Antenatal Management of Severe Feto-Maternal Alloimmune Thrombocytopenia: HLA Incompatibility May Affect Responses to Fetal Platelet Transfusions

By M.F. Murphy, P. Metcalfe, A.H. Waters, J. Ord, H. Hambley, and K. Nicolaides

In feto-maternal alloimmune thrombocytopenia (FMAIT), severe hemorrhage, particularly intracranial haemorrhage (ICH), may occur before delivery. Management strategies to prevent ICH in high-risk pregnancies include maternal administration of intravenous Ig with or without steroids and fetal platelet transfusions. This report describes a patient who lost three fetuses with ICH because of FMAIT due to anti–HPA-1a. ICH occurred earlier in successive pregnancies (at 28, 19, and 16 weeks of gestation) despite maternal treatment with intravenous Ig and steroids from 14 weeks of gestation in the third pregnancy. The fourth pregnancy was managed by administering weekly intraperitoneal injections of Ig to the fetus from 12 to 18 weeks of gestation. At 18 weeks, there was no evidence of ICH, but the fetal platelet count was only 12 × 10^9/L. Serial fetal platelet transfusions were started, but there were poor responses because of immune destruction of the transfused platelets by maternal HLA antibodies. There were improved responses to transfusions prepared from the mother and from HLA-compatible HPA-1a-negative donors. At 35 weeks of gestation, a normal infant was delivered by Caesarean section after 20 platelet transfusions. There was prolonged thrombocytopenia in the baby for 15 weeks after birth, probably due to transfer of HPA-1a antibodies in the transfusions of unwashed maternal platelets. The optimal management of pregnancies likely to be severely affected by FMAIT is still evolving. Intensive management was successful in this case, but a successful outcome cannot be guaranteed in severely affected cases. This is the first time that HLA incompatibility has been found to complicate fetal transfusion therapy.

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FETO-MATERNAL alloimmune thrombocytopenia (FMAIT) is due to incompatibility for human platelet antigens (HPA), most frequently HPA-1a. The exact incidence of FMAIT is unknown, but is in the range of 1 in 1,000 to 5,000 births. The most serious complication is intracranial hemorrhage (ICH); in a study of 137 cases of FMAIT, Kaplan et al reported death in 6.5% and neurologic sequelae in 19%. ICH is most likely to occur during or soon after delivery, but there is increasing evidence of spontaneous ICH in utero in up to 10% of cases. Most cases of in utero ICH have occurred between 30 and 35 weeks of gestation, but there have been recent reports of ICH before 20 weeks of gestation.

There is presently no routine antenatal screening for FMAIT; in most cases, the first affected infant is unexpected. The recurrence rate in subsequent pregnancies is very high and the condition tends to become more severe. The previous history allows some assessment of the likelihood of severe hemorrhage. If a previous sibling has had an ICH, the risk of antenatal ICH is high in a subsequent pregnancy. In cases in which the previous infant was thrombocytopenic, but did not have a major hemorrhage, the risk of antenatal hemorrhage is more difficult to assess. The use of fetal blood sampling to measure the fetal platelet count has allowed the diagnosis and severity of FMAIT in utero to be made with certainty. In addition, this technique provides a means for transfusing compatible platelets to severely affected fetuses.

Therapeutic options for pregnancies at high risk for ICH due to FMAIT include maternal administration of intravenous (IV) Ig with or without steroids and fetal platelet transfusions. Two platelet transfusion strategies are being investigated: a single transfusion before delivery and serial transfusions administered at approximately weekly intervals during the latter part of pregnancy.

This report describes a patient who lost three fetuses with ICH due to FMAIT. ICH occurred earlier with successive pregnancies (at 28, 19, and 16 weeks of gestation). Intensive management of her fourth affected pregnancy was successful.

CASE REPORT

First Pregnancy

The patient (SR) was 29 years of age. A raised level of alphafetoprotein was detected, leading to concern that the fetus might have spina bifida. Ultrasound examination did not show any spina bifida, but was suggestive of severe fetal anaemia, which was confirmed by fetal blood sampling (hemoglobin [Hb], 3 g/dL). There was no evidence for hemolytic disease and the fetus was transfused on 10 occasions, although the Hb level decreased quickly after each transfusion. The fetus eventually developed hydrocephaly and died at 28 weeks of gestation. The diagnosis of FMAIT was not considered at this time.

Second Pregnancy

The patient was 30 years of age. Hydrocephaly was diagnosed at 19 weeks of gestation. The patient was referred to one of the investigators (K.N.). Cordocentesis was performed and the fetal platelet count was less than 10 × 10^9/L. The pregnancy was terminated.

Serologic investigations showed that the mother's platelets typed as HPA-1a negative and that her serum contained HLA and HPA-1a antibodies (Table 1). The father's platelets typed as homozygous HPA-1a positive.

Third Pregnancy

The patient was 31 years of age. The strategy for managing this pregnancy was to use prednisolone (20 mg/d) and IV Ig (1 g/kg once
Table 1. Platelet Antigens and Platelet-Reactive Antibodies Present in the Mother, Fetus, and Father

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th>Fetus</th>
<th>Father</th>
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<tbody>
<tr>
<td>HLA type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HPA-1a</td>
<td>1b/1b</td>
<td>1a/1b</td>
<td>1a/1a</td>
</tr>
<tr>
<td>2nd pregnancy</td>
<td>Present</td>
<td>ND</td>
<td>NT</td>
</tr>
<tr>
<td>3rd pregnancy</td>
<td>Present</td>
<td>ND</td>
<td>NT</td>
</tr>
<tr>
<td>4th pregnancy</td>
<td>Present</td>
<td>ND</td>
<td>NT</td>
</tr>
<tr>
<td>HLA type</td>
<td>A1 A19 (29)</td>
<td>A1 A19 (30)</td>
<td>A1 A19 (30)</td>
</tr>
<tr>
<td></td>
<td>B8 B12 (44)</td>
<td>B8 B13</td>
<td>B8 B13</td>
</tr>
<tr>
<td></td>
<td>Bw4 Bw6</td>
<td>Bw4 Bw6</td>
<td>Bw4 Bw6</td>
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Specificity of HLA antibodies

| 2nd pregnancy (week 19) | A2 | ND | NT |
| 3rd pregnancy (week 16) | A2 + A28 | ND | NT |
| 4th pregnancy (week 22) | A2 + A28 | Present | NT |

Abbreviations: ND, not detected; NT, not tested.

The patient was 32 years of age. In view of the increasing severity of FMAIT in successive pregnancies and the occurrence of ICH at 16 weeks of gestation in the previous pregnancy, it was decided to administer Ig to the fetus by ultrasound-guided intraperitoneal (IP) injections. Endobulin (Immuno) was administered. The volume transfused was 7 mL and the concentration of platelets was 2,800 × 10^9/L. There were no complications resulting in platelet consumption. However, it was subsequently found that responses to transfusions from the mother (SR) and from one HPA-la-negative donor (DD) were better than from other HPA-la-negative donors (DK, RM, MD, and PN) (Fig 1). This suggested an immune mechanism for the poor transfusion responses.

The mother's serum was tested against platelets from each HPA-la-negative platelet donor using the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) technique (see Results). No additional platelet-specific antibodies were detected, but positive results due to HLA antibodies were found when crossmatching the maternal serum and fetal plasma against platelets from all of the HPA-la-negative donors used for the initial fetal transfusions except donor DD.

Once HLA incompatibility had been identified as the cause of the poor responses to fetal platelet transfusions, platelets from the mother or HLA-compatible HPA-la-negative donors, usually donor DD, were used. Good responses to fetal transfusions were achieved and transfusions were administered at approximately weekly intervals (Fig 1).

At 35 weeks of gestation, a fetal platelet transfusion was administered, raising the fetal platelet count to 216 × 10^9/L and delivery was planned for the following day. A 2.5-kg infant was delivered by Cæsarean section and the postdelivery platelet count was 185 × 10^9/L.

However, there was prolonged thrombocytopenia after birth and no sign of spontaneous recovery until 13 weeks (Fig 2). A bone marrow aspirate performed during this period on day 40 showed increased numbers of megakaryocytes in a hypercellular marrow. Fourteen compatible platelet transfusions were administered in the postnatal period. It is noteworthy that the transfusion of unwashed platelets from the mother was followed by a prolonged thrombocytopenia in the infant’s platelet count.

**Materials and Methods**

**Platelet Serology**

Lymphocytotoxicity test (LCT). A comprehensive panel of 90 typed lymphocytes was used in the LCT to determine the specificity of HLA antibodies.

MAIPA. MAIPA was used to detect HLA antibodies in fetal plasma and maternal serum, and for HPA-1 typing and detection of platelet-specific antibodies. The assay was modified to prevent false-positive reactions due to antimouse antibodies in human sera by first incubating the platelets with the human serum or plasma to be tested and then washing twice with PBS/EDTA before incubating with a mouse monoclonal antibody (MoAb). Results are expressed as absorbance at 405 nm; increasing absorbance indicates increasing strength of reaction.

The following MoAbs against platelet glycoproteins (Gp) were used in the MAIPA: anti-GpIb/IIa (NIB 85/661; N.I.B.S.C., Potters Bar, UK), anti-GpIa/IIa (G19; Immunotech, Marseille, France), anti-GpIIb/IX (FMC 25; Chemicon; and AN51; Dako, High Wycombe, UK), and anti-HLA class I (W6.32; Dakopatts).

HPA-1a typing was performed using known antisera from patients with posttransfusion purpura. HPA-1b typing was performed using serum from patient IT (kindly provided by Dr E. Taaning).
Fetal Blood Sampling

Fetal blood sampling and platelet transfusions were performed using ultrasound-guided cordocentesis as previously described.\(^1\)

Platelet Concentrates

Platelet concentrates were prepared by plateletpheresis of the mother and unrelated HPA-1a-negative donors using the Cobe Spectra cell separator (Cobe, Lakewood, CO), which was programmed to reduce the final volume of the concentrate. This increased the concentration of platelets by nearly four times that obtained using the standard technique (up to \(5,400 \times 10^9/L\)). The advantage of this procedure was that no further concentration of the platelet concentrates was required, even for the very early fetal platelet transfusions. All platelet donors were cytomegalovirus (CMV) seronegative. Each platelet concentrate was gamma-irradiated with 1,500 cGy to reduce the risk of transfusion-associated graft-versus-host disease. Platelet concentrates were transfused within 24 hours of collection, using a standard platelet-giving set.

The volume of the platelet concentrate for each transfusion was calculated using the formula:

\[
\text{Volume} = \frac{\text{Desired Increment} \times \text{Fetoplacental Blood Volume}}{\text{Platelet Count of Concentrate} \times \text{Recovery Factor}}
\]

A recovery factor of 2 was used, based on the findings of a previous study in which the immediate platelet increment was approximately 50% of that expected.\(^1\) The fetoplacental blood volume for gestational age was derived from previously constructed charts.\(^1\)

RESULTS

Platelet and HLA Typing

The HPA-1 and HLA types of the mother, father, and fetus are shown in Table 1.

Maternal and Fetal HPA-1a Antibodies

HPA-1a antibodies of weak to moderate strength were detectable in maternal serum from the time the mother was first investigated after her second pregnancy. The strength of the maternal HPA-1a antibodies did not change during the third and fourth pregnancies.

Anti–HPA-1a was not detectable in any of the fetal samples tested.

Maternal and Fetal HLA Antibodies

Specificity. Maternal HLA antibodies in the second and third pregnancies had specificity for only nonpaternal HLA antigens (Table 1), suggesting that they had formed in response to the fetal red blood cell transfusions administered in the first pregnancy. In the fourth pregnancy, the specificity of the maternal HLA antibodies against nonpaternal HLA antigens broadened due to the fetal platelet transfusions. In addition, antibodies developed against HLA-B13, which was present on paternal and fetal cells.

Fetal plasma samples were tested for HLA antibodies using the MAIPA, as fetal serum was not available for testing in the LCT. HLA antibodies were not detectable in fetal samples...
at the end of the second and third pregnancies (Table 1). In the fourth pregnancy, HLA antibodies were detectable in fetal plasma tested at 22, 28, and 34 weeks of gestation. The fetal HLA antibodies had similar specificity in the MAIPA to the maternal antibodies. However, anti-B13 (against B13 on fetal cells) present in the mother was not detectable in the fetal plasma. It should be noted that all of the fetal samples were taken after a previous transfusion of unwashed maternal platelets (Fig 1).

**Strength of reaction.** The maternal HLA antibodies increased in strength from when she was first tested at the end of her second pregnancy to the end of the fourth pregnancy (Fig 3).
In the fetus, HLA antibodies were not detected during the second and third pregnancies. The strength of reaction increased during the fourth pregnancy and decreased after delivery.

**HLA Crossmatches**

The mother’s serum and the fetal plasma were crossmatched in the MAIPA for HLA antibodies with platelets from the first nine HPA-la-negative donors used for the fetal transfusions (Fig 4). Positive reactions and poor responses were obtained with all donors except for donor DD and the mother herself. The mother [A1 A19(29) B8 B12(44) Bw4 Bw6] and the donor DD [A1 A3 B8 B16(39) Bw6] shared the antigens A1 and B8. However, she did not produce antibodies against the mismatched antigens A3 and B16(39) despite the repeated fetal platelet transfusions.

Platelets from donor JW were crossmatched with maternal serum and fetal plasma taken at 24 and 28 weeks of gestation. The results showed a weak reaction with the early samples and a stronger reaction with the later samples; these results correlated with a better response to the platelet transfusion from this donor on the first occasion at 24 weeks (see JW1, Fig 4) than on the second occasion at 28 weeks (see JW2, Fig 4).

**DISCUSSION**

Although FMAIT has no long-term adverse effects in about 75% of cases, it does nevertheless cause considerable morbidity and mortality, due mainly to ICH. It had been assumed that the risk of ICH was greatest at birth, but it is now realized that ICH may occur before delivery. Intrauterine ICH is most likely to occur in the last trimester, but this report and two previous reports have documented ICH before 20 weeks of gestation. The occurrence of severe thrombocytopenia at such an early stage of pregnancy is not surprising as it is known that HPA-la is already expressed by 16 weeks of gestation. Management of the pregnancy at risk of FMAIT is therefore more exacting than previously envisaged.

This report describes a patient who lost three fetuses with ICH because of FMAIT due to anti–HPA-la. ICH occurred earlier in successive pregnancies (at 28, 19, and 16 weeks of gestation). In the fourth pregnancy, treatment was started at 12 weeks of gestation, with weekly IP injections of Ig to the fetus. Fetal blood sampling was performed as early as possible (at 18 weeks of gestation) and the fetus was found to be severely thrombocytopenic, although there was no evidence of ICH. It is uncertain as to what extent this treatment contributed to the successful outcome in this case.

Fetal platelet transfusions were started at 18 weeks of gestation, as early in the pregnancy as possible. There were no complications with the 20 procedures up to the time of delivery, except on the first occasion when bleeding from the cord puncture site occurred, necessitating transfusion of maternal red blood cells. Initially, the main problem was the unexpectedly poor responses to transfusions from unrelated HPA-la-negative donors. The responses were better using maternal platelets and serologic testing showed that the poor responses to unrelated HPA-la-negative donors were due to HLA incompatibility. Subsequent transfusions from HLA-compatible HPA-la-negative donors resulted in improved responses.

HLA antibodies have a high incidence in pregnant women, and although they are usually IgG, there is no evidence that they harm the fetus. This is because antibodies against fetal HLA antigens are absorbed by HLA antigens on the placenta; only HLA antibodies against HLA antigens not expressed by the fetus will pass into the fetal circulation. In the present study the mother’s serum contained HLA antibodies against both paternal and nonpaternal antigens. As the mother had not been transfused herself, the antibodies against nonpaternal antigens were presumably stimulated by
the fetal red blood cell transfusions in her first pregnancy and by platelet transfusions in the last pregnancy. The poor responses to platelet transfusions from unrelated HPA-1a-negative donors before the transfusion of maternal platelets suggests that HLA antibodies had already crossed the placenta by 18 weeks of gestation.

This is the first time that HLA incompatibility has been found to complicate fetal platelet transfusion support. It may cause great difficulty in selecting HLA-compatible HPA-1a-negative donors. In the case reported here, only 4 of 75 HPA-1a-negative donors were found to be HLA compatible, and some of these were unsuitable for other reasons, such as CMV seropositivity or poor venous access. On several occasions it was necessary to use the mother as the donor because compatible donors were unavailable. The maternal platelet concentrates were not washed because of the difficulties in manipulating small volumes of highly concentrated platelets and the consequent risk of inducing irreversible aggregation and impaired platelet function.

Another feature of this case was the prolonged thrombocytopenia in the neonate lasting for up to 12 weeks before the onset of spontaneous recovery. The cause of this is uncertain, and may have been related to the prolonged transfusion support in utero. Notwithstanding this, the transfusion of unwashed maternal platelets at about 4 weeks almost certainly contributed to the subsequent decrease and delayed recovery in the baby’s platelet count. This was presumably due to the transfer of maternal HPA-1a antibodies.

In summary, the antenatal management of pregnancies at high risk for ICH due to FMAIT is still evolving. Further clinical studies are needed to determine the most effective management strategy, which may prove to be a combination of noninvasive treatment, such as maternal Ig and steroids, and fetal platelet transfusions. This report shows that it is possible to consider treatment from a very early stage of pregnancy in those cases at high risk for ICH. Although there was a successful outcome in this case, no guarantees can be given at this stage when counselling parents about the likely outcome in similar high-risk pregnancies.

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