Maternal HLA Panel Reactive Antibodies in Early Gestation Positively Correlates with Chronic Chorioamnionitis: Evidence in Support of the Chronic Nature of Maternal Anti-fetal Rejection

JoonHo Lee[1], **Roberto Romero**[1,*], **Yi Xu**[1], **Jung-Sun Kim**[2], **Ji Young Park**[3], **Juan Pedro Kusanovic**[1,4,5], **Tinnakorn Chaiworapongsa**[1,6], **Sonia S. Hassan**[1,6], and **Chong Jai Kim**[1,7,*]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, Maryland, and Detroit, Michigan, USA

[2]Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[3]Department of Laboratory Medicine, Hallym University College of Medicine, Seoul, Republic of Korea

[4]Department of Obstetrics and Gynecology, Sótero del Rio Hospital, Santiago, Chile

[5]Department of Obstetrics and Gynecology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

[6]Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

[7]Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan, USA

Abstract

Problem—Maternal tolerance of the fetus is essential for viviparity, yet anti-fetal rejection occurs in several pregnancy complications. Chronic chorioamnionitis (CCA) is a feature of anti-fetal cellular rejection. There is a robust association between CCA and maternal seropositivity for anti-HLA panel reactive antibodies (PRA) at the time of delivery. This longitudinal study was performed to assess maternal HLA PRA status in early gestation and the temporal evolution of maternal HLA PRA in the context of CCA and thereby to determine whether HLA PRA during the course of pregnancy is useful for the detection of anti-fetal rejection.

Method of Study—Maternal sera obtained before 16 weeks of gestation and at delivery were analyzed for HLA panel reactive antibodies (PRA) in cases with (*N*=100) and without (*N*=150) CCA.

Results—IgG but not IgM HLA class I and II PRA positivity at delivery was higher in cases with CCA than in those without CCA. IgG HLA class I PRA positivity before 16 weeks of gestation was higher in cases with CCA than in those without (30.3% vs. 13.3%; *p*=0.001). Positive conversion (negative HLA PRA before 16 weeks of gestation but positive at delivery) of IgG HLA class I and II PRA was significantly associated with CCA. Fetal HLA class I antigen-specific antibodies were confirmed in 12 of 16 mothers tested who were sensitized to HLA class I antigens before 16 weeks of gestation.

*Correspondence to: Chong Jai Kim, MD, PhD, Department of Pathology, Wayne State University School of Medicine, Hutzel Women's Hospital, 3990 John R St, Detroit, MI 48201, USA. Tel: (313) 577 6404. Fax: (313) 577 5686. cjkim@med.wayne.edu. Roberto Romero, MD, Perinatology Research Branch, NICHD, NIH, DHHS, Hutzel Women's Hospital, 3990 John R St, Detroit, MI 48201, USA. Tel: (313) 993 2700. Fax: (313) 993 2694. prbchiefstaff@med.wayne.edu.

Conclusion—Positive maternal HLA PRA before 16 weeks of gestation and the temporal evolution of maternal HLA PRA are associated with the presence of CCA at the time of delivery. Maternal IgG HLA PRA has potential to be a monitoring tool of anti-fetal rejection. Furthermore, the findings herein indicate that subsets of fetuses are exposed to alloimmune HLA antibodies for months, especially in cases with CCA.

Keywords

chronic chorioamnionitis; human leukocyte antigen; panel reactive antibody; pregnancy; preterm birth; rejection

Introduction

Maternal tolerance of the fetal semi-allograft is considered essential for viviparity,[1–16] and several investigators have proposed associations between abnormal maternal allogeneic responses and certain obstetrical syndromes such as recurrent pregnancy loss, fetal growth restriction, preeclampsia, and spontaneous preterm birth.[17–27] However, a specific definition of maternal anti-fetal rejection in placental pathology has not yet been formulated. Recently, we proposed that chronic chorioamnionitis (CCA; infiltration of maternal T cells in the chorioamniotic membranes) represents the placental manifestation of maternal anti-fetal cellular rejection and that it is the most common lesion found in the placentas of women delivering after spontaneous preterm labor (with intact or ruptured membranes).[28] This lesion is frequently associated with villitis of unknown etiology, which also represents the same immunological process in the villous placenta.[29–33]

Preterm birth is a major health care problem, as there were 13 million preterm births worldwide in 2005, and its frequency is increasing.[34,35] It is the leading cause of perinatal morbidity and mortality,[36] and in the United States, annual costs related to preterm birth are estimated to be $26 billion.[37] Preterm birth is categorized according to two clinical circumstances: spontaneous and indicated preterm births.[36] However, a precise mechanism of preterm birth still remains elusive in most cases, although infection and/or inflammation, uteroplacental ischemia, and cervical disorders have been proposed as mechanisms responsible for preterm birth.[38,39] Therefore, there is an urgent need to understand the mechanisms of disease responsible for spontaneous or indicated preterm births with the hope of developing primary or secondary prevention.

While histopathological demonstration of leukocyte infiltration is the main diagnostic tool in patients with clinically suspected ongoing rejection of solid organ transplants such as heart, liver, and kidney,[40–42] the clinical value of chronic chorioamnionitis is quite limited during the course of pregnancy because it is impossible to take serial biopsies of the placenta or the chorioamniotic membranes. This presents a major challenge for identification and monitoring of anti-fetal rejection in pregnant women.

Generation of donor-specific human leukocyte antigen (HLA) antibodies (DSA) is an important feature of humoral antibody-mediated rejection after allograft transplantation, and DSA can be used for screening or diagnostic purposes.[43–45] HLA sensitization is a risk factor for poor graft outcome, and HLA panel reactive antibodies (PRA) are commonly used in clinical practice to assess the HLA sensitization of recipients[46,47] and the likelihood of graft rejection in patients who undergo transplantation.[48–51] HLA PRA screenings are being done before and after organ transplantation, and clinical approaches to reduce the degree of sensitization such as immunoglobulin administration and plasmapheresis are available.[46,47] It also has been shown that *de novo* HLA antibodies (negative PRA before transplantation

and positive PRA after transplantation) are associated with a significant decrease in a five-year graft survival rate for living related kidney transplantation.[51]

We have recently reported a strong association between chronic chorioamnionitis and maternal IgG HLA class I PRA positivity.[26] Furthermore, the correlation between maternal and umbilical cord plasma HLA PRA positivity is also robust,[26] as maternal IgG antibodies cross the placenta.[52] Therefore, positive maternal HLA PRA, which can be a consequence of either preformed antibodies before pregnancy or *de novo* generation during the index pregnancy, is a marker of maternal sensitization which is required for humoral anti-fetal rejection.

In this study, we sought to analyze maternal HLA PRA status in early gestation and the temporal evolution of maternal HLA PRA according to the presence or absence of chronic chorioamnionitis by undertaking a longitudinal analysis of spontaneous preterm births and term births.

Materials and Methods

Study population

To determine maternal HLA PRA status in early gestation and the evolution of maternal HLA PRA during the course of pregnancy according to the presence or absence of chronic chorioamnionitis subsequently diagnosed at the time of delivery, the pregnant women who underwent blood sampling before 16 weeks of gestation and at the time of delivery and whose placentas were available for histopathologic examination were selected from the Bank of Biological Materials of the 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 were Hispanic women who delivered at the Sótero del Rio Hospital, Santiago, Chile. The study population was divided according to the presence or absence of chronic chorioamnionitis and gestational age at delivery: women with chronic chorioamnionitis (*N*=100: 50 term and 50 spontaneous preterm deliveries) and women without chronic chorioamnionitis (*N*=150: 100 term and 50 spontaneous preterm deliveries). Cases of fetal congenital anomalies and multiple gestations were excluded from this study. Spontaneous preterm deliveries included cases of preterm labor or preterm prelabor rupture of membranes. Among 250 'at the time of delivery' samples, 242 samples (96.8 %) were obtained within 1 week, 3 samples (1.2 %) within 2 weeks, 3 samples (1.2 %) within 3 weeks, and the remaining 2 samples (0.8 %) within 4 weeks (24 and 25 days) before delivery. Serum samples were kept at −80°C until use. All patients provided written informed consent. The Institutional Review Boards of the participating institutions approved the collection and use of biological materials and clinical data for research purposes.

Placental pathology

Histopathological lesions of the placenta were defined according to diagnostic criteria proposed by the Perinatal Section of the Society for Pediatric Pathology, which include lesions consistent with amniotic fluid infection, maternal vascular underperfusion, and fetal vascular thrombo-occlusive disease.[53] Villitis of unknown etiology was diagnosed based on the histologic criteria previously described,[33] and the diagnosis of chronic chorioamnionitis was made when lymphocytic infiltration into the chorionic trophoblast layer or chorioamniotic connective tissue occurred as described previously.[28] Chronic deciduitis with plasma cells was defined when lymphoplasmacytic infiltrate was observed in the decidua basalis.[54]

Flow cytometry for HLA panel reactive antibodies

Flow cytometric analyses of HLA class I and class II PRA in maternal sera were performed using the FlowPRA®-I screening test (One Lambda, Canoga Park, CA, USA) and the FlowPRA®-II screening test (One Lambda), according to the manufacturer's instructions. Class I or class II microbeads were mixed with 20 μl of serum, and incubated for 30 min at room temperature with gentle rotation. The beads were washed 3 times with 1 ml of FlowPRA wash buffer by centrifugation at 9,000xg for 2 min, followed by incubation with 100 μl of FITC-conjugated F(ab)2 fragment of Fcγ fragment specific goat anti-human IgG for 30 min for IgG HLA PRA or with 100 μl of FITC-mouse anti-human IgM at 1:10 dilution for IgM HLA PRA (BD Biosciences, San Jose, CA, USA). After washing the beads twice with 1 ml of wash buffer and adding 0.5 ml of fixing solution (PBS with 0.5% formaldehyde), the FL1 fluorescence of 5,000 events was analyzed using BD LSRII flow cytometry (BD Biosciences). A sample with reactivity of 10% or more was considered positive for HLA PRA.[47,55] For the analysis of IgG isotypes, a biotin-labeled mouse anti-human IgG1, IgG2, IgG3, or IgG4 antibody (Invitrogen Corp., Carlsbad, CA, USA) was used instead of FITC-conjugated F(ab)2 fragment of Fcγ fragment specific goat anti-human IgG in the same kits used above for the second antibody and followed by washing and incubation with 1μg/ml Streptavidin-APC (eBioscience, San Diego, CA, USA) for another 30 min before flow cytometry analysis.

Fetal HLA genotyping and anti-fetal specificity of maternal HLA antibodies

As maternal-fetal HLA-specific antibodies can be considered analogous to donor-specific antibodies in organ transplantation settings, we also assessed whether maternal HLA antibodies were specific for fetal HLA antigens by analyzing fetal HLA genotypes and by testing maternal sera for fetal HLA-specific antibodies in 16 IgG HLA class I PRA positive cases before 16 weeks of gestation, whose genomic DNA from fetal cord blood were available. Fetal HLA specificity of maternal HLA PRA was determined using LABTypeR SSO typing kits (One Lambda) for fetal HLA genotype and LABScreenR Single Antigen (One Lambda) for maternal HLA antibody detection with Luminex assay (Luminex Corporation, Austin, TX, USA), according to the manufacturers' instructions. Briefly, for fetal HLA genotyping, locus-specific polymerase chain reaction (PCR) amplification was performed with 2 μl of genomic DNA from fetal cord blood (20 to 50 ng/μl), D-mix (One Lambda), locus-specific amplification primers (One Lambda) and Taq polymerase (Applied Biosystems, Carlsbad, CA, USA). The PCR was conducted in the following cycles: 1 cycle at 96 °C for 3 min; 5 cycles at 96 °C for 20 s, 60 °C for 20 s, and 72 °C for 20 s; 30 cycles at 96 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s; 1 cycle at 72 °C for 10 min. Thereafter, the amplified PCR products were denatured and neutralized, followed by hybridization with LABType SSO beads at 60°C for 15 min. After 3 washes with wash buffer, the beads were labeled with R-PE Conjugated Streptavidin and washed twice. Data acquisition was performed with Luminex 100 (Luminex) and data analysis was conducted with HLA Fusion 2.0 software (One Lambda). For the epitope assessment of maternal HLA antibody, 20 μl of maternal sera were mixed with 5 μl of Class I or Class II LABScreen beads (One Lambda), followed by incubation for 30 min at room temperature with gentle shaking. After 3 washes with wash buffer, the beads were incubated with PE-conjugated anti-human IgG (One Lambda) for another 30 min at room temperature and washed twice. Thereafter, the samples were analyzed for data acquisition with Luminex 100 (Luminex). Data analysis was conducted with HLA Fusion 2.0 software (One Lambda).

Statistical analysis

Medians and ranges for continuous variables and frequencies and percentages for categorical variables were reported. The Kruskal-Wallis analysis of variance test and the Mann-Whitney U test or one-way analysis of variance and post-hoc tests were performed to compare

differences for continuous variables. Proportions were compared using the χ^2 test or Fisher's exact test for categorical variables. Statistical analyses were performed using the SPSS version 15.0 (SPSS, Inc., Chicago, IL, USA). All *P* values were two-sided, and a value of less than 0.05 was considered statistically significant.

Results

Maternal IgG HLA PRA positivity and chronic chorioamnionitis

Demographic and clinical characteristics of patients in the different study groups are summarized in Table I. Representative histological features of chronic chorioamnionitis showing lymphocytic infiltration into the chorionic trophoblast layer or chorioamniotic connective tissue are shown in Figure 1A and 1B.

At the time of delivery, IgG HLA class I PRA positivity in maternal serum was higher in patients whose placenta had chronic chorioamnionitis than in those without this lesion (70.0% vs. 24.0%, *P* < 0.001) (Figure 2A). In samples obtained before 16 weeks of gestation, maternal serum IgG HLA class I PRA positivity was also higher in cases with chronic chorioamnionitis than in those without chronic chorioamnionitis (30.0% vs. 13.3%, *P* = 0.001) (Figure 2A). The IgG HLA class II PRA positivity at the time of delivery was higher in patients with chronic chorioamnionitis than in those without this lesion (36.0% vs. 16.7%, *P* < 0.001) (Figure 2B). Before 16 weeks of gestation, chronic chorioamnionitis tended to be associated with a higher rate of positive maternal serum IgG HLA class II PRA, although the difference did not reach statistical significance (12.0% vs. 6.0%, *P* = 0.094) (Figure 2B). Subgroup analyses of patients with term and preterm deliveries showed a similar difference for the IgG HLA class I and class II PRA positivity in maternal serum at the time of delivery according to the presence or absence of chronic chorioamnionitis (*P* < 0.05, for each) However, in the samples obtained before 16 weeks of gestation, a significant difference in maternal serum IgG HLA class I PRA positivity between the cases with and without chronic chorioamnionitis was found only in preterm births (34.0% vs. 10.0%, *P* < 0.01)

We also determined if there is an association between chronic deciduitis with plasma cells and maternal IgG HLA PRA positivity. Women with chronic deciduitis with plasma cells had a significantly higher rate of chronic chorioamnionitis than those without (70.5% vs. 33.5%, *P* < 0.001). As is the case with chronic chorioamnionitis, women with chronic deciduitis with plasma cells had higher serum IgG HLA class I and class II PRA positivity at the time of delivery than those without this lesion (HLA class I: 88.6% vs. 30.9%, *P* < 0.001; HLA class II: 45.5% vs. 19.9%, *P* < 0.001). Even in samples collected before 16 weeks of gestation, chronic deciduitis with plasma cells was associated with higher positive rates of maternal IgG HLA class I and class II PRA (HLA class I: 43.2% vs. 15.0%, *P* < 0.001; HLA class II: 18.2% vs. 6.3%, *P* = 0.017)

Evolution of HLA PRA seropositivity as a function of gestational age and chronic chorioamnionitis

To determine the effect of advancing gestational age on the development of IgG HLA PRA positivity, we compared IgG HLA PRA positivity between samples taken before 16 weeks of gestation and those obtained at the time of delivery. IgG HLA class I and class II PRA positivity were significantly higher in the samples obtained at the time of delivery than in those obtained before 16 weeks of gestation (HLA class I: 42.4% vs. 20.0%, *P* < 0.001; HLA class II: 24.4% vs. 8.4%, *P* < 0.001). Such an evolution of IgG HLA class I and class II seropositivity as a function of gestational age at blood sampling was observed in all subgroups stratified according to the presence or absence of chronic chorioamnionitis and

gestational age at delivery ($P < 0.05$, for each), except in patients who delivered preterm and had no chronic chorioamnionitis. Figure 3 shows maternal HLA class I PRA reactivity for each case. Most patients showed an increase in the reactivity of HLA PRA for samples obtained at the time of delivery than in those obtained before 16 weeks. This observation was consistent among the four study groups. Similar results were obtained for HLA class II PRA (Figure 4).

There were also significant differences in the temporal changes of IgG HLA class I and class II PRA positivity according to the presence or absence of chronic chorioamnionitis ($P < 0.01$, for each) (Figure 5). When negative to positive conversion of maternal IgG HLA PRA (*de novo* HLA PRA) was defined as negative IgG HLA PRA in a serum sample obtained before 16 weeks of gestation and positive IgG HLA PRA in the sample obtained at the time of delivery, a negative to positive conversion of IgG HLA class I PRA was found in 42.0% of cases with chronic chorioamnionitis, while it was found in 11.3% of cases without chronic chorioamnionitis ($P < 0.001$). Negative to positive conversion of IgG HLA class II PRA was observed in 26.0% of cases with chronic chorioamnionitis, and in 10.7% of cases without chronic chorioamnionitis ($P = 0.001$). Only 2% (5/250) of mothers showed negative conversion for either IgG HLA class I or class II PRA. At the time of delivery, the proportion of highly sensitized patients (panel-reactivity of 80% or more) was significantly greater in patients with persistent HLA class I PRA positivity than in those with *de novo* positive conversion (46.8% vs. 25.4%, $P < 0.05$). Similar differences in the temporal changes of IgG HLA class I and class II PRA positivity according to the presence or absence of chronic chorioamnionitis were also shown in the subgroups including term and preterm delivery cases ($P < 0.05$, for each).

To adjust for the effect of a previous pregnancy as a confounding factor which could have influence on the HLA PRA positivity, we stratified the study population according to the presence or absence of a history of previous pregnancy. Both primigravida mothers and multigravida mothers showed similar differences in the temporal changes of IgG HLA class I and class II PRA positivity according to the presence or absence of chronic chorioamnionitis ($P < 0.05$, for each).

Maternal IgM HLA PRA positivity and chronic chorioamnionitis

As the presence of IgM HLA antibodies and an IgM-to-IgG HLA antibody switch have also been associated with poor graft outcome in some instances of solid organ transplantation such as kidney and heart,[56,57] we examined whether IgM HLA PRA is generated in the mother during pregnancy, and whether it is associated with chronic chorioamnionitis. IgM HLA class I PRA positivity is shown in Figure 6. In samples obtained at the time of delivery, IgM HLA class I PRA was present in 38.0% and 34.0% of cases with and without chorioamnionitis, and before 16 weeks of gestation, the frequency was 26.0% and 28.7%, respectively. Overall, in stark contrast to IgG HLA class I and class II PRA, IgM HLA class I and class II PRA positivity did not show significant differences according to the gestational age at maternal blood sampling and with the presence or absence of chronic chorioamnionitis in both term and preterm births.

Insofar as an IgM-to-IgG HLA antibody switch, among 50 cases positive for IgM but not IgG HLA class I PRA before 16 weeks of gestation, 19 cases (38%) showed IgG HLA class I PRA positivity at the time of delivery and 31 cases (62%) did not. Interestingly, cases with chronic chorioamnionitis had a higher rate of IgM-to-IgG antibody switch (IgG HLA class I PRA positivity at the time of delivery) than those without chronic chorioamnionitis (71.4% vs. 25.0%, $P < 0.01$). Furthermore, among 150 mothers negative for both IgM and IgG HLA class I PRA before 16 weeks of gestation, 26.7% (n=40) showed IgG HLA class I PRA positivity at the time of delivery and the cases with chronic chorioamnionitis had higher IgG

HLA class I PRA positivity at the time of delivery than those without chronic chorioamnionitis (57.1% vs. 8.5%; *P* < 0.001)

IgG HLA antibody isotype

In all HLA PRA positive cases, IgG isotypes were further determined by flow cytometry. The predominant IgG isotypes were IgG1 and IgG3 for both HLA class I and class II PRA. Among HLA class I PRA positive mothers (n=156), the IgG1 isotype was found in 91.0%, IgG2 in 18.6%, IgG3 in 18.6%, and IgG4 in 10.9% of the cases. For HLA class II PRA, IgG1 was found in 70.7%, IgG2 in 1.2%, IgG3 in 18.3%, and IgG4 in 4.9% of the cases.

Fetal HLA specificity of maternal HLA PRA

We further determined whether fetal HLA specific antibodies are detected in HLA PRA positive mothers. HLA genotyping using genomic DNA identified specific HLA class I and class II alleles of the fetus as shown in Table II. Overall, 12 of 16 (75.0%) HLA class I PRA positive mothers had fetal HLA class I specific antibodies. Interestingly, six cases (37.5%) had fetal HLA class II specific antibodies despite all tested had negative HLA class II PRA (panel-reactivity < 10%). The frequency that mothers tested had HLA antibodies specific to each of the fetal HLA class I antigens was 37.5% for HLA-A, 68.8% for HLA-B, and 25.0% of HLA-C. For HLA class II antigens, 31.3% of cases had antibodies specific against HLA-DQ, 12.5% against HLA-DR, and 37.5% against HLA class II antigens. When the cases with chronic chorioamnionitis (*N*=9) and those without chronic chorioamnionitis (*N*=7) were compared, the cases with chronic chorioamnionitis tended to have more chances for maternal HLA antibodies specific to fetal HLA class I antigens (88.9% vs. 57.1%, *P* = 0.262) and HLA-A antigen (55.6% vs. 14.3%, *P* = 0.145).

Discussion

Principal findings

The principal findings of this study show: 1) a significant proportion of mothers have HLA antibodies early in pregnancy (before 16 weeks of gestation), which can either be preformed antibodies before conception or antibodies generated *de novo*. This is particularly the case in spontaneous preterm births; 2) chronic chorioamnionitis is associated with a significantly higher maternal IgG HLA PRA positive rate and a seropositive conversion rate during the course of pregnancy; 3) HLA PRA negative mothers can be highly sensitized (HLA PRA reactivity > 80%) during pregnancy; 4) maternal IgG HLA PRA is predominantly of the IgG1 isotype or IgG3 isotype; and 5) maternal HLA antibodies were specific against fetal HLA antigens (fetus-specific antibody) in 14 of 16 cases tested in this study.

Maternal HLA PRA in early gestation and chronic chorioamnionitis

In the current study, we present evidence that fetal HLA-specific maternal antibodies can be detected in a subset of women from early pregnancy until the time of delivery. We found that women whose placentas had chronic chorioamnionitis were more frequently sensitized to HLA antigens than those without chronic chorioamnionitis. Importantly, such sensitization was observed more frequently even before 16 weeks of gestation in chronic chorioamnionitis cases. Of note, although an IgM-to-IgG HLA antibody switch was not detected in all mothers who had positive IgM HLA PRA before 16 weeks of gestation, mothers who had an IgM-to-IgG antibody switch were more likely to have chronic chorioamnionitis. Such data suggest that IgG HLA PRA status in early gestation (a part of anti-fetal humoral rejection) has promise for predicting subsequent development of chronic chorioamnionitis. Another implication of our findings is that the immunological process of

rejection may be of considerable duration in nature as the antibodies can be detected months before delivery and the diagnosis of chronic chorioamnionitis.

Chronic chorioamnionitis, a feature of maternal anti-fetal rejection

Chronic chorioamnionitis, by definition, is characterized by predominantly lymphocytic infiltration regardless of etiological agent, and there are examples associated with infection such as rubella[58] and toxoplasmosis.[59] However, Gersell et al could not find any evidence of infection in all of the 17 cases they have reported even by meticulous examinations including immunohistochemical, bacteriological, and serological studies,[60,61] and Jacques and Qureshi suggested an immunologic nature of chronic chorioamnionitis.[62] Its close association with villitis of unknown etiology[28] having features of maternal anti-fetal rejection[29–33] and with humoral antibody-mediated rejection (anti-HLA antibodies and C4d deposition)[26] is also consistent with the concept of anti-fetal rejection. The current study further supports this concept by demonstrating an association between chronic chorioamnionitis and maternal serum HLA PRA positivity in early gestation and its evolutionary patterns during the course of pregnancy.

Nosological considerations of chronic placental inflammation

It seems evident that the vast majority of cases with chronic chorioamnionitis, villitis of unknown etiology, and chronic deciduitis are placental manifestations of anti-fetal rejection. Therefore, there is a nosological problem with the current terminology for these lesions as allograft rejection in the liver or renal transplants is not defined as 'hepatitis' or 'nephritis', and refined schema and grading systems of allograft rejection pertinent to each organ have been developed.[63,64] Accordingly, we propose that chronic chorioamnionitis, villitis of unknown etiology, and chronic deciduitis of an alloimmune nature need to be diagnosed as anti-fetal rejection of the chorioamniotic membranes, placental disc, and the decidua basalis, respectively. The terms 'chronic chorioamnionitis', 'chronic villitis', and 'chronic deciduitis' should be reserved for those cases in which certain associations with other etiological agents such as virus and other microbial organisms have been documented. The term 'villitis of unknown etiology' also needs to be abandoned. This approach embracing the pathogenesis of placental inflammatory lesions would minimize problems in the communications between the clinicians and the scientists and, more importantly, between the obstetricians and the patients, which would have quite different effects upon the clinical management of the patients.

While there are differences in the clinicopathological characteristics between chronic placental inflammation of an alloimmune nature and infectious etiology,[31] Benirschke et al have emphasized the importance of clinical suspicion and meticulous work-up in differentiating those two different categories of lesions.[65] To address this issue, clinicians and reproductive immunologists would need to work together to develop common guidelines and the criteria for the diagnosis of anti-fetal rejection in the placenta. In particular, the extent of basic and clinically relevant work-ups to rule out possibilities of chronic inflammation of non-alloimmune or infectious etiologies should be clearly defined.

Mechanisms of maternal HLA sensitization during pregnancy

Pregnancy[66] is one of the three major risk factors for HLA sensitization along with organ transplantation[67,68] and blood transfusion.[69] The prevalence of maternal HLA antibodies increases as a function of advancing gestation[70] and the number of previous pregnancies.[71] The placenta has been considered an immuno-privileged organ because trophoblasts do not express classical HLA class I molecules, except HLA-C.[72–74] Instead, they express non-classical, less polymorphic HLA-E, -F, and –G,[75,76] and this accounts, at least in part, for tolerance at the maternal-fetal interface.

If HLA PRA is generated as a result of maternal immune response against fetal trophoblast antigens at the feto-maternal interface, HLA PRA should be largely against HLA-C, which has been reported to be associated with the development of adverse pregnancy outcomes such as preeclampsia, fetal growth restriction, and miscarriage through the interaction with maternal leukocytes (natural killer cells and T cells) in the decidua.[25,77,78] However, maternal fetal HLA-specific antibodies against non-trophoblastic fetal HLA antigens (HLA-A, -B, and –DR) were found more commonly in the present study, which clearly indicates that maternal antibody generation in response to fetal HLA antigens is not merely against trophoblasts which express HLA-C and -G but against different cells expressing classical HLA antigens.

Potential routes of maternal exposure to non-trophoblastic fetal cells include feto-maternal hemorrhage[79] or retained fragments of placental tissue which may contain non-trophoblastic cells after delivery.[80] However, feto-maternal hemorrhage would be a more plausible explanation in the cases showing *de novo* positive HLA PRA conversion across gestation in the current study. Cohen and Zuelzer have shown that transplacental passage of fetal erythrocytes increases as a function of gestational age as it was detected in 50% of pregnant women at the time of delivery.[81] In addition, disruption to the integrity of the placental chorionic villous surface is considered a regular event during pregnancy due to the dynamic nature of placental circulation.[82] It is also known that even during normal pregnancy there is a variable degree of granulocyte traffic from the fetus to the mother.[83] This fetal cell trafficking,[84–86] as well as the presence of cell-free fetal nucleic acids in maternal circulation,[87] have been well-documented. Therefore, variable degrees of continuous or intermittent influx of non-trophoblastic HLA class I or class II positive fetal cells into the maternal circulation is easily expected during pregnancy.

Anti-fetal HLA antibodies and the fetus

Intrauterine infection and/or inflammation causes spontaneous preterm parturition, which is associated with the fetal inflammatory response syndrome and neonatal morbidity.[88–90] The presence of fetal HLA specific antibodies in the majority of the cases tested (75.0%) using maternal sera obtained before 16 weeks of gestation in this study indicates that there is chronic fetal exposure to these antibodies, the implications of which are yet to be determined. In mice, IgG antibodies cross the placenta and localize in several fetal tissues; their highest levels are found in the blood, thymus, and liver.[91] Interestingly, antibodies persist for a longer period in the fetus than in the placenta. In humans, neonatal alloimmune neutropenia (NAIN) and neonatal alloimmune thrombocytopenia (NAIT) are well-known examples of alloimmune reactions, typically presenting against paternal/fetal antigens such as human neutrophil antigens[92,93] and human platelet antigens.[94,95] Interestingly, cases of NAIN and NAIT associated with maternal HLA class I antibodies have also been well-documented, indicating that HLA sensitization of the mother is a mechanism of disease in the fetus.[96–99]

Strengths and Weaknesses

This is the first longitudinal study of maternal HLA PRA status in the context of chronic chorioamnionitis (anti-fetal cellular rejection) during the course of pregnancy. Fetal HLA specificity of maternal HLA antibodies was also tested. There are, however, limitations to this study. We could not determine HLA PRA positivity of patients before pregnancy, as blood samples were not available for analysis. Therefore, it is possible that panel-reactive antibodies detected in some pregnant women represent sensitization before the current pregnancy. Another limitation is that eight samples (3.2 %) among the 'at the time of delivery' samples were obtained between 2–4 weeks before delivery, and it could be argued that they might not reflect an *in vivo* situation around delivery. When we analyzed the data

of the eight samples, five cases represented preterm birth with chronic chorioamnionitis, and they were negative for both HLA class I and class II PRA. Therefore, it is possible that a positive conversion occurred late in gestation in some cases, and it is unlikely that the results of our analyses would have been significantly affected.

Conclusion

Collectively, our study confirms an association between chronic chorioamnionitis and maternal HLA PRA positivity across gestation. Since maternal anti-fetal rejection in some cases is persistent, HLA PRA deserves further testing as a monitoring tool for fetal well-being. Such an approach has potential to assist in exploring antibody-mediated rejection as a mechanism of disease in the "great obstetrical syndromes", notably "preterm birth".

Acknowledgments

Reference List

1. Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol. 1953; 7:320–338.

2. Bystry RS, Aluvihare V, Welch KA, Kallikourdis M, Betz AG. B cells and professional APCs recruit regulatory T cells via CCL4. Nat Immunol. 2001; 2:1126–1132. [PubMed: 11702067]

3. Mor G, Abrahams VM. Potential role of macrophages as immunoregulators of pregnancy. Reprod Biol Endocrinol. 2003; 1:119. [PubMed: 14651752]

4. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat Immunol. 2006; 7:241–246. [PubMed: 16482172]

5. Aluvihare VR, Betz AG. The role of regulatory T cells in alloantigen tolerance. Immunol Rev. 2006; 212:330–343. [PubMed: 16903924]

6. Guleria I, Sayegh MH. Maternal acceptance of the fetus: true human tolerance. J Immunol. 2007; 178:3345–3351. [PubMed: 17339426]

7. Kallikourdis M, Betz AG. Periodic accumulation of regulatory T cells in the uterus: preparation for the implantation of a semi-allogeneic fetus? PLoS ONE. 2007; 2:e382. [PubMed: 17440618]

8. Terness P, Kallikourdis M, Betz AG, Rabinovich GA, Saito S, Clark DA. Tolerance signaling molecules and pregnancy: IDO, galectins, and the renaissance of regulatory T cells. Am J Reprod Immunol. 2007; 58:238–254. [PubMed: 17681041]

9. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, Tirado I, Markert UR, Klapp BF, Poirier F, Szekeres-Bartho J, Rabinovich GA, Arck PC. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med. 2007; 13:1450–1457. [PubMed: 18026113]

10. Blois SM, Kammerer U, Alba SC, Tometten MC, Shaikly V, Barrientos G, Jurd R, Rukavina D, Thomson AW, Klapp BF, Fernandez N, Arck PC. Dendritic cells: key to fetal tolerance? Biol Reprod. 2007; 77:590–598. [PubMed: 17596562]

11. Makrigiannakis A, Karamouti M, Drakakis P, Loutradis D, Antsaklis A. Fetomaternal immunotolerance. Am J Reprod Immunol. 2008; 60:482–496. [PubMed: 19032609]

12. von Rango U. Fetal tolerance in human pregnancy--a crucial balance between acceptance and limitation of trophoblast invasion. Immunol Lett. 2008; 115:21–32. [PubMed: 18055021]

13. Kalkunte SS, Mselle TF, Norris WE, Wira CR, Sentman CL, Sharma S. Vascular endothelial growth factor C facilitates immune tolerance and endovascular activity of human uterine NK cells at the maternal-fetal interface. J Immunol. 2009; 182:4085–4092. [PubMed: 19299706]

14. Mold JE, Venkatasubrahmanyam S, Burt TD, Michaelsson J, Rivera JM, Galkina SA, Weinberg K, Stoddart CA, McCune JM. Fetal and adult hematopoietic stem cells give rise to distinct T cell lineages in humans. Science. 2010; 330:1695–1699. [PubMed: 21164017]

15. Betz AG. Immunology. Have you seen your mother, baby. Science. 2010; 330:1635–1636. [PubMed: 21164004]

16. Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. Ann N Y Acad Sci. 2011; 1221:80–87. [PubMed: 21401634]

17. Redman CW. Immune factors and recurrent abortion: a review. Am J Reprod Immunol. 1983; 4:179–181. [PubMed: 6588765]

18. Kilpatrick DC. Immune mechanisms and pre-eclampsia. Lancet. 1987; 2:1460–1461. [PubMed: 2892017]

19. Aksel S. Immunologic aspects of reproductive diseases. JAMA. 1992; 268:2930–2934. [PubMed: 1433710]

20. Kohut KG, Anthony MN, Salafia CM. Decidual and placental histologic findings in patients experiencing spontaneous abortions in relation to pregnancy order. Am J Reprod Immunol. 1997; 37:257–261. [PubMed: 9127648]

21. Matthiesen L, Berg G, Ernerudh J, Ekerfelt C, Jonsson Y, Sharma S. Immunology of preeclampsia. Chem Immunol Allergy. 2005; 89:49–61. [PubMed: 16129952]

22. Riley JK, Yokoyama WM. NK cell tolerance and the maternal-fetal interface. Am J Reprod Immunol. 2008; 59:371–387. [PubMed: 18405308]

23. Sharp AN, Heazell AE, Crocker IP, Mor G. Placental apoptosis in health and disease. Am J Reprod Immunol. 2010; 64:159–169. [PubMed: 20367628]

24. Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol. 2010; 63:534–543. [PubMed: 20331588]

25. Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, Claas FH, Walker JJ, Redman CW, Morgan L, Tower C, Regan L, Moore GE, Carrington M, Moffett A. Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. J Clin Invest. 2010; 120:4102–4110. [PubMed: 20972337]

26. Lee J, Romero R, Xu Y, Kim J-S, Topping V, Yoo W, Kusanovic JP, Chaiworapongsa T, Hassan SS, Yoon BH, Kim CJ. A signature of maternal anti-fetal rejection in spontaneous preterm birth: chronic chorioamnionitis, anti-human leukocyte antigen antibodies, and C4d. PLoS ONE. 2011; 6:e16806. [PubMed: 21326865]

27. Jin LP, Fan DX, Li DJ. Regulation of costimulatory signal in maternal-fetal immune tolerance. Am J Reprod Immunol. 2011; 66:76–83. [PubMed: 21276120]

28. Kim CJ, Romero R, Kusanovic JP, Yoo W, Dong Z, Topping V, Gotsch F, Yoon BH, Chi JG, Kim JS. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. Mod Pathol. 2010; 23:1000–1011. [PubMed: 20348884]

29. Redline RW, Patterson P. Villitis of unknown etiology is associated with major infiltration of fetal tissue by maternal inflammatory cells. Am J Pathol. 1993; 143:473–479. [PubMed: 8342596]

30. Labarrere CA, Faulk WP. Maternal cells in chorionic villi from placentae of normal and abnormal human pregnancies. Am J Reprod Immunol. 1995; 33:54–59. [PubMed: 7619234]

31. Redline RW. Villitis of unknown etiology: noninfectious chronic villitis in the placenta. Hum Pathol. 2007; 38:1439–1446. [PubMed: 17889674]

32. Kim JS, Romero R, Kim MR, Kim YM, Friel L, Espinoza J, Kim CJ. Involvement of Hofbauer cells and maternal T cells in villitis of unknown aetiology. Histopathology. 2008; 52:457–464. [PubMed: 18315598]

33. Kim MJ, Romero R, Kim CJ, Tarca AL, Chhauy S, LaJeunesse C, Lee DC, Draghici S, Gotsch F, Kusanovic JP, Hassan SS, Kim JS. Villitis of unknown etiology is associated with a distinct pattern of chemokine up-regulation in the feto-maternal and placental compartments: implications for conjoint maternal allograft rejection and maternal anti-fetal graft-versus-host disease. J Immunol. 2009; 182:3919–3927. [PubMed: 19265171]

34. Hamilton BE, Martin JA, Ventura SJ. Births: preliminary data for 2005. Natl Vital Stat Rep. 2006; 55:1–18.

35. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, Rubens C, Menon R, Van Look PF. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ. 2010; 88:31–38. [PubMed: 20428351]

36. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008; 371:75–84. [PubMed: 18177778]

37. Russell RB, Green NS, Steiner CA, Meikle S, Howse JL, Poschman K, Dias T, Potetz L, Davidoff MJ, Damus K, Petrini JR. Cost of hospitalization for preterm and low birth weight infants in the United States. Pediatrics. 2007; 120:e1–e9. [PubMed: 17606536]

38. Romero R, Sepulveda W, Baumann P, Yoon BH, Brandt F, Gomez R, Mazor M, Sorokin Y, Cotton DB. The preterm labor syndrome: biochemical, cytologic, immunologic, pathologic, microbiologic, and clinical evidence that preterm labor is a heterogeneous disease. Am J Obstet Gynecol. 1993; 168:288.

39. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, Chaiworapongsa T, Mazor M. The preterm parturition syndrome. BJOG. 2006; 113 (Suppl 3):17–42. [PubMed: 17206962]

40. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology. 1997; 25:658–663. [PubMed: 9049215]

41. Tan CD, Baldwin WM III, Rodriguez ER. Update on cardiac transplantation pathology. Arch Pathol Lab Med. 2007; 131:1169–1191. [PubMed: 17683180]

42. Solez K. History of the Banff classification of allograft pathology as it approaches its 20th year. Curr Opin Organ Transplant. 2010; 15:49–51. [PubMed: 19949334]

43. McKenna RM, Takemoto SK, Terasaki PI. Anti-HLA antibodies after solid organ transplantation. Transplantation. 2000; 69:319–326. [PubMed: 10706035]

44. Ho EK, Vlad G, Colovai AI, Vasilescu ER, Schwartz J, Sondermeijer H, Burke E, Marboe CC, Itescu S, Suciu-Foca N, Mancini D. Alloantibodies in heart transplantation. Hum Immunol. 2009; 70:825–829. [PubMed: 19555730]

45. Smith JD, Banner NR, Hamour IM, Ozawa M, Goh A, Robinson D, Terasaki PI, Rose ML. De Novo Donor HLA-Specific Antibodies after Heart Transplantation Are an Independent Predictor of Poor Patient Survival. Am J Transplant. 2011; 11:312–319. [PubMed: 21219570]

46. Gebel HM, Bray RA. Sensitization and sensitivity: defining the unsensitized patient. Transplantation. 2000; 69:1370–1374. [PubMed: 10798756]

47. Betkowski AS, Graff R, Chen JJ, Hauptman PJ. Panel-reactive antibody screening practices prior to heart transplantation. J Heart Lung Transplant. 2002; 21:644–650. [PubMed: 12057697]

48. Terasaki PI, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. Am J Transplant. 2004; 4:438–443. [PubMed: 14961999]

49. Panigrahi A, Deka R, Bhowmik D, Tiwari SC, Mehra NK. Immunological monitoring of posttransplant allograft sensitization following living related donor renal transplantation. Transplant Proc. 2004; 36:1336–1339. [PubMed: 15251326]

50. Campos EF, Tedesco-Silva H, Machado PG, Franco M, Medina-Pestana JO, Gerbase-DeLima M. Post-transplant anti-HLA class II antibodies as risk factor for late kidney allograft failure. Am J Transplant. 2006; 6:2316–2320. [PubMed: 16925566]

51. Li X, Ishida H, Yamaguchi Y, Tanabe K. Poor graft outcome in recipients with de novo donor-specific anti-HLA antibodies after living related kidney transplantation. Transpl Int. 2008; 21:1145–1152. [PubMed: 18803618]

52. Simister NE. Placental transport of immunoglobulin G. Vaccine. 2003; 21:3365–3369. [PubMed: 12850341]

53. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation--a workshop report. Placenta. 2005; 26 (Suppl A):S114–S117. [PubMed: 15837060]

54. Khong TY, Bendon RW, Qureshi F, Redline RW, Gould S, Stallmach T, Lipsett J, Staples A. Chronic deciduitis in the placental basal plate: definition and interobserver reliability. Hum Pathol. 2000; 31:292–295. [PubMed: 10746670]

55. Bartel G, Wahrmann M, Exner M, Regele H, Schillinger M, Horl WH, Bohmig GA. Determinants of the complement-fixing ability of recipient presensitization against HLA antigens. Transplantation. 2007; 83:727–733. [PubMed: 17414705]

56. Lietz K, John R, Burke E, Schuster M, Rogers TB, Suciu-Foca N, Mancini D, Itescu S. Immunoglobulin M-to-immunoglobulin G anti-human leukocyte antigen class II antibody switching in cardiac transplant recipients is associated with an increased risk of cellular rejection and coronary artery disease. Circulation. 2005; 112:2468–2476. [PubMed: 16230499]

57. Stastny P, Ring S, Lu C, Arenas J, Han M, Lavingia B. Role of immunoglobulin (Ig)-G and IgM antibodies against donor human leukocyte antigens in organ transplant recipients. Hum Immunol. 2009; 70:600–604. [PubMed: 19375468]

58. Garcia AG, Marques RL, Lobato YY, Fonseca ME, Wigg MD. Placental pathology in congenital rubella. Placenta. 1985; 6:281–295. [PubMed: 3903730]

59. Garcia AG. Congenital toxoplasmosis in two successive sibs. Arch Dis Child. 1968; 43:705–710. [PubMed: 5702232]

60. Gersell DJ. Chronic villitis, chronic chorioamnionitis, and maternal floor infarction. Semin Diagn Pathol. 1993; 10:251–266. [PubMed: 8210775]

61. Gersell DJ, Phillips NJ, Beckerman K. Chronic chorioamnionitis: a clinicopathologic study of 17 cases. Int J Gynecol Pathol. 1991; 10:217–229. [PubMed: 1917272]

62. Jacques SM, Qureshi F. Chronic chorioamnionitis: a clinicopathologic and immunohistochemical study. Hum Pathol. 1998; 29:1457–1461. [PubMed: 9865833]

63. Demetris A, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga H, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neil D, Pappo O, Parizhskaya M, Randhawa P, Rasoul-Rockenschaub S, Reinholt F, Reynes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. Hepatology. 2000; 31:792–799. [PubMed: 10706577]

64. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant. 2008; 8:753–760. [PubMed: 18294345]

65. Benirschke K, Coen R, Patterson B, Key T. Villitis of known origin: varicella and toxoplasma. Placenta. 1999; 20:395–399. [PubMed: 10419804]

66. Rebibou JM, Chabod J, Alcalay D, Coussediere MC, Deteix P, Touchard G, Dupont I, Thevenin C, Chalopin JM, Tiberghien P. Flow cytometric evaluation of pregnancy-induced anti-HLA immunization and blood transfusion-induced reactivation. Transplantation. 2002; 74:537–540. [PubMed: 12352915]

67. Sanfilippo F, Vaughn WK, Bollinger RR, Spees EK. Comparative effects of pregnancy, transfusion, and prior graft rejection on sensitization and renal transplant results. Transplantation. 1982; 34:360–366. [PubMed: 6760495]

68. Braun WE. The alloimmunized patient: monitoring and therapeutics. Am J Med Sci. 1997; 313:279–282. [PubMed: 9145037]

69. Scornik JC, Ireland JE, Howard RJ, Pfaff WW. Assessment of the risk for broad sensitization by blood transfusions. Transplantation. 1984; 37:249–253. [PubMed: 6367163]

70. Regan L, Braude PR, Hill DP. A prospective study of the incidence, time of appearance and significance of anti-paternal lymphocytotoxic antibodies in human pregnancy. Hum Reprod. 1991; 6:294–298. [PubMed: 2056027]

71. Triulzi DJ, Kleinman S, Kakaiya RM, Busch MP, Norris PJ, Steele WR, Glynn SA, Hillyer CD, Carey P, Gottschall JL, Murphy EL, Rios JA, Ness PM, Wright DJ, Carrick D, Schreiber GB. The effect of previous pregnancy and transfusion on HLA alloimmunization in blood donors: implications for a transfusion-related acute lung injury risk reduction strategy. Transfusion. 2009; 49:1825–1835. [PubMed: 19453983]

72. Mor, G. Immunology of implantation: an introduction. In: Mor, G., editor. Immunology of pregnancy. 1. New York, NY: Springer; 2006. p. 1-6.

73. King A, Hiby SE, Gardner L, Joseph S, Bowen JM, Verma S, Burrows TD, Loke YW. Recognition of trophoblast HLA class I molecules by decidual NK cell receptors--a review. Placenta. 2000; 21 (Suppl A):S81–S85. [PubMed: 10831129]

74. Tilburgs T, Scherjon SA, Claas FH. Major histocompatibility complex (MHC)-mediated immune regulation of decidual leukocytes at the fetal-maternal interface. J Reprod Immunol. 2010; 85:58–62. [PubMed: 20347157]

75. Hunt JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. FASEB J. 2005; 19:681–693. [PubMed: 15857883]

76. Gonen-Gross T, Goldman-Wohl D, Huppertz B, Lankry D, Greenfield C, Natanson-Yaron S, Hamani Y, Gilad R, Yagel S, Mandelboim O. Inhibitory NK receptor recognition of HLA-G: regulation by contact residues and by cell specific expression at the fetal-maternal interface. PLoS One. 2010; 5:e8941. [PubMed: 20126612]

77. Tilburgs T, Scherjon SA, van der Mast BJ, Haasnoot GW, Versteeg V, Roelen DL, van Rood JJ, Claas FH. Fetal-maternal HLA-C mismatch is associated with decidual T cell activation and induction of functional T regulatory cells. J Reprod Immunol. 2009; 82:148–157. [PubMed: 19631389]

78. Faridi RM, Agrawal S. Killer immunoglobulin-like receptors (KIRs) and HLA-C allorecognition patterns implicative of dominant activation of natural killer cells contribute to recurrent miscarriages. Hum Reprod. 2011; 26:491–497. [PubMed: 21159685]

79. Sebring ES, Polesky HF. Fetomaternal hemorrhage: incidence, risk factors, time of occurrence, and clinical effects. Transfusion. 1990; 30:344–357. [PubMed: 2190367]

80. Wilczynski JR. Immunological analogy between allograft rejection, recurrent abortion and pre-eclampsia - the same basic mechanism? Hum Immunol. 2006; 67:492–511. [PubMed: 16829304]

81. Cohen F, Zuelzer WW. Mechanisms of isoimmunization. II. Transplacental passage and postnatal survival of fetal erythrocytes in heterospecific pregnancies. Blood. 1967; 30:796–804. [PubMed: 4965622]

82. Watson AL, Burton GJ. A microscopical study of wound repair in the human placenta. Microsc Res Tech. 1998; 42:351–368. [PubMed: 9766430]

83. Sunku CC, Gadi VK, de Laval de LB, Guthrie KA, Nelson JL. Maternal and fetal microchimerism in granulocytes. Chimerism. 2010; 1:11–14. [PubMed: 21327147]

84. Bianchi DW. Fetomaternal cell trafficking: a new cause of disease? Am J Med Genet. 2000; 91:22–28. [PubMed: 10751084]

85. Berry SM, Hassan SS, Russell E, Kukuruga D, Land S, Kaplan J. Association of maternal histocompatibility at class II HLA loci with maternal microchimerism in the fetus. Pediatr Res. 2004; 56:73–78. [PubMed: 15128924]

86. Yan Z, Aydelotte T, Gadi V, Guthrie KA, Nelson JL. Acquisition of the rheumatoid arthritis hla shared epitope through microchimerism. Arthritis Rheum. 2011; 63:640–644. [PubMed: 21360493]

87. Maron JL, Johnson KL, Slonim D, Lai CQ, Ramoni M, Alterovitz G, Jarrah Z, Yang Z, Bianchi DW. Gene expression analysis in pregnant women and their infants identifies unique fetal biomarkers that circulate in maternal blood. J Clin Invest. 2007; 117:3007–3019. [PubMed: 17885688]

88. Romero R, Gomez R, Ghezzi F, Yoon BH, Mazor M, Edwin SS, Berry SM. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. Am J Obstet Gynecol. 1998; 179:186–193. [PubMed: 9704786]

89. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol. 1998; 179:194–202. [PubMed: 9704787]

90. Madsen-Bouterse SA, Romero R, Tarca AL, Kusanovic JP, Espinoza J, Kim CJ, Kim JS, Edwin SS, Gomez R, Draghici S. The transcriptome of the fetal inflammatory response syndrome. Am J Reprod Immunol. 2010; 63:73–92. [PubMed: 20059468]

91. Adeniyi-Jones SC, Ozato K. Transfer of antibodies directed to paternal major histocompatibility class I antigens from pregnant mice to the developing fetus. J Immunol. 1987; 138:1408–1415. [PubMed: 3805722]

92. Curtis BR, Reno C, Aster RH. Neonatal alloimmune neutropenia attributed to maternal immunoglobulin G antibodies against the neutrophil alloantigen HNA-1c (SH): a report of five cases. Transfusion. 2005; 45:1308–1313. [PubMed: 16078917]

93. Porcelijn L, Abbink F, Terraneo L, Hoogen LO, Huiskes E, de Haas M. Neonatal alloimmune neutropenia due to immunoglobulin G antibodies against human neutrophil antigen-5a. Transfusion. 2011; 51:574–577. [PubMed: 20735765]

94. Rothenberger S. Neonatal alloimmune thrombocytopenia. Ther Apher. 2002; 6:32–35. [PubMed: 11886574]

95. Koh Y, Taniue A, Ishii H, Matsuyama N, Amakishi E, Hayashi T, Furuta RA, Fukumori Y, Hirayama F, Yoshimura K, Nagamine T, Tamai S, Nakano S. Neonatal alloimmune thrombocytopenia caused by an antibody specific for a newly identified allele of human platelet antigen-7. Transfusion. 2010; 50:1276–1284. [PubMed: 20070614]

96. Sasaki M, Yagihashi A, Kobayashi D, Watanabe N, Fujikawa T, Chiba S, Sato S, Morishita K, Sekimoto T, Ikeda H. Neonatal alloimmune thrombocytopenia due to anti-human leukocyte antigen antibody: a case report. Pediatr Hematol Oncol. 2001; 18:519–524. [PubMed: 11764101]

97. Tomicic M, Starcevic M, Bux J, Zach V, Hundric-Haspl Z, Drazic V, Grahovac B. Severe neonatal neutropenia due to anti-human leucocyte antigen B49 alloimmunization only: a case report. Transfus Med. 2003; 13:233–237. [PubMed: 12880394]

98. Gramatges MM, Fani P, Nadeau K, Pereira S, Jeng MR. Neonatal alloimmune thrombocytopenia and neutropenia associated with maternal human leukocyte antigen antibodies. Pediatr Blood Cancer. 2009; 53:97–99. [PubMed: 19229975]

99. Starcevic M, Tomicic M, Malenica M, Zah-Matakovic V. Neonatal alloimmune thrombocytopenia caused by anti-HLA-A24 alloantibodies. Acta Paediatr. 2010; 99:630–632. [PubMed: 19912145]
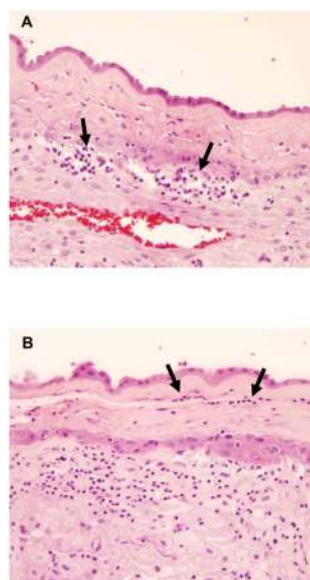
Figure 1.
Histological features of chronic chorioamnionitis. Maternal lymphocytic infiltration into the fetal compartments (chorionic trophoblast layer or chorioamniotic connective tissue layer) is a hallmark of chronic chorioamnionitis. *A*, A case of preterm labor shows infiltration of lymphocytes in clusters (arrows) into the chorionic trophoblast layer [Hematoxylin & Eosin (H&E), X200]. *B*, Another case of preterm labor in which lymphocytic infiltration (arrows) involves the chorioamniotic connective tissue layer (H&E, X200).

Figure 2.
IgG HLA class I and class II PRA positivity according to the presence or absence of chronic chorioamnionitis. *A*, Cases with chronic chorioamnionitis had higher rates of IgG HLA class I PRA positivity than those without chronic chorioamnionitis both before 16 weeks of gestation and at the time of delivery ($P < 0.01$, for each). B, Cases with chronic chorioamnionitis had a higher rate of IgG HLA class II PRA positivity than those without chronic chorioamnionitis at the time of delivery ($P < 0.01$), but the difference was not significant before 16 weeks of gestation.
HLA, human leukocyte antigen; *PRA*, panel reactive antibodies; *CCA*, chronic chorioamnionitis
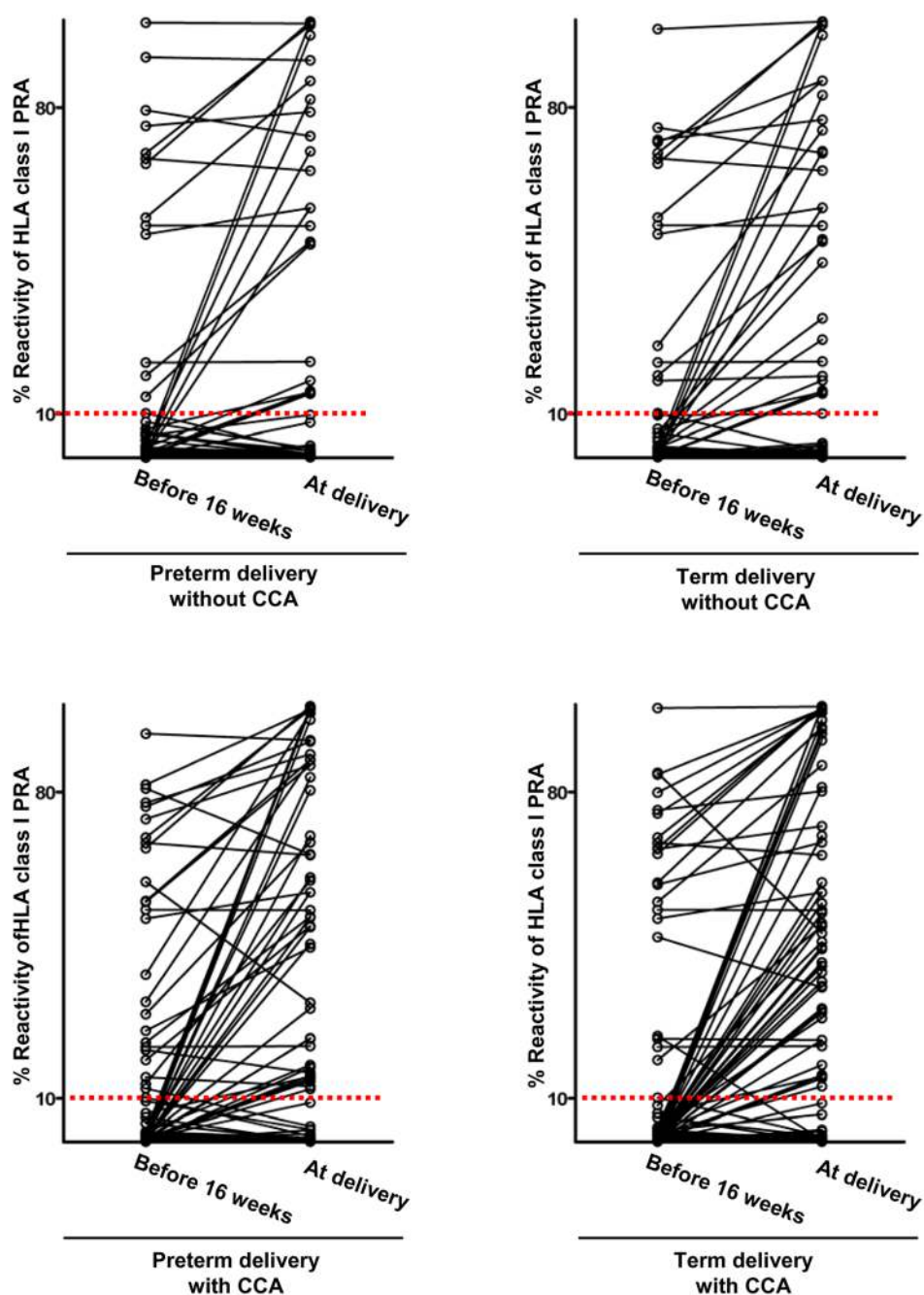
Figure 3.
Temporal changes in HLA class I PRA reactivity in study groups stratified according to the presence or absence of chronic chorioamnionitis and gestational age at birth. A majority of cases had a tendency toward increase in the panel-reactivity of HLA class I PRA as a function of advancing gestational age in all study groups. Dashed lines represent the cut-off value (10%) of panel-reactivity for positive HLA PRA.
HLA, human leukocyte antigen; *PRA*, panel reactive antibodies; *CCA*, chronic chorioamnionitis
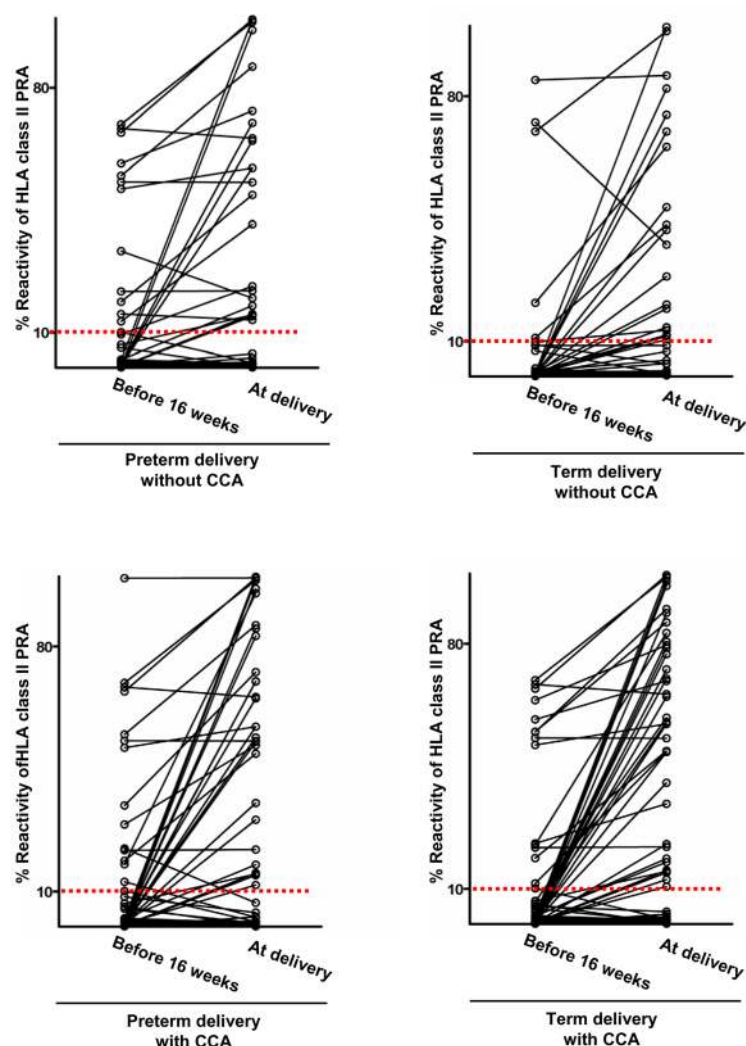
Figure 4.
Temporal changes in HLA PRA class II reactivity in study groups stratified according to the presence or absence of chronic chorioamnionitis and gestational age at birth. A majority of cases had a tendency toward increase in the panel-reactivity of HLA class II PRA as a function of advancing gestational age in all study groups. Dashed lines represent the cut-off value (10%) of panel-reactivity for positive HLA PRA.
HLA, human leukocyte antigen; *PRA*, panel reactive antibodies; *CCA*, chronic chorioamnionitis
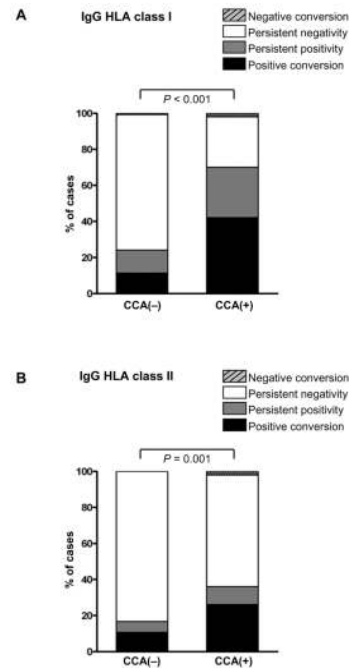
Figure 5.
Differences in the temporal changes of HLA PRA positivity according to the presence or absence of chronic chorioamnionitis. A, There were differences in the pattern of temporal changes in HLA class I PRA positivity according to the presence or absence of chronic chorioamnionitis ($P < 0.001$). B, Similar differences in the temporal changes of HLA class II PRA positivity were observed between the two groups ($P = 0.001$).
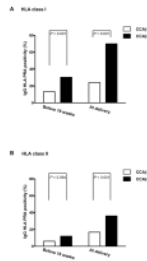*HLA,* human leukocyte antigen; *PRA*, panel reactive antibodies; *CCA*, chronic chorioamnionitis
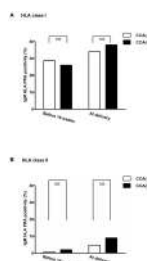
Figure 6.
IgM HLA class I and class II PRA positivity according to presence or absence of chronic chorioamnionitis. There were no differences in the rates of IgM HLA PRA positivity according to the presence or absence of chronic chorioamnionitis– *A*, HLA class I PRA, and *B*, HLA class II PRA.
HLA, human leukocyte antigen; *PRA*, panel reactive antibodies; *CCA*, chronic chorioamnionitis

Table I

Demographics of study population

	PTB (n=50)	TB (n=100)	PTB-CCA (n=50)	TB-CCA (n=50)	P value
Maternal age (year) *	23.5 (17–44)	22.5 (15–38)	25.5 (15–45)	27.5 (16–43)	NS
Gestational age at delivery (weeks) *	36.1 (23.1–36.9)	39.5 (37.3–41.6)	35.1 (28.1–36.9)	39.2 (37.0–41.4)	<0.001
Gestational age at blood sampling (weeks) *					
Before 16 weeks	12.2 (8.3–16.0)	12.5 (7.0–16.0)	13.5 (6.6–16.0)	12.5 (7.7–15.9)	NS
At the time of delivery	35.8 (22.4–36.9)	39.5 (37.3–41.4)	34.7 (24.7–36.9)	39.2 (37.0–41.4)	<0.001
Birthweight (g) *	2605 (580–3840)	3445 (2720–4140)	2405 (1160–3180)	3380 (2680–4080)	<0.001
SGA (%)	2.0	0.0	6.0	0.0	0.035
Baby gender (male, %)	52.0	43.0	70.0	42.0	0.01
Cesarean delivery (%)	18.0	15.0	20.0	10.0	NS
Primigravida (%)	44.0	40.0	38.0	26.0	NS
Nullipara (%)	44.0	45.0	40.0	28.0	NS
Alcohol (%)	2.0	9.0	2.0	2.0	NS
Cigarette smoking (%)	18.0	16.0	16.0	12.0	NS
Drug (%)	0.0	1.0	2.0	0.0	NS
ACA (%)	16.0	14.0	14.0	8.0	NS
MVU (%)	10.0	1.0	10.0	4.0	0.038
FVTOD (%)	4.0	7.0	14.0	12.0	NS
VUE (%)	12.0	12.0	40.0	38.0	<0.001
CDP (%)	6.0	10.0	38.0	24.0	<0.001

*
median (range)

ACA, acute chorioamnionitis; CDP, chronic deciduitis with plasma cells; FVTOD, fetal vascular thrombo-occlusive disease; MVU, maternal vascular underperfusion; NS, not significant; PTB, spontaneous preterm birth without chronic chorioamnionitis; PTB-CCA, spontaneous preterm birth with chronic chorioamnionitis; SGA, small-for-gestational–age neonates; TB, normal term birth without chronic chorioamnionitis; TB-CCA, normal term birth with chronic chorioamnionitis; VUE, villitis of unknown etiology

Table II

Fetal HLA specificities of maternal HLA antibodies

Case	CCA	Fetal Genotyping (HLA-)						HLA PRA*		HLA antibodies identified	
		A	B	C	DR	DR 51/52/53	DQ	Class I	Class II	Class I	Class II
1	Yes	29, 31	39, 44	7, 16	2, 8	53	4, 7	+	–	A1, A23, A24, A25, A32, A80, B27, B35, B37, B38, B41, **B44**†, B45, B46, B47, B49, B50, B51, B52, B53, B56, B57, B58, B59, B60, B61, B62, B63, B71, B72, B75, B76, B77, B78, B82, Cw5	**DQ7**†
2	Yes	1, 68	41, 51	15, 17	8, 10	–	4, 5	+	–	**A1**†, A11, A23, A24, A36, A80, B8, B13, **B41**†, B42, B44, B45, B47, B49, B50, B55, B60, B61, B76, B82, Cw6, **Cw17**†, Cw18	**DQ5**†, DQ6, DR4, **DR10**†, DR53
3	No	26, 68	39, 44	7, 17	4, 7	53	2, 8	+	–	A23, A24, A25, A32, A66, A74, B7, B8, B13, B27, B37, B38, **B41**†, B44, B45, B46, B47, B48, B49, B50, B51, B52, B53, B57, B58, B59, B60, B61, B62, B63, B73, B75, B76, B77, B81, Cw2, **Cw17**†	DP1, DP2, DP13, DP14, DP15, DP23
4	No	2, 31	7, 39	7	1, 14	52	5, 7	+	–	A1, **A2**†, A11, A23, A24, A25, A26, A32, A36, A66, A68, A69, **B7**†, B8, B13, B27, B37, B41, B42, B44, B45, B46, B47, B48, B49, B51, B52, B53, B54, B55, B56, B57, B58, B59, B60, B61, B62, B63, B67, B71, B72, B73, B76, B77, B81, B82, Cw1, Cw2, Cw9, C10, Cw14, Cw15, Cw17	DR9, DR51, DR103
5	Yes	1, 30	7, 27	2, 7	8, 17	52	2, 7	+	–	A11, A25, A32, B13, **B27**†, B37, B38, B44, B47, B49, B51, B52, B53, B57, B58, B59, B63, B82	
6	Yes	2, 23	35, 44	4, 7	4, 7	53	2, 8	+	–	A1, **A23**†, A24, A25, A32, A80, B13, B27, B37, B38, B41, **B44**†, B45, B47, B49, B50, B51, B52, B53, B57, B58, B59, B60, B61, B63, B76, B77, B82	
7	No	29, 68	44, 51	15, 16	7, 8	53	2, 4	+	–	A2, A23, B57, B58	
8	No	2, 68	15, 35	1, 4	8, 14	52	4, 7	+	–	B7, B13, B27, B41, B47, B48, B60, B61, B73, B81, Cw17	DP1, DP20, **DQ7**†
9	No	68	35, 51	3, 8	4	53	8	+	–	B7, B8, B49, **B51**†, B52, B53, B57, B58, B60, B61	
10	No	23, 68	7, 50	5, 7	13, 14	52	5, 7	+	–	A29, A32, A66, A74, **B7**†, B8, B13, B18, B27, B37, B38, B39, B41, B42, B44, B45, 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, DQ4, **DR13**†, DR51
11	No	2, 3	7, 39	7	16, 17	51, 52	2, 7	+	–	A1, A30, A31, A36, A43, B13, B41, B44, B45, B47, B49, B50, B60, B61, B62, B71, B72, B75, B76, Cw17, Cw18	DP1, DP20, DQ4, **DQ7**†, DR11, DR53
12	Yes	2, 29	7, 58	7	1, 8	–	4, 5	+	–	**A2**†, A25, A68, A69, B37, B76, Cw2, Cw4, Cw5, Cw6, Cw15, Cw17, Cw18	
13	Yes	23, 68	39, 44	4, 7	7, 8	53	2, 4	+	–	A1, A11, **A23**†, A24, A25, A30, A31, A32, A33, A80, B7, B13, B27, B37, B38, B41, **B44**†, B45, B46, B47, B49, B50, B51, B52, B53, B57, B58, B59, B60, B61, B63, B76, B77, B82, Cw1, Cw2, **Cw4**†, Cw5, Cw6, Cw8, Cw9, Cw10, Cw12, Cw14, Cw15, Cw16, Cw17, Cw18	DP1, DP10, DR1, DR11, DR16

Case	CCA	Fetal Genotyping (HLA-)						HLA PRA*		HLA antibodies identified	
		A	B	C	DR	DR 51/52/53	DQ	Class I	Class II	Class I	Class II
14	Yes	3, 24	39, 56	1, 7	8, 12	52	4, 7	+	−	A33, A34, A68, B8, B18, B38, $\mathbf{B39}^\dagger$, B41, B64, B65, Cw5	
15	Yes	1, 3	7, 37	6, 7	10, 15	51	5, 6	+	−	A66, B13, B41, B44, B45, B47, B49, B50, B60, B61, Cw1, Cw15	DR4
16	Yes	2, 24	35, 39	4, 7	13, 16	51, 52	6, 7	+	−	A23, $\underline{\mathbf{A24}}^\dagger$, A25, A30, A31, B13, B18, B27, $\underline{\mathbf{B35}}^\dagger$, B37, B38, B44, B47, B49, B50, B51, B52, B53, B57, B58, B59, B62, B63, B71, B72, B75, B77, B78, Cw1, Cw2, $\underline{\mathbf{Cw4}}^\dagger$, Cw5, Cw6, Cw9, Cw10, Cw14, Cw15, Cw17, Cw18	DP1, DQ2, $\underline{\mathbf{DQ6}}^\dagger$, DR4, DR53

*, A sample with reactivity of 10% or more was considered positive for HLA panel reactive antibodies

† specific against fetal

HLA antigens HLA, human leukocyte antigen; PRA, panel reactive antibodies