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Chronic villitis in untreated neonatal alloimmune thrombocytopenia: An etiology for severe early intrauterine growth restriction and the effect of intravenous immunoglobulin therapy

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KEY WORDS
Neonatal alloimmune thrombocytopenia
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Objective: The objective of the study was to examine placental histopathology in intravenous immunoglobulin-treated and untreated neonatal alloimmune thrombocytopenia and correlate pathological findings with clinical outcomes.

Study design: Placentas from 14 neonatal alloimmune thrombocytopenia–affected pregnancies were identified. Maternal antepartum treatment with intravenous immunoglobulin and pregnancy outcomes were abstracted from medical records. Placental histopathology and clinical outcomes were compared between intravenous immunoglobulin and no intravenous immunoglobulin treatment groups using Fisher's exact test. One subject, treated only after an intracranial hemorrhage (ICH) was diagnosed, was excluded from the analysis. $P < .05$ was considered significant.

Results: Untreated pregnancies demonstrated a lymphoplasmacytic chronic villitis not seen in the intravenous immunoglobulin–treated pregnancies ($P = .005$). Intrauterine growth restriction and intrauterine fetal demise occurred as frequently as ICH in the untreated group. No ICH, intrauterine growth restriction, or intrauterine fetal demises occurred in the treated group, although the P value was not significant.

Conclusion: Chronic villitis is frequently manifest in neonatal alloimmune thrombocytopenia, with intravenous immunoglobulin alleviating this inflammatory immunologic response. We suspect a more universal role for the maternal antibody, such as fetal endothelial cell damage, in the sequelae of neonatal alloimmune thrombocytopenia.

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Neonatal alloimmune thrombocytopenia (NAIT) is an immune-mediated process whereby fetal platelets are destroyed by maternal antibodies directed against fetal platelet antigens. Although many couples display genotypic differences in their platelet surface antigens that could lead to NAIT, only a fraction results in sensitized pregnancies. The reported incidence of NAIT is 1 in 1000 to 2000 pregnancies, although it is likely higher, because of incomplete ascertainment.[1] Increasing use of assisted reproductive technologies may ultimately lead to an increased incidence of NAIT by exposing gestational carriers to additional foreign antigens.[2] Unlike the natural history of red cell alloimmunization, NAIT can affect a first pregnancy,[3] with the diagnosis often made only after a severe hemorrhagic complication has occurred. Intrauterine fetal demise is also a recognized complication of this disorder.[4]

Current treatment for NAIT is administration of maternal antepartum intravenous immunoglobulin (IVIG) with or without steroids, but optimal dosing regimens have yet to be established.[5] Moreover, although IVIG treatment has greatly reduced the incidence of severe sequelae, little is known about immunoglobulin's mechanism of action, and much remains to be learned.[6]

Few studies of the placenta in NAIT-affected pregnancies exist, with most research efforts focused on treatment of NAIT,[1,7] applicability of population screening,[8,9] and prevention of the devastating fetal hemorrhages that can occur.[10] Placental findings are frequently reported within the context of case reports, but no systematic analysis of placental findings has been reported to date.[11] This study's purpose was two-fold: to describe histological findings in NAIT-affected placentas and determine what if any effect IVIG exerts on the process.

Material and methods

A search of clinical files in the Department of Gynecology and Obstetrics at the Johns Hopkins Hospital identified 14 consecutive cases of pregnancies that were complicated by NAIT between 1989 and 2003. The medical records of each case were comprehensively reviewed, and in all cases, demographic characteristics as well as clinical and laboratory variables potentially associated with NAIT were abstracted and entered into an Excel database (Microsoft, Redmond, WA). The diagnosis of NAIT was established by the documentation of neonatal hemorrhage and/or thrombocytopenia and the subsequent detection of fetal-maternal alloantigen incompatibility and associated maternal antiplatelet antibodies. Clinical and laboratory variables included maternal age and ethnicity, gestational age at delivery, fetal growth indices, fetal hemorrhagic events, fetal platelet counts, maternal antiplatelet antibody identification, fetal adverse outcomes, and treatment with weekly maternal IVIG (1 g/kg).

Paraffin-embedded placental tissues from all 14 patients were retrieved from the pathology archives of the Johns Hopkins Hospital or from outside sources if the delivery occurred at another hospital. Hematoxylin and eosin-stained histologic sections were prepared for each patient and all samples reviewed by experienced pathologists who were blinded to treatment and clinical outcomes (E.W. and F.A.). Histologic findings of 10 placentas from uncomplicated term pregnancies served as controls. STATA software (7.0 Stata Corporation, College Station, TX) was used for data analysis. We sought to determine IVIG's effect on preventing hemorrhagic sequelae and any differences that prophylactic use of IVIG might have on placental histopathology; therefore, 1 subject, not treated with maternal IVIG until after fetal intracranial hemorrhage was diagnosed at 21 weeks, was excluded from IVIG treatment analysis. $P < .05$ was considered significant. The Johns Hopkins University Internal Review Board approved all methods of data acquisition and analysis.

Results

Fourteen NAIT-affected pregnancies were identified in 8 different women (Table I). Eleven of 14 patients resulted in a second- or third-trimester delivery of a live-born infant; 1 subsequently died at 18 days of life. There were 3 intrauterine fetal demises (IUFDs). Six of the pregnancies had been treated with maternal IVIG as described earlier, 4 beginning at 13 weeks, and 1 at 17 weeks; in 1 case, weekly IVIG was begun at 21 weeks after referral for in utero intracranial hemorrhage. Although we cannot rule out with absolute certainty for 3 of our patients' prior pregnancies (delivered elsewhere) the possibility of any additional fetal hemorrhages, there were none described in the relevant medical records. No other fetal or neonatal hemorrhages were found in patients under our care other than those cited (Table II).

A substantial chronic inflammatory infiltrate was seen in the villous stroma surrounding the fetal vessels of 5 of 14 (35.7%) placentas (Figure). The presence of chronic villitis did not correlate with maternal age, gestational age at delivery, ethnicity, or fetal gender. Other placental findings included advanced villous maturation (5 of 14), increased syncytial knots (2 of 14), infarctions (4 of 14), and necrosis (1 of 14). Five placentas had no specific pathologic changes. The particular maternal platelet alloantibody involved did not appear to alter the findings.

The effect of treatment with weekly IVIG was striking. All placentas with chronic villitis were in the untreated group, a statistically significant difference

Table I Summary of 14 cases of NAIT

Patient number	IVIG (GA)	Ab	IUGR	IUFD	ICH	Placental pathology
1	No	HPA-1a	No	No	Yes	NSPC
2	No	HPA-5b	Yes	Yes	No	CV, AVM, ISK, infarcts
3	Yes (22)	HPA-3b	No	No	Yes	Patchy CV, AVM, infarcts
4	No	HPA-3a	No	*	No	Severe CV, AVM, infarcts
5	No	HPA-3a	Yes	Yes	No	Severe CV, villous necrosis
6	No	HPA-3a	No	No	No	Florid CV
7	No	HPA-3a	No	Yes	No	Severe CV
8	Yes (12)	HPA-1a	No	No	No	ISK, small infarct
9	Yes (18)	HPA-5b	No	No	No	AVM
10	Yes (12)	HPA-1a	No	No	No	NSPC
11	Yes (11)	HPA-3b	No	No	No	NSPC
12	Yes (17)	HPA-1a	No	No	No	Mild AVM
13	Yes (13)	HPA-1a	No	No	No	NSPC
14	Yes (13)	HPA-1a	No	No	No	NSPC

Ab, Maternal alloantibody; *NSPC,* no specific pathological changes; *CV,* chronic villitis; *AVM,* accelerated villous maturation; *ISK,* increased syncitial knots; *GA,* gestational age in weeks at which weekly maternal IVIG therapy was begun.
* Infant died at 1 month of age.

Table II Presence or absence of chronic villitis and of adverse fetal outcomes by IVIG status

	IVIG (absent) (n = 6)	IVIG (present) (n = 7)	*P* value
Chronic villitis	5 (83.3%)	0	.005
Other histopathology	5 (83.3%)	3 (42.9%)	.266
ICH	1 (16.7%)	0	.46
IUGR	2 (33.3%)	1 (14.3%)	.56
IUFD	3 (50%)	0	.07

($P = .005$; Table II). No placentas in patients treated beginning from the first to early second trimester with maternal IVIG displayed this finding. In addition, none of the other histologic findings in IVIG-treated placentas were described as severe. The 1 placenta in which weekly IVIG was begun at 22 weeks, after a fetal intracranial hemorrhage had been identified, demonstrated infarcts, patchy chronic villitis, and accelerated villus maturation.

Intrauterine growth restriction (IUGR) occurred in 3 of 14 fetuses. There were 3 IUFDs and 1 neonatal death. IUGR and IUFD were seen with the same frequency as fetal hemorrhage. Again, administration of IVIG greatly impacted these outcomes. Two of the 3 IUGR fetuses were in the untreated group, as were all IUFDs and the neonatal death. There were no deaths in the treated group, and none of the previously mentioned categories reached statistical significance.

Comment

Chronic villitis of unknown etiology has been well documented as being strongly associated with IUGR[12-15] and more recently with later neurological impairment.[16] Of note, the majority of cases of chronic villitis has no corroborative evidence for congenital infections and demonstrates a lymphohistiocytic infiltrate as opposed to a plasma cell infiltrate.[17-20] The presence of a marked lymphoplasmacytic infiltrate that is predominantly plasma cells in untreated NAIT-affected pregnancies is intriguing. We suspect these cells are maternal, and by hematoxylin and eosin they appear to be plasma cells that would be of B-cell origin rather than T lymphocytes. In any event, NAIT should be considered in the differential diagnosis of IUGR, including early severe IUGR, particularly when placental histology reveals chronic villitis with a preponderance of plasma cells in the infiltrate.

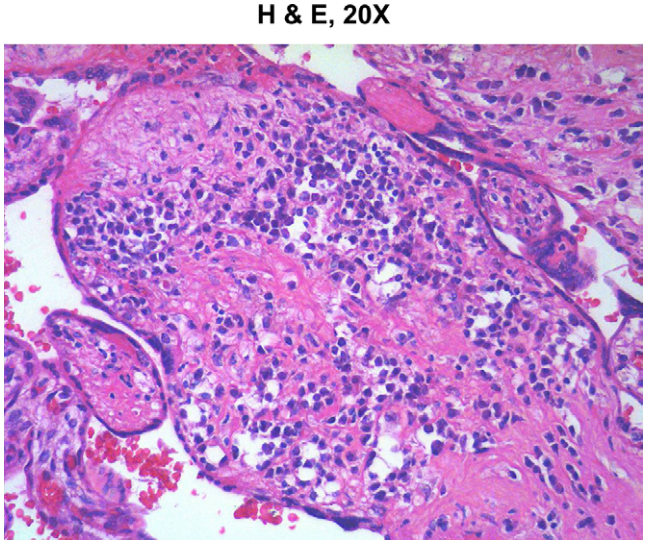

Figure Chronic villitis in an untreated NAIT-affected placenta.

We speculate that the finding of chronic villitis indicates another mechanism of injury in NAIT in addition to thrombocytopenia. Specifically, we hypothesize that fetal endothelial cells are being actively damaged in NAIT, thereby injuring the fetal vessel wall, through the maternal-fetal platelet antibody response. In addition, platelet glycoproteins IIIa and Ia, targets of the maternal antiplatelet antibodies, are also expressed by endothelial cells, thereby providing a potential site for maternal antibody recognition[21,22] One might speculate that a more direct maternal alloantibody-fetal endothelial cell interaction exists for HPA-1 and HPA-5 alloimmunization because it is glycoproteins IIIa and Ia that are involved.

Chronic villitis in placentas with perivillous tissue damage could explain the findings of IUGR and IUFD through impaired maternal-fetal gas and nutrient exchange. Fetal platelet activation and aggregation through conformational changes in glycoprotein IIb/IIIa[23] can lead to intravillous thrombosis, as has been reported by DeTar et al[11] for NAIT because of human leukocyte antigen alloimmunization. Endothelial cell damage per se could explain, moreover, why it has been historically difficult to accurately predict adverse fetal hemorrhagic outcomes from platelet counts alone. Compromise of the fetal vascular wall integrity may play an important role in the pathogenesis of NAIT's hemorrhagic sequelae by compounding the risk from thrombocytopenia. Endothelial cell activation and subsequent damage may begin to explain NAIT's propensity for intraparenchymal intracranial hemorrhage as opposed to the intraventricular hemorrhage more commonly seen in prematurity and other conditions.

IVIG was introduced as a therapy to prevent hemorrhagic sequelae. We sought to compare both outcomes and the placental histopathology between a cohort treated prophylactically using IVIG (from late first to early second trimesters) with a cohort of patients who did not receive maternal IVIG. Therefore, because 1 subject did not have IVIG begun until after an ICH had already occurred, her data were excluded from the analysis. Our observation that maternal IVIG therapy obviated the perivascular plasma cell infiltrate suggests that IVIG confers protection to not only fetal platelets but also possibly fetal endothelial cells as well. This might in turn explain IVIG's ability to decrease the risk of fetal hemorrhage, even in the face of fetal thrombocytopenia.[6] It has long been suggested that IVIG exerts its beneficial effects by blocking free antibody, but recent in vitro studies suggest it may in fact play a role in modifying endothelial cell activation, which would be consistent with our findings and hypothesis.[24] In vivo, IVIG may interfere with the interaction of maternal B cells with their antigen and, thus, their subsequent proliferation and differentiation into antibody-secreting plasma cells.

Many questions still remain. This study did not aim to clarify the optimal regimen for IVIG, nor did it answer whether there is a crucial gestational age window in which IVIG must be started, beyond which the cascade of injury has already been set in motion. Nonetheless, our findings suggest that IVIG begun in the late first or early second trimester is prophylactic in eliminating what we suspect is a transplacental infiltration by maternal B lymphocytes seen in untreated pregnancies. Because the only patient treated with IVIG and steroids was the one in which intracranial hemorrhage had already been diagnosed, steroids' effect on placental pathology is unknown. Finally, the small sample size prohibits us from making more definitive conclusions.

Our study has disclosed histological observations in NAIT-affected placentas that may prove to be a hallmark of this disorder and lend support to our hypothesis that alterations in fetal endothelial cell integrity may plays a compounding role to that of thrombocytopenia in the hemorrhagic sequelae of NAIT. In addition, IUGR, may be a significant associated adverse outcome. Further study is warranted to better delineate whether IUGR should be added to the list of NAIT sequelae and conversely whether NAIT should be on the list of differential diagnoses for idiopathic IUGR. Our study's methodology has permitted us to examine the effect of maternal IVIG on the disease process and raises new questions surrounding the observation of a perivascular B-cell response within the villous stroma of cases of untreated pregnancies. What pathologic role this infiltrate may play in the process and sequelae of platelet alloimmunization will be the subject of future investigation.

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