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Amniotic Fluid Soluble HLA-G in Term and Preterm Parturition, and Intra-amniotic Infection/Inflammation

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Abstract

Objective—Circulating soluble HLA-G (sHLA-G) has been associated with pregnancy complications, and determination of sHLA-G concentrations in amniotic fluid (AF) has been reported in normal pregnancies. Our aim was to determine if the AF concentrations of sHLA-G change with advancing gestation, spontaneous labor at term, and in patients with spontaneous preterm labor (PTL) with intact membranes, as well as in those with preterm prelabor rupture of membranes (PROM), in the presence or absence of intraamniotic infection/inflammation (IAI).

Study design—This cross-sectional study included the following groups: 1) midtrimester (n=55); 2) normal pregnancy at term with (n=50) and without (n=50) labor; 3) spontaneous PTL with intact membranes divided into: a) PTL who delivered at term (n=153); b) PTL who delivered preterm without IAI (n=108); and c) PTL with IAI (n=84); and 4) preterm PROM with (n=46) and without (n=44) IAI. sHLA-G concentrations were determined by ELISA. Non-parametric statistics were used for analysis.

Results—1) Among patients with PTL, the median AF sHLA-G concentration was higher in patients with IAI than in those without IAI or women that delivered at term ($p<0.001$ for both comparisons); 2) Similarly, patients with preterm PROM and IAI had higher median AF sHLA-G concentrations than those without IAI ($p=0.004$); 3) Among patients with PTL and delivery, those with histologic chorioamnionitis and/or funisitis had a higher median AF sHLA-G concentration than those without histologic inflammation ($p<0.001$); and 4) The median AF sHLA-G concentration did not change with advancing gestational age.

Conclusions—AF sHLA-G concentrations are elevated in preterm parturition associated to IAI as well as in histologic chorioamnionitis. We propose that sHLA-G may participate in the regulation of the host immune response against intra-amniotic infection.

Keywords

sHLA-G; preterm labor; preterm delivery; preterm prelabor rupture of membranes; pregnancy; amniocentesis; microbial invasion of the amniotic cavity; chorioamnionitis

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INTRODUCTION

Spontaneous preterm labor (PTL) with intact membranes and preterm prelabor rupture of membranes (PROM) are syndromes caused by multiple etiologies that can activate the common terminal pathway of parturition,[1] and intrauterine infection is one of the most important mechanisms of disease causally linked to preterm birth.[2–12] Intraamniotic infection and/or inflammation (IAI) is present in approximately one third of patients with spontaneous preterm labor[13] and in almost half of patients with preterm PROM,[14] it is associated with development of fetal inflammatory response syndrome,[15,16] and is considered an important risk factor for development of fetal injury.[17–30] Other pathological processes implicated in the preterm parturition syndrome include cervical insufficiency,[31,32] uterine overdistension,[33] allergy,[34–37] endocrine disorders,[38–41] vascular insults,[42–45] and abnormal allograft reaction.[46] It has been suggested that abnormalities in the recognition and adaptation to a set of foreign antigens, such as fetal antigens, may be a mechanism of disease responsible for intrauterine growth restriction, preeclampsia and recurrent pregnancy loss.[47–49]

HLA-G is a molecule that belongs to the class I region of the major histocompatibility complex, which includes the HLA class Ia genes that encode the classical high polymorphic transplantation antigens (HLA-A, -B, -C), and the nonclassical class Ib genes (HLA-E, -F, and -G).[50] HLA-G is called “nonclassic” because it also differs from classic HLA class Ia molecules by its genetic diversity, expression, structure, and functions.[51] HLA-G is characterized by an expression pattern of seven isoforms: four membrane-bound isoforms (HLA-G1, G2, G3 and G4) and three soluble isoforms (HLA-G5, G6 and G7)[52–55] that are generated by alternative splicing of a unique primary transcript.[56–58] HLA-A, -B and -C show broad tissue expression, whereas HLA-G expression is highly tissue-restricted, placenta being one of the most important organs for HLA-G expression.[59–63]

Soluble HLA-G (sHLA-G) isoforms were found to function as immuno-tolerant molecules by inhibiting NK cells function,[64] and a solid body of evidence supports a role for sHLA-G concentrations in maternal blood in the pathophysiology of recurrent spontaneous abortion, preeclampsia, intrauterine growth restriction, placental abruption, spontaneous preterm labor and preterm PROM.[65–69] Of interest, increased expression of HLA-G has been reported in infectious and autoimmune/inflammatory diseases,[70] as well as in patients with septic shock.[71] In pregnancy, sHLA-G has been detected in amniotic fluid of normal pregnant women in the second and third trimesters.[72–76] The objective of this study was to determine if the amniotic fluid concentrations of sHLA-G change with advancing gestation, spontaneous labor at term, and in patients with spontaneous PTL as well as in those with preterm PROM, in the presence or absence of intra-amniotic infection/inflammation.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was designed by searching our clinical database and bank of biological samples including 590 patients in the following groups: 1) women in the mid-trimester of pregnancy (14–18 weeks) who underwent amniocentesis for genetic indications and delivered a normal neonate at term (n=55); 2) normal pregnant women at term with (n=50) and without (n=50) spontaneous labor; 3) patients with an episode of spontaneous preterm labor (PTL) and intact membranes who were classified into: a) PTL without IAI who delivered at term (n=153); b) PTL without IAI who delivered preterm (<37 weeks gestation) (n=108); and c) PTL with IAI (n=84); and 4) women with preterm PROM with (n=46) and without (n=44) IAI. Information regarding the racial composition of patients

included in this study was available in 91% (534/590) of cases, and included: 66% (389/590) Hispanic, 22% African-American (130/590), and 2.5% (15/590) Caucasian.

All women provided written informed consent prior to the collection of amniotic fluid. The collection of amniotic fluid and its utilization for research purposes was approved by the Institutional Review Boards of participating institutions and by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, DHHS. Many of these samples have been previously used to study the biology of inflammation, hemostasis, and growth factor concentrations in normal pregnant women and those with pregnancy complications.

Definitions

The definitions of normal pregnancy, spontaneous preterm labor and preterm PROM have been previously described.[77] Intra-amniotic infection was defined as a positive amniotic fluid culture for microorganisms. Intra-amniotic inflammation was diagnosed by an amniotic fluid interleukin (IL)-6 concentration ≥2.6 ng/mL.[13] Histologic chorioamnionitis was diagnosed based on the infiltration by neutrophils of the chorionic plate or of the extraplacental membranes. Acute funisitis was diagnosed by the presence of neutrophils in the wall of the umbilical vessels and/or Wharton's jelly using the criteria previously described.[78]

Amniotic fluid collection

Collection and processing of amniotic fluid samples have been described elsewhere.[77] White blood cell (WBC) count, glucose concentration and Gram stain were performed shortly after collection.[79–81] The results of these tests were used for clinical management, while amniotic fluid IL-6 concentrations were used only for research purposes. Among patients with spontaneous preterm labor with intact membranes who delivered within 72 hours of amniocentesis, placenta, umbilical cord, and chorioamniotic membranes were collected and the presence or absence of histologic chorioamnionitis and/or funisitis was assessed. This period of time was selected to preserve a meaningful temporal relationship between amniotic fluid sHLA-G concentration and placental pathologic findings.

Soluble Human Leukocyte Antigen G immunoassay

Specific and sensitive enzyme-linked immunoassay was utilized to determine concentrations of sHLA-G in human amniotic fluid. This immunoassay reacts with the soluble HLA-G5 isoform. Soluble HLA-G immunoassays were obtained from BioVendor, LLC (Chandler, NC, USA) and EXBIO Praha (Vestec, Czech Republic) were specifically validated in our laboratory for human amniotic fluid prior to the conduction of this study. The calculated inter- and intra-assay coefficients of variation for sHLA-G immunoassays in our laboratory were 7.2% and 4.2%. The sensitivity was calculated to be 1.67 U/ml.

Statistical analysis

The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test for normal distribution of the data. Since amniotic fluid sHLA-G concentrations were not normally distributed, non-parametric tests were used for analyses. Comparisons between proportions were performed with Chi-square test. The Kruskal-Wallis with post-hoc Mann-Whitney U test was used for comparisons of continuous variables. Adjustment for multiple comparisons was performed using the Bonferroni method.[82] Spearman rank correlation was utilized to assess correlations between amniotic fluid concentration of sHLA-G, glucose, IL-6 and WBC count. A p-value of <0.05 was considered statistically significant. The statistical package used was SPSS v.15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and clinical characteristics of the study population

Table I shows the demographic and clinical characteristics of patients in the midtrimester, term no labor and term in labor groups. Tables II and III display the demographic and clinical characteristics of patients with spontaneous preterm labor and intact membranes and those with preterm PROM, respectively. The median gestational age at amniocentesis was significantly lower in patients with spontaneous preterm labor with intact membranes with IAI than in those without IAI who deliver preterm, and than that of those who deliver at term (Table II). Similar results were observed between patients with preterm PROM with IAI when compared to those without IAI (Table III).

Amniotic fluid sHLA-G in normal pregnancy and term parturition

Soluble HLA-G was detected in 99.7% (588/590) of amniotic fluid samples. There were no significant differences in the median sHLA-G concentration in amniotic fluid between women in the mid-trimester and that of those with a normal pregnancy at term not in labor [60.7 U/mL vs. 47.6 U/mL, respectively; p=0.5] (Figure 1). Patients with spontaneous labor at term had a significantly lower median amniotic fluid sHLA-G concentration than those without labor (36.2 U/mL vs. 47.6 U/mL, respectively; p=0.02) (Figure 2).

Information of fetal gender was available in 488 cases. No differences were found in the median amniotic fluid concentration of sHLA-G between male and female fetuses (57.3 U/mL vs. 50.7 U/mL, respectively; p=0.8).

Amniotic fluid sHLA-G concentrations in spontaneous preterm labor with intact membranes and preterm PROM

Among patients with spontaneous preterm labor, those with IAI had a significantly higher median amniotic fluid concentration of sHLA-G than patients who delivered preterm without IAI (88.4 U/mL vs. 46.5 U/mL, respectively; p<0.001) and than those with spontaneous preterm labor with intact membranes who delivered at term (88.4 U/mL vs. 50.4 U/mL, respectively; p<0.001) (Figure 3). There were no differences in the median amniotic fluid sHLA-G concentration between patients with spontaneous preterm labor without IAI who delivered preterm and those who delivered at term (46.5 U/mL vs. 50.4 U/mL, respectively; p=0.4) (Figure 3).

Similarly, patients with preterm PROM with IAI had a significantly higher median amniotic fluid sHLA-G concentration than those without IAI (preterm PROM with IAI: 76.7 U/mL vs. preterm PROM without IAI: 46.6 U/mL; p=0.004) (Figure 4).

A significant but weak correlation was observed between amniotic fluid concentration of sHLA-G and those of IL-6 and WBC count in patients with spontaneous preterm labor and those with PPROM (Spearman's rho coefficient: IL-6: 0.31, p<0.001 and WBC count: 0.2, p<0.001). There was no significant correlation between the amniotic fluid concentrations of sHLA-G and glucose (Spearman's rho coefficient: glucose: −0.025, p=0.6).

Amniotic fluid sHLA-G concentrations in patients with histologic chorioamnionitis

Among patients with spontaneous preterm labor who delivered within 72 hours from amniocentesis, 63 patients had placental pathology available and 51% (32/63) had evidence of placental inflammation. Patients with histologic chorioamnionitis and/or funisitis had a significantly higher median sHLA-G concentration in amniotic fluid than those without histologic inflammation (122.7 U/mL vs. 47.7 U/mL, respectively; p<0.001) (Figure 5).

DISCUSSION

Principal findings of the study

1) sHLA-G is a physiologic constituent of amniotic fluid, and its concentration did not change with gestational age; 2) in the presence of intra-amniotic infection/inflammation, patients with spontaneous PTL with intact membranes as well as those with preterm PROM had a significantly higher median amniotic fluid concentration of sHLA-G than that of those without intra-amniotic infection/inflammation; 3) similarly, patients with histologic chorioamnionitis and/or funisitis had a higher median amniotic fluid sHLA-G concentration than those without evidence of placental inflammation; 4) amniotic fluid sHLA-G concentrations were significantly correlated with indirect amniotic fluid markers for intra-amniotic infection/inflammation (WBC count and IL-6 concentrations); and 5) spontaneous labor at term was associated with a significant decrease in the median amniotic fluid sHLA-G concentration.

What is HLA-G?

The class I region of the major histocompatibility complex includes the HLA class Ia genes, that encode the classical high polymorphic transplantation antigens (HLA-A, -B, -C), and the nonclassical class Ib genes (HLA-E, -F, and -G).[50] HLA-G is similar in organization to the classical HLA-A, -B, and -C encoded proteins except that an in-frame termination codon prevents translation of a majority of the cytoplasmic region of the HLA-G polypeptide. The full-length HLA-G transcript encodes a protein with an overall homology of 86% to the HLA-A, -B, and -C protein sequence.[83–86] Although HLA-G has a similar structure to classic HLA Ia molecules, it has much lower polymorphism[87–90] and restricted capacity to translate to proteins.

The *HLA-G* gene was first reported by Geraghty and coworkers in 1987,[83] but it was not discovered that this gene encoded the class I molecule expressed in human trophoblasts until 1990.[91,92] The *HLA-G* gene is located within the major histocompatibility complex in the short arm of chromosome 6[93] and contains eight exons: 1) the first exon encodes the leader peptide; 2) the second, third and fourth exons encode the alpha-1, alpha-2 and alpha-3 regions of the protein, respectively; 3) the fifth exon encodes the transmembrane region, 4) the sixth and seventh exons encode the cytoplasmic tail; and 5) the eighth exon encodes the 3′ untranslated region.[85] HLA-G is characterized by an expression pattern of seven isoforms: four membrane-bound isoforms (HLA-G1, -G2, -G3 and -G4) and three soluble isoforms (HLA-G5, -G6 and -G7)[52–55] that are generated by alternative splicing of a unique primary transcript.[56–58] HLA-G binds to three inhibitory receptors: immunoglobulin-like transcript-2 (ILT-2) and -4 (ILT-4) and killer immunoglobulin-like receptor (KIR) 2DL4. These receptors are differentially expressed depending of the immune cell type:[94] 1) ILT-2 is expressed in myeloid and lymphoid cells;[95–97] 2) ILT-4 only by myeloid cells;[98] and 3) KIR2DL4 is expressed by NK cells and CD8+ T cells.[99,100]

HLA-A, -B and -C show broad tissue expression, whereas HLA-G expression is highly tissue-restricted, placenta being one of the most important organs for HLA-G expression.[59–63] Extravillous trophoblast,[91,92,101] Hofbauer cells,[102] and endothelial cells from chorionic villi[103] express HLA-G. Interestingly, HLA-G expression has been observed in complete and partial hydatidiform moles as well as in ectopic pregnancies, suggesting that HLA-G expression in trophoblast cells is independent of embryonic development.[104] Besides expression in fetal tissues such as trophoblast cells, HLA-G constitutive expression was found only in adult thymic medulla,[105] cornea,[106] pancreatic islets,[107] and erythroid and endothelial-cell precursors.[108] However, HLA-G expression can be induced in cancers,[109,110] organ transplant,[111] multiple sclerosis,[112] inflammatory diseases,

[113,114] and viral infections.[115,116] The mRNA for HLA-G has been detected in several embryonic an adult tissues, including adult and fetal thymus,[117,118] fetal liver and eye,[56,119] adult spleen,[120] skin and keratinocytes,[121] but the protein has been reported to be restricted to trophoblast and choriocarcinoma cells.[92,122,123]

HLA-G and immune response

Maternal decidua and fetal trophoblasts contribute to create a tolerant environment for the fetus.[65,124,125] Specific mechanisms must exist to modulate and escape the maternal immune system in order to avoid fetal rejection,[126] however, these mechanisms are still poorly understood.

The HLA-G molecules may play a role regulating immune cells and potentially be an essential part in this immune interaction between mother and fetus.[65,125,127–133] Pazmany et al[134] demonstrated that expression of HLA-G is sufficient to protect trophoblast from lysis by activated natural killer (NK) cell lines and clones. Rouas-Freiss et al[135] demonstrated that cytotrophoblast cells strongly inhibit maternal uterine NK cell-mediated lysis in both semiallogeneic and allogeneic combinations, and that this HLA-G-mediated protection was inhibited by treatment of cytotrophoblasts with an HLA-G-specific monoclonal antibody. Le Gal et al[64] demonstrated that HLA-G inhibits cytotoxic T lymphocytes. HLA-G seems to be able to inhibit both cytotoxic T-lymphocyte response and NK cells,[64,136,137] as well as prevent proliferation of CD4+T cells[138–141] and induce CD8+T apoptosis through the Fas/FasL pathway.[126,140,142,143] Similarly, Fournel et al[142] showed that binding of sHLA-G induces CD8+T cells death through the Fas/FasL pathway. Feger et al[144] identified a novel population of CD4 and CD8 T cells characterized by cell surface expression of HLA-G with regulatory properties that may have implications for the mechanisms of inflammation, autoimmunity, and also tumor immune surveillance. The correlation of HLA-G T cells with acute central nervous system inflammation, such as multiple sclerosis relapse, supports the hypothesis of HLA-G T cells presumed immunoregulatory role in modulating inflammatory responses.[144]

Soluble HLA-G present at the maternal-fetal interface may contribute to immune tolerance by killing T-cells. Elevated concentration of circulating NK cells have been linked to reproductive failure.[145,146] Soluble HLA-G isoforms, likely membrane-bound HLA-G molecules, were found to function as immuno-tolerant molecules by inhibiting NK cells function.[64] Moreover, Petroff et al[147] were the first to identify the two HLA class I-binding inhibitory receptors, ILT2 and ILT4, on macrophages within maternal decidua, suggesting that class I HLA molecules expressed at the maternal-fetal interface could impact the function of decidual macrophages. This immunomodulator function led to the suggestion that HLA-G may play a role in tolerance preventing immunorejection of the semiallogeneic fetus by the maternal immune system, probably contributing to graft tolerance and tumor escape.[65]

sHLA-G in normal pregnancy

Contradictory results have been reported regarding plasma/serum sHLA-G concentrations in normal pregnancy. A decade ago, Rebmann et al[74] found sHLA-G in almost all samples tested. There were no differences in the plasma sHLA-G concentration between men and non-pregnant women. Similarly, no differences were observed between non-pregnant and pregnant women at delivery, although information about gestational age and the presence or absence of labor at the time of sample collection was not provided. The umbilical cord plasma concentration of sHLA-G was significantly lower compared to that of maternal plasma. Puppo et al[73] did not find differences in serum sHLA-G concentrations between non-pregnant women, pregnant women at different gestational ages, and umbilical cord

samples. In contrast, Hunt and Ober et al[148] reported that non-pregnant women had significantly lower serum sHLA-G concentrations than pregnant women at any gestational age, and that serum sHLA-G concentrations do not change with advancing gestational age. These findings are consistent with those published in two recent studies including serum[76] and plasma[58] samples. In contrast, Athanassakis et al[149] reported that non-pregnant women have significantly higher serum sHLA-G concentrations than pregnant women, and that serum concentrations of sHLA-G progressively increase as gestation progresses. However, in a large cohort of non-pregnant and normal pregnant women in each trimester, Steinborn et al[69] found that plasma concentrations of sHLA-G significantly decreases from the first trimester until the end of pregnancy. It has been proposed that the use of plasma or serum for determination of sHLA-G concentrations may account, in part, for discrepancies observed among studies.[150]

HLA-G and sHLA-G in pregnancy complications

Inadequate maternal immune recognition and response may be associated with maternal-fetal histocompatibility.[128] There is a solid body of evidence supporting a role for HLA-G/sHLA-G in the pathophysiology of several obstetric complications[65–69] such as recurrent spontaneous abortion,[58,149,151–167] preeclampsia, intrauterine growth restriction, placental abruption, preterm labor and preterm PROM.

Preeclampsia—It has been proposed that defective HLA-G expression may be related to the vascular and immune abnormalities characteristics of preeclampsia.[168,169] Hara et al[170] studied HLA-G protein expression by immunohistochemistry in extravillous trophoblasts from patients with preeclampsia and those with a normal pregnancy. All the extravillous trophoblasts from normal pregnancies were stained for HLA-G protein, whereas attenuated expression of HLA-G protein was observed in patients with preeclampsia. Similar results were observed for placental lysates[171] and RNA expression[168] in cases with preeclampsia. Moreover, an association between fetal HLA-G genotype (e.g. *+14bp*/*+14bp*) and the higher risk of preeclampsia has been observed in primiparas.[172]

Several studies have found that the maternal plasma/serum concentrations of sHLA-G of patients with preeclampsia are significantly lower than that of normal pregnant women at the time of the disease.[66,171] Of interest, these differences can be observed even before the clinical diagnosis of preeclampsia. Yie et al[173] demonstrated that patients who subsequently will develop preeclampsia have plasma sHLA-G concentrations in the first, second and third trimesters significantly lower than that of matched normal pregnant women, and suggested that sHLA-G may be a useful molecule for predicting preeclampsia. Steinborn et al[69] reported similar results for the second trimester of pregnancy but, unexpectedly, did not find significant differences in the maternal plasma sHLA-G concentration between normal pregnant women and those with preeclampsia at the time of diagnosis of the disease. This finding is in agreement with the results of a study that did not find significantly different HLA-G expression by immunohistochemistry on chorionic and extravillous cytotrophoblast between patients with severe preeclampsia delivered preterm and patients with normal pregnancies delivered at term.[174]

Intrauterine growth restriction—Steinborn et al[69] also found lower maternal plasma sHLA-G concentrations in the second trimester in patients who subsequently developed intrauterine growth restriction compared to normal pregnancies; however, no significant differences were observed in the third trimester.[69] A significant association between an HLA-G genotype homozygous for the presence of a 14 base pair polymorphism and increased birth weight has been reported.[175] Yet, a null mutation in exon 3 of HLA-G has

not been associated with increased risk for preeclampsia or intrauterine growth restriction.[176]

Placental abruption—Patients with placental abruption have a significantly lower plasma concentration of sHLA-G than patients with normal pregnancies.[68]

Preterm labor and Preterm PROM—Steinborn et al[69] also measured sHLA-G in patients with spontaneous preterm labor and in those with preterm PROM. The authors reported significantly higher plasma concentrations of sHLA-G in patients with preterm labor compared to normal pregnancies and those with preterm PROM. No differences were observed between normal pregnant women and patients with preterm PROM.

HLA-G and sHLA-G in amniotic fluid

The results reported herein demonstrate that sHLA-G is a physiological constituent of the amniotic fluid, since it was detected in all but two amniotic fluid samples included in this study. This finding is in agreement with previous reports. McMaster et al[72] reported the presence of HLA-G in all amniotic fluid samples collected at 16–18 weeks of gestation and in the third trimester. Rebmann et al[74] measured sHLA-G concentrations in amniotic fluid samples obtained from mid-trimester amniocentesis, as well as in plasma from maternal and cord blood obtained at term, and found that sHLA-G concentrations in amniotic fluid in the mid-trimester are significantly lower than that of maternal plasma. Similarly, the maternal serum concentrations of sHLA-G were significantly higher than that of amniotic fluid from normal pregnancies and patients with preeclampsia.[66] In contrast, Puppo et al[73] found that the concentration of sHLA-G in the amniotic fluid is significantly higher than in maternal serum, and also significantly higher than the amniotic fluid concentration of sHLA-ABC. The authors suggested that amniotic epithelial cells, which express both HLA-ABC and HLA-G antigens, may preferentially secrete sHLA-G molecules.[73]

In amniotic fluid from mid-trimester amniocentesis obtained due to advanced maternal age, Emmer et al[75] found that the concentrations of sHLA-G were higher in women with male fetuses that that of those with female fetuses. These differences were not observed in the present study. The same authors also reported that the amniotic fluid concentrations of sHLA-G were significantly lower in patients with neural tube defects than that of controls,[177] and proposed that this finding may reflect an altered cell-mediated immunity in those pregnancies, which might be associated to folate deficiency or thymus dysplasia.

In the study reported herein, although there was a trend toward reduction by the end of pregnancy, no significant differences were observed in the amniotic fluid concentrations of sHLA-G between patients in the mid-trimester and that of those at term not in labor. This is in contrast with the results of Hackmon et al,[76] who found that amniotic fluid sHLA-G1 concentrations were lower in samples obtained from patients at term who underwent cesarean section compared to that of those from mid-trimester amniocentesis. The reasons for the discrepancies among studies are unclear, although it is possible that the sample size and methods used to determine sHLA-G may explain the conflictive results. The authors proposed that lower amniotic fluid concentrations of sHLA-G toward term may reflect a maternal immune response against fetal trophoblast, which may lead to parturition. This finding has not been observed in maternal blood, since there are no significant changes in sHLA-G serum concentrations among first, second and third trimester.[73,148] Interestingly, we found that spontaneous labor at term was associated with lower amniotic fluid concentrations of sHLA-G than that of those from women not in labor. It is possible that sHLA-G plays a role as immunomodulator in early pregnancy, and that declining

amniotic fluid sHLA-G concentrations near term may contribute to the initiation of parturition.

Although the main source of maternal serum sHLA-G is probably the extravillous cytotrophoblast, the sources of sHLA-G in the amniotic fluid still needs to be elucidated.[66] It has been proposed that production of soluble HLA-G may occur in amnion and decidual trophoblast cells[177] and amnion epithelium.[178] Recently, it has been shown that fresh and cultured amniotic fluid cells express soluble forms of HLA-I and HLA-G.[179,180] Interestingly, both shed HLA-G1 and HLA-G5 have been detected in different body fluids such as serum from pregnant and non-pregnant women, patients with cancer or transplants, and even in plasma from men.[73,74,111,181,182] This suggest that, beside trophoblast, circulating HLA-G may have other sources. Monocytes have a very low HLA-G expression, but it can be induced to some extent in these cells by cytokines such as Interferon (IFN)-γ. [102,183]

sHLA-G is significantly elevated in intra-amniotic infection and inflammation

This study is the first to demonstrate significantly higher amniotic fluid concentrations of sHLA-G in pregnancies associated with intra-amniotic infection/inflammation, which is one of the most important mechanisms of disease associated to spontaneous preterm labor with intact membranes or preterm PROM. The findings reported herein suggest that sHLA-G participates in the host response to microbial invasion of the amniotic cavity. Indeed, among patients with spontaneous preterm labor and intact membranes and those with preterm PROM who had intra-amniotic infection/inflammation, the median amniotic fluid sHLA-G concentration was almost 2-fold higher than that of those without intra-amniotic infection/ inflammation. These findings are supported by the results of patients with histologic chorioamnionitis and/or funisitis, who had a higher median amniotic fluid sHLA-G concentration than those without evidence of placental inflammation.

Increased expression of HLA-G has been reported in infectious diseases,[70] mainly in viral infections.[115,116,184] In addition, Monneret et al[71] measured plasma sHLA-G5 concentrations in consecutive patients with septic shock. The authors found that sHLA-G5 concentrations increased 24–48 hours from the onset of septic shock, and a significant difference between survivors and non-survivors was observed at this time, remaining significant until day seven. Interestingly, non-survivors were associated with lower concentrations of sHLA-G5 than that of those who survived. The authors concluded that sHLA-G5 may have an important role in negative feedback signals that limit the process of inflammation during septic shock.[71]

A solid body of evidence supports an involvement of HLA-G in autoimmune and inflammatory diseases.[70] In fact, HLA-G expression has been described in the skin, pancreas, digestive tract, muscle and central nervous system: 1) HLA-G expression was demonstrated in patients with atopic dermatitis and psoriasis;[57,113,114,185] 2) HLA-G expression is enhanced in cases of celiac disease[186] and ulcerative colitis, while there is almost no expression in cases of Crohn's disease.[187,188] It has been proposed that differential expression of HLA-G provides a potential way to distinguish between ulcerative colitis and Crohn's disease;[187] 3) HLA-G expression in the human pancreas is restricted to insulin, glucagon, and ductal cells, and is upregulated in the presence of an inflammatory stimulus;[107] and 4) a role for HLA-G has been proposed in multiple sclerosis,[189–191] acute neuroinflammatory disorders,[144] asthma,[192–194] rheumatoid arthritis,[195] and myositis.[144,196,197]

HLA-G5 and HLA-G6 stimulate TGF-β1, an anti-inflammatory cytokine, in monocyte and monocyte-derived macrophage models,[198,199] and low doses of HLA-G5 induce IL-10

secretion.[198,199] In cultured trophoblast cells, Moreau et al[200] showed that IL-10 enhances HLA-G transcription and up-regulate HLA-G cell surface expression in peripheral blood monocytes. Thus, induction of HLA-G expression by IL-10 on monocytes may play a role in down-regulation of the immune response.[200] Our group demonstrated that spontaneous term and preterm parturition, as well as intra-amniotic infection/inflammation, is associated with increased amniotic fluid concentrations of IL-10.[201] We suggest that IL-10 has a role in the regulation of the immune response *in vivo* by initiating actions that dampen inflammation.[201] Kapasi et al[202] demonstrated that exposure to HLA-G is associated with *in vitro* suppression of allo-cytotoxic T lymphocytes and the development of a Th2-type cytokine response, leading the authors to propose that HLA-G may contribute to a putative protective role in pregnancy.[202] HLA-G participates in the imbalance toward a Th2-type immune response during pregnancy[185] by inducing the secretion of anti-inflammatory cytokines such as IL-3, IL-4[203] and IL-10,[202] and down-regulating the production of IFN-γ and TNF-α.[203] Therefore, HLA-G has been proposed as a negative feedback signal that limits the process of inflammation.[185]

In conclusion, amniotic fluid concentrations of sHLA-G are elevated in preterm parturition associated intra-amniotic infection/inflammation as well as histologic chorioamnionitis. We propose that sHLA-G may participate in the regulation of the host immune response against intra-amniotic infection.

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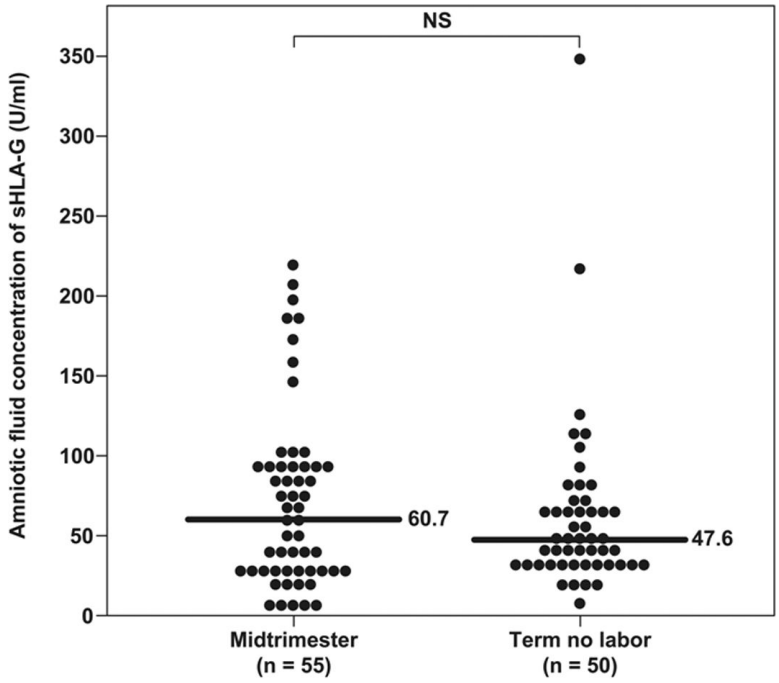

Figure 1. Amniotic fluid concentration of sHLA-G in normal pregnancies at mid-trimester and in those at term not in labor

No significant differences were observed in the median sHLA-G concentration in amniotic fluid between women in the mid-trimester and those with a normal pregnancy at term not in labor [60.7 U/mL, interquartile range (IQR) 28.6–96 vs. 47.6 U/mL, IQR 33.5–69, respectively; p=0.5].

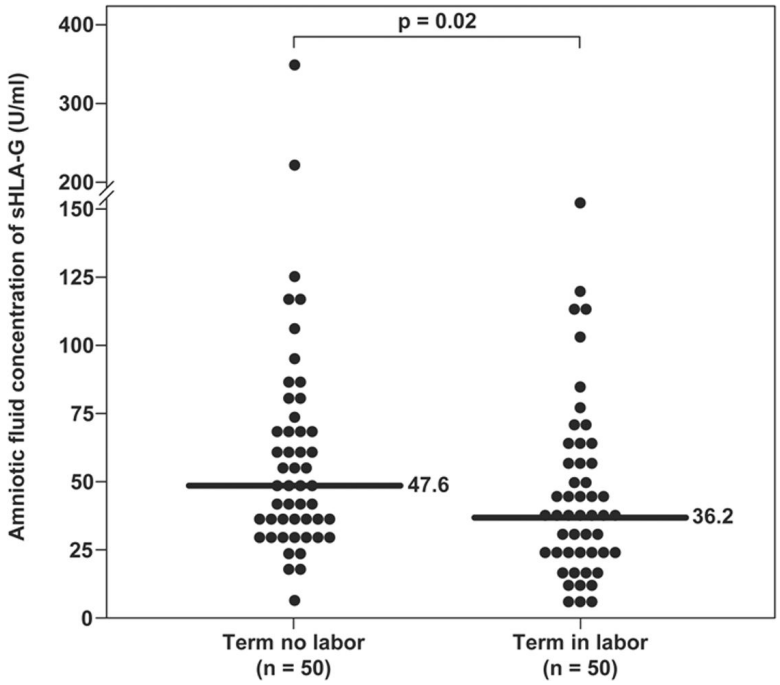

Figure 2. Amniotic fluid concentration of sHLA-G in normal pregnancies with and without spontaneous labor at term

Women with spontaneous labor at term had a significantly lower median amniotic fluid sHLA-G concentration than those without labor (36.2 U/mL, IQR 22.9–57.8 vs. 47.6 U/mL, IQR 33.5–69, respectively; p=0.02).

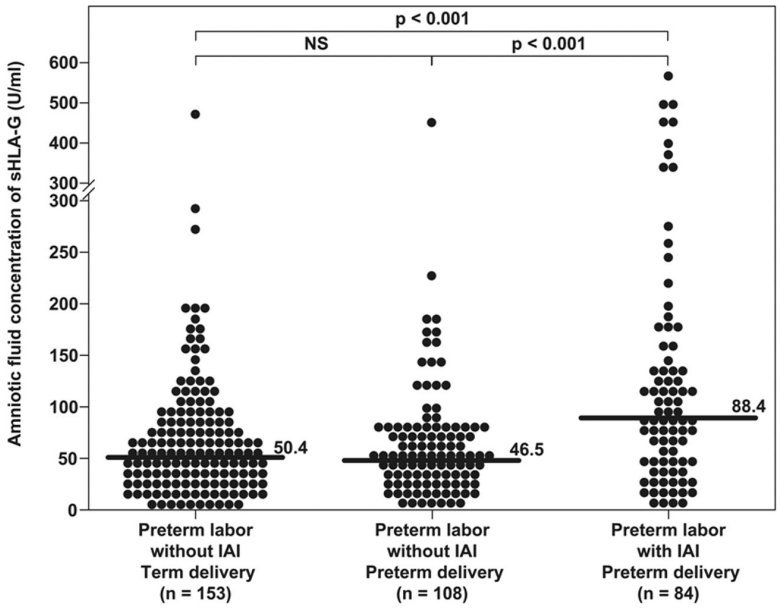

Figure 3. Amniotic fluid concentration of sHLA-G among patients with spontaneous preterm labor and intact membranes (PTL)

Patients with spontaneous preterm labor with intact membranes with intra-amniotic infection/inflammation (IAI) had a significantly higher median amniotic fluid concentration of sHLA-G than those who delivered preterm without IAI (PTL with IAI: 88.4 U/mL, IQR 43.5–142.9 vs. PTL without IAI: 46.5 U/mL, IQR 26.9–73.9; $p<0.001$) and those with spontaneous preterm labor with intact membranes who delivered at term (PTL with IAI: 88.4 U/mL, IQR 43.5–142.9 vs. PTL delivered at term: 50.4 U/mL, IQR 29.8–85.9; $p<0.001$). There were no differences in the median amniotic fluid sHLA-G concentration between patients with spontaneous preterm labor without IAI who delivered preterm and those who delivered at term.

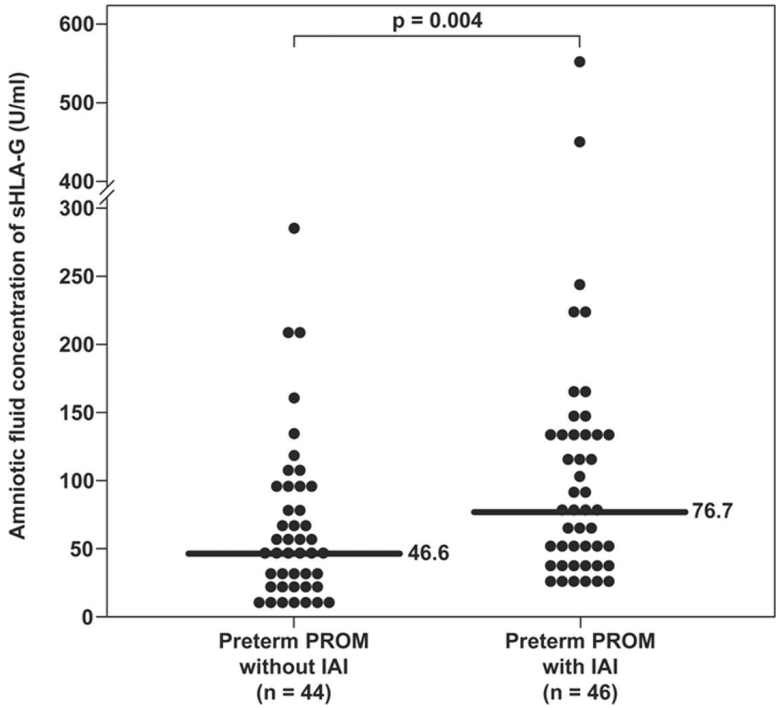

Figure 4. Amniotic fluid concentration of sHLA-G in patients with preterm prelabor rupture of the membranes (preterm PROM)

Patients with preterm PROM with IAI had a significantly higher median amniotic fluid sHLA-G concentration than those with preterm PROM without IAI (76.7 U/mL, IQR 39.7–135.4 vs. 46.6 U/mL, IQR 25.8–94.9, respectively; p=0.004).

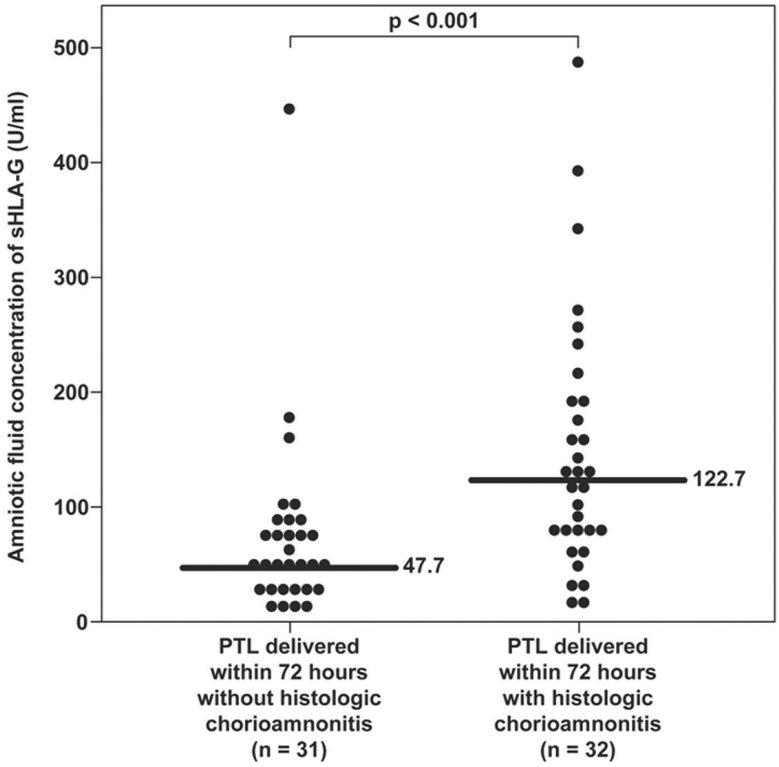

Figure 5. Amniotic fluid concentration of sHLA-G in patients with spontaneous preterm labor with and without histologic chorioamnionitis who delivered within 72 hours from amniocentesis

Patients with histologic chorioamnionitis and/or funisitis had a significantly higher median sHLA-G concentration in amniotic fluid than those without histologic inflammation (122.7 U/mL, IQR 79–190.1 vs. 47.7 U/mL, IQR 28.3–83.7, respectively; $p<0.001$).

Table I

Demographic and clinical characteristics of patients in the mid-trimester and those at term with and without spontaneous labor

	Mid-trimester (n=55)	p[a]	Term No labor (n=50)	Term In labor (n=50)	p[b]
Maternal age (years)	36 (35–38)	<0.01	27 (21–32)	22 (19–27)	<0.01
Gestational age at amniocentesis (weeks)	16 (16–17)	<0.01	39 (38–39)	38.6 (37.9–39.4)	NS
Gestational age at delivery (weeks)	39 (38–40)	NS	39 (38–39)	38.5 (37.9–39.4)	NS
Birthweight (grams)	3,344 (3,113–3,604)	NS	3,260 (3,080–3,630)	3,375 (3,093–3,563)	NS

Values are expressed as percentage (number) or median (interquartile range).

NS: not significant.

p[a]: comparison between patients in the mid-trimester and those at term not in labor

p[b]: comparison between patients at term not in labor and those at term in labor

Table II

Demographic and clinical characteristics of patients presenting with spontaneous preterm labor with intact membranes

	PTL without IAI Term delivery (n=153)	p	PTL without IAI Preterm delivery (n=108)	p[a]	PTL with IAI Preterm delivery (n=84)	p[b]
Maternal age (yrs)	22 (19–30)	NS	22 (19–30)	NS	23 (20–28)	NS
Smoking	18.4 (28/152)	NS	10.4 (11/106)	0.001	30 (24/80)	0.04
BMI (Kg/m^2)	22.7 (20.1–25.5)	NS	22.3 (20.1–25.2)	<0.01	25 (21.8–30)	<0.01
GA at amniocentesis (wks)	31.9 (29.4–33.3)	NS	31.9 (29.8–33.1)	<0.01	28.8 (25.1–33)	<0.01
GA at delivery (wks)	38.7 (38–39.7)	<0.01	34.6 (33.3–35.6)	<0.01	29.8 (25.6–33.3)	<0.01
Birthweight (grs)	3,170 (2,900–3,515)	<0.01	2,330 (1,940–2,678)	<0.01	1,310 (735–2,118)	<0.01

Values expressed as percentage (number) or median (interquartile range)

PTL: preterm labor; GA: gestational age; BMI: body mass index; IAI: intra-amniotic infection/inflammation NS: not significant p[a]: comparison between PTL who delivered preterm without IAI and PTL with IAI; p[b]: comparison between PTL who delivered at term without IAI and PTL with IAI

Table III

Demographic and clinical characteristics of patients presenting with preterm prelabor rupture of membranes

	Preterm PROM without IAI (n=44)	Preterm PROM with IAI (n=46)	p
Maternal age (yrs)	24.5 (20–32.8)	30 (23.5–37.5)	0.008
Smoking	18.2 (8/44)	17.8 (8/45)	NS
BMI (Kg/m^2)	24.5 (21.5–28.8)	25.3 (23.5–27.7)	NS
GA at amniocentesis (wks)	32.6 (29.4–33.8)	30.5 (27.9–32.4)	0.02
GA at delivery (wks)	33.2 (31.4–34.4)	30.8 (28.7–33.1)	<0.001
Birthweight (grs)	2,020 (1,678–2,323)	1,645 (1,385–2,120)	0.02

Values expressed as percentage (number) or median (interquartile range)

PROM: prelabor rupture of membranes; GA: gestational age; BMI: body mass index; IAI: intra-amniotic infection/inflammation NS: not significant.