scores were 6 and 7 at one and five minutes. He was admitted to our neonatal intensive care unit shortly after birth due to decreased oxygen saturation and clinical features of DS. Laboratory data demonstrated metabolic acidosis, polycythemia, and thrombocytopenia; chromosomal analysis confirmed 47, XY, + 21 (Table 1).

Table 1 Laboratory values on admission (day 0)

Hemogram		Blood chemistry	
RBC	524×10^4/μL	TP	6.6 g/dL
Hb	19.8 g/dL	Alb	3.7 g/dL
WBC	14,300 /μL	AST	40 IU/L
Neu	61%	ALT	8 IU/L
Lym	34%	LDH	399 IU/L
Mo	3%	ALP	565 IU/L
Eo	1%	T-Bil	1.6 mg/dL
PLT	109×10^9/L	D-Bil	0.2 mg/dL
MPV	10.6 fL	BUN	11 mg/dL
		Cr	0.87 mg/dL
		UA	6.1 mg/dL
Coagulation systems		CRP	< 0.03 mg/dL
PT-INR	1.42	IgG	1428 mg/dL
APTT	58.3 s	IgA	3 mg/dL
Fibrinogen	76 mg/dL	IgM	2 mg/dL
AT-3	41%		
D-dimer	2.6 μg/mL	Karyotype	47, XY, + 21

RBC red blood cells, *Hb* hemoglobin, *Neu* neutrophils, *Lym* lymphocytes, *Mo* monocytes, *Eo* eosinophils, *WBC* white blood cells, *PLT* platelet, *MPV* mean platelet volume, *PT-INR* prothrombin time/international normalized ratio, *APTT* activated partial thromboplastin time, *AT* antithrombin, *TP* total protein, *Alb* albumin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *ALP* alkaline phosphatase, *T-Bil* total bilirubin, *D-Bil* direct bilirubin, *BUN* blood urea nitrogen, *Cr* creatinine, *UA* uric acid, *CRP* C-reactive protein, *Ig* immunoglobulin

There was no evidence of hemorrhage or other abnormal findings by ultrasound of the head and abdomen. As shown in Fig. 1, the initial PLT count on the peripheral venous blood sample was 109×10^9/L. On day 2, the patient had no signs of bleeding, such as petechiae; however, the PLT count declined to 13.0×10^9/L. Therefore, random-donor PLT concentrates were transfused on days 2 and 3. On day 4, the PLT count increased to 107×10^9/L after PLT transfusion. On day 3, immunoserological tests for NAIT were conducted, with positive results on day 4. Intravenous immunoglobulin (IVIG) with 500 mg/kg/day was administered for 3 days. The PLT count increased to 76.0×10^9/L by day 6 and to 225×10^9/L by day 14. Thereafter, the thrombocytopenia did not relapse without clinical complications, and he was discharged on day 26.

Materials and methods

Preserved blood samples from the mother at 36 weeks' gestation and the cord blood at birth were further investigated on day 3. The blood samples from the patient on day 3 and those from the parents on day 3 and 8 months after childbirth were collected, after obtaining written informed consent from the parents for immunoserological testing. Additional informed consent was obtained from both parents to report this case.

HLA genotyping

The blood samples collected from the parents on day 3 and the cord blood at birth were screened for HLA genotyping using the polymerase chain reaction reverse

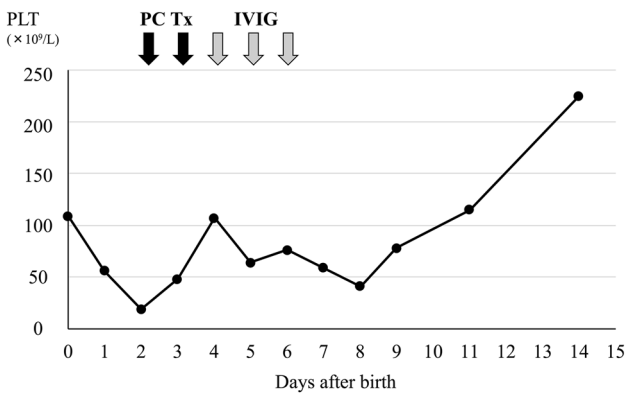

Fig. 1 Platelet counts during the patient's clinical course. The vertical axis shows platelet count and the horizontal axis marks the days after birth. Inverted filled arrow random-donor platelet concentrates (10 mL/kg) were transfused on days 2 and 3. Inverted filled arrow intravenous immunoglobulin was administrated at a dose of 500 mg/kg/day on days 4, 5 and 6. *IVIG* intravenous immunoglobulin, *PC* platelet concentrate, *PLT* platelet(s)

sequence-specific oligonucleotide-Luminex method (Genosearch HLA Kit, MBL, Nagoya, Japan) with a Luminex 100 IS fluoroanalyzer (Luminex Inc., Austin, TX, USA), in accordance with the manufacturer's instructions.

HPA and Nak^a (CD36) antigen genotyping

The blood samples collected from the parents on day 3 and the cord blood at birth were screened for HPA and Nak^a antigen genotyping using a commercially available kit (WAK-Flow HPA typing kit, Wakunaga, Hiroshima, Japan). Platelet antigens of the panel were genotyped for HPA-1 through HPA-7, HPA-15, HPA-21, and Nak^a.

HLA antibody screening, identification, titration, and cross-matching

Serum samples of the mother collected during pregnancy and those of patient collected on day 3 were screened for anti-HLA class I antibodies using the Luminex® method with fluorescent beads coated with HLA-A, -B, and -Cw antigens (One Lambda LABScreen Single Antigen Class I & Supplement, Veritas Corporation, Tokyo, Japan). Maternal serum samples during pregnancy were screened for the antibodies against a panel of platelets using a commercial mixed passive hemagglutination assay (MPHA) kit (ANTI-HPA·MPHA Panel, Beckman Coulter, USA). Maternal serum samples collected during pregnancy were tested against platelets from the paternal blood sample collected on day 3 using MPHA. To distinguish between anti-platelet and anti-HLA class I antibodies, the reactivity of serum against chloroquine-treated platelets was determined. The titer of each serum sample was based on serial dilutions in PBS until a negative test result was obtained. To detect anti-HLA antibody and analyze its specificity, maternal serum samples collected during pregnancy were tested against lymphocytes from the paternal blood sample collected on day 3 with an anti-human immunoglobulin lymphocytotoxicity test using a panel of lymphocytes. Neonatal platelets were not available for testing.

HPA antibody screening and cross-matching

MPHA, enzyme-linked immunosorbent assay (ELISA), and a modified rapid monoclonal antibody (mAb)-specific immobilization of platelet antigens (MAIPA) assay were performed to detect the HPA antibodies for HPA-1 through HPA-7, HPA-15, HPA-21, and Nak^a. Maternal serum samples collected 8 months after childbirth and the preserved cord blood were screened for HPA antibodies using the MPHA, ELISA, and modified rapid MAIPA. Maternal serum collected 8 months after childbirth and the cord blood preserved from the time of childbirth were tested against paternal platelets collected 8 months after childbirth using the modified rapid MAIPA. The preserved blood samples of the mother that were collected during pregnancy were also tested for HPA-15. MPHA was performed using a commercially available kit (ANTI-HPA·MPHA Panel, Beckman Coulter, USA) to detect HPA-1 through -6 and Nak^a. ELISA was performed using a commercially available kit (PAKPLUS, Immucor, Norcross, GA) to detect HPA-1 through -5 and Nak^a. The modified rapid MAIPA protocols were based on the original protocol [11, 12] with four capture mAbs: the antibodies against GP-IIb/IIIa, Ib/IX, Ia/IIa, and CD109.

Results

From HPA and HLA genotyping (Table 2a), maternal-paternal or neonatal mismatch of HLA-A31, B61, Cw15 and HPA-15a were observed. It was confirmed that the paternal platelets or white blood cells strongly reacted to the maternal serum in HLA cross-matching (Table 2b). No anti-HPA antibody was detected in the maternal or neonatal serum and no reactions were observed in HPA cross-matching between paternal platelets and maternal or neonatal serum (Table 2b). These results did not suggest HPA antibody involvement in NAIT. Multispecific anti-HLA class I antibodies were detected in maternal and neonatal serum samples, and the antibodies were specific to HLA-A31 and B61 among the father-derived HLA antigens in maternal serum sample (Table 2c). Hence, along with the results of cross-matching, this patient was diagnosed as NAIT owing to the presence of anti-HLA A31 and B61 antibodies.

Discussion

NAIT is diagnosed based on the presence of IgG alloantibodies in the maternal serum that react with neonatal platelet antigens. In Japan, the most frequently occurring cases of NAIT are caused by HPA-4b [6]. The ratio of positive anti-HLA antibodies increases per pregnancy with raising their titers [5]. However, the incidence of NAIT is about 20 times higher with anti-HPA antibodies than with anti-HLA antibodies [6]. This may be in part explained by the fact that maternal anti-HLA antibodies tend to be attenuated and are difficult to transfer to the baby because they are neutralized by the HLA antigens of the baby that are expressed in the placenta [13].

In the present case, there were HLA-A31, HLA-B61, HLA-Cw15, and HPA-15a mismatches between the mother and the patient. However, the HPA antibody screening and HPA crossmatching did not show the suggestive result of HPA antibody involvement in NAIT. In contrast, anti-HLA

Table 2 Immunoserological investigation

a. HLA genotyping

Antigen	HLA-A		HLA-B		HLA-C	
Mother	A2	A11	B62	B59	Cw1	Cw4
Father	A24	A31	B62	B61	Cw4	Cw15
Patient	A2	A31	B61	B59	Cw1	Cw15

b.. HLA and HPA antibody screening and cross-matching

	Maternal serum	Neonatal serum
Anti-HLA antibody	Positive	Positive
HLA cross-matching against paternal platelets	Positive, titer 512	Not tested
HLA cross-matching against paternal lymphocytes	Positive	Not tested
Anti-HPA antibody	Negative	Negative
HPA cross-matching against paternal platelets	Negative	Negative

c.. HLA antibody identification

	Anti-HLA specificity
Maternal serum	A-23, 29, 30, 31, 32, 33, 66, 74, 80
	B-7, 8, 13, 27, 4005, 41, 42, 47, 48, 55, 60, 61, 73, 81
	Cw-2, 7, 8, 10, 17
Neonatal serum	B-48, 60, 81, 7

HLA human leukocyte antigen, *HPA* human platelet antigen

class I antibodies were detected both in maternal and neonatal serum, although specificities to HLA A31 and B61 antibodies were determined only in the maternal serum. Together with the result of cross-matching, this patient was diagnosed as NAIT due to the anti-HLA A31 and B61 antibodies. Little sample could be taken from the baby, and the antibody was assumed to be consumed by neonatal tissue [14], resulting in the limitation to determine specificities of anti-HLA antibodies in this small patient.

Growth retardation in trisomy 21 fetuses is partly attributable to placental structural abnormalities [15]. The chorionic villi in trisomy 21 were reported to be bigger and more asymmetric and irregular in shape than were normal villi [15]. Dysmorphic villi of trisomy 21 show villous vascular growth. Therefore, maternal anti-HLA antibodies may not be attenuated and may be transferred to the baby because of no neutralization by the baby's HLA antigens that are expressed in the placenta owing to the placental structural abnormalities in trisomy 21. This could be explained by the fact that maternal HLA antibodies result in the development of NAIT. In the present case, the mother had prior pregnancies, and the anti-HLA antibody titer was likely to increase due to HLA antigen stimulation from them [5]. But that alone did not lead to onset. It is thought that placental dysfunction may have existed, and the damaged placenta may have facilitated the leakage of maternal blood.

The incidence of thrombocytopenia in neonates with DS is higher than that in the general population. DS is associated with several hematological disorders, such as TAM. Pediatricians who are involved in the care of patients with DS need to take care of the bleeding and other clinical findings due to hematological abnormalities. NAIT is often asymptomatic or presents with only petechial hemorrhages in the absence of complications, such as intracranial hemorrhage, making it difficult to establish a diagnosis based on the symptoms. As mentioned earlier, DS may have placental structural abnormalities that may predispose the patient to NAIT. If DS is complicated by thrombocytopenia, NAIT should be considered as a differential diagnosis from the beginning, and medical history-taking and a careful clinical examination are crucial for establishing a correct diagnosis. Very few DS case with NAIT due to anti-HLA antibodies has been reported, but NAIT should figure prominently in the differential diagnosis of thrombocytopenia in this patient population.

Acknowledgements We are grateful to the technical staff of the Department of Blood Transfusion and Transplantation Immunology, Fukushima Medical University School of Medicine and Japanese Red Cross Kinki Block Blood Center for their exceptional competence with immunoserological testing.

Compliance with ethical standards

Conflicts of interest None to declare.

References

1. Bassler D, Greinacher A, Okascharoen C, Klenner A, Ditomasso J, Kiefel V, et al. A systematic review and survey of the management of unexpected neonatal alloimmune thrombocytopenia. Transfusion. 2008;48:92–8.
2. Kamphuis MM, Paridaans N, Porcelijn L, De Haas M, Van Der Schoot CE, Brand A, et al. Screening in pregnancy for fetal or neonatal alloimmune thrombocytopenia: a systemic review. BJOG. 2010;117:1335–43.
3. Murphy MF, Williamson LM, Urbaniak SJ. Antenatal screening for fetomaternal alloimmune thrombocytopenia: should we be doing it? Vox Sang. 2002;83:409–16.
4. Taaning E, Skibsted L. The frequency of platelet alloantibodies in pregnant women and the occurrence and management of neonatal alloimmune thrombocytopenic purpura. Obstet Gynecol Surv. 1990;45:521–5.
5. Triulzi DJ, Kleinman S, Kakaiya RM, Busch MP, Norris PJ, Steele WR, et al. The effect of previous pregnancy and transfusion on HLA alloimmunization in blood donors: implications for a transfusion-related acute lung injury risk reduction strategy. Transfusion. 2009;49:1825–35.
6. Ohto H, Miura S, Ariga H, Ishii T, Fujimori K, Morita S. The natural history of maternal immunization against foetal platelet alloantigens. Transfus Med. 2004;14:399–408.
7. Sarah A, Krishna A, Nghiem-Rao TH, Leuthner S, Clark R, Lagatta J. NICU management and outcomes of infants with trisomy 21 without major anomalies. J Perinatol. 2018;38(8):1068–73.
8. Klusmann JH, Creutzig U, Zimmermann M, Dworzak M, Jorch N, Langebrake C, et al. Treatment and prognostic impact oftransient leukemia in neonates with Down syndrome. Blood. 2008;111:2991–8.
9. Ishigaki H, Miyauchi J, Yokoe A, Nakayama M, Yanagi T, Taga T, et al. Expression of megakaryocytic and myeloid markers in blasts of transient abnormal myelopoiesis in a stillbirth with Down syndrome: report of histopathological findings of an autopsy case. Hum Pathol. 2011;42:141–5.
10. Nakamura T, Nomura T, Kamohara T, Takahashi H, Hatanaka D, Kusakari M, et al. Down's syndrome with neonatal alloimmune thrombocytopenia due to HLA-A2 antibody. Fukushima J Med Sci. 2015;61:149–54.
11. Campbell K, Rishi K, Howkins G, Gilby D, Mushens R, Ghevaert C, et al. A modified rapid monoclonal antibody-specific immobilization of platelet antigen assay for the detection of human platelet antigen (HPA) antibodies: a multicentre evaluation. Vox Sang. 2007;93:289–97.
12. Hayashi T, Amakishi E, Matsuyama N, Yasui K, Furuta RA, Hori Y, et al. Detection of antibodies against human platelet antigens 15a and 15b by using a cell line panel. Br J Haematol. 2010;151:402–4.
13. Peterson JA, McFarland JG, Curtis BR, Aster RH. Neonatal alloimmune thrombocytopenia: pathogenesis, diagnosis and management. Br J Haematol. 2013;161:3–14.
14. Okubo M, Nishida E, Watanabe A, Nishizaki N, Obinata K, Azuma F, et al. Marked thrombocytopenia in a neonate is associated with anti-HPA-5b, anti-HLA-A31, and anti-HLA-B55 antibodies. Pediatr Blood Cancer. 2019;66(3):e27555.
15. Qureshi F, Jacques SM, Johnson MP, Hume RF Jr, Kramer RL, Yaron Y, et al. Trisomy 21 placentas: histopathological and immunohistochemical findings using proliferating cell nuclear antigen. Fetal Diagn Ther. 1997;12:210–5.