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BRIEF REPORT
Neonatal Alloimmune Thrombocytopenia due to HPA-9b Incompatibility

Ashok Raj, MD,[1] Vasundhara P. Kailasnath, MD,[2] Salvatore Bertolone, MD,[1] and Janice G. McFarland, MD[3]*

Neonatal alloimmune thrombocytopenia (NAIT) is one of the most frequent causes of both severe thrombocytopenia and intracranial hemorrhage (ICH) in fetuses and term neonates. The diagnosis is established by demonstrating antibodies against human platelet antigens (HPA) and discordance in platelet antigen typing between parents or between the mother and neonate. We report a case of NAIT that was likely due to maternal sensitization to HPA-9b (Max[a]), a recently recognized, rare platelet-specific antigen. Pediatr Blood Cancer 2009;53: 459–461. © 2009 Wiley-Liss, Inc.

Key words: alloimmune thrombocytopenia; alloimmunization; neonate; platelet antigen HPA-9b

INTRODUCTION

Neonatal alloimmune thrombocytopenia (NAIT) is caused by transplacental maternal antibody directed at paternally derived platelet antigens on fetal platelets. The majority of implicated antibodies target platelet-specific alloantigens. Sensitization to the platelet alloantigen human platelet antigens (HPA)-1a accounts for over 80% of serologically defined cases while incompatibility for at least 10 additional alloantigens have been found in the remainder. In cases of fetal/neonatal thrombocytopenia with clinical features consistent with NAIT where incompatibilities for the most commonly implicated antigens have been ruled out, the syndrome may be due to sensitization to rare or private specificities present on paternal and fetal platelets. We report a case of NAIT due to a recently recognized, rare platelet alloantigen.

CASE REPORT

Our patient was a male baby born to a 19-year-old primigravida African-American mother at 41 weeks gestation via spontaneous vaginal delivery. Prenatal evaluation included testing for human immunodeficiency virus and hepatitis B surface antigen, which were negative and VDRL which was non-reactive. The mother was immune to rubella virus, had a positive group B Streptococcus screening test, and was treated with intravenous penicillin prior to delivery. There were no significant prenatal medical or obstetric complications. The family history was negative for any bleeding disorders. The patient was born with Apgar scores of 9 and 10 at 1 and 5 min, respectively, and with a birth weight of 3,448 g. He was active, vigorous, and feeding well. Two hours after delivery, generalized petechiae were noted. On physical examination, he had skull molding, caput succedaneum and petechiae over the face, trunk, and upper and lower extremities. Examination of all other organ systems was unremarkable with absence of hepatosplenomegaly, normal neurologic examination, and absence of signs of infection including temperature instability, respiratory distress, lethargy, poor perfusion, or poor feeding.

Laboratory investigation revealed severe thrombocytopenia with a platelet count of 5×10^9/L and normal white blood cell and red cell counts. The blood group of both mother and baby was A Rh positive. Computerized tomography scan of the head showed a mild right scalp hematoma without evidence of intracranial hemorrhage (ICH). The maternal platelet count was 188×10^9/L. A clinical algorithm showing the differential diagnoses that were considered is shown in Supplemental Figure 1. Random donor platelets were transfused whenever the platelet count was below 50×10^9/L (Fig. 1). Intravenous immunoglobulin G 400 mg/kg/day was given for a total of 5 days and Prednisolone 2 mg/kg/day was given for 7 days. The platelet count at the time of discharge was 88×10^9/L. The patient continues to do well on follow up at 3 months of age with a platelet count of 274×10^9/L.

Samples from both parents were sent to the Platelet and Neutrophil Immunology Laboratory of Blood Center of Wisconsin for detection and identification of platelet-reactive antibodies and platelet antigen genotyping. Maternal serum was tested against both panel and paternal cells in the flow cytometry assay to detect both IgG and IgM platelet-reactive antibodies [1]. Panel cells were selected to represent the majority of platelet-specific alloantigens implicated in NAIT, including HPA-1a and-1b; HPA-2a and -2b; HPA-3a and -3b; HPA-4a; HPA-5a and -5b; HPA-15a and-15b. Platelet glycoprotein (GP) specific antibodies were sought in maternal serum using the Pak-2MP assay (GTI, Brookfield, WI) which detects reactivity against platelet GP Ib-IX, GP IV, and class I HLA; and the Modified Antigen Capture ELISA (MACE) which detects antibodies reactive with GPs IIb/IIIa, and Ia/IIa [2–4]. In addition, maternal serum was tested against paternal platelet GPIIb/IIIa in the MACE to detect reactivity against rare or private specificities. Genotyping of parental and baby's DNA for platelet-specific antigens of the HPA-1, -2, -3, -4, -5, -6, -9, and -15 systems was performed with a multicode PLX assay developed with Eragen Biosciences (Madison, WI) [5–7].

Maternal serum was reactive in flow cytometry against one of two panel cells tested for IgG only and with paternal cells for both IgG and IgM. Testing in the Pak-2 MP assay indicated the presence of antibodies to HLA class I antigens. Testing in the MACE, using immobilized GPs IIb/IIIa and Ia/IIa from panel cells and GP IIb/IIIa

Additional Supporting Information may be found in the online version of this article.

[1]Division of Hematology Oncology and Bone Marrow Transplantation, Department of Pediatrics, University of Louisville, Louisville, Kentucky; [2]Department of Pediatrics, University of Louisville, Louisville, Kentucky; [3]Platelet and Neutrophil Immunology Laboratory, Blood Center of Wisconsin, Milwaukee, Wisconsin

*Correspondence to: Janice G. McFarland, Director, Platelet and Neutrophil Immunology Laboratory, Blood Center of Wisconsin, PO box 2187, 638 N 18th Street, Milwaukee, WI 53201-2178.
E-mail: janicemcfarland@bcw.edu

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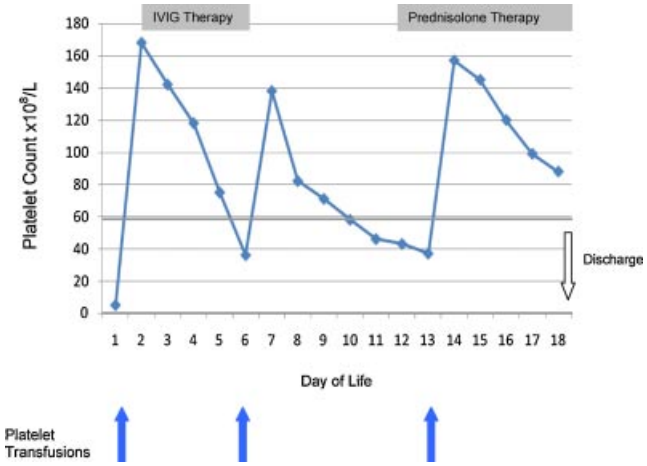

Fig. 1. Platelet counts ($\times 10^9$/L) of the baby during first 18 days of life. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

from paternal platelets was negative. Both parents were blood group type A_1. Platelet alloantigen genotyping revealed incompatibilities for HPA-1b, -3b, and -9b with paternal DNA and for HPA-3b and -9b with the child's DNA (Table I). Maternal serum was absorbed with HPA-3a/a and HPA-9a/a platelets to remove HLA antibody and retested using flow cytometry against in tact HPA-3b/b and paternal platelets. The absorption removed all reactivity against the HPA-3b/b cells but reactivity persisted against the paternal HPA-9a/b platelets, suggesting the presence of anti-HPA-9b antibody.

DISCUSSION

NAIT is the most common cause of severe fetal/neonatal thrombocytopenia and one of the most frequent causes of ICH in this population [8,9]. Testing for this disorder should be performed for any neonate with unexplained thrombocytopenia, regardless of the presumed cause. NAIT is caused by alloimmunization due to feto–maternal platelet antigen incompatibility. Maternal IgG alloantibodies to platelet antigens cross the placenta and bind to fetal platelets [10]. The diagnosis is established by demonstrating antibodies against platelet-specific antigens and discordance in platelet antigen typing between the parents or between the mother and the neonate. Similar to Rh alloimmunization, this disorder tends to worsen in subsequent pregnancies and also as an affected gestation progresses [11,12] (Supplemental Fig. 2).

In Caucasians, HPA-1a and HPA-5b incompatibilities are the most common causes of NAIT [13,14]. Advances in the understanding of HPA and improvements in diagnostic techniques have made it possible to identify maternal–fetal incompatibility for other alloantigens in many NAIT cases, but others go unresolved despite use of the best available diagnostic techniques [13]. Inconclusive serologic evaluation of clinically compelling cases may be due to the absence of rare platelet polymorphisms on laboratory control platelets [15–18]. Moreover, if the testing is performed against whole platelets, other maternal antibodies that are not thought to cause NAIT (e.g., anti-HLA or -ABO) may be detected. These, in turn, might obscure reactivity to the more relevant platelet-specific antigens.

The HPA-9b determinant, discovered in the evaluation of an apparent NAIT case [15], is created by a single nucleotide polymorphism (SNP) in the GPIIb gene, a guanine-to-adenine substitution at position 2602, resulting in a valine-to-methionine substitution in the protein. The inheritance pattern, as that of the other HPA antigens, is autosomal co-dominant [15].

Ethnicity may play a role in the incidence of HPA-9b. With the exception of the current case, the family involved being African-American; all of the reported HPA-9b positive individuals have been Caucasian. However, testing of larger numbers of non-Caucasian individuals will be necessary before any firm conclusions can be drawn about the differences in the incidence of this marker in various ethnic groups.

Demonstration of both an incompatibility for a platelet-specific antigen between the mother and father or neonate and the relevant antibody in maternal serum is generally required to serologically confirm a diagnosis of NAIT. Sensitive and reliable platelet alloantigen genotyping methods are used for platelet typing, and usually, the antibody can be detected using platelet antibody tests that utilize isolated platelet GPs as targets, eliminating possible interfering non-platelet-specific antibodies (e.g., HLA, ABO) that may obscure relevant platelet-specific antibody. However, in some instances the relevant antibody is not detected in GP specific assays, and reactivity in an intact platelet test is used to support the diagnosis, provided steps are taken to remove possible interfering non-platelet-specific antibodies. In the present case, no specific anti-GPIIb/IIIa reactivity was detected in maternal serum against paternal platelets in the GP-specific assay (MACE), as might be expected if anti-HPA-9b were present. However, incompatibility with both the child's and the father's DNA for this rare platelet GPIIb polymorphism was documented, and moreover, testing of maternal serum after absorption to remove anti-HLA reactivity continued to demonstrate reactivity against intact paternal (HPA-9a/b) platelets, but not against HPA-3b/b platelets, suggesting that anti-HPA-9b was indeed present. Previous studies have also reported difficulty in detecting anti-HPA-9b using isolated GPIIb/IIIa in clinically compelling cases of NAIT where other platelet antigen incompatibilities had been excluded [18]. Due to the low-frequency of HPA-9b in the population (<0.5%) and its presence on both paternal and baby's platelets in this case, together with the flow cytometry reactivity in absorbed maternal serum against the father's HPA-9b positive platelets; we believe that the likelihood that this case of NAIT was due to anti-HPA-9b is quite high.

Maternal immunization against HPA-9b may have serious clinical consequences. A recent study reported ICH in three of

TABLE I. Platelet Antigen Genotyping of Parents and Baby

	HPA-1	HPA-2	HPA-3	HPA-4	HPA-5	HPA-6	HPA-9	HPA-15
Mother	1a/1a	2a/2a	3a /3a	4a/4a	5a/5a	6a/6a	9a/9a	15a/15b
Father	1a/1b	2a/2a	3b/3b	4a/4a	5a/5a	6a/6a	9a/9b	15a/15b
Baby	1a/1a	2a/2a	3a/3b	4a/4a	5a/5a	6a/6a	9a/9b	15a/15b

HPA, human platelet antigen. Shading indicates incompatibilities between mother and baby that are possible causes of NAIT in this case.

five cases in which clinical findings were available, suggesting that this severe complication is at least as common in NAIT due to anti-HPA-9b as it is in the more commonly recognized cases due to sensitization to HPA-1a [18]. In conclusion, maternal sensitization against HPA-9b is an important cause of NAIT and should be considered, along with other rarely implicated platelet alloantigens, in cases of apparent NAIT not explained by maternal–fetal incompatibility for more commonly recognized platelet markers.

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