Would it be possible to prevent HPA-1a alloimmunization to reduce the incidence of fetal and neonatal alloimmune thrombocytopenia?

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) has been considered the platelet (PLT) equivalent of hemolytic disease of the fetus and newborn (HDFN).[1] These conditions are caused by destruction of fetal PLTs or red blood cells (RBCs), respectively, by maternal alloantibodies that have crossed the placenta. Knowledge and awareness of FNAIT and treatments for this disease have lagged behind those for HDFN because of the relatively low incidence of FNAIT (86 per 100,000 live births in a recent review[2] of data[2-8]) compared to HDFN (approx. 1%, before it became preventable) and early discovery of the cellular pathogenesis of HDFN that led to introduction of Rh immune globulin prophylaxis (RhIG) in 1968.[9] This therapy has been incredibly successful at preventing HDFN, which raises the question of whether it would be possible to do the same for FNAIT?

The following discussion is restricted to the antibodies responsible for the great majority of cases of HDFN and FNAIT among Caucasian populations, anti-D and anti-human PLT antigen (HPA)-1a, respectively. Both diseases can result in death or lifelong disability. With HDFN, severe neurologic complications are due to the destruction of brain stem basal ganglia by bilirubin toxicity occurring with severe jaundice in neonates (kernicterus). More variable neurologic impairment such as blindness and cerebral palsy is caused by intracranial hemorrhage (ICH) in FNAIT. In countries with stringent anti-D prophylaxis programs, the incidence of these encephalopathies is now similar with HDFN[10] and FNAIT,[11] approximately 2 per 100,000 births. Underreporting occurs with both diseases.[11,12] Despite some similarities, there are contrasting features of the clinical presentation, antibody response, and current therapies of these two alloimmune cytopenias, listed in Table 1.

Several curious differences between the generation of maternal anti-HPA-1a and anti-D responses are as yet unexplained. First, in several studies, FNAIT often occurs in primigravidae,[13] while this is rare for HDFN.[9] This observation suggests immunizing factor(s) are present during HPA-1a–incompatible pregnancies, in addition to HPA-1a–positive fetal PLTs.[14] Second, unusually for an alloantibody response, HPA-1a immunization and FNAIT are highly restricted to women with HLA Class II DRB3*0101 (91%) or DQB1*0201 (94%).[15] (Approximately one third of the population have these alleles, which are also associated with some autoimmune diseases.) Third, anti-HPA-1a concentrations in alloimmunized women persist after pregnancy,[16] whereas anti-D gradually declines within months or years of immunizing events.[17] Fourth, although anti-HPA-1a is responsible for approximately 80% of cases of FNAIT,[18] it is rarely stimulated by transfusion.[19] In contrast, anti-D is the most frequent antibody produced after mismatched (but ABO-compatible) transfusion and pregnancy. Finally, while IVIG administration to pregnant women can prevent ICH in FNAIT, it has little or no effect in ameliorating HDFN.[20]

FNAIT is usually diagnosed after the birth of an affected baby, by the presence of widespread petechiae or purpura but occasionally after ICH or a perinatal death. Subsequent pregnancies are managed predominantly by IVIG infusion to the mother.[21] The dose and duration of IVIG are dependent on the likely severity of fetal thrombocytopenia, assessed by previous history. Administration of steroids and/or intrauterine transfusion (IUT) of HPA-1a–negative PLTs (after fetal blood sampling to determine PLT count) is performed only in a few selected cases.[22] The management options have changed little in 20 years but the choice has greatly shifted toward the increased use of IVIG that has largely replaced fetal interventions except for the most severe cases.[23,24] There is as yet no primary prevention for FNAIT.

To introduce antenatal screening programs to detect either HPA-1a–alloimmunized women (0.1%-0.2%) or at-risk women (HPA-1a negative [≥2%], perhaps also being DRB3*0101 positive, i.e., approx. 0.7% of all pregnancies), treatment must be available. The current therapy given to women with antibodies would be IVIG.

IVIG is expensive. Currently it is only used for HPA-alloimmunized women identified as a result of affected pregnancies. The cost per pregnancy treated (approx. $100,000-$400,000) depends on the dose (1 or 2 g/kg/week), weeks of treatment (10-25 weeks), and manufacturer of IVIG.[22] If a screening program were adopted and a cheap low dose were to be given to all alloimmunized women (2 per 1000) this therapy would cost $800 million in the United States (£100 million in the United Kingdom).

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TABLE 1. Clinical features, antibody characteristics, and current treatments for anti-HPA-1a FNAIT and anti-D HDFN

Factors that differ	FNAIT caused by anti-HPA-1a	HDFN caused by anti-D
Susceptible (at-risk) pregnancies	Approx. 2% of women (HPA-1a–negative) (<1% also DRB3*0101 positive)	Approx. 10% of women (D–)
Occurrence of disease	First pregnancy, frequently	Second and subsequent pregnancies, usually
Antibody response after incompatible transfusion	Anti-HPA-1a is rarely formed; anti-HPA-5b > -1b > -5a predominate	Anti-D is most frequent
Effect of alloantibodies	Thrombocytopenia	Anemia, hemolysis
Causes of fetal death	Intracranial hemorrhage	Heart failure, hydrops
Causes of neonatal death	Intracranial hemorrhage	Kernicterus (bilirubin encephalopathy)
HLA association with antibody response	>90% HLA DRB3*0101 >90% HLA DQB1*0201	Weak or no association (conflicting data)
Routine screening of pregnant women	None	First trimester; D phenotype
Antibody detection	Postnatally, usually, after birth of thrombocytopenic baby	First and third trimester, by routine testing
Antibody concentration during pregnancy	Some correlation with severity of FNAIT	Good correlation with severity of HDFN
Postnatal antibody	Remains for years	Declines after months
Noninvasive fetal diagnosis of cytopenia	None	Doppler ultrasonography; fetal hematocrit (anemia)
Treatment given to alloimmunized pregnant women	IVIG with or without steroids; dose and duration determined by previous history	None
Treatment of babies in severe cases	Fetal IUT and neonatal transfusion	Fetal IUT, neonatal exchange transfusion, phototherapy
Prevention of immunization	None	Antenatal and postnatal RhIG (anti-D prophylaxis)

At-risk women would have to be screened more than once during pregnancy to detect those with new antibodies. Additional hidden costs include time and personnel required in a clinic or home health care service for the infusions, which require approximately 6 hours, administered once or twice a week. Often the IVIG is not well tolerated. The immune system of pregnant women is adapted so they do not become immunosuppressed while being actively tolerant of their semiallogeneic fetuses and is in a state of mild systemic inflammation with heightened innate immunity and enhanced antibody responses.[25] These patients may therefore be at greater risk of adverse effects of high-dose IVIG than nonpregnant patients.[26] Headaches are not uncommon and, rarely, inflammation of the brain occurs that may lead to aseptic meningitis. Prednisone, given to counter side effects of IVIG, has additional adverse effects including hyperglycemia, which could lead to secondary effects in the fetus. The use of IVIG in pregnant women is thus not inexpensive or benign. Would increased use be desirable or affordable?

The benefit of IVIG is that it prevents severe bleeding in fetuses and neonates with FNAIT, thus usually avoiding risks associated with invasive procedures such as amniocentesis, fetal blood sampling, and IUT.

An alternative, more progressive therapy, would be a prophylactic antibody, mimicking anti-D prophylaxis (RhIG) for preventing HDFN. This approach would remove or destroy fetal PLTs present in the maternal circulation. Primigravidae would need to be screened early to detect susceptible patients that are HPA-1a negative and without anti-HPA-1a. The prophylactic antibody would be a small dose (milligrams) of IgG anti-HPA-1a, given antenatally in the second and third trimesters and also postnatally, with the aim of preventing immunization of the mother by fetal HPA-1a–positive PLTs acquired by fetomaternal hemorrhage.

This idea is behind the work of Tiller and colleagues[27] presented in this issue of **TRANSFUSION**. The authors describe experiments using GPIIIa-deficient mice. They found that after transfusion of human HPA-1a–positive PLTs, production of mouse anti-human PLT antibodies was reduced approximately 50% by concurrent intravenous injection of human anti-HPA-1a IgG. Similar results were obtained when injecting murine anti-GPIIIa antisera just after transfusion of murine wild-type (GPIIIa-positive) PLTs, with approximately 80% reduction of anti-GPIIIa. The passively administered antibodies also ameliorated disease severity by increasing PLT counts and preventing ICH in newborn pups, when compared to pups from immunized mothers not given antisera. A dose–response effect was observed in these experiments, however, unlike the complete prevention of D immunization achieved with anti-D prophylaxis. Nevertheless, this article on antibody-mediated immunosuppression marks the beginning of research toward prevention of FNAIT.

Despite these encouraging results, development of a prophylactic anti-HPA-1a will not be straightforward. Information accrued from clinical studies of polyclonal, monoclonal, and recombinant anti-D has shown some of the problems that may arise. In addition, important differences in the pathogenesis of FNAIT and HDFN will affect the conduct of clinical trials.

There are insufficient numbers of HPA-1a–negative women immunized by pregnancy to provide enough high-

titer plasma to fractionate anti-HPA-1a immunoglobulin for treatment of patients at risk. This shortfall could not be made up by immunizing HPA-1a–negative individuals with HPA-1a–positive PLTs, because they would very rarely make anti-HPA-1a.[19] (The reason for this could be because GPIIIa on placental syncytiotrophoblast may be required to initiate the anti-HPA-1a response.[14]) Therefore, a monoclonal anti-HPA-1a would be required.

That in itself is not a problem, except that glycosylation of antibodies has a major effect on their functional activity in vivo. This issue was apparent after 20 years of clinical studies with anti-D; none of the anti-Ds produced from animal cell lines were shown to clear D+ RBCs with appropriate kinetics or to prevent D immunization. Presumably, nonhuman sugars or sequences of the oligosaccharide component of these antibodies are incompatible with the human innate immune system, which can detect foreign sugars with great sensitivity by a variety of cellular receptors, lectins, and antibodies.[28] In contrast, monoclonal anti-D produced by human cell lines and having human glycosylation was safe and effectively prevented D immunization in a similar manner to RhIG.[29] Unfortunately, despite this early success, these human monoclonal antibodies (MoAbs) are not being used because regulatory authorities currently favor rodent cell lines for manufacture of recombinant glycoproteins for clinical use.

It is not known what the optimum glycosylation of an antibody is for mediating rapid but not inflammatory clearance of blood cells by IgG Fc receptors on splenic macrophages.[30,31] Production, characterization, and testing of glycoform variants of the IgG would be a lengthy project. Several in vitro bioassays such as phagocytosis and antibody-dependent cell-mediated cytotoxicity assays have been used to study the functional activity of anti-Ds,[32] but not for PLT antibodies, so these would have to be devised.

Once candidate anti-HPA-1a MoAbs are produced, autologous and allogeneic studies of their ability to clear PLTs could be performed in humans. Testing their effectiveness at preventing HPA-1a alloimmunization in normal volunteers would, however, be more difficult, because antibody responses to HPA-1a–positive PLTs are seldom encountered in transfused patients.[19]

Currently, insufficient RhIG is available worldwide to meet current demand,[33] which is likely to increase with the introduction of more antenatal prophylaxis programs.[34] Fortunately, implementation of noninvasive free fetal *RHD* screening could exclude approximately 40% of pregnant D– women from unnecessary RhIG.[35] Provision of inexpensive, safe, and effective monoclonal anti-D and anti-HPA-1a for antenatal and postnatal prophylaxis against both HDFN and FNAIT would require a multidisciplinary approach, international collaboration, and very considerable investment. However, this therapy would be a major advance in reducing both fetal disease and the adverse effects of current management of pregnant women. If the obstacles outlined above could be surmounted, prevention of these two diseases may become more equivalent.

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CONFLICT OF INTEREST

None.

Belinda M. Kumpel, PhD
e-mail: belinda.kumpel@nbs.nhs.uk
Bristol Institute for Transfusion Sciences
NHS Blood and Transplant
Bristol, UK

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