Case Report

Neonatal Alloimmune Thrombocytopenia Associated with HLA-A11 Alloantibody

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Abstract: Neonatal alloimmune thrombocytopenia (NAIT) is a result of platelet destruction by maternal antibodies directed against paternally-inherited antigens on fetal platelets. NAIT is mostly caused by antibodies to human platelet antigens (HPA). This is a report of a term newborn with NAIT associated with an antibody to the human leukocyte antigens (HLA). The newborn presented with cutaneous bleeding at 5 days of age. The lowest platelet count was 36.8x10⁹/L. An antibody to HLA-A11 was present in maternal serum while HPA antibodies were not detected. The HLA-A11 antigen was identified in the patient, sister, and father, but not the mother. The newborn received intravenous immunoglobulin and had a favorable outcome.

Keywords: • HLA antibody • Human leukocyte antigen • NAIT • Neonatal alloimmune thrombocytopenia

Introduction

Neonatal alloimmune thrombocytopenia (NAIT) is a result of platelet destruction by maternal antibodies directed against paternally-inherited antigens on fetal platelets. The incidence of NAIT is 1:1,000-1:2,000 pregnancies.¹² The most serious complication of NAIT is intracranial hemorrhage, which occurs in 10-30% of patients.¹³⁻⁵ NAIT can occur in the first pregnancy, and recur in approximately 90% of subsequent pregnancies.⁵⁻⁷ The most common cause of NAIT is antibodies to one of the human platelet antigens (HPA). In the western population, antibodies to HPA-1a and HPA-5b are the most common causes, while in the Japanese population antibody to HPA-4b is the most common.¹² HLA antibodies frequently occur in pregnant women, although their role in NAIT has been controversial.⁵ We herein report a case of NAIT associated with HLA-A11 antibody. The antibody reacting to paternal HLA phenotype specific cells was detected in maternal serum. The patient was treated with intravenous immunoglobulin (IVIg) and had a favorable outcome.

Case report

A five-day-old female newborn presented with petechiae scattering over her face and body. She was the second child of the family, born by Caesarean section at 39 weeks of gestation. Her birth weight was 3,344 g. The Apgar scores were 9 and 10 at 1 and 5 minutes, respectively. The 31-year-old mother was healthy and had not received any blood transfusion. Her 2-year-old sister was healthy with no history of NAIT. Physical examination showed a normal newborn with generalized petechiae. She was otherwise well, without hepatosplenomegaly, dysmorphic features or signs of infection.

A complete blood count showed a hemoglobin (Hb) of 15.3 g/dL, hematocrit (Hct) 46.4%, white blood cell...
(WBC) count $7.4 \times 10^9/L$ (neutrophil 71%, eosinophil 3.4%, basophil 1.3%, lymphocyte 15.3%, monocyte 9.2%), and a platelet count of $63.5 \times 10^9/L$. Maternal platelet count was $298 \times 10^9/L$. Later at 6 days of age, the platelet count decreased to $36.8 \times 10^9/L$. The patient was then treated with 5 g (1.5 g/kg) of IVIg. Platelet transfusion was not given. Her platelet count increased to $155.1 \times 10^9/L$ on the next day. After a discharge from the hospital, the patient was clinically well without further bleeding. A cranial ultrasound showed no evidence of intracranial hemorrhage. The platelet count was $529 \times 10^9/L$ and $434 \times 10^9/L$ at 14 and 51 days of age, respectively.

**Materials and Methods**

**Screening for platelet antibodies**

The maternal and the patient’s sera were collected at 5 days after delivery. The sera were screened for platelet antibodies by a qualitative solid-phase enzyme-linked immunosorbent assay (ELISA) (Pakplus, Gen-Probe Incorporated, Waukesha, WI, USA). The test screened for antibodies to HLA class I antigens and to epitopes on platelet glycoproteins IIb/IIIa, Ib/IX, Ia/IIa, and IV. The maternal serum was also screened for platelet-associated immunoglobulin G (IgG) by a solid phase red cell adherence (SPRCA) assay.

**Screening and identification of HLA antibodies**

The maternal serum was screened for HLA antibodies by microlymphocytotoxicity test (LCT), with known panels of T and B lymphocytes which covered the common HLA antigens in Thai populations, and was subsequently tested by the flow-based bead assays (Luminex, One Lambda Inc., Canoga Park, CA, USA) to determine the HLA class I specificity. The patient’s serum was also tested by the flow-based bead assays.

**HPA Typing**

Genotyping of HPA-1 to HPA-7 and HPA-15 of the patient, her parents and sister were performed by using a multiplex polymerase chain reaction as previously described. HLA typing and crossmatching

HLA typing was performed in the patient, her parents and sister. HLA-A and HLA-B were serologically typed by micro LCT with qualified anti-HLA sera (Department of Transfusion Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand). HLA-A, B, and HLA-C alleles were determined by the polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSO) method (Innogenetics, Gent, Belgium). The crossmatching between maternal serum and the patient’s, sister’s, and paternal lymphocytes was performed by micro LCT. The crossmatching between patient’s serum and the sister’s, and paternal lymphocytes was performed by the same method. The crossmatching between maternal serum and paternal platelets was also performed by a SPRCA assay.

**Results**

The results of platelet HPA and HLA genotyping and antibody identification from the patient and her family were summarized in Tables 1 and 2. The ELISA assay and platelet-associated IgG screening showed no detectable antibodies. There were HPA-3 and HPA-15 incompatibilities between the newborn and her mother. HLA antibody screening and identification showed anti-HLA antibodies to HLA class I (HLA-A11) in the mother. HLA crossmatching in the newborn initially showed positive result, but after the serum was treated with dithiothreitol (DTT), the antibody was not detected. This may be explained by a low level of antibody.

Similarly, HLA-A11 antigen was found in the patient (HLA-A*11:01/31:01), sister (HLA-A*11:02/31:01) and father (HLA-A*11:01/11:02), but not in the mother. The crossmatching between maternal serum and the patient’s, sister’s, and paternal lymphocytes by micro LCT showed strongly positive results. The crossmatching between maternal serum and paternal platelets by SPRCA assay also showed positive result. The mother’s blood group was O, Rh(D) positive. The patient’s, sister’s, and paternal blood group were B, Rh(D) positive. To exclude a
Discussion

This is a report of NAIT associated with an HLA antibody. The newborn presented with early-onset thrombocytopenia with cutaneous bleeding at five days of age. The diagnosis was made by the presence of HLA antibody directed to the newborn HLA-A11 antigen. An anti-HLA-A11 antibody detected in maternal but not in the patient’s serum may be explained by a delay in serum collection, at five days of age. The father had HLA-A11 (HLA-A*11:02/11:01), and the elder daughter and the patient had different alleles of HLA-A11. The HLA-A11 antigen presented in both daughters likely caused repeated sensitization and alloimmunization in the mother. The crossmatching between maternal serum and the patient’s, sister’s, and paternal lymphocytes showed positive results. The patient did not have infections or hepatosplenomegaly that could explain thrombocytopenia. She responded well to IVlg.
Of note, our case had HPA-3 and HPA-15 incompatibilities. Although HPA antibodies were not detected, the HPA incompatibilities may weaken our conclusion that the NAIT in this case was caused solely by the HLA antibody. Antibody to HPA-3a was reported to react with labile component of HPA-3a, and only with fresh, unfixed platelets by SPRCA, immunofluorescence test and mixed passive hemagglutination test. The MAIPA assay showed negative result.\(^9\) An ELISA test performed in our case may have failed to detect an antibody to labile component of HPA-3a.

Antibodies to human leukocyte antigen (HLA) are seen in 7-39% of pregnancies.\(^1\) As platelets express class I HLA on the surface,\(^12\) it is conceivable that HLA antibodies are a cause of NAIT. Nevertheless, several large studies have shown that the antibodies are not associated with NAIT.\(^13\) In a prospective study by King \textit{et al}, HLA-antibodies were seen in 31% of 447 maternal sera at delivery, but the presence of antibodies was not associated with the newborns’ platelet count.\(^13\) Sharon \textit{et al} showed that HLA antibodies were detected in 33% of 1,507 maternal sera. Although the antibodies reacted with paternal lymphocytes, none of the newborns had thrombocytopenia.\(^14\) Reasons refuting the role of HLA antibodies include the presence of HLA antigens on fetal placental tissue and all nucleated cells that can adsorb the antibodies, and the reduced HLA expression on neonatal platelets.\(^8\)

On the other hand, Mueller-Eckhardt \textit{et al} reported that in 6.9% of 144 HPA-1A negative mothers of newborns with presumed NAIT, only HLA antibodies were present.\(^1\) This implicated the role of HLA antibodies in NAIT. There are also several case reports of NAIT caused by HLA antibodies.\(^11,16-22\) The evidences are especially strong for the antibody to HLA-A2 and NAIT.\(^11,16\) HLA-A2 is the common HLA antigen in most populations. In this case, the antibody is directed to HLA-A11, which is the common HLA antigen in Thai population (frequency of 27.7%).\(^23\)

In NAIT associated with HLA antibodies, the onset is usually shortly after birth, but may be later to within a few days after birth.\(^16-22\) The platelet counts are moderately to severely decreased. Cases with concurrent neutropenia were reported.\(^21,22\) Treatments of choice for those with severe thrombocytopenia or bleeding are IVIg and maternal or cross-matched platelet transfusion.

In our case, the patient responded well to IVIg without platelet transfusion. There have been several reports of mild cases of NAIT associated with antibodies to HLA who resolved spontaneously or responded to IVIg alone and not needing platelet transfusion.\(^13-21\) Although the mother and patient were not tested for autoimmune diseases, neonatal autoimmune thrombocytopenia was less likely as her mother had no history of autoimmune disease and had a normal platelet count.

Anti-HLA antibody was reported to cause a refractoriness to random platelet transfusion in a newborn who had NAIT from antibody to HPA-1a.\(^24\) Transfusion of maternal platelets was effective in increasing the platelet count. It was suggested that anti-HLA antibodies, although not associated with NAIT, may complicate the treatment by causing refractoriness, and transfusion of maternal platelet was a treatment of choice.

Our case supports the role of HLA antibodies as a cause of NAIT. The condition should be considered in newborns with isolated thrombocytopenia, who have HLA antibodies directed to paternal antigens, no HPA incompatibilities, and other causes of thrombocytopenia excluded.

References


ภาวะเกล็ดเลือดต่ำาจากการทำาลายดวยอัลโลอิมมูนในทารกแรกเกิดที่เกี่ยวของกับแอนติบอดีชนิด HLA-A11

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และ สมพร  โชตินฤมล

1 ภาควิชากุมารเวชศาสตร  งานธนาคารเลือด คณะแพทยศาสตร  มหาวิทยาลัยเชียงใหม
2 งานบริการโลหิตแห  นชาติ สภากาชาดไทย

บทคัดย่อ ภาวะเกล็ดเลือดต่ำาจากการทำาลายดวยอัลโลอิมมูนในทารกแรกเกิด (neonatal alloimmune thrombocytopenia, NAIT) เปนผลจากการทำาลายเกล็ดเลือดโดยแอนติบอดีจากมารดาซึ่งจับกับแอนติเจนบนผิวเกล็ดเลือดของทารกที่รับถายทอดจาบิดา NAIT ส่วนใหญ่เกิดจากแอนติบอดีตองแอนติเจนชนิด human platelet antigen (HPA) งานวิจัยนี้ไดรายงานผูปวยทารกแรกเกิดที่เกี่ยวของกับแอนติบอดีตองแอนติเจนชนิด human leukocyte antigen (HLA)-A11 โดยทางเลือกการเลือกออกที่ผิวหนังเมื่ออายุ 5 วัน จำนวนเลือดต่ำาที่สูง 36.8 x 10^9 ตอลิตร พบแอนติบอดีตอง HLA-A11 ในซีรัมของมารดาโดยไมพบแอนติบอดีตอง HPA พบแอนติเจนชนิด HLA-A11 ในทารก ที่สาว และบิดา แตไมพบในมารดา ทารกไดรับอิมมูนในกลุ่มตินทางหลอดเลือดต่ำาและตอบสนองตอการรักษา

Keywords:
* แอนติบอดีตอง HLA
* แอนติเจน human leukocyte antigen
* NAIT

วารสารโลหิตวิทยาและเวชศาสตรบริการโลหิต 2558;25:149-54.