Review of Fetal and Neonatal Immune Cytopenias

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Abstract: The fetoplacental interface plays a unique role in pathologies of the fetus and neonate, and is increasingly being recognized for effects on fetal and neonatal development that resonate into adulthood. In this review, we will use several exemplary disorders involving each of the 3 types of blood cells to explore the effect of perinatal insults on subsequent development of the affected cell line. We will present new data regarding outcomes of infants treated prenatally for fetal and neonatal alloimmune thrombocytopenia (FNAIT) and contrast these with outcomes of infants affected by hemolytic disease of the fetus and newborn. We also will explore the differences between FNAIT and passively transferred antibodies, as seen in maternal idiopathic thrombocytopenic purpura. Neonatal hemochromatosis is an example of a disease that previously was largely fatal, but whose newly discovered etiology as an immune-mediated perinatal disorder has resulted in development of highly effective treatment. Finally, we will examine the interplay between lymphopoiesis and the placenta in an effort to further explore the phenomenon of neutropenia in preeclampsia, whose etiology remains unknown.

Introduction

Hematologic pathologies in the neonatal period are a unique and particularly fascinating group of disorders owing to their multifaceted origins. In some cases, they arise from abnormalities inherent in fetal hematopoiesis. In other cases, they result from an abnormal fetoplacental interface, which may be caused by insults from the placenta itself or by culprits of maternal origin that manage to circumnavigate the placental protective mechanisms and reach their fetal targets. This review focuses on pathologies that stem largely from the latter mechanism. In addition to exploring the various immune-mediated disorders commonly found in the fetus and neonate, we will discuss the effects of these diseases and their treatment on subsequent hematologic development in the neonate.

Neonatal alloimmune thrombocytopenia (NAIT), in which maternal antibodies traverse the placenta and destroy fetal platelets,

is a devastating disease when it leads to intracranial hemorrhage. Fortunately, effective treatment is available using antenatal intravenous immunoglobulin (IVIG) and corticosteroids. We will present original data demonstrating the excellent outcomes in infants treated prenatally for NAIT with IVIG and/or corticosteroids. These treatments are less effective in hemolytic disease of the fetus and newborn (HDFN), a disease of seemingly similar pathophysiology, in which maternal antibodies against the Rh antigen result in the destruction of fetal red blood cells (RBCs). We will illustrate the effects of the established treatment of HDFN on fetal and neonatal hemoglobin using a complex case seen at our center. In maternal idiopathic thrombocytopenic purpura (ITP) affecting the neonate, the role of breastfeeding in perpetuating or worsening the ITP will be considered. Shifting to neonatal leukopenia, we will briefly discuss the effect of preeclampsia on the developing fetal white blood cell lineage. Finally, we will discuss neonatal hemochromatosis, a disorder with nearly universal mortality rates that recently has entered the realm of autoimmune diseases based on the remarkable results seen with IVIG treatment.

Overview of Fetal Hematolymphopoiesis

In order to provide a background for understanding neonatal hematologic pathology, we will start with a review of fetal hematopoiesis. This intricate and highly sophisticated process involves several different embryonic structures, including the thymus, bone marrow, liver, and extraembryonic yolk sac. The most primitive hematopoietic cells first appear as erythroid cells in the yolk sack on day 18 of gestation, emerging from hemangioblast precursors that also can differentiate into endothelial cells. These "first wave" progenitor cells are considered *primitive erythroblasts*, and differ significantly from later RBCs in terms of both size—the estimated mean corpuscular volume is greater than 400 fL—and persistence of the nucleus. The second wave of yolk sac–derived progenitor cells are known as *burst-forming units*. They are seen in the yolk sac as early as 4 weeks, and then seed the fetal liver by 5 weeks of gestation. Beyond 7 weeks of gestation, hematopoiesis takes place primarily in the liver.[1]

Unlike the yolk sac, the fetal liver houses multiple lineages. Megakaryocytes and granulocyte precursors can be found in the liver by 6 weeks of gestation. Between weeks 7 and 9 of gestation, platelets and leukocytes can be found in the fetal circulation. Lymphopoiesis occurs in the thymus and liver starting at 9 weeks of gestation, with B cells preceding T cells in appearance in the circulation. The bone marrow begins to play a role in hematopoiesis in weeks 10 to 11, with a progressively increasing ratio of myeloid to erythroid cells; the adult ratio of 3 to 1 is achieved by 21 weeks of gestation. Beyond the 24th week of gestation, the bone marrow remains the primary site of hematopoiesis.

Thrombopoiesis occurs via unique mechanisms involving cells of different ploidy and derivation of many mature cells from 1 large ancestral cell. Megakaryocytes are derived from the basic pluripotent stem cell precursor. Various cytokines stimulate differentiation into the *megakaryocyte–erythroid progenitor* cell, capable of further differentiating into erythroid or megakaryocyte lineage cells. Once the cell is committed to thrombopoiesis, it initially produces low-ploidy megakaryocytes. Recent work has suggested that in the setting of rapid growth occurring in the fetus and then neonate, it is more efficient to have 2n to 4n megakaryocytes produce platelets than to have them further progress to 32n or 64n megakaryocytes and produce more platelets per megakaryocyte.[2] It is not yet known whether platelets produced by these megakaryocytes are fundamentally different than those made by higher-ploidy megakaryocytes, nor whether they are more susceptible to cytokines and chemokines that are stimulatory or inhibitory.

Hemolytic Disease of the Fetus and Newborn

HDFN has long been the paradigm of a disease in which maternal antibodies are formed against fetal antigens and cause destruction of the targeted cells. First characterized in 1941 by Levine and colleagues, HDFN occurs when maternal immunoglobulin G (IgG) antibodies develop against fetal RBC antigens that are not present on the maternal RBCs and cross the placenta, resulting in destruction of fetal RBCs and causing anemia and hyperbilirubinemia. Fortunately, with the advent of anti-D immunoglobulin—the most commonly used preparation in the United States being RhoGam—and the institution of universal testing, severe hemolytic disease before and after birth has become relatively uncommon[3] and occurs in 3 unique settings. In the first setting, red cell antigens other than D are involved. Severe fetal anemia almost always involves sensitization to the other components of the Rh complex: Cc and Ee. Although ABO antigen incompatibility is more common, it rarely results in fetal anemia. In the second setting, the woman was sensitized to D during a prior exposure in another country. In the third setting, sensitization to D occurs in a woman who has had multiple children, as in our example case. This increases the chances that the woman would have an undetected fetomaternal hemorrhage large enough to overcome the effect of routinely administered anti-D. However, the mechanisms underlying the disease and the outcomes of treatment such as in utero transfusions provide a fascinating example of how perinatal exposures can alter—or "program"—the postnatal milieu and subsequent response to similar challenges.

The most common antigen incompatibility that causes HDFN is the Rhesus D (RhD) antigen. Thus, a woman who is RhD-negative (that is, who lacks the D antigen on her own RBCs) who is exposed to RhD-positive blood from her fetus may go on to develop anti-D IgG. The most common cause of exposure to fetal blood is fetomaternal hemorrhage, usually in the third trimester or during delivery.[4] Although the index fetus may not be affected by the maternal antibodies, subsequent pregnancies with an RhD-positive fetus are at high risk for HDFN; once the maternal immune system has been primed, anti-D is ineffective and the mother's anti-D antibodies will cross the placenta and destroy the fetal RBCs. The second most common antigen is the ABO blood group antigen, which occurs almost exclusively in infants with mothers of blood type O. These mothers, who lack A or B antigens on their own RBCs, have naturally occurring anti-A and anti-B IgG type antibodies and therefore, fetomaternal hemorrhage is not a prerequisite for antibody development. The clinical presentation of hemolytic disease due to ABO incompatibility is predominantly early hyperbilirubinemia, which is successfully treated with phototherapy; the degree of anemia is mild.[5] Studies have shown that IVIG infusion given to the baby after birth will reduce the peak bilirubin level.[6] In contrast, HDFN secondary to RhD antigen can cause significant hemolytic anemia, heart failure, portal vein obstruction, ascites, pleural and pericardial effusions, and hydrops fetalis. A myriad of fetal and maternal events increase the risk for maternal immunization by increasing the chances of a large fetomaternal hemorrhage; these include ectopic pregnancy, fetal demise, spontaneous or induced abortion, maternal abdominal trauma, chorionic villus sampling, and amniocentesis.

Currently, guidelines from the United States Preventive Services Task Force and the American College of Obstetricians and Gynecologists recommend screening all pregnant women for antibodies and RBC antigen typing at the first prenatal visit, followed by a repeat screening at 24 to 28 weeks of gestation.[7,8] If patients are Rh-negative and anti-D–negative at the second screen, they should receive anti-RhD immunoglobulin at 28 weeks of gestation, with additional doses as needed for high-risk events.[9] For the first pregnancy of an RhD-negative mother, serial anti-RBC antibody titers are monitored if the fetus or its father is RhD-positive. Above a certain titer threshold, particularly if there is any cause to suspect fetal anemia, serial fetal middle cerebral artery (MCA) Doppler ultrasound is used to monitor for worsening anemia, which correlates with high velocity on MCA Doppler ultrasound. If mild anemia is detected, the fetus continues to be monitored by ultrasound until it is sufficiently mature for delivery. In cases of severe anemia identified by MCA Doppler ultrasound, cordocentesis with planned transfusion is performed to confirm fetal anemia, which is defined as fetal hemoglobin less than 10 g/dL or hematocrit less than 30%.[10]

The standard management of severely affected cases of HDFN was to give red cell transfusions in utero approximately every 3 weeks in order to maintain the fetal hemoglobin level until such time as the fetus could be safely delivered. This could be as many as 4 to 6 in utero transfusions starting from 20 weeks of gestation until 36 weeks of gestation. Of note, antenatal treatment with IVIG was previously evaluated based on the model of its successful utilization in neonatal alloimmune thrombocytopenia, a disease with similar pathophysiology.[11] In the case of Rh disease, however, treatment with IVIG did not appear to modify the disease severity; there was no reduction in the frequency or volume of intrauterine transfusions, the severity of hemolysis, or the development of hydrops fetalis.[11,12]

Several years ago, our center treated an Rh-negative woman who had experienced 6 normal, unaffected pregnancies followed by a seventh pregnancy that resulted in a jaundiced and anemic neonate, presumably owing to a missed fetomaternal hemorrhage that sensitized her during the previous pregnancies. Her eighth pregnancy ended tragically, with intrauterine fetal demise at 18 weeks of gestation secondary to hydrops fetalis. When she expressed the desire to become pregnant again, it was clear that any future babies were likely to be severely affected by HDFN, and at less than 18 weeks of gestation. In general, 20 weeks has been the earliest gestational age at which fetal blood sampling and fetal transfusion can be performed. Therefore, during this ninth pregnancy we started a treatment program at 12 weeks that was developed by Dr Kenneth Moise based on our studies in fetal alloimmune thrombocytopenia. The woman underwent 3 plasmaphereses in 1 week (ie, Monday, Wednesday, and Friday), and after the third one she received 2 consecutive infusions of IVIG 1 g/kg intended to block rebound production of anti-D. This was followed by twice weekly 1 g/kg IVIG infusions and 30 mg of prednisone daily in a continued attempt to block anti-D transfer for the fetus. Using MCA Doppler ultrasound to determine fetal hematocrit status, we were able to continue this treatment without fetal blood sampling until the MCA velocity started to increase at 25 weeks of gestation, indicating the development of fetal anemia. The initial fetal blood sampling demonstrated a hemoglobin concentration of 6 g/dL. In utero red cell transfusions with Rh-negative RBCs were initiated, and maternal treatment with IVIG and prednisone was stopped. At 33 weeks of gestation, after receiving 5 in utero RBC transfusions, the neonate was delivered via cesarean section after commencement of spontaneous labor. The baby was mildly anemic at birth, with a hemoglobin concentration of 12.4 g/dL; this is low

for a newborn but not severely so. He was not jaundiced initially, but at 10 days of life he developed hyperbilirubinemia and required an exchange transfusion.

Further on in this review, we will discuss the effects of antenatal IVIG treatment in the discussion on alloimmune thrombocytopenia and illustrate that this maternally administered treatment has been relatively benign in terms of its effects on the fetus. In contrast, the effects of serial in utero RBC transfusions have been shown to have a significant impact on subsequent erythropoiesis in the infants. In 1990, a study by Millard and colleagues tracked 12 babies who had received multiple in utero transfusions for Rh disease.[13] Essentially all of the babies experienced a drop in hemoglobin to a level of 6 g/dL within 1 to 2 months after delivery. One infant became severely anemic, with a hemoglobin concentration of 2 g/dL. Additionally, the average reticulocyte count was 0.8% and the average erythropoietin level was 23 mg/dL, and the level of circulating antibody remained high.[13] These findings suggested that after in utero red cell transfusions, neonates failed to produce red cells normally after birth, especially in the setting of worsening anemia. Subsequently, a small study performed at our center found marked erythroid hypoplasia in the bone marrow of infants who underwent bone marrow biopsy as part of the diagnostic workup of their hypoproductive anemia.[14] This is in marked contrast to the general understanding of Rh disease, as illustrated by one of its other well-known names: *erythroblastosis fetalis*.

In our previously cited study of 4 patients and in another study of 2 patients by Ohls and colleagues,[15] erythropoietin was used to treat the severe anemia that developed postnatally. All 4 infants experienced an increase in hemoglobin and reticulocyte count 2 to 4 weeks after initiation of erythropoietin therapy. These results illustrated that erythropoietin was able to successfully stimulate erythropoiesis and allowed the newborn to avoid further postnatal transfusions. However, the effect was slow in onset (2-4 weeks, even at relatively high doses of erythropoietin). A randomized trial was initiated but unfortunately, only 8 patients were entered (4 on erythropoietin and 4 not). Because eligible sensitized patients were no longer available, the trial was closed.

The exact mechanism by which in utero transfusions create a hypoplastic bone marrow is unknown. We suspect it involves a combination of the anti-D antibody and a suppressive effect to lower in utero erythropoietin levels. This may be especially true because typically the fetal RBCs would contain 100% hemoglobin F, whereas the multiply transfused fetus would contain 100% hemoglobin A and therefore would be able to off-load oxygen more effectively to tissues at a given hemoglobin concentration. Furthermore, in view of the risks involved in providing in utero transfusions, they would be done as infrequently as possible (approximately every 3 weeks). This would result in relatively high hemoglobin concentrations after transfusion, thereby contributing to the suppressive effects. Another hypothesis considered is the potential destruction of erythrocyte precursors—such as cells in the colony-forming unit-erythroid stage—that could express the D antigen and therefore be targets for maternal antibodies. Generally, however, this antigen appears very late in erythropoiesis, thereby excluding erythrocyte progenitor cells as potential targets for maternal anti-D IgG.[13]

Owing to the rarity of sensitization amid better worldwide utilization of anti-D prophylaxis in the pregnancy of Rh-negative women, the number of these cases has fallen significantly. Therefore, despite the interesting phenomena seen in HDFN owing to Rh incompatibility, further studies are not possible. Of note, it is believed that at a hemoglobin concentration of 6 g/dL, the production of erythropoietin by the liver would be sufficiently stimulated to compensate for the absence of erythropoietin production by the kidney. However, in the cases described by Millard and colleagues, this compensatory mechanisms did not occur, as evidenced by the very low hemoglobin concentrations these children reached.

Fetal and Neonatal Alloimmune Thrombocytopenia

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the platelet equivalent of HDFN for RBCs. In an untreated state it is estimated that it would occur approximately one-tenth as often as sensitization to D, which at its peak prior to anti-D affected 1 in every 100 births in the United States.[16] FNAIT is caused by maternal sensitization to paternally derived antigens on fetal platelets (human platelet antigen 1a; HPA-1a). Depending upon the definition of severity and whether cases are accrued by screening or clinical recognition of neonatal thrombocytopenic bleeding, this disorder affects approximately 1 in 1000 to 1 in 5000 live births. It is the most common cause of severe thrombocytopenia at any age and of intracranial hemorrhage in full-term newborns.[17] Currently, there is no routine screening for NAIT; diagnosis is made following the birth of a severely thrombocytopenic neonate and antenatal management is possible only in subsequent pregnancies. Intracranial hemorrhage occurs in up to 20% of affected infants. Unfortunately, the only predictor of intracranial hemorrhage in subsequent pregnancies is history of one in a previous affected sibling.[18] Maternal administration of IVIG with or without corticosteroids has emerged as the mainstay of antenatal management of FNAIT and has been supported by numerous studies.[19] IVIG alone at 1 mg/kg/week has been shown to effectively increase platelet counts in fetuses with platelet counts greater than 20,000 per µL

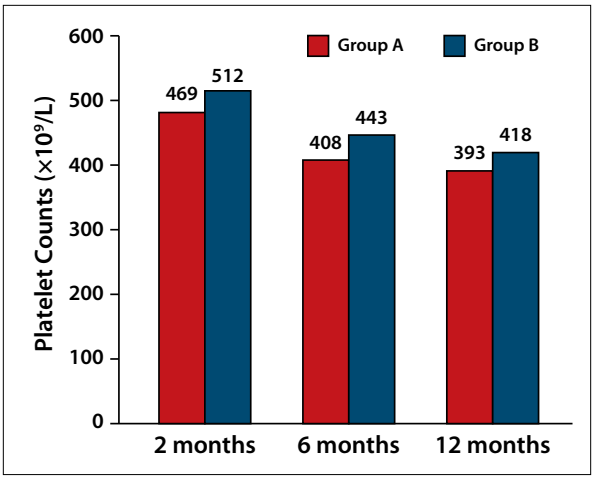

Figure 1. Platelet counts over 1 year. Platelet counts at 2, 6, and 12 months did not differ significantly between the 2 groups, although it can be seen than group B had higher platelet counts on average.

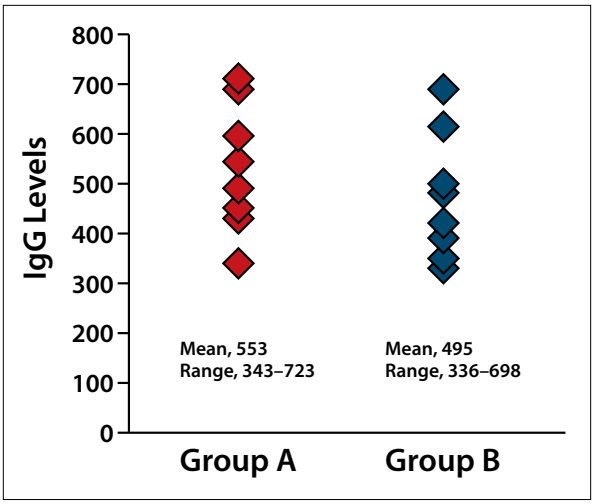

Figure 2. IgG levels at 12 months of age did not differ significantly between the 2 groups. The average IgG level was 553 for group A and 495 for group B, with similar ranges.

IgG, immunoglobulin G.

prior to therapy, but not in those with lower platelet counts or with a previous sibling affected by intracranial hemorrhage.[20] Studies have demonstrated that risk stratification enables tailored therapy with higher doses of IVIG (2 mg/kg/week) and additional prednisone (0.5-1 mg/kg/day), leading to improved outcomes.[17,21]

Our center conducted an unpublished study examining the long-term effects of antenatal treatment with IVIG in children with a sibling previously affected by FNAIT. We followed a cohort of standard-risk maternal-fetal pairs, defined as those with pretreatment platelet counts greater than 20×10^9/L and no history of intracranial hemorrhage in a previous sibling. Participants were randomly assigned to receive antenatal treatment with either IVIG at 2 g/kg/week (group A) or IVIG 1g/kg/week plus prednisone 0.5 mg/kg/week (group B) starting at 23 weeks of gestation on average. We sought to determine whether any differences in infection rates, growth parameters, platelet counts, or immunoglobulin levels would exist between the 2 groups at 2, 6, and 12 months of age. Questionnaires were sent to pediatricians and parents at the appropriate age, and follow-up calls were made to acquire the missing data. We were able to acquire data on 30 of the 51 infants in group A and 26 of the 45 infants in group B. There were no significant differences in growth parameters and platelet counts at 2, 6, and 12 months between the groups. Mean height and weight were in the 25th to 50th percentiles at 2, 6, and 12 months. Mean head circumference fell within the 10th to 25th percentiles at 2 months and the 50th to 75th percentiles at 6 and 12 months. Mean platelet counts were 469, 408, and 393 (group A) and 512, 443, and 418 (group B) at 2, 6, and 12 months, respectively (Figure 1). Although group B counts were slightly higher, the difference between the 2 groups was not statistically significant. Furthermore, we found no significant difference between the 2 groups in immunoglobulin G (IgG) levels at 12 months of age (Figure 2).

We also explored rates of common infections including otitis media, upper respiratory infections, gastroenteritis, conjunctivitis, and bronchiolitis at 2, 6, and 12 months of age. Although preliminary results suggested a higher rate of otitis media infections in children in group B (prednisone treated) over the course of 1 year, collection of additional data and subsequent analysis failed to demonstrate any significant difference in infection rates between the 2 groups (Table 1). A larger percentage of children were affected by otitis media in group B than in group A (Figure 3), although the difference was not statistically significant.

Our study did not find any significant differences in postnatal infection rates, platelet counts, or IgG levels in infants treated prenatally with high doses of IVIG or lower-dose IVIG in combination with corticosteroids. We can therefore surmise that both of these perinatal challenges, although different from one another in other respects, exerted equal effects—if any—on the developing immune and hematopoietic systems. Future studies comparing these parameters in prenatally treated children with normal controls would help to further illuminate the effects of prenatal exposure to different doses of IVIG on the postnatal immune system.

Maternal ITP and the Role of Breastfeeding

Contrasting the neonatal course of infants of mothers with ITP with those with FNAIT who did not receive

Table 1. Total Number of Patients and Infection Rates by Type Over the Course of 12 Months

	Group A	Group B
Total no. of patients	51	45
Total no. of patients with data returned	30	26
Total no. of infections	91	97
Average no. of infections per patient	3	3.7
% of patients affected by infections over 12 months	87%	92%
No. of infections treated with antibiotics	24	37
No. of upper respiratory	42	43
No. of conjunctivitis	5	6
No. of gastroenteritis	6	3
No. of bronchiolitis	6	8
No. of pneumonias	1	1
No. of otitis media	24	36

No., number.

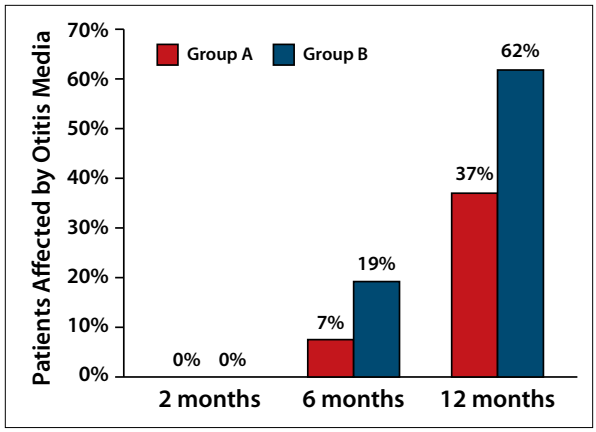

Figure 3. Percent of patients with otitis media at 2, 6, and 12 months. Although Group B had a higher incidence than Group A of otitis media infections at 6 and 12 months, the differences were not significantly different.

antenatal treatment brings forth some interesting similarities and differences. As background, infants of mothers with ITP generally are not severely affected by thrombocytopenia. The only study that did fetal blood sampling in fetuses of mothers with ITP demonstrated low fetal platelet counts, in one instance as early as 21 weeks of gestation.[22] However studies of newborns have been relatively consistent, with approximately 15% of neonates having platelet counts lower than 50,000/μL at birth and just 5% having counts lower than 20,000/μL.[23,24] These counts are higher than those seen in FNAIT, in which 90% of neonates have counts less than 50,000/μL and 50% have counts less than 20,000/μL.[16]

More surprising is the ongoing course after birth. In FNAIT, despite the severity of the fetal and neonatal thrombocytopenia and the absence of maternal treatment for this unexpected condition, the thrombocytopenia resolves relatively quickly and rarely lasts more than 1 to 2 weeks. In contrast, the infants of mothers with ITP typically have their counts worsen in the 2 to 4 days after birth and therefore need close neonatal monitoring of their counts. Furthermore, in certain cases the platelet counts can persist at low levels for months. One possibility is that somehow the maternal ITP is transferred to the fetus. However, a more plausible explanation is that in certain cases, breastfeeding perpetuates the fetal thrombocytopenia. The most obvious explanation would be that antiplatelet antibodies are transferred to the fetus. The immaturity of the fetal gastrointestinal tract potentially allows for preservation of the antibodies in the stomach and intact absorption through a porous intestinal tract. No studies have begun to clarify either explanation, although it appears that cessation of breastfeeding for several days is often enough to allow recovery of the neonatal platelet counts.

Neonatal Hemochromatosis

Neonatal hemochromatosis (NH) is another interesting example of the delicate interplay between the maternal and fetal immune systems. Recently classified as an immune-mediated disorder, NH is the most common cause of liver failure in neonates and is associated with multiorgan iron overload. Often fatal, with survival rates of only 20% to 30%, NH originates early in gestation and often results in hydrops fetalis or fetal death. Affected infants who are born viable typically have end-stage liver failure at birth, as well as iron deposition in the pancreas, thyroid, heart, and adrenal glands, resulting in multiorgan failure shortly after birth.

The unusual inheritance pattern—characterized by high recurrence rates, sporadic occurrence in pregnancies following an unaffected pregnancy, and occurrence in siblings with the same mother but different fathers—suggests that this is primarily a disease of maternal origin.[25] In 2004, Whitington and colleagues hypothesized that the etiology of NH is maternal alloantibodies against a fetal hepatocyte antigen, similar to NAIT and Rh disease.[26] He postulated that a previously sensitized woman, upon reexposure to a fetal hepatocyte antigen, develops anti–fetal hepatocyte IgG, which crosses the placenta and binds to fetal hepatocytes, injuring the cells and altering iron metabolism. Although the specific fetal hepatocyte antigen and maternal antibody target remain unknown, the degree of liver injury and disease severity varies. Fetal attributes such as sex, differences in human leukocyte antigen, and hepatocyte antigen polymorphisms may contribute to the phenotypic expression of the disease.

Prior to the consideration of an alloimmune etiology, NH was treated with iron chelation and/or liver transplant, both with poor results and significant associated morbidity and mortality. In 2004, Whitington and Hibbard reported on the use of weekly IVIG treatment administered to the mother beginning at 18 weeks of gestation until delivery, with astonishing results. Of the 30 infants with previously affected siblings who were treated prenatally with IVIG, only 6 had clinical evidence of liver disease, and most responded to antioxidants. None of the treated infants had intrauterine growth restriction, oligohydramnios, or hydrops fetalis, all of which are typically seen in pregnancies affected by NH disease. Interestingly, 80% of the neonates had biochemical markers of NH such as elevated serum ferritin and alpha fetoprotein, but none developed end-stage liver disease and required liver transplant. These findings of significantly decreased disease severity confirm the potentially important role of perinatal immune modulation in the setting of autoantibody-mediated disease.

Neutropenia and Preeclampsia

The best-defined cause of neonatal neutropenia is alloimmune neonatal neutropenia (AAN), in which maternal antibodies against paternally-derived antigens on fetal neutrophils cross the placenta and lead to destruction of the target neutrophils. This disease is the neutrophil parallel to HDFN and to FNAIT for RBCs and platelets, respectively. Multiple different antigens have been identified as potential targets, but the causative antigen remains unknown in approximately 50% of cases. Interestingly, antineutrophil antibodies are found in approximately 20% of pregnant and postpartum women but only 0.2% to 2% of newborns are affected by AAN, further proof of the mysterious nuances of the fetomaternal interface.[27]

Although neutrophil-specific antigens have been identified (eg, NA1, NA2, NB1), a relatively common cause is an isoantibody rather than an alloantibody. This occurs when mothers are FcγRIII null. Coincidentally and confusingly, NA1 and NA2 are on FcRIII. Because the antibody is directed against FcγRIII, it will react with all neutrophils; not just NA1-positive ones, for example. Furthermore, mannose-binding protein is also associated with FcγRIII but the mothers generally are asymptomatic. Because limited screening studies have been performed to identify the incidence of sepsis in neonates affected by AAN, the impact of the transient neutropenia—even though it is often severe—is relatively unknown.[28]

Turning to leukopenia in neonates of mothers with preeclampsia, it has been known since the original work by Koenig and Christensen in 1989 that maternal preeclampsia results in neonatal leukopenia more than in thrombocytopenia (even with HELLP syndrome [hemolysis, elevated liver enzymes, and low platelet count], in which the mother is thrombocytopenic).[29] Fortunately, this is usually transient (lasting less than 3 to 4 days), rendering its significance as a factor in sepsis and mortality in the newborn relatively low. However, a concept developed by LaGamma (among others) of so-called "late sepsis" describes the idea that transient leukopenia—and specifically, neutropenia—could result in the development of a nidus of infection such that actual sepsis could develop later, following count recovery.[30] In an article from our center by Sharma, it appeared that leukopenia would be more severe and longer lasting if there were a multiple gestation pregnancy and if delivery were at 32 weeks or earlier. In this pilot study of a limited number of patients, an increased incidence of late sepsis was seen.[31]

The increase in both maternal and perinatal morbidity and mortality in pregnancies affected by more severe forms of pregnancy-induced hypertension, such as preeclampsia and HELLP syndrome, is well established. Koenig and Christensen's study illustrated that the presence of severe hypertension and HELLP also was correlated with a higher incidence of neonatal neutropenia. Subsequent studies have confirmed these observations,[32,33,34] noting also that frequency and severity of neutropenia were greater in very-low–birth weight infants and in those infants who were small for gestational age.[35,36] The significance of this transient neutropenia and its role in sepsis and mortality risk remains unclear. Although some studies did find an increased incidence of sepsis in neutropenic infants of preeclamptic mothers,[34] normal reference ranges of absolute neutrophil counts were modified in 1994 for infants weighing less than 1500 g[37] and most studies done subsequently using these adjusted values failed to find a significant correlation between neutropenia and mortality. One large-scale study conducted in Brazil in 2007 included more than 900 infants and explored the rates of sepsis and neutropenia specifically in very-low–birth weight infants born to mothers with preeclampsia.[38] They confirmed that the incidence of neutropenia in infants of mothers with preeclampsia was higher than in control infants when the newer criteria for neutropenia were used. Their data demonstrated no association between late-onset sepsis and neutropenia, but they did find a significant association between neutropenia and early-onset sepsis. They also found that neutropenia in the first 72 hours of life was associated with death—independently of sepsis—in infants at less than 32 weeks of gestation. In those infants who were at 32 weeks of gestation or more, there was no difference in mortality rates between neonates with and without neutropenia.

The exact mechanism underlying neonatal neutropenia in the setting of maternal preeclampsia remains elusive. Koenig and Christensen reported evidence of hypoproliferation of myeloid precursors as a possible

etiology in their original work in 1989; examination of bone marrow aspirates obtained from neutropenic infants of mothers with preeclampsia demonstrated a significantly decreased percentage of myeloid precursors as compared with historical control data. Their subsequent study in 1991 linked the decreased myelopoeisis with a soluble factor secreted by the placenta into the fetal circulation.[39]

Multiple studies have been done looking at neutrophil activation as a contributing factor to placental pathophysiology that results in preeclampsia.[36,40,41] Wang and colleagues proposed that agents generated by the placenta could be released into the maternal circulation and affect the neutrophil-endothelial interaction.[40] Their in vitro studies have demonstrated hyperadhesiveness of both neutrophils and endothelium derived from placentas of preeclamptic women as compared with those from normal placenta, as well as altered expression of neutrophil integrins such as CD11b when neutrophils are exposed to preeclamptic placenta medium. More recently, in 2011, Faulhaber and colleagues compared plasma levels of chemokines interleukin 8 (IL-8) and GRO-alpha, both critical in leukocyte chemotaxis. They found that levels of IL-8 were significantly lower in neonates of preeclamptic pregnancies than in neonates born to mothers without preeclampsia, regardless of whether the infant was neutropenic.[41] Zook and colleagues explored the role of placental pathology in preeclampsia-associated neonatal neutropenia and found no correlation between neonatal neutropenia and histologic changes such as placental infarction or vasculopathy.[36]

The Fas/Fas ligand (FasL) pathway has also been a recent topic of investigation as a potential link between a potential immune-mediated cause of preeclampsia and its associated complications, such as neonatal neutropenia. The Fas-FasL pathway has been implicated in establishment of normal maternal-fetal tolerance, as well as mediation of neutrophil activation and neutropenia. Kuntz and colleagues found that there are elevated soluble Fas ligand concentrations in maternal and cord blood sera of gestations complicated by preeclampsia. The group's earlier studies had demonstrated enhanced Fas ligand expression in trophoblasts in pregnancy-induced hypertension, and FasL has previously been associated with neutrophil-mediated injury and apoptosis, as well as inhibition of hematopoiesis. They now hypothesized that the high expression of FasL on trophoblasts in the presence of elevated soluble FasL may play a role in neonatal neutropenia.[42]

Another area of great interest has been serum granulocyte colony-stimulating factor (G-CSF) levels in both preeclamptic mothers and their neonates, and whether the neutropenia is due to transiently decreased production of neutrophil growth factors, the presence of inhibitors, as Koenig and Christensen proposed, or decreased responsiveness to growth factors. Matsubara and colleagues found that serum G-CSF concentrations were significantly higher in women affected by preeclampsia compared with those of women with unaffected pregnancies at a similar gestational age.[43] However, studies looking at neonatal serum show conflicting results. A study in 2007 by Guner and colleagues found no statistically significant difference between G-CSF levels measured at 12 hours of life in infants of preeclamptic mothers vs those whose mothers had normal pregnancies.[44] In contrast, a larger study in 1999 by Tsao and colleagues found that the levels of G-CSF in cord blood were significantly lower in infants of hypertensive mothers as compared with those of normotensive mothers.[45] Interestingly, infants of preeclamptic mothers demonstrated a muted response to exogenous G-CSF. Zuppa and colleagues compared the absolute neutrophil counts of premature neonates with sepsis and neutropenia whose gestation was complicated by preeclampsia with age- and weight-based septic and neutropenic controls without maternal hypertension. He found that administration of recombinant human G-CSF daily for 3 consecutive days resulted in a slower response by the neonates of preeclamptic mothers, with a lag of 2 to 3 days, as well as achievement of lower peak absolute neutrophil counts. He hypothesized that the as yet unidentified placental inhibitor of neutrophil production may delay and blunt the effect of recombinant human G-CSF on neonatal progenitor cells.[46] Thus, although this soluble factor remains elusive, one can postulate that it—or other factors released by the preeclamptic placenta—can play a role in fetal neutropenia associated with maternal preeclampsia. Much remains to be learned about the pyelopoietic environment in infants of hypertensive pregnancies.

Conclusion

The hemochorial histoarchitecture of the human placenta is the most invasive type of placentation and is characterized by direct contact of maternal blood and the fetal trophoblast.[47] This intimate relationship between the maternal and fetal circulation confers some benefits, such as optimized nutrient uptake, but also renders the fetus relatively unprotected from any disruption in the placental barrier and subsequent harm from maternally transmitted pathogens or immune responses. The exact mechanisms that underlie the intricate trafficking of blood cells and antibodies at the fetomaternal interface remain incompletely understood. The immune-mediated cytopenias discussed above illustrate the clinical manifestations of abnormalities in these traffic patterns. Indeed, as in the case of neonatal hemochromatosis, the pathophysiology is elucidated retrospectively, after serendipitous success in treatment with IVIG. Particularly in view of the burgeoning interest in epigenetics and the prenatal origins of adult disease, there is much to be learned from the intricate and sophisticated fetomaternal unit.

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