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

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In feto-maternal alloimmune thrombocytopenia (FMAIT), severe hemorrhage, particularly intracranial hemorrhage (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 intrauterine 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 $12 \times 10^9$/$L$. 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 a prolonged thrombocytopenia in the baby for 15 weeks after birth, probably due to transfer of HPA-1a antibodies in the transfections 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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a week) from 14 weeks of gestation. However, an ultrasound scan at 16 weeks showed that ICH had already occurred. The fetal platelet count was less than \(10 \times 10^9/L\) and the pregnancy was terminated.

At this stage, the woman was advised that it was extremely unlikely that she could have an unaffected fetus even using all currently known forms of treatment. Undeterred by this, she became pregnant again.

**Fourth Pregnancy**

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 88 mL (0.5 g) at 14 weeks, 8.0 mL (0.4 g) at 16 weeks, and 10 mL (0.5 g) at 17 weeks of the pregnancy. There were no apparent complications of this treatment.

At 18 weeks of gestation, the ultrasound scan showed no evidence of ICH. A further dose of 10 mL (0.5 g) Ig was administered IP. Fetal blood sampling was then performed (platelet count, \(12 \times 10^9/L\)) and a transfusion of platelets from an HPA-la-negative donor was administered. The volume transfused was 5 mL and the concentration of platelets was \(5,200 \times 10^9/L\). There was some bleeding from the puncture site and the transfusion.

Further fetal blood sampling was performed 7 days later and the fetal platelet count was only \(8 \times 10^9/L\) (Fig 1), much lower than expected from our previous experience. 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 trough in the infant's platelet count.

**MATERIALS AND METHODS**

**Platelet Serology**

*lymphocytotoxic 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-la 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-GplIib/IIIa (NIB 85/661; N.I.B.S.C., Potters Bar, UK), anti-GplIa (Gi9; Immunotech, Marseille, France), anti-Gplb/IX (FMC 25; Chemicon; and AN51; Dako, High Wycombe, UK), and anti-HLA class I (W6.32; Dakopatts).

HPA-la typing was performed using known antisera from patients with posttransfusion purpura. HPA-la typing was performed using serum from patient IT (kindly provided by Dr E. Taaning).
Fig 1. Fetal platelet transfusions during the fourth pregnancy of patient SR, showing pretransfusion and posttransfusion fetal platelet counts. The donors (initials) were all HPA-1a negative; donor SR was the mother.

**Fetal Blood Sampling**

Fetal blood sampling and platelet transfusions were performed using ultrasound-guided cordocentesis as previously described.17

**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 x 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.13 The fetoplacental blood volume for gestational age was derived from previously constructed charts.18

**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, and the occurrence of severe thrombocytopenia at such an early stage of pregnancy is not surprising as it is known that HPA-1a 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
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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.

ACKNOWLEDGMENT

We thank Prof T. A. Lister and Sister Emily Okukenu (ICRF Department of Medical Oncology, St Bartholomew’s Hospital), Dr E. Caffrey (Regional Blood Transfusion Centre, Cambridge), and Dr L. Paton (Regional Blood Transfusion Centre, Tooting) for providing compatible donors and undertaking plateletelapheresis. We thank Olwyn Edmondson (Regional Blood Transfusion Centre, Brentwood) for assistance with the HLA serology. We also thank the many platelet donors who contributed to the successful outcome of this case, particularly those who donated on several occasions.

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