Int J Hematol (2014) 100:398–401
DOI 10.1007/s12185-014-1620-0

CASE REPORT

# A case of neonatal alloimmune thrombocytopenia in the presence of both anti-HPA-4b and anti-HPA-5b antibody: clinical and serological analysis of the subsequent pregnancy

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Received: 7 March 2014 / Revised: 10 June 2014 / Accepted: 11 June 2014 / Published online: 28 June 2014


**Abstract** Neonatal alloimmune thrombocytopenia (NAIT) is induced by maternal alloantibodies raised against fetal platelet antigens inherited from the paternal parent. In contrast to Caucasians, in Asians, predominantly in Japanese, most frequently detected antibodies in NAIT are anti-HPA-4b and anti-HPA-5b. In some NAIT cases multiple alloantibodies are detected. In such cases it is very difficult to determine which antibody is the dominant antibody in NAIT. In this case report, we describe a NAIT case (first sibling) with severe thrombocytopenia and cephalhematoma in the presence of both anti-HPA-4b and anti-HPA-5b antibodies in the maternal serum. We carefully examined titers of anti-HPA antibodies during the subsequent pregnancy with HPA-4b-positive and HPA-5b-negative fetus determined by amniocentesis at gestational week 16. We administered IVIG (1 g/kg/w) to the mother from gestational week 32 to 35. The mother subsequently delivered a second sibling with normal platelet count by cesarean section. Although we could not completely rule out the involvement of anti-HPA-4b, our findings suggested that anti-HPA-5b was implicated in the NAIT in the first sibling.

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**Keywords** NAIT · Anti-HPA-5b · Anti-HPA-4b · Multiple alloantibodies · Amniocentesis · IVIG

## Introduction

Neonatal alloimmune thrombocytopenia (NAIT) is caused by transplacental transfer of maternal alloantibodies raised against fetal platelet antigens inherited from the paternal parent [1]. Antigens capable of triggering NAIT due to a biallelic polymorphism are carried on platelet membrane glycoprotein (GP) Ib-IX-V, GPIIb-IIIa, GPIa-IIa, and CD109. In Caucasians, the most frequent and clinically relevant cause of NAIT is the incompatibility of human platelet antigen (HPA)-1 due to the leucine33/proline33 polymorphism of GPIIIa, and previous analyses on NAIT cases demonstrated that anti-HPA-1a was the dominant alloantibody, and anti-HPA-5b due to the glutamic acid505/lysine505 polymorphism of GPIa was the second most commonly implicated antibody [2, 3]. In contrast, in Asians HPA-1 incompatibility is extremely rare because almost all Asians possess HPA-1a antigen. A prospective study demonstrated that the most common anti-HPA alloantibody raised against fetal HPA was anti-HPA-5b in Japanese. Although anti-HPA-5b antibody induced NAIT in only a few of affected infants, NAIT cases with intracranial hemorrhage due to anti-HPA-5b have been reported [4, 5]. Occasionally, multiple alloantibodies were detected in NAIT cases. In such cases it is very difficult to determine which antibody is the dominant antibody in NAIT.

We have detected both anti-HPA-4b and anti-HPA-5b antibodies in the maternal serum in a case of NAIT with a severe thrombocytopenia and cephalhematoma. Careful examination of the subsequent pregnancy with HPA-4b-positive and HPA-5b-negative fetus determined by

amniocentesis and the delivery of the neonate with normal platelet count suggested that the dominant antibody in the previously affected sibling with NAIT was anti-HPA-5b.

## Methods

### Anti-platelet and anti-human leukocyte antibodies

Anti-platelet antibodies were initially examined by anti-PLT MPHA screen (Beckman Coulter, Tokyo, Japan). Titers of anti-HPA-4b and anti-HPA-5b alloantibodies were further examined by serial dilution in modified monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay employing anti-CD61 and anti-CD49b monoclonal antibodies, respectively. HPA-transfectants instead of platelets were used as the source of HPA, as previously described [6, 7]. The signal to noise (S/N) ratio, defined as the reactivity of alloantibodies with HPA-transfectants (signal)/the reactivity of alloantibodies with mock-transfectants (noise), greater than 2.0 was considered as positive [6].

Anti-HLA antibodies were examined by LABScreen PRA Class I (One Lambda, California, USA).

### HPA and HLA typing

HPA and human leukocyte antigens (HLA) were genotyped using WAKFlow HPA Typing kit (Wakunaga, Hiroshima, Japan) and GenoserachHLA kit (MBL, Nagoya, Japan), respectively, based on the polymerase chain reaction-reverse sequence specific oligonucleotide (PCR-rSSO) method.

## Results

### Case report

A 20-year-old healthy Japanese woman with gravida 3 para 0 delivered a baby (male) weighing 3,032 g with an Apgar score of 8/9 with vacuum extraction at week 38 of gestation. She had no history of transfusion. The first sibling showed huge cephalhematoma (7.5 cm) but no intracranial hemorrhage, and was referred to a city hospital because of anemia (hemoglobin 8.4 g/dL) at day 4. He also showed a severe thrombocytopenia ($12.0 \times 10^9$/L), and was transfused with platelet concentrates as well as red cell concentrates. He was discharged from the hospital, because his anemia and thrombocytopenia gradually improved (hemoglobin 10.1 g/dL, platelet count $91.0 \times 10^9$/L) on day 16. NAIT was considered as the cause of his thrombocytopenia, and the analyzed data were shown in detail in the following section. Eight months later, the mother was referred to Osaka University Hospital to manage her subsequent pregnancy (gravida 4 para 1) because of high risk of NAIT. The HPA and HLA of the second fetus were genotyped employing cells obtained by amniocentesis at week 16 of gestation with written informed consent.

### Alloantibodies in the maternal serum and typing of HPA and HLA

Because of the possibility of NAIT as a cause of the severe thrombocytopenia observed in the first sibling, we examined alloantibodies in the maternal serum. As an initial step, we confirmed that the cross-match test between maternal serum and paternal platelets was positive in mixed passive hemagglutination (MPHA) assay. Anti-HPA antibodies were then screened by anti-PLT MPHA screen kit, and the titers of the antibodies were examined by MAIPA assay employing HPA-transfectants as a source of HPA. Our assays revealed that the maternal serum obtained 5 days after the delivery contained anti-HPA-4b ($\times$1), anti-HPA-5b ($\times$64), and anti-HLA antibodies. MAIPA assay using intact platelets showed that the titer of anti-HPA-5b was $\times$128, which was comparable with the data obtained MAIPA using transfectants ($\times$64). Consistent with the detection of anti-HPA antibodies, significant differences in genotypes of HPA exist between mother and the first sibling: HPA-3, HPA-4, and HPA-5 incompatibilities. In addition, genotyping of rare HPA, such as HPA-6, -7, -15, and -21, denied the possibilities of these rare HPA incompatibilities (Table 1). There was no anti-HPA-3b antibody detected despite HPA-3-incompatibility. Anti-HPA-3b antibody was still undetectable even by MAIPA assay using intact platelets instead of HPA-3b transfectants or by magnetic-MPHA assay using intact HLA-matched platelets instead of fixed platelets. Anti-HLA antibodies contained anti-HLA A24, anti-HLA B54, and additional unspecified antibodies. Any anti-HPA or anti-HLA antibody was undetectable in the first sibling's serum in our assay. Nonetheless, it is probable that the thrombocytopenia of the first sibling was due to NAIT caused by anti-HPA-4b and/or anti-HPA-5b.

Since the subsequent pregnancy after NAIT runs the high risk of NAIT [8], we analyzed HPA and HLA genotypes of the second fetus employing amniotic cells at week 16 of gestation, which was confirmed employing blood cells obtained from the neonate after birth. HPA-3 and HPA-4 incompatibility between mother and the second baby existed, whereas their HPA-5 antigens were compatible (Table 1). As shown in Fig. 1, at gestational week 10 the mother contained anti-HPA-4b with $\times$4 titer and anti-HPA-5b with $\times$32 titer, and the titer of anti-HPA-4b elevated to $\times$16 at gestational week 19. To reduce the

**Table 1** HPA and HLA typing of the family

| HPA type | HPA-1 | HPA-2 | HPA-3 | HPA-4 | HPA-5 | HPA-6 | HPA-7 | HPA-15 | HPA-21 | Nak[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| Mother | a/a | a/a | a/a | a/a | a/a | a/a | a/a | a/a | a/a | + |
| Father | a/a | a/a | b/b | a/b | a/b | a/a | a/a | a/b | a/a | + |
| First sibling | a/a | a/a | a/b | a/b | a/b | a/a | a/a | a/a | n.t. | n.t. |
| Second sibling | a/a | a/a | a/b | a/b | a/a | a/a | a/a | a/a | n.t. | n.t. |

| HLA type | HLA-A | | HLA-B | | HLA-Cw | |
|---|---|---|---|---|---|---|
| Mother | 2 | 2 | 7 | 46 | 1 | 7 |
| Father | 11 | 24 | 54 | – | 1 | – |
| First sibling | 2 | 11 | 46 | 54 | 1 | – |
| Second sibling | 2 | 11 | 7 | 54 | 1 | 7 |

*n.t.* not tested

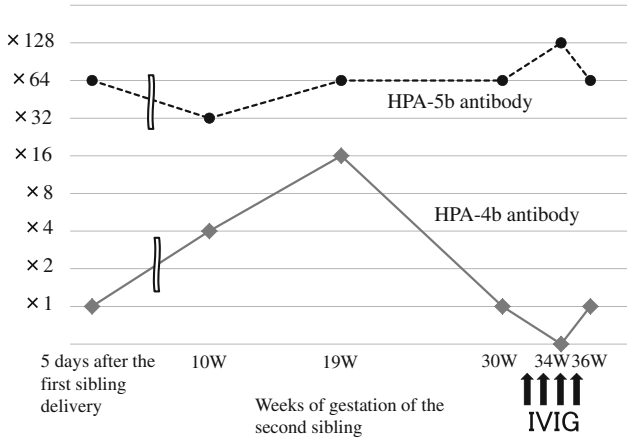


**Fig. 1** Transition of anti-HPA antibodies during pregnancy of the second sibling. Titers of Anti-HPA-4b and anti-HPA-5b alloantibodies were examined by serial dilution in modified monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay employing anti-CD61 and anti-CD49b monoclonal antibodies, respectively. HPA-transfectants instead of platelets were used as the source of HPA

severity of NAIT in the second fetus, we started intravenous immunoglobulin (IVIG, 1 g/kg/w) from gestational week 32 to 35 despite a spontaneous reduction in the titer of anti-HPA-4b to ×1 at week 30 [9]. At 36 weeks the mother was delivered of second sibling by cesarean section. Platelet count of the second sibling (female) in her peripheral blood was within normal range (214 × $10^9$/L), and thrombocytopenia was not developed during follow-up (up to 7 days after birth). In the serum obtained from the second neonate, we detected anti-HPA-5b antibody with ×32 titer, but not anti-HPA-4b antibody.

## Discussion

NAIT cases with severe thrombocytopenia due to either anti-HPA-4b or anti-HPA-5b have been well documented as case reports [5, 10]. In the prospective study in Asians, predominantly in Japanese, anti-HPA antibodies were detected in 0.91 % (223/24,630) of pregnant women's samples, and the most frequently detected antibody was anti-HPA-5b (168 samples), followed by anti-HPA-4b (49 samples). However, the presence of these anti-HPA antibodies did not necessarily induce NAIT. Only 12 % of HPA-5b-positive neonates developed thrombocytopenia (<150 × $10^9$/L) even in the presence of anti-HPA-5b antibodies, while 54 % of HPA-4b-positive neonates developed thrombocytopenia in the presence of anti-HPA-4b antibodies [4]. Thus, anti-HPA-4b antibody appears more potent than anti-HPA-5b to induce NAIT. By contrast, in Caucasians anti-HPA-1a is the dominant antibody, followed by anti-HPA-5b in NAIT, while NAIT due to anti-HPA-4b antibody was very rare [3]. Multiple anti-HPA antibodies were detected in some NAIT cases [3, 4]. In such cases, it is extremely difficult to determine which antibody is dominant in NAIT. In this case report, we demonstrated a NAIT patient whose mother had both anti-HPA-4b and anti-HPA-5b antibodies. In the subsequent pregnancy, the mother delivered a HPA-4b-positive and HPA-5b-negative neonate with normal platelet counts despite the presence of anti-HPA-4b antibody. Although we could not completely rule out the possibility of the incompatibilities of other rare HPA, there were no incompatibilities regarding HPA-6, -7, -15, -21 and Nak[a] (Table 1). Anti-HLA A24 and B54 antibodies were detected in the maternal serum. However, it is unlikely that anti-HLA antibodies contributed to the NAIT, because in our case HLA B54 from the father was present in both siblings. Although both siblings did not possess HLA A24, the mother had anti-HLA A24 antibody without any transfusion history. Thus, the anti-HLA A24 antibody was probably induced by previous 2 pregnancies (2 abortions). Taken together, although the involvement of anti-HPA-4b could not completely be ruled out, the severe thrombocytopenia in the elder sibling is likely due to anti-HPA-5b antibodies.

Administration of IVIG (1 g/kg/w) from gestational week 32 to 35 to the mother in our case may increase the platelet count in the second neonate. However, the effect of IVIG on increasing the fetal platelet count was inconsistent, and mean platelet increase was only $35.7 \times 10^9$/L after 4–6 doses of IVIG [11, 12]. In addition, in our case the titer of anti-HPA4b decreased spontaneously before starting IVIG and maintained low level until the delivery. Thus, the effect of IVIG is only minimal, if any, and it is unlikely that anti-HPA-4b alone has a capacity to induce NAIT in our case. Interestingly, the titer of anti-HPA-5b was $\times 64$ after the birth of the NAIT neonate and maintained high levels ($\times 32$ to $\times 128$) during the subsequent pregnancy even with the HPA-5b-negative fetus, which is consistent with the data that high titer of anti-HPA-5b may be related to the development of NAIT [13]. As the mother possessed anti-HLA A24 antibody in the absence of HLA A24 in both siblings, it is possible that the mother may already be immunized by HPA-5b before the pregnancy of first sibling through previous abortions, which may lead to severe thrombocytopenia in the first sibling.

In addition to the incompatibility of HPA-4 and HPA-5, HPA-3 incompatibility exists between the mother and fetuses (Table 1). Because the genotype of the mother and the father were HPA-3a/a and HPA-3b/b, respectively, fetuses should be HPA-3a/b. However, anti-HPA-3b was never induced during multiple pregnancies (gravida 4 para 2). Neither MAIPA assay using intact platelets instead of HPA-3b-transfectants nor magnetic-MPHA assay using intact HLA-matched platelets instead of fixed platelets detected any anti-HPA-3b. In the prospective study, anti-HPA-3b was not detected in pregnant women's samples with HPA-3b incompatibility (0/3308) [4]. Although anti-HPA-3b induced NAIT cases have been reported, the development of anti-HPA-3b is extremely rare in Asians [3, 4].

In summary, we have reported NAIT with anti-HPA-4b and anti-HPA-5b antibodies. Although anti-HPA-4b appears more potent to induce NAIT, our careful examination of the subsequent pregnancy with HPA-4b-positive and HPA-5b-negative fetus and the delivery of the neonate with normal platelet count suggested that anti-HPA-5b was implicated in the NAIT in the first sibling.

The identification of anti-HPA-5b as the dominant antibody regarding our NAIT case with multiple alloantibodies would contribute to a better understanding of the pathogenesis of NAIT as well as the management of subsequent pregnancies of the mother.

**Acknowledgments** This study was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology in Japan and from the Ministry of Health, Labor and Welfare in Japan.

**Conflict of interest** All authors have no conflict of interest.

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