Delayed detectability of anti-HPA-3a by the MAIPA assay in a severe neonatal alloimmune thrombocytopenia, but successful transfusion of incompatible donor platelets: a case report

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Introduction
Fetal/neonatal alloimmune thrombocytopenia (F/NAIT) is the most frequent cause of haemorrhagic morbidity and mortality in otherwise healthy term infants and occurs in 1 in 1000 to 1 in 2000 pregnancies [1].

Maternal HPA-1a alloimmunization is responsible for the majority of NAIT [1], and only a few cases of NAIT caused by anti-HPA-3a have been published [2–4]. However, based on gene frequencies of HPA-3 in Caucasoids [5], HPA-3 alloimmunization is potentially the most frequent cause of NAIT. Thus, this antigen system may be of minor antigenecity. Alternatively, the frequency of HPA-3 antibodies may be underestimated as a result of difficulties in their detection [2,3].

We describe a term newborn with NAIT caused by anti-HPA-3a; however, serological proof from the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay was delayed for 6 months. Although compatible donor platelets are recommended for NAIT, unmatched platelets were transfused without adverse effects and this led to a transient increase of platelet counts. A second transfusion of a platelet concentrate, matched for the maternal HPA-3 allotype, led to a persistent platelet increment above 50,000/µl. Thus, in suspected NAIT, platelet transfusions may not need to be delayed until serological results are available.

Case report
A 25-year-old healthy primigravida gave birth in the 38th week of pregnancy to a male child with Small for Date Syndrome (1620 g, 44 cm). Petechiae and severe thrombocytopenia (9000/µl) were diagnosed (Fig. 1). Inflammation markers were normal and a connate infection was ruled out. NAIT was assumed, and a dose of 3·6×1010 platelets of a donor genotyped HPA-1b/b, -3a/b was transfused, together with intravenous immunoglobulin (IVIG) (two doses of 0·5 g/kg). Platelets were 119,000/µl 8 h after transfusion, but 19,000/µl on the following day. A paternal–maternal HPA-3a mismatch was revealed by genotyping, and HPA-3b/b platelets were transfused (3·3×1010). Platelet counts rose to 106,000/µl, were maintained at 50,000–64,000/µl during the following 10 days and were normal thereafter. Repeated ultrasound examinations revealed no intracranial or abdominal bleedings during the time of severe thrombocytopenia. The child was discharged with a normal platelet count on day 22.

Materials and methods
The parents and the newborn were typed for HPA-1, -2, -3, -4, -5 and -15 by a polymerase chain reaction sequence-specific primer (PCR-SSP) kit (Inno-Train, Kronberg/Taunus,
Germany). Human leucocyte antigen (HLA) class I and HLA-DRB3 were typed by SSP-PCR using Olerup SSP Kits (GenoVision; Qiagen, Vienna, Austria). Maternal serum was tested for alloantibodies against HPA and HLA class I antigens by commercially available kits: PAKPLUS® and QuikScreen® (GTI, Waukesha, WI, USA), and Capture-P Ready-Screen® (Immucor Inc., Norcross, GA, USA). MAIPA was performed to detect maternal auto- and alloantibodies, as described previously [6], using the following monoclonal antibodies: anti-CD41a (GPIIb/IIIa, clone P2; Immunotech, Marseille, France), anti-CD42a (GPIb/IX, clone FMC-25; Serotec, Oxford, UK), anti-CD49b (GPIa/IIa, clone G19; Immunotech), and anti-β₂-microglobulin (clone B1G6; Immunotech). Donor platelets homozygous for HPA-1a/b, -2a/a, -3a/b, -4a/a, -5a/a, -15a/b; newborn HPA-1a/b, -2a/a, -3a/b, -4a/a, -5a/a, -15b/b; suggesting HPA-3 alloimmunization.

The mother’s HLA class I type was A*24,*31; B*35,*44; HLA DRB3*0202; the newborn was A*24,*33; B*35,*58.

Maternal antibodies after delivery are shown in Table 1. No platelet-reactive antibodies were detectable by the capture enzyme-linked immunosorbent assay (ELISA) (PAKPLUS® and QuikScreen®) immediately after delivery. However, alloantibodies were detected with platelets typed HPA-3a/a or HPA-3a/b by a solid-phase assay with intact platelets (Capture-P Ready-Screen®). In addition, cross-matching maternal serum with paternal platelets and donor platelets typed HPA-3a/a, -3a/b and -3b/b in a solid-phase assay with intact platelets (MASPAT® Kit) revealed strong reactions with HPA-3a/a, weaker reactions with HPA-3a/b, and was negative with HPA-3b/b platelets. These reactions were not caused by HLA class I antibodies.

By MAIPA, only HLA class I antibodies against paternal and donor platelets were detectable immediately after delivery and 8 weeks later. Eight weeks after delivery, weakly reacting antibodies against HLA class I antigens were also detected by the ELISA PAKPLUS®.

**Results**

A feto–maternal mismatch for HPA-3a was revealed (father HPA-1a/b, -2a/a, -3a/a, -4a/a, -5a/a, -15a/b; mother HPA-1a/b, -2a/a, -3b/b, -4a/a, -5a/a, -15b/b; newborn HPA-1a/b, -2a/a, -3a/b, -4a/a, -5a/a, -15b/b; suggesting HPA-3 alloimmunization.

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MAIPA with serum obtained 6 months after birth revealed weakly positive reactions with three different homozygous HPA-3a donor platelets (absorbances at 492 nm = 0.307, 0.220 and 0.288) and negative reactions with a homozygous HPA-3b donor (absorbance at 492 nm = 0.089). Furthermore, antibodies against HLA class I antigens were detectable. The cross-match with paternal platelets was positive for HLA class I antibodies, but not for GPIIb/IIIa.

Discussion

This case report illustrates the difficulties of serological proof of HPA-3 alloimmunization in NAIT. The clinical picture strongly suggested NAIT and we therefore transfused HPA-1bb platelets because HPA-1a alloantibodies are the most important causes of severe NAIT [1]. The results of genotyping governed our further clinical procedures, as only HPA-3 incompatibility needed further consideration, even though the MAIPA assay did not provide serological proof.

Strong evidence for difficulties in the detection of HPA-3 antibodies comes from various workshops on platelet immunobiology and from other reports [2,7,8]. As shown by Goldberger et al. [9] and Calvete et al. [10], the critical component for immune recognition is a three-dimensional structure consisting of the isoleucine-serine polymorphism at position 843 and an O-glycosylation site at serine 847. This carbohydrate residue seems to be a labile component of the epitope because treatment of HPA-3a-typed platelets with sodium dodecyl sulphate and/or neuraminidase modifies GPIIb and thus has been shown to reduce the binding affinity of some HPA-3a antibodies [11]. Our report on a newborn with severe thrombocytopenia caused by HPA-3a antibodies confirms the challenge of verifying alloimmunization against HPA-3a.

The transfusion of incompatible platelets was well tolerated, and the subsequent transfusion from an HPA-3-matched donor was successful. The maternal antibody level seemed to recover early due to the autologous thrombopoiesis. However, multiple serological tests are necessary for the specification of the involved alloantibody as results may influence advice on future pregnancies.

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