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Detection of anti-HLA antibodies in maternal blood in the second trimester to identify patients at risk for antibody-mediated maternal anti-fetal rejection and spontaneous preterm delivery

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Abstract

Problem—Maternal anti-fetal rejection is a mechanism of disease in spontaneous preterm labor. The objective of this study was to determine whether the presence of human leukocyte antigen (HLA) panel-reactive antibodies (PRA) during the second trimester increases the risk for spontaneous preterm delivery.

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Methods of Study—This longitudinal case-control study included pregnant women with spontaneous preterm deliveries (n=310) and control patients with normal term pregnancies (n=620), matched for maternal age and gravidity. Maternal plasma samples obtained at 14-16, 16-20, 20-24, and 24-28 weeks of gestation were analyzed for HLA Class I and Class II PRA positivity using flow cytometry. The fetal HLA genotype and maternal HLA alloantibody epitope were determined for a subset of patients with positive HLA PRA.

Results—1) Patients with spontaneous preterm delivery were more likely to exhibit HLA Class I (adjusted OR=2.54, p<0.0001) and Class II (adjusted OR=1.98, p=0.002) PRA positivity than those delivering at term; 2) HLA Class I PRA positivity for patients with spontaneous preterm delivery between 28-34 weeks (adjusted OR=2.88; p=0.001) and after 34 weeks of gestation (adjusted OR=2.53; p<0.0001) was higher than for those delivering at term; 3) HLA Class II PRA positivity for patients with spontaneous preterm delivery after 34 weeks of gestation was higher than for those delivering at term (adjusted OR=2.04; p=0.002); 4) multiparous women were at higher risk for HLA Class I PRA positivity than nulliparous women (adjusted OR=0.097, p<0.0001 for nulliparity); 5) nulliparous women had a higher rate of HLA Class I PRA positivity with advancing gestational age (p=0.001); and 6) 78% of women whose fetuses were genotyped had allo-antibodies specific against fetal HLA class I antigens.

Conclusions—Pregnant women with positive HLA class I or class II PRA during the second trimester are at an increased risk for spontaneous preterm delivery due to antibody-mediated maternal anti-fetal rejection.

Keywords

Flow cytometry; preterm birth; rejection; transplantation

Introduction

Preterm birth occurs in 5 to 13% of deliveries, and is the leading cause of perinatal mortality and morbidity worldwide.[1-5] The economic impact cannot be over-emphasized, costing $26 billion annually in the United States alone.[6] Preterm birth can be the result of the spontaneous onset of labor in intact/pre-labor rupture of membranes or indicated for maternal or fetal complications such as preeclampsia and intrauterine growth restriction.[2,7]

Spontaneous preterm parturition is syndromic in nature,[7-12] which means that activation of the common pathway of parturition can be caused by multiple pathologic processes such as intrauterine infection/acute inflammation,[13-38] vascular lesions leading to relative utero-placental ischemia,[39-46] uterine over-distension in multiple gestations,[47] stress,[48, 49] cervical disease,[37,38,50-56] a decline in progesterone action,[57-64] and immune-related disorders.[65-69]

Among immune-related disorders, two distinct categories have been identified: 1) an allergic-like phenomenon[69] and 2) maternal anti-fetal rejection.[70-72] Recently, our group reported that chronic chorioamnionitis, a placental lesion reflecting maternal anti-fetal cellular rejection, is common in spontaneous preterm delivery.[70,73] This evidence suggests that rejection is associated with preterm parturition in a fraction of cases. A major challenge is to identify patients with ongoing maternal anti-fetal rejection during pregnancy. The

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diagnosis of chronic chorioamnionitis can only be made after the delivery of the placenta, and therefore, biomarkers in maternal blood are highly desirable to identify patients at risk for this pathologic process.

Allograft rejection results from cellular and humoral (antibody-mediated) immune response by the recipient of a graft.[74-79] The major histocompatibility complex (MHC) Class I and Class II molecules include the human leukocyte antigen (HLA) and have been implicated in the rejection of solid organs,[77,79-83] as well as bone marrow.[84] The presence of pre-formed donor-specific HLA antibodies in the circulation of the recipient is a risk factor for graft rejection.[79,85-88] Hence, the detection of such circulating antibodies is undertaken before transplantation for donor selection to assess the likelihood of a successful graft.[88-90] In addition, the detection of these antibodies after transplantation has been used to implement therapies to reduce the degree of sensitization such as immunoglobulin administration and plasmapheresis.[88, 89]

The standard method which consisted of a serologically based complement cytotoxicity assay has been replaced with solid phased assays, such as the HLA panel-reactive antibodies (PRA) using flow cytometry analysis.[91, 92] The HLA PRA assay detects anti-HLA Class I and Class II antibodies. We have reported a strong association between chronic chorioamnionitis and maternal IgG HLA Class I PRA positivity.[93] Moreover, we have shown a strong correlation between maternal and umbilical cord plasma HLA PRA positivity, indicating that circulating antibodies in the mother can cross the placenta.[93]

Recently, we found that HLA PRA in maternal and fetal sera can be associated with C4d deposition on the umbilical vein endothelium and that this occurs more frequently after spontaneous preterm labor/delivery than in normal delivery at term.[70] Activation of complement by the detection of C4d (a degradation product of the C4b) is indicative of acute humoral graft rejection.[94-98] We also reported an association between positive maternal HLA PRA and the diagnosis of chronic chorioamnionitis by histopathological examination of the placenta.[70, 93]

The objective of this study was to determine whether the presence of anti-HLA antibodies detected by HLA PRA in maternal blood in the second trimester of pregnancy is a risk factor for spontaneous preterm delivery in asymptomatic pregnant women.

Methods

Study population

A case-control study was designed to include pregnant women whose plasma samples were obtained one or more times during the second trimester (between 14 and 28 weeks of gestation). Cases consisted of pregnant women who subsequently developed spontaneous preterm labor with intact or ruptured membranes and had a preterm birth. All women in the control group delivered at term.

Three hundred and ten patients with spontaneous preterm delivery were matched for maternal age (within 3 years) and gravidity (primigravidae vs. multigravidae) with 620

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pregnant women with normal term deliveries (ratio=1:2). Two or more serial plasma samples were available in 255 patients who had a spontaneous preterm delivery and in 578 who delivered at term (controls). Spontaneous preterm labor was diagnosed as the presence of regular uterine contractions (at least 3 in 30 minutes) associated with cervical dilatation followed by a preterm delivery. Preterm prelabor rupture of membranes (PPROM) was diagnosed by sterile speculum examination when pooling of amniotic fluid in the vagina was observed or when positive nitrazine and ferning tests, performed if necessary, were confirmed before 37 completed weeks of gestation in the absence of labor. Women with hypertensive disorders during pregnancy, small-for-gestational-age neonates, multiple gestations, and fetal congenital anomalies were excluded from this study. All plasma samples were stored at -80°C until use.

All patients were Hispanic women who delivered at the Sótero del Río Hospital, Santiago, Chile. Maternal plasma samples were retrieved from the Bank of Biological Materials of Wayne State University/Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U. S. Department of Health and Human Services. All patients provided written informed consent, and the Institutional Review Boards of the Sótero del Río Hospital (an affiliate of the Pontificia Universidad Católica de Chile), Wayne State University, and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U. S. Department of Health and Human Services approved the use of clinical data and the collection and utilization of biological samples for research purposes.

Flow cytometry of panel-reactive anti-HLA antibodies

The FlowPRA®-I screening test (One Lambda Inc., Canoga Park, CA, USA) and FlowPRA®-II screening test (One Lambda) were used for flow cytometric analyses of HLA Class I and Class II PRA in maternal plasma, according to the manufacturer's instructions. Serum and plasma samples gave concordant results for these tests.[99] Briefly, HLA class I or class II microbeads were mixed with 20 μL of plasma, and incubated for 30 min at room temperature with gentle rotation. The beads were washed, centrifuged, and subsequently incubated with 100 μl of FITC-conjugated F (ab) 2 fragment of Fcγ fragment-specific goat anti-human IgG for 30 min. After 2 washes, 0.5 mL of fixing solution was added. The FL1 fluorescence of 5,000 events was analyzed using BD LSRII flow cytometry (BD Biosciences, San Jose, CA, USA). A sample with reactivity of 10% or more was considered to be positive for HLA PRA.[88, 100]

Luminex assay for fetal HLA specificity of maternal HLA antibodies

As maternal HLA antibodies specific to fetal HLA antigens can be considered analogous to specific antibodies in the setting of organ transplantation, fetal HLA specificity of maternal HLA antibodies was analyzed in 14 HLA Class I PRA-positive cases during the second trimester. This group was selected based on the availability of genomic DNA from umbilical cord blood. LABType® SSO typing kits (One Lambda) were used for the fetal HLA genotype and LABScreen® Single Antigen (One Lambda) for the epitope assessment of maternal HLA antibodis with Luminex assay (Luminex Corporation, Austin, TX, USA), according to the manufacturers' instructions.

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Statistical analysis

For continuous variables, the Kolmogorov-Smirnov tests were used to examine distributions for normality. When data were not normally distributed, a Kruskal-Wallis analysis of variance test was used, and the Mann-Whitney U test was performed for multiple comparisons among groups. When the distribution was normal, one-way analysis of variance tests were used to compare differences, and post hoc tests were performed for the multiple comparisons. For categorical variables, proportions were compared with the χ^2 test or Fisher's exact test. Medians and ranges were reported for continuous variables, whereas frequencies and percentages were calculated for categorical variables. Logistic regression analysis was performed to determine the magnitude of association between positive HLA PRA and the occurrence of spontaneous preterm delivery, adjusting for gestational age at sampling, fetal gender, parity, and history of previous preterm delivery which could influence spontaneous preterm delivery development. Maternal age and gravidity were adjusted in the matching process between case and control groups. Effect modification terms were used to assess subgroup effects.

The HLA PRA Class I reactivity level was also evaluated as a dichotomous variable (above or below 10%) using a generalized linear model assuming a binomial distribution with a logit link function. The effect and significance of the fixed effects were determined by generalized estimating equations,[101] therefore allowing for longitudinal (and eventually correlated) HLA PRA Class I reactivity observations from the same individuals. Explanatory variables included as covariates in this model were the group factor (disease or normal), parity, and gestational age (GA); effect modification between parity, gestational age, baby gender and history of preterm delivery was also investigated. Model selection was performed using the ANOVA function in the Geepack package which allowed comparison of the quality of the data fit among different models. Basic statistical analyses were conducted using the SPSS Version 15.0 (SPSS, Inc., Chicago, IL, USA). Longitudinal analysis was conducted using the Geepack of R statistical software.[102] All *P* values are two-sided, and a value of $P<0.05$ is considered to be statistically significant.

Results

Patient characteristics

Table I shows the demographics and clinical characteristics of the study population. The frequency of previous preterm delivery was significantly higher in women who had a spontaneous preterm delivery than in those who delivered at term ($P<0.001$).

HLA Class I PRA positivity in maternal blood as a risk factor for spontaneous preterm delivery

Figure 1A displays the differences in the rates of positive HLA Class I and Class II PRA between the two groups. The first maternal blood sample was used for a cross-sectional analysis. Patients who had a spontaneous preterm delivery had a higher rate of sero-positivity for HLA Class I PRA than those who delivered at term [34.5% (107/310) vs. 20.5% (127/620), $p<0.001$, see Figure 1A). However, the proportion of positive HLA Class

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II PRA was not significantly different between the two groups [14.8% (46/310) vs. 11.8% (73/620) p=0.18].

A positive maternal HLA Class I PRA during the second trimester was an independent risk factor for spontaneous preterm delivery after adjusting for confounding factors including gestational age at sampling, fetal gender, parity, and history of previous preterm delivery (odds ratio [OR] 2.40, 95% confidence interval [CI] 1.69-3.39). The sensitivity and specificity of positive HLA class I PRA in the identification of patients who subsequently had a spontaneous preterm delivery were 34.5% (107/310) and 79.5% (493/620), respectively. The rate of positive HLA Class I PRA did not differ between women who eventually had a spontaneous preterm delivery with intact membranes and those with preterm PPROM (35.1% vs. 33.3%).

Relationship between positive HLA PRA Class I and gestational age at delivery

Patients with a spontaneous preterm delivery were subdivided into three groups according to the gestational age at birth: 1) extremely preterm (<28 weeks), 2) moderately preterm (28-33 weeks), and 3) late preterm (34-37 weeks of gestation).[2, 103] The rate of maternal HLA PRA Class I positivity did not differ between patients who had a spontaneous preterm delivery before 28 weeks of gestation [14.3% (2/14)] and those who delivered at term [20.5% (127/620) (p=0.7)]. However, patients who delivered between 28 and 34 weeks of gestation [43.1% (25/58)] and after 34 weeks of gestation [33.6% (80/238)] had a higher rate of positive maternal HLA PRA Class I than patients who delivered at term [20.5% (127/620)] (p<0.001, for each) (Figure 1B).

Temporal changes in maternal HLA PRA positivity: longitudinal analysis

To determine whether there is a difference in the rate of positive maternal HLA PRA Class I and Class II between patients who subsequently had a spontaneous preterm delivery and those who delivered at term according to gestational age at maternal blood sampling, a longitudinal analysis was conducted. Spontaneous preterm delivery and parity status were associated with maternal sero-positivity for HLA PRA Class I after adjusting for fetal gender and a history of preterm delivery [(adjusted OR=2.54, p<0.0001) for spontaneous preterm delivery and an adjusted OR = 0.097, p<0.0001 for nulliparity)]. The association between spontaneous preterm delivery and sero-positivity status for HLA PRA Class I did not change with gestational age (no significant interaction). In contrast, nulliparous women were at a higher risk to have sero-positivity for HLA PRA Class I as gestational age increased (p<0.001) (Figure 2). Fetal gender and a history of preterm delivery did not significantly change sero-positivity status for HLA PRA Class I.

There were no effect modification terms identified for patients with HLA PRA Class II positivity (cut-off level above 10%) using mixed-effects models, including gestational age, parity, and pregnancy outcome variables (preterm or term birth). Therefore, only the main effects were tested. Spontaneous preterm delivery and parity status were associated with HLA PRA class II sero-positivity after adjusting for fetal gender and a history of preterm delivery (adjusted OR=1.98, p=0.002 for spontaneous preterm delivery and an adjusted OR

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= 0.11, p<0.0001 for nulliparity). There was no significant change in the sero-positivity status as a function of gestational age.

HLA PRA Class I positivity was significantly associated with late preterm deliveries (34-36 6/7 weeks) (adjusted OR = 2.53, p<0.0001) and moderate preterm deliveries (28-33 6/7 weeks) (adjusted OR = 2.88, p=0.001) relative to those who delivered at term. HLA PRA Class I and II positivity did not significantly differ between extreme preterm deliveries relative to term deliveries. Women with late preterm deliveries were more likely to exhibit HLA PRA Class II positivity compared to those who delivered at term (adjusted OR = 2.04, P=0.002). Moderate preterm delivery was not statistically significant when associated with HLA class II PRA positivity (adjusted OR = 1.7, p=0.23).

Fetal HLA specificity of maternal PRA

Table II summarizes the information for fetal HLA genotyping and the assessment of fetal HLA specificity of maternal HLA antibodies. For HLA class I antigens, 78.6% (11/14) positive maternal HLA PRA Class I cases had HLA antibodies specific against fetal HLA class I antigens. For HLA PRA Class II, 66.7% (4/6) of positive maternal HLA PRA Class II cases and none of the 8 negative maternal HLA PRA Class II cases (0%) had HLA antibodies specific against fetal HLA class II antigens (p=0.015).

Discussion

Principal findings of the study

(1) women with HLA PRA Class I or Class II positivity in the second trimester were significantly more likely to have a spontaneous preterm delivery than those who were HLA PRA negative;[64] (2) the rate of HLA PRA Class I was higher in women who had a spontaneous preterm delivery after 28 weeks of gestation than in patients who delivered at term; (3) the rate of HLA PRA Class II positivity was higher in women who had a spontaneous preterm delivery after 34 weeks (late preterm birth) than in patients who delivered at term; and (4) multiparous women had a higher risk of sero-positivity for HLA PRA Class I and II than nulliparous women. However, nulliparous women were at higher risk for sero-positivity for HLA class I as gestational age increased.

Maternal anti-fetal rejection as a mechanism of disease in human pregnancy

The central paradigm of reproductive immunology is that the fetus is a semi-allograft, which is tolerated during pregnancy.[104-113] The mechanisms for tolerance have been the subject of intensive investigation for decades and remain to be elucidated, although a considerable body of evidence supports a role for T-regulatory cells,[110, 114-116] expression of non-classical MHC molecules of trophoblast cells,[117, 118] tryptophan catabolism,[119, 120] T-cell apoptosis,[121] complement,[122] and co-stimulatory molecules such as the programmed death ligand, among others.[123-125]

Our group has been concerned with the role of maternal anti-fetal rejection as a mechanism of disease in human pregnancy.[70-72,93,126] Transplant rejection occurs when the recipient's immune system responds to antigenic differences between self and the graft.[79] Antigenic

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differences encoded by MHC genes are the major cause of transplant rejection.[79] *Allo-recognition* refers to immune responses to MHC antigens. Such antigens can elicit a B-cell response with the production of antibodies which mediates humoral rejection, as well as T-cell-mediated rejection (cellular rejection).[79] Both mechanisms – humoral and cellular – have been described by our group in the context of maternal anti-fetal rejection.[71] Chronic chorioamnionitis is an example of a cellular-mediated maternal anti-fetal rejection in which maternal T cells are in direct contact with the fetal trophoblast.[73] Antibody-mediated rejection occurs when the mother develops antibodies against fetal antigens in the MHC system.[127] The antibodies can cross the placenta and damage fetal cells by activating complement.[128, 129]

When fetal cells cross into the maternal circulation, they can elicit a maternal immune response to paternal antigens expressed in fetal cells. Although tolerance has been attributed to the expression of monomorphic MHCs by trophoblast, this cannot be invoked to explain tolerance to fetal white blood cells that cross the placenta into the maternal circulation. Humoral rejection requires the generation of antibodies that can cross the placenta and activate complement. We have previously demonstrated that these events occur in patients with histopathological evidence of maternal anti-fetal rejection in the placenta.

How can we identify the mother at risk for maternal anti-fetal rejection?

In transplant medicine, two major steps are taken to prepare recipients and potential donors.[79,130] Genotyping for MHC is routinely performed to characterize the degree of incompatibility between potential donors and recipients.[79,130] Also, the presence of anti-HLA antibodies is determined before transplantation because the presence of specific antibodies against antigens in the graft is associated with poor graft survival.[79, 130] This is accomplished by testing with HLA PRA sero-positivity. This test is also used to monitor the development of antibodies by the recipient after transplantation and the results guide therapy.[95, 130]

HLA PRA sero-positivity can be employed during pregnancy to identify the mother sensitized against the MHC antigens. Indeed HLA PRA testing is equivalent to an indirect Coombs test to assess whether the mother has antibodies against red blood cell antigens. HLA PRA positivity needs to be followed by characterization of antibodies to determine their HLA antigen specificity. Moreover, for rejection to occur, these antigens must be present in the fetus and the antibodies able to cross the placenta. We have previously documented that HLA PRA positivity is associated with histological evidence of maternal anti-fetal rejection at the time of birth by examination of the placenta. However, it is unknown whether HLA PRA positivity in the mid-trimester is associated with adverse pregnancy outcome, and specifically, spontaneous preterm labor.

Maternal HLA PRA status and the risk of preterm delivery

Although the clinical significance of maternal HLA antibodies has been studied in the setting of recurrent spontaneous abortions,[131,132] this is the first study to examine the relationship between second-trimester maternal HLA PRA status and subsequent spontaneous preterm delivery. Our findings reported herein indicate that mothers who are

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HLA PRA positive are more likely to have a spontaneous preterm delivery than those who are HLA PRA negative, and this is especially true for late preterm deliveries which account for the majority of all preterm births. The HLA PRA sero-positivity which conferred risk for spontaneous preterm delivery was both against Class I and Class II HLA. The magnitude of risk for preterm delivery conferred by HLA PRA positivity was greater for Class I than for class II.

In the present study, the HLA class I PRA positive rate in spontaneous preterm deliveries before 28 weeks of gestation was not different from that of pregnant women who delivered at term. However, patients with spontaneous preterm deliveries after 28 weeks of gestation had a higher rate of positive HLA class I PRA than women who delivered at term. This finding strongly suggests an association between maternal antibody-mediated anti-fetal rejection and late spontaneous preterm delivery. This is consistent with previous reports indicating that early preterm labor is associated with intra-amniotic infection and acute chorioamnionitis and funisitis.[133-137] Our findings are also consistent with previous observations that mean gestational age at delivery for patients with chronic chorioamnionitis (a manifestation of maternal anti-fetal cellular rejection) is higher than in those with acute chorioamnionitis among spontaneous preterm deliveries.[73]

HLA PRA positivity, however, indicates only the presence of antibodies in the maternal circulation against a wide range of potential antigens belonging to the HLA family. In a subset of patients, we were able to demonstrate that the anti-HLA antibodies were against specific fetal antigens as demonstrated by characterization of the fetal genotype. These antigens are paternally inherited. The clinical utility and predictive value of maternal HLA alloantibodies for spontaneous preterm delivery need to be determined in future studies. The sensitivity of HLA PRA positivity to detect spontaneous preterm delivery was 34.5%. This is not unexpected as only a fraction of all preterm deliveries results from maternal anti-fetal rejection.

When do pregnant women become sensitized against fetal HLA antigens?

HLA sensitization can occur after a blood transfusion,[138] organ transplantation,[79,83] feto-maternal hemorrhage,[139, 140] and fetal cell trafficking into the maternal circulation.[140-142] It is possible, therefore, that sensitization occurred before or during the index pregnancy. Our finding that HLA PRA positivity was more common in parous than in non-parous women suggests that the likelihood of sensitization increases with a prior pregnancy. The observation that a positive sero-conversion of HLA PRA class I during the index pregnancy is more frequent with advancing gestational age indicates that the risk of sensitization increases with the duration of pregnancy. This is consistent with a previous report that the positive rate of anti-paternal/fetal cytotoxic antibody test increases as a function of gestational age in normal pregnant women.[143] The finding that a large proportion of HLA PRA Class I positive pregnant women (77.7%) already had antibodies at the time of initial sampling indicates that sensitization begins before the second trimester.

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Strengths and limitations of the study

There are two major strengths of this study: 1) It is the first longitudinal examination of HLA PRA positivity during pregnancy, and 2) there was a large number of patients studied. A limitation of this study is that the HLA PRA status prior to pregnancy or during the first trimester could not be evaluated. Because most HLA PRA Class I positive cases had HLA PRA Class I at their first blood sampling, HLA alloantibodies might not be related to the index pregnancy in a fraction of HLA PRA positive women in this study. HLA sensitization prior to pregnancy is likely to have immunological effects on the index pregnancy considering that the presence of either donor-specific or non-donor-specific HLA antibodies in the recipients is associated with the development of humoral and cellular rejection and poor graft outcome in organ transplantation.[144, 145] In addition, the observation in this study that 78.6% of HLA PRA positive cases tested had fetal HLA-specific antibodies indicates that maternal HLA antibodies may have an effect on the fetuses regardless of the gestational age at which they are detected.

Conclusion

A positive HLA PRA Class I or Class II during the second trimester of pregnancy is a risk factor for spontaneous preterm delivery, especially for late preterm birth which represents 70% of all preterm births. These observations provide support for the concept that maternal anti-fetal rejection is a mechanism of disease in late spontaneous preterm births.

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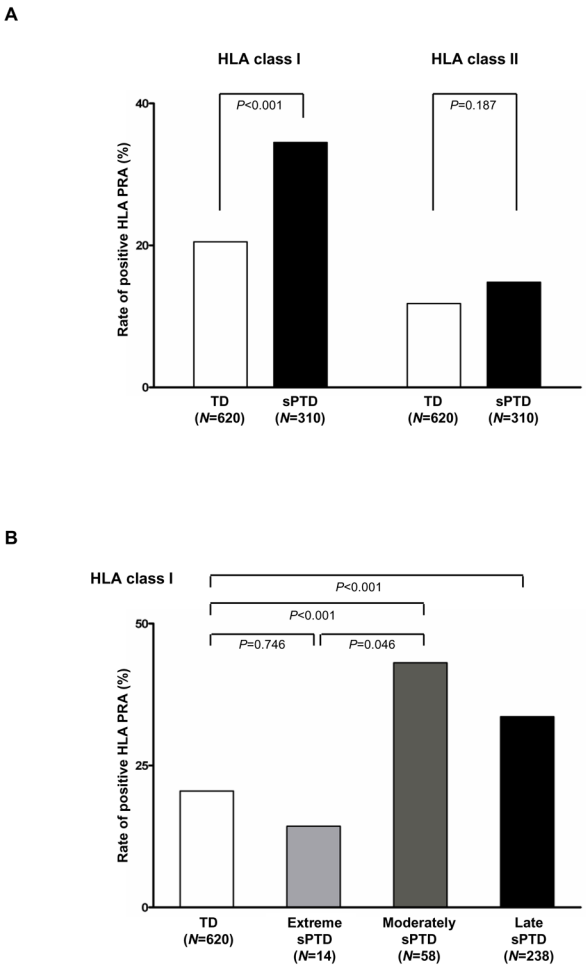

Figure 1. HLA PRA in maternal plasma

(A) HLA class I and class II PRA positive rates in normal term delivery and spontaneous preterm delivery cases, (B) HLA class I PRA positive rate according to gestational age at delivery. HLA, human leukocyte antigen; PRA, panel-reactive antibody; TD, normal term delivery; sPTD, spontaneous preterm delivery; extreme sPTD, delivered before 28 + 0 weeks of gestation; moderate sPTD, delivered between 28 + 0-33 + 6 weeks of gestation; late sPTD, delivered between 34 + 0-36 + 6 weeks of gestation.

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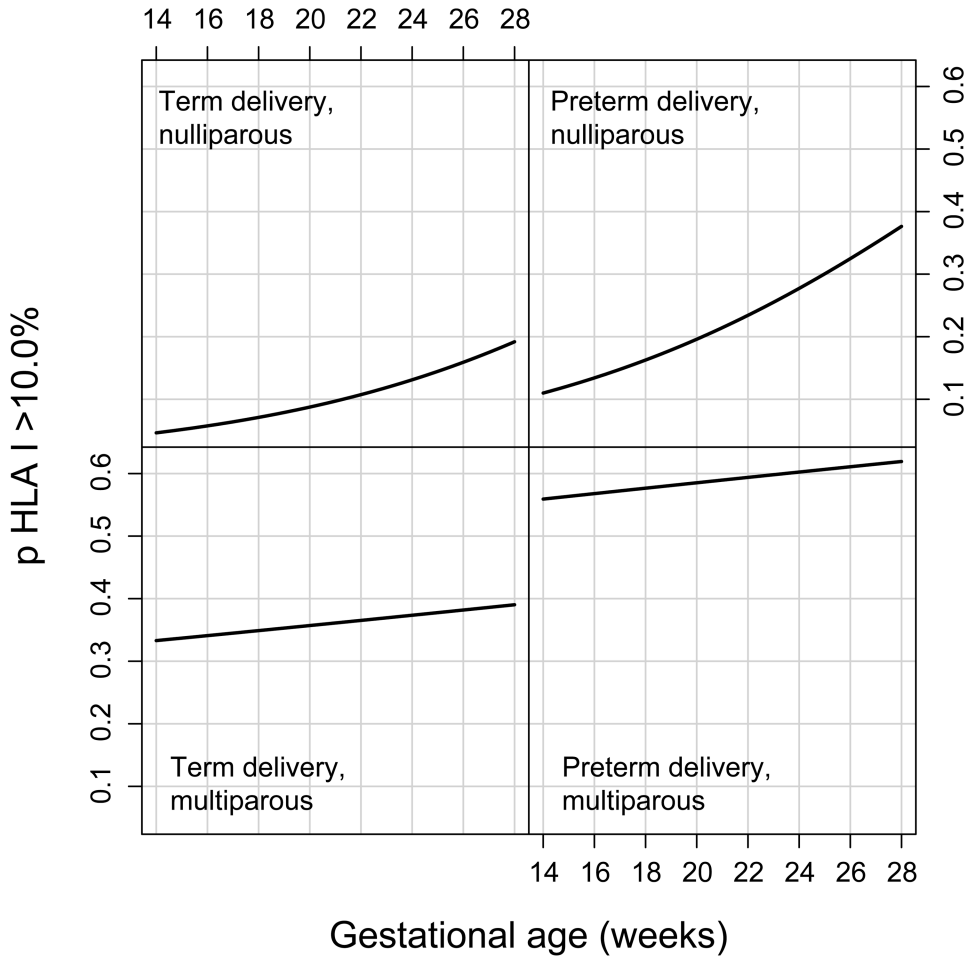

Figure 2. Illustration of effect modification between parity and case status over time

The likelihood of a HLA PRA class I > 10% elevating among women with spontaneous preterm delivery compared to women with normal term delivery over time differed significantly by parity in multiparous women with spontaneous preterm delivery, exhibiting the greatest risk relative to nulliparous women over time. TD, normal term delivery; sPTD, spontaneous preterm delivery; OR, odds ratio.

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Table I

Demographics of the study population

	Term delivery (*N*=620)	Spontaneous preterm delivery (*N*=310)	*P* value
Maternal age (year)*	25 (15-44)	26 (15-46)	0.939
Gravida (Primigravida) (%)	36.8	36.8	1.000
Para (Nullipara) (%)	39.4	40.3	0.776
Gestational age at 1st sample (weeks)*	16.7 (14.1-25.3)	17.1 (14.1-27.6)	0.044
Number of serial sampling*	3 (1-4)	2 (1-4)	<0.001
Number of cases at 14-16 weeks	219	98	
Number of cases at 16-20 weeks	446	215	
Number of cases at 20-24 weeks	476	199	
Number of cases at 24-28 weeks	509	213	
History of previous preterm delivery (%)	3.5	17.7	<0.001
Cigarette smoking (%)	12.3	12.9	0.779
Alcohol use (%)	4.0	3.9	0.906
Drug abuse (%)	0.5	1.3	0.230
Cesarean delivery (%)	16.1	21.6	0.040
Baby gender (Male) (%)	54.4	58.1	0.283
Gestational age at delivery (weeks)*	39.7 (37.0-42.0)	35.6 (22.0-36.9)	<0.001
Birth weight (gm)*	3400 (2530-4150)	2590 (480-3930)	<0.001

* Median (range)

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Table II

Fetal HLA specificity of maternal HLA antibodies

Case		HLA PRA*		Fetal Genotyping (HLA-)						HLA antibodies identified	
		Class I	Class II	A	B	C	DQ	DR	DR 51/ 52/ 53	Class I	Class II
1	sPTD	73.9	5.1	33, 68	14, 51	3, 8	2, 7	14, 17	52	A23, A24, A25, A32, B13, B18, B27, B35, B37, B38, B44, B45, B46, B47, B49, B50, **B51**†, B52, B53, B57, B58, B59, B62, B63, B71, B72, B75, B76, B77, B78, B82, Cw9	
2	sPTD	98.5	0.5	2, 68	39, 48	1, 7	4, 9	8, 9	53	A1, A3, A11, A23, A24, A25, A26, A29, A30, A31, A32, A33, A34, A36, A43, A66, **A68**†, A69, A74, A80, B7, B13, B27, B35, B37, B41, B42, B47, **B48**†, B51, B52, B55, B60, B61, B62, B67, B72, B73, B75, B78, B81, Cw2, Cw17	
3	TD	40.4	1.1	3, 24	38, 39	7, 12	5, 7	1, 16	51	B7, B13, B27, B42, B48, B54, B55, B56, B60, B61, B67, B72, B73, B81, B82	DP14, DR52
4	TD	85.1	60.9	1, 31	40	1, 2	6, 8	4, 13	52, 53	**A1**, A3, A11, A23, A24, A25, A26, A29, A34, A36, A43, A66, A80, B44, B45, B73, B76, B82, **Cw2**†, Cw4, Cw5, Cw6, Cw7, Cw15, Cw17, Cw18	DR8, DR11, DR12, **DR13**†, DR14, DR17, DR18, **DR52**†
5	sPTD	94.2	1.5	29, 31	39, 44	7, 16	2, 8	4, 7	53	A1, A23, A24, A25, A32, A80, B27, B35, B37, B38, B41, **B44**†, B45, B46, B47, B49, B50, B51, B52, B53, B57, B58, B59, B60, B61, B62, B63, B71, B72, B75, B76, B77, B82, Cw5	DP11, DQ7, DR13
6	TD	99.9	1.5	3, 11	7, 49	7	2, 6	7, 13	52, 53	A66, **B7**, B8, B13, B18, B27, B35, B38, B39, B41, B42, B45, B46, B47, B48, B50, B51, B52, B53, B54, B55, B56, B57, B59, B60, B61, B62, B63, B64, B65, B67, B71, B72, B73, B75, B76, B78, B81, B82, Cw2, Cw15, Cw16, Cw17	
7	TD	35.1	16.0	2	15, 35	1, 4	8, 9	4, 9	53	B7, B13, B41, B42, B44, B45, B47, B49, B50, B55, B56, B60, B61, B62, B67, B81, B82	DR7, DR18
8	sPTD	90.0	95.0	1, 24	18, 52	5, 12	2, 6	3, 15	51, 52	**A1**†, A3, A11, A25, A26, A29, A30, A31, A32, A33, A34, A36, A43, A66, A74, A80, B44, B45, B46, B73, B76, Cw1, Cw6, Cw7, Cw8, Cw9, Cw10, **Cw12**†, Cw14, Cw16, Cw18	DQ4, DQ5, **DQ6**†, DQ8, DQ9, DR1, DR4, DR7, DR9, DR10, **DR15**†, DR16 **DR51**†, DR53, DR103
9	sPTD	94.8	57.7	1, 23	8, 14	6, 8	2, 5	1, 3	52	A2, **A23**, A24, A25, A32, A68, A69, B13, B27, B35, B37, B38, B41, B44, B45, B46, B47, B49, B50, B51, B52, B53, B55, B56, B57, B58, B59, B60, B61, B62, B63, B71, B72, B75, B77, B76, B77, B78, Cw10	DQ7, **DR1**†, DR4, DR9, DR10, DR14, DR15, DR51, DR53, DR103
10	sPTD	66.0	17.2	29, 68	39, 44	7, 16	7, 8	4, 14	52, 53	A1, A23, A24, A25, A32, B13, B18, B27, B35, B37, B38, **B44**†, B45, B47, B49, B50, B51, B52, B53, B57, B58, B59, B62, B63, B71, B72, B75, B76, B77, B78, B82	**DR4**†, DR13
11	sPTD	84.3	1.0	23, 68	7, 50	5, 7	5, 7	13, 14	52	A32, A66, A74, **B7**†, B8, B13, B18, B27, B37, B38, B39, B41, B42, B44, B46, B47, B48, B51, B52, B54, B55, B56, B57, B59, B60, B61, B62, B64, B65, B67, B71, B72, B73, B75, B76, B77, B78, B81, B82, Cw1, Cw2, Cw9, Cw10, Cw14, Cw15, Cw17	DP11

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Case		HLA PRA*		Fetal Genotyping (HLA-)						HLA antibodies identified	
		Class I	Class II	A	B	C	DQ	DR	DR 51/ 52/ 53	Class I	Class II
12	sPTD	99.6	99.6	2, 31	39, 49	7	5	1	–	**A2**†, A11, A23, A24, A25, A26, A32, A33, A34, A43, A66, A68, A69, B13, B18, B27, B35, B37, B38, B41, B42, B44, B45, B46, B47, **B49**†, B50, B51, B52, B53, B54, B55, B56, B57, B58, B59, B60, B61, B62, B63, B64, B65, B71, B67, B71, B72, B73, B75, B76, B77, B78, B82, Cw2, Cw5, Cw6, Cw9, Cw10, Cw15, Cw17, Cw18	DP1, DP2, DP3, DP4, DP5, DP6, DP9, DP10, DP11, DP13, DP14, DP17, DP18, DP19, DP20, DP28, DQ2, DQ4, DQ7, DQ8, DQ9, DR4, DR7, DR8, DR9, DR11, DR12, DR13, DR15, DR16, DR51, DR52, DR103
13	TD	16.5	1.0	2, 24	39, 40	7, 15	6, 8	4, 15	51, 53	B44, B45, B49, B50, B52, B62, B71, B72, B75, B76, B82	DP2, DR8, DR9, DR13, DR18
14	sPTD	66.8	0.5	1, 29	8, 51	7, 16	7, 8	4, 13	52, 53	A23, A24, A25, A32, B13, B18, B27, B35, B37, B38, B44, B45, B47, B49, **B51**†, B52, B53, B57, B58, B59, B63, B71, B72, B75, B77, B78, Cw1, Cw12, Cw15	DQ2

*, panel-reactivity (%)

†, specific against fetal HLA antigens

HLA, human leukocyte antigen; *PRA*, panel-reactive antibodies; *TD*, normal term delivery; *sPTD*, spontaneous preterm delivery

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