Mechanisms of fetal and neonatal alloimmune diseases

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A B S T R A C T

For more than 60 years, HLA antigens have been known to be highly polymorphic and responsible for immune reactions towards foreign cells and tissues. It has always been a mystery how a semi-allogeneic fetus can be accepted by the mother for nine months. Allografts are usually rejected, and continuous immunosuppression is necessary to keep the allograft accepted by the host. In 1953, Medawar suggested that there was a protective barrier between mother and fetus that hindered any contact between fetal and maternal cells. It is now well established that there is bilateral passage of cells and organelles between the fetus and the mother. A normal pregnancy, therefore, requires a natural suppression inducing tolerance, both in the fetal and maternal immune systems. If such tolerance is incomplete, alloimmunity may be induced. The manifestation of the clinical conditions caused by alloimmunization depends on the target of the maternal alloantibodies. Here, maternal alloimmune responses toward fetal antigens on red cells, platelets and neutrophils will be discussed.

Learning goals

At the conclusion of this activity, participants should:
- have an understanding of the fetal immune system;
- understand the changes in the maternal immune system upon pregnancy;
- be able to describe the brake of tolerance related to alloimmunization in pregnancy;
- understand the mechanism of RhD immunization and immunization with platelet and neutrophil antigens.

Normal immune function during pregnancy

The mother

The physical barrier between the mother and fetus consists of just one layer of endothelial cells. In addition to nutrients, molecules related to metabolism and IgG antibodies, and even intact cells can easily pass in both directions. Analyses of fetal lymph nodes from the first trimester show maternal microchimerism inducing fetal regulatory T cells (Tregs) towards maternal alloantigens.[1] Maternal microchimerism in lymphoid organs of children may also be detectable years after birth.[2]

The many changes that take place during a normal pregnancy include alteration of the maternal immune response toward an active state of tolerance, especially in the decidua where the maternal and fetal tissues meet.[3] Pregnant women respond normally to vaccines and the level of IgG antibodies in plasma does not change significantly during pregnancy.[4] Failure of immune modulation during the 1st trimester is associated with spontaneous miscarriage.[3] In women with underlying immune-related diseases, changes in the maternal immune system may be clinically evident, affecting the mother's health for better or worse. It is generally observed that pregnant women with multiple sclerosis or rheumatoid arthritis have less disease activity during pregnancy and that the condition of mothers with systemic lupus erythematosus often worsens during pregnancy.[5,6] All three diseases have immune-related pathophysiology.

Expectant mothers are generally not more prone to infections than non-pregnant women. However, certain infections may have a more severe course and may have detrimental consequences for the pregnant mother or the fetus.[7,8] It has also been shown that pregnant mice reject skin grafts from the father, although slightly later than non-pregnant controls.[9] The immune tolerance of pregnancy, therefore, does not completely prevent alloimmunity.

Immune modulation during pregnancy can be divided into either local changes in the fetal-maternal interface in the uterus or changes detectable in the peripheral circulation. Natural killer cells (NK cells) are important players in the pregnant uterine environment. NK cells are normally categorized as part of the innate immune system and are typically involved in the immune response against tumor cells, viruses and allogeneic cells.[10] NK cells can either be killer cells [antibody-dependent cell cytotoxicity (ADCC), cytokine-mediated cytotoxicity] or modulate the immune response to avoid the killing of target cells. Interactions between molecules on target cells and on NK cells direct the function of the NK cells [killer cell immunoglobulin-like receptors (KIRs)]. Uterine NK cells are more numerous in decidua at the time of ovu-

lation, and are crucial for normal implantation of a fertilized egg and placentation during the 1st trimester.[11] In decidua, maternal NK cells are exposed to invading trophoblasts of fetal origin. Trophoblast cells do not express the highly polymorphic HLA A and B antigens, but rather the less polymorphic HLA C, G and E antigens.[12] Normally, NK cells kill target cells lacking HLA class 1 antigens. During pregnancy, interaction between HLA C, E and G on invading fetal trophoblasts and KIR receptors on maternal NK cells inhibit an aggressive immune response and killing of target cells.[12,13] Certain combinations of maternal decidual NK cells and fetal HLA-C allotypes are thought to be important to ensure fetal tolerance.[14] Cytokines produced by the NK cells induce Tregs upon stimulation with fetal alloantigens. The normal maternal immune response to fetal alloantigens is, therefore, hyporesponsiveness and tolerance induced by NK cells and T cells. The increased number of Tregs both in the peripheral circulation and, in particular, around the membranes in samples from expectant mothers indicate local tolerogenic immunity in the uterine environment.[3]

Fetus

Fetal blood cells are already detectable from the first weeks of gestation, initially in the yolk sac and, from five weeks gestation, in the aorto-gonado-mesonephros region. All immune cells develop from hematopoietic stem cells; B cells mature in the bone marrow and T cells mature in the thymus from late in the 1st trimester onwards. This maturation is necessary for mature B and T cells to become functional as immune cells. Antigen-presenting cells are also necessary for the immune response to take place, and the most efficient antigen-presenting cells are dendritic cells originating from myeloid precursor cells.

In 2008, Mold *et al*. published a study of cells extracted from fetal lymph nodes showing that T cells populate fetal lymph nodes from as early as ten weeks of gestation. Highly efficient alloreactive Tregs have also been shown to be present at an early stage inducing tolerance to maternal alloantigens.[1] The overall fetal immune response may be considered as tolerogenic.[3] Exposure to maternal alloantigens during fetal life may, theoretically, induce fetal alloimmune reactions towards maternal antigens if there is a break in tolerance.[15]

The fetus does not produce significant amounts of antibodies, and IgG antibodies present in the fetus and newborn are mainly of maternal origin. IgG antibodies can cross the placenta upon binding to FcRn receptors in syncytiothrophoblasts. The different IgG subclasses have different affinity for the receptor (IgG_1> IgG_4>IgG_3>IgG_2)[16] (see also Vidarsson in this Education Program). By term, the level of IgG_1 in fetal circulation exceeds the maternal level. Since the half-life of IgG is 21 days, the neonate is protected by maternal-derived antibodies until its own immune system has matured. The drawback of such transplacental IgG transportation is that harmful maternal alloantibodies also have access to the fetus.

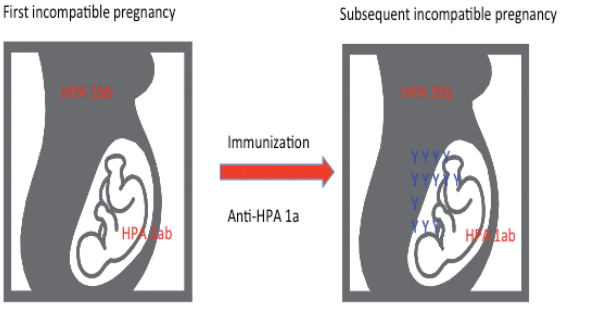
Fetal and neonatal alloimmune diseases

Fortunately, most of the time, the maternal immune system tolerates semi-allogeneic fetal antigens, and no signs of alloimmune responses can be detected after pregnancy. This is quite surprising, considering the presence of paternally-derived allogenic fetal cells in the mother during pregnancy, particularly during delivery when fetal maternal hemorrhage regularly occurs.[17] In the minority of pregnancies where alloimmunization does occur, alloantibodies targeting fetal antigens may be detected in maternal plasma. These are IgG class antibodies. From this we can conclude that antigen presentation has taken place, maternal T cells have been stimulated, and that maternal B cells have differentiated into long-lived IgG-producing plasma cells. If the immune response takes place during pregnancy, the antibodies may lead to disease in the fetus and/or neonate. On the other hand, if the mother is immunized during delivery, the antibodies may affect the fetus and neonate in future antigen incompatible pregnancies (Figure 1).

Hemolytic disease of the fetus and newborn

When the fetus has inherited a paternal red blood cell antigen that the mother lacks, the mother may become alloimmunized and produce alloantibodies toward the 'foreign' red blood cell antigen. The most common example is RhD alloimmunization. Maternal anti-D IgG antibodies can cross the placenta and opsonize fetal red blood cells for destruction in the reticuloendothelial system. In severe cases, the fetus may develop hemolytic disease of the fetus and newborn (HDFN), characterized by severe anemia, possibly progressing to fetal hydrops and fetal death if untreated.[18]

Hemolytic disease of the fetus and newborn was probably already described in the 16th century, but it was not until the 1930s that the idea of antibody-mediated disease was discussed, and ten years later the description of Rhesus (Rh) blood group system with the exact pathophysiology of HDFN was elucidated.[19,20] The overall natural risk of RhD immunization is around 13% in RhD-negative women.[21] In the 1940s, 50% of children born with HDFN died. ABO incompatibility was found to pro-



Cell type	Most frequent antibody specificity	Sign	Severe clinical symptom
Red cell	Anti- RhD	Anemia	Kernicterus
Platelet	Anti-HPA 1a	Thrombocytopenia	Hemorhage (ICH)
Neutrophil	Anti-HNA 1a-d, -2, 4a, 5a	Granulocytopenia	Infections

Figure 1. Immunization takes place most often at time of delivery and antibodies will affect the next incompatible fetus. The consequences of antibodies towards different blood cells are listed.

tect from severe consequences of anti-D, probably due to the decreased half-life of D-positive cells destroyed by maternal naturally occurring anti-A or anti-B IgM antibodies. In these mothers, only 2% were immunized by the RhD antigen.[21] This led scientists to believe that fast removal of the antigen in women at risk, but who were not immunized during pregnancy, would decrease the chance of immunization and thereby the frequency of HDFN. In the late 1960s, it was clearly shown that post-delivery injection of anti-D to RhD-negative women who had given birth to an RhD-positive child, effectively prevented immunization.[22] One per cent of women at risk were immunized probably due to immunization during pregnancy. The risk of immunization was shown to be further decreased to 0.1%-0.2% upon introduction of antenatal anti-D injection (gestational week 28-32) to RhD-negative women.[23] As a result of worldwide use of anti-D prophylaxis, the number of fetuses and neonates affected by HDFN is nowadays very small. In most Western countries, there are screening programs to identify RhD-negative women early in pregnancy and screening for anti-D antibody development in those found to be RhD negative. In pregnancies where anti-D antibodies are detected, the pregnancy is monitored carefully with repeated measurement of anti-D antibody level and ultrasound examination by fetal medicine specialists in cases with high and/ or rising antibody levels to look for signs of fetal anemia. In severe cases, fetal anemia may be detected using ultrasound by measuring middle cerebral artery (MCA) peak blood flow: MCA flow 1.5 or more times higher than normal is often a trigger for consideration of intrauterine transfusion (IUT) of RhD-negative blood.[24] IUT must be repeated every 2-3 weeks until delivery at 35-36 weeks gestation. In a few cases, exchange transfusion is needed in neonates with severe anemia and increased bilirubin from hemolysed red blood cells in order to prevent kernicterus.[18,25] The mechanism of anti-D in prevention of immunization has not yet been fully elucidated.[26-28] The anti-D prophylaxis program is one of the most successful stories of preventive medicine in the fetus and newborn. It should not be forgotten that other red cell alloantigens are sometimes the cause of HDFN.[29] In many countries, all pregnant women have a blood sample screened for alloantibodies to red blood cell antigens.

Fetal and neonatal alloimmune thrombocytopenia

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is discussed in detail by Ghevaert in this Education Program. Here, a brief overview and some new data are provided. In some cases of miscarriage, stillbirth and severe neonatal thrombocytopenia, with or without intracranial hemorrhage (ICH), antibodies targeting fetal platelet antigens are detected in the mother. In more than 80% of cases involving Caucasian women, the antibodies react with human platelet antigen (HPA)-1a. The mechanism of immunization and the pathophysiology of FNAIT has been carefully studied[30,31] and resembles that of HDFN in many ways.[32]

In the platelet membrane, there are many receptors that are essential for the hemostatic process. These receptors have amino acid substitutions (allotypes) defined by single nucleotide polymorphisms (SNPs). Around 17 antigen systems with two allotypes (HPA-a as the most frequent, HPA-b as the less frequent), have been defined to date.[33] HPA-1a is by far the most immunogenic. Two percent of Caucasians have the platelet type HPA-1bb and women with this platelet type are at risk of immunization in a pregnancy where the fetus carries the HPA-1a antigen. Characteristically, the women at risk also carry the HLA class II antigen DRB3*01:01. It has been carefully studied how HPA-1a peptides bind to these HLA class II molecules and why HPA-1b molecules do not bind.[31] The HPA-1a antigen is not only present on platelets; the vitronectin receptor ($\alpha V\beta 3$), which is present on invading fetal trophoblasts, also houses the HPA-1 antigen epitope. It has been suggested that fetal trophoblast may also be a source of antigenic stimulation.[34] Whether the placental function could be affected by the binding of anti-HPA-1a antibodies to HPA-1a antigen in the placenta is currently being studied. Reduced birth weight in boys from HPA-1a alloimmunized pregnancies may reflect this.[35] It is presumed that all pregnant women at risk are exposed to the HPA-1a antigen during pregnancy and delivery. Nevertheless, in women carrying HPA-1b and DRB3*01:01, immunization with anti-HPA-1a production is only detectable in 30% of the women at risk,[36] meaning that tolerance to fetal antigens is the normal situation even in HPA-incompatible pregnancies. Immunization and antibody production can thereby be considered as a break of tolerance. HPA-1bb and HLA DRB3*01:01 are clearly not the only factors determining the risk of immunization. There is growing evidence that in many pregnant women there is a subclinical inflammation present in the placental villae.[37] This may represent a danger signal for the break of tolerance to fetal antigens and maternal immunization. A large prospective screening study showed that 25% of women are immunized during pregnancy and 75% during delivery.[38] Whether those who are immunized post-partum elicit the same tolerogenic state compared to those immunized during pregnancy is not yet clear.[39]

HPA-1a immunization is present in 1:1-2000 pregnancies in the Caucasian population.[36,40,41] In 10% of the severe cases symptoms and signs of hemorrhage can be detected, ICH being the most feared complication (1:10-25,000 neonates).[41,42] ICH may occur as early as the 2nd trimester or after delivery, but most bleeding episodes have been reported to occur in the latter half of pregnancy.[43] Most children suffering ICH do not survive, or they survive with severe life-long neurological disabilities.[43,44] Prophylaxis based on the same principle as for prevention of HDFN, is about to go into clinical trials (www.profnait.eu) (see Ghevaert in this Education Program). One approach is to give anti-HPA-1a hyperimmune IgG within 72 h after delivery to HPA-1a-negative women who are not already immunized, with the aim of preventing immunization as a result of delivery. If this is successful, the next step would be to add antenatal prophylaxis to prevent the remaining 25% of immunizations. All HPA-a antigens have been identified as potentially immunogenic in pregnancies, although HPA-1a immunization is the most frequent and results in the most severe thrombocytopenia.[45]

Anti-HLA class I antibodies are detected in approximately 30% of women who have been pregnant before.[46,47] This means that break of tolerance in the mother for fetal HLA class I antigens is the most common finding related to alloimmunization in pregnancy. Maternal anti-HLA class 1 antibodies show associations to recurrent miscar-

riage,[48] but have otherwise not received attention as a source of pathology during pregnancy. Platelets have HLA class I molecules on their surface. In some cases of FNAIT, no other cause of thrombocytopenia except high levels of maternal anti-HLA class I antibodies can be detected.[47,49] Studies are ongoing to clarify whether or not HLA class I antibodies can induce FNAIT, and if the specificity and level of antibodies are important for developing alloimmune thrombocytopenia.

Since systematic screening for platelet-specific antibodies is not carried out, most FNAIT cases will be diagnosed upon birth of a child with severe thrombocytopenia or bleeding. Compatible platelet transfusions are the treatment of choice, with or without additional IVIg. Random donor platelet concentrates may be used until compatible platelet concentrates are available.[50] In the next pregnancy, high doses of IVIg may be given to the mother during pregnancy if the previous child had severe symptoms of FNAIT.[51,52] Intrauterine platelet sampling and intrauterine transfusions are generally not recommended due to the risk of bleeding complications and further immunization.[52] The ultimate goal is to develop prophylaxis to avoid HPA-1a immunization. If FNAIT prophylaxis for HPA-1a is shown to be efficient, antenatal screening that can identify the women at risk must be introduced as part of the general perinatal health care program. These points are discussed in more detail by Ghevaert in this Education Program.

Fetal and neonatal alloimmune neutropenia

Around 8% of neonates in neonatal intensive care units have neutropenia on admission (ANC $<0.5 \times 10^9$/L).[53] One cause of neonatal neutropenia is alloantibodies to human neutrophil antigens (HNA). The frequency of fetal and neonatal alloimmune neutropenia (FNAIN) is probably around 1:1000, but no large prospective studies have been performed. Anti-neutrophil antibodies develop as a consequence of HNA-alloimmunization during pregnancy or delivery. The most common specificities are anti-HNA-1a-d, -HNA-2, -HNA-4a and -HNA-5a. In the HNA-1 system, 4 alleles have been described while in the HNA-2 system HNA-2a is the only allele described and those who become immunized possess 2 null alleles. In the other HNA systems, two alleles are present. The mechanism of immunization has not been further studied, but maternal exposure to alloantigens during pregnancy or delivery and a break of natural tolerance to fetal antigens are probable explanations.

There is no screening for HNA antibodies during pregnancy. Neutropenia is most often detected as a result of neonatal infection or as a result of the routine differential white blood cell count in the newborn. Treatment is related to infection (antibiotics) and in some cases granulocyte-colony stimulating factor is used. Neutrophil counts normalize within days to weeks.[54]

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