Soluble HLA-G changes in maternal blood serum during the progression of labor

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Abstract

BACKGROUND: The expression of the HLA-G antigen from implantation toward term is crucial for the growth of a semiallogenic fetus as it shields this fetus from the maternal cytotoxic response. Little is known, however, about the potential role of soluble HLA-G isoforms during delivery. The initiation of labor is associated with a complex molecular response leading to a brief activation of the maternal immune system with an accompanying capacity to restrict this activation, and HLA-G seems to be an important factor in enabling the proper immune response at the maternal fetal interface.

METHODS: In our study the levels of soluble HLA-G concentration were evaluated in the blood serum samples obtained from 47 pregnant women who either underwent cesarean sections or delivered vaginally. The patients were divided into three subgroups according to the progression of labor at the time of the cesarean or, in cases of vaginal delivery, according to the duration of the pregnancy.

RESULTS: We have observed that the progression of labor is associated with a continuous increase in the sHLA-G plasma level. The sHLA-G levels were statistically significantly higher in the blood sera obtained from the women in advanced labor than from the women who were at the beginning of labor.

CONCLUSION: The changes in sHLA-G concentration levels observed during the stages of labor may indicate that this isoform participates in maintaining reproductive tract homeostasis.

INTRODUCTION

HLA-G is a non-classical human leukocyte antigen of Class Ib. It plays an important role in setting up and maintaining the immune tolerance that is achieved by the inhibition of immunocompetent cells [1]. As a result of an alternative splicing of a primary HLA-G transcript, seven isoforms of this antigen are generated. Four of them (G1-G4) are bound to a cell surface, while three others (G5-G7) that lack the exons for cytoplasmatic and transmembrane domain constitute the soluble isoforms [2,3]. The latter may be either secreted (HLA-G5) or proteolytically cleaved from the cell surface

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(sHLA-G1). The function of the HLA-G molecule is firstly related to the fetal–maternal tolerance where its expression by cytotrophoblast prevents the fetus from attack by maternal natural killer (NK) cells [4]. As the non-classical HLA Class I molecule,HLA-G exerts its function by binding to inhibitory receptors-such as the leukocyte-inhibitory receptors LILRB1/ILT-2/CD85j, ILT-4/LILRB2/CD85d, and KIR2DL4 [5,6]-this interaction leads to the inhibition of the following processes: CD4+ T cell proliferation [5,7], the cytotoxic activity of CTL and NK cells [5,8,9], dendritic cell maturation [10], and the cell cycle progression of alloreactive T cells [11]. Furthermore, through the mechanism known as "trogocytosis"[12], HLA-G upregulates its own receptors [13] and shifts the cytokine TH1/Th2 balance toward Th2 [14,15].

As with the membrane-bound HLA-G, the soluble isoforms exhibit the immunosuppressive activities [5] that are characteristic of HLA-G1 and the secreted HLA-G5 proteins. In addition, the soluble HLA-G isoforms (both shed HLA-G1 and HLA-G5) can induce Fas-dependent apoptosis of activated $CD8^+$ T and NK cells [16,17] and can also inhibit angiogenesis by their interaction with the CD160/BY55 receptor [18]. The suppressive functions of soluble HLA-G isoforms may be seen not only at the local site of their expression but also – unlike their membrane-bound counterparts – at distant sites throughout the peripheral circulation.

In pregnancy, it has been found that villous cytotrophoblast cells produce just one HLA-G isoform, HLA-G5 [19]. In contrast, the extravillous cytotrophoblast that is close to the decidua, not only expresses the membrane HLA-G isoform, HLA-G1, but also possibly a second isoform, HLA-G2 [19,20]. Although HLA-G is produced by these cells throughout pregnancy, its expression is the highest in the first trimester and declines toward term. Soluble HLA-G isoform has been detected in the supernatant culture media from *in vitro* cultured embryos [21] and in maternal and cord blood [20] as well as in amniotic fluid. [22,23]

The function of HLA-G and its soluble isoform in pregnancy has already been established in many studies. Expression of the HLA-G antigen, from implantation to term, is crucial for the growth of a semiallogenic fetus as it shields this fetus from the maternal cytotoxic response [19]. Disturbances in HLA-G expression are therefore thought to be associated with the development of various obstetric complications, such as, preeclampsia, placental abruption, or IUGR [24–26]. Little is known, however, about the potential role of HLA-G isoforms during delivery. The initiation of labor is associated not only with an increase in endocrine signals from the fetal adrenals, but also with a complex molecular response leading to a brief activation of the maternal immune system with an accompanying capacity to restrict this very activation [27–44], and HLA-G seems to be an important factor enabling the proper immune response at the maternal-fetal interface. In order to verify this hypothesis we have aimed in this study to measure the concentration level of the soluble HLA-G isoform with respect to the progression of labor at term.

2. MATERIALS AND METHODS

2.1 Patients

The blood serum samples evaluated in our study were obtained from 47 pregnant women who either underwent cesarean sections or delivered vaginally. The women included in our study were selected from those who delivered in 2007 at the Department of Gynecology, Obstetrics and Oncology of the Jagiellonian University, Krakow, Poland. The patients were divided into three subgroups either according to the progression of labor at the time of the cesarean or in cases of vaginal delivery, according to the duration of pregnancy. The following groups were distinguished: Group One consisted of 24 women, 12 of whom underwent elective cesearean section without labor (i.e., with cervical external *os* closed), and 12 women on whom cesarean sections were performed after the spontaneous beginning of labor (i.e., whose cervical dilation was less than 3 cm at the time of the cesarean). Group Two comprised 7 women on whom cesarean sections were performed during advanced labor (i.e., whose cervical dilation was more than 3 cm at the time of the cesarean) and 9 women who experienced spontaneous, uncomplicated vaginal delivery at term. Finally, Group Three consisted of 7 women with preterm delivery (25–36 wks). Preterm delivery at the time of diagnosis was accompanied by uncontrollable progressive cervical dilation and pathological findings in the CTG reading indicating fetal stress or the preterm rupture of membranes. The patients included in these subgroups delivered vaginally. The various indications for cesarean section were as follows: fetal distress syndrome, malpresentation, uterine myoma, previous cesarean delivery, and fetal heart defect. We have also used this division in our recent studies concerning the regulation of immune system activity during labor[27,28,35,45]. Patients with multiple pregnancies or existing complications of pregnancy (such as hypertension and diabetes mellitus) were excluded from this study, as were cases of fetal demise. Additionally, patients with intra-amniotic infection, as confirmed by histopathological examination of the fetal membranes and chorionic plate or other symptoms of intrauterine infections, were excluded from the study.

The patient's consent was obtained in each case. Prior to the present study we also obtained the approval of the Jagiellonian University Ethical Committee for our research program (KBET/135/B/2007).

2.2. ELISA

The blood was collected in a serum collection tube prior to surgery in patients on whom cesarean sections were performed and, in cases of patients who delivered

Table 1. Clinical characteristic of patients

Variables	Group I (n=24)	Group II (n=16)	Goup III (n=7)	p-value
Maternal age (median, IQR)	30 (7)	30 (6)	30.5 (8)	0.76
Parity (median, IQR)	2 (1.5)	1 (2)	2 (2)	0.33
Gestational age (median, IQR)	38 (2)	39 (2)	33 (6)	<0.01
Newborn mass (median, IQR)	3145 (580)	3220 (560)	1920 (1100)	0.02
Newborn length (median, IQR)	53 (5)	54 (6)	47 (8)	0.01
Apgar score (median, IQR)	10 (1.5)	10 (0.0)	7 (2)	<0.01

IQR – intraquartile range

vaginally, during the active phase of stage I of labor. A clot was allowed to form at room temperature for 30–60 minutes. The tube was placed on ice for 30 minutes in order to contract the clot. The serum samples were then centrifuged at 3000 xg for 10 minutes at room temperature. The supernatants 1.0–2.0 ml were collected and stored at –80°C. The soluble human leukocyte antigen-G (sHLA-G) was determined using the sHLA-G sandwich ELISA kit (BioVendor-Exibo, Czech Republic). Stated briefly, the blood plasma samples were diluted twice and incubated for 1 hour in the 96-well microplate precoated with the monoclonal anti-sHLA-G antibodies. Following incubation the wells were washed and filled with the monoclonal anti-human beta-2-microglobulin antibodies labeled with horseradish peroxidase. After an additional 1 hour of incubation, the wells were again washed and the color reaction was developed using tetramethyl benzidine (TMB) substrate. The absorbance values were measured at 450 nm on a microplate reader followed by calculation of the sHLA-G concentrations. Finally, the assay was calibrated using a set of sHLA-G standards provided by the producer of the kit.

2.3 Statistical analysis

The distribution of variables in the study groups of women checked with the use of the Shapiro-Wilk test showed that each of them was different from normal. The statistical significance between the groups was determined by the Kruskal-Wallis analysis of variance (ANOVA) test. The Mann-Whitney U test was then used as applicable.

RESULTS

3.1 Clinical comparison of analyzed groups of patients

Since the indications for performing a cesarean section vary, it would seem important to compare the parameters characterizing the course of pregnancy and labor in the groups of patients considered (Table 1).

We did not observe any statistically significant differences in maternal clinical parameters among the groups examined. Since the examined group of patients included those with preterm delivery, the differences in clinical parameters such as gestational age, newborn mass, newborn length, and Apgar score are understood from a clinical point of view. This profile of clinical parameters characterizing the course of pregnancy enabled us to compare the respective sHLAG blood serum concentration levels in these groups.

3.1 Soluble SHLA-G levels in maternal bood serum with respect to the progression of labor

During labor at term, the blood serum sHLA-G concentration level significantly increases. The levels of sHLAG blood serum concentration in group I ranged from 0 to 26.6 U/ml (32% positive cases); in group II they ranged from 0 to 59.18 U/ml (75% positive cases), and in group III from 4.42 to 70.41 U/ml (all cases were positive). The highest level of sHLA-G concentration was observed in group III, that is, in the women with preterm delivery. The sHLA-G levels were statsitically significantly higher in the blood sera obtained from the women in advanced labor than in the sera from those women who were at the beginning of labor (Figure 1).

DISCUSSION

In the present study we have focused on the maternal sHLA-G plasma levels during the course of labor, and we have observed that the progression of labor is associated with a continuous increase in the sHLA-G plasma level. To our knowledge (based on a literature search in PubMed), this is the first investigation to concentrate on the sHLA-G level in patients who delivered by cesarean at term according to the progression of labor at the time of the procedure.

During pregnancy, the HLA-G antigen is expressed in high levels by an extravillous trophoblast [19,46]. HLA-G protein is also present in maternal compartment-decidua where its expression has been found to be dependent on hormones, mainly progesterone and certain cytokines [47]. The soluble isoform-sHLA-G is present in villous cytotrophoblast [48,49] and in maternal and fetal circulation as well as in the amniotic fluid [19,22,23]. In the maternal plasma, the sHLA-G level is the highest during the first trimester of pregnancy

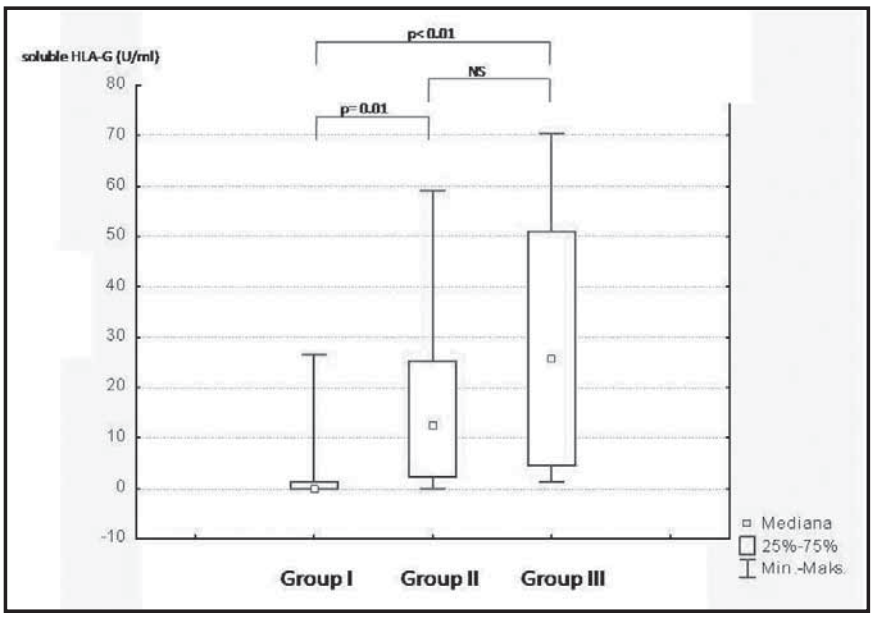

Figure 1. The analysis of the maternal blood serum concentration level according to the progression of labor at the time of the cesarean or, in cases of vaginal delivery, according to the duration of pregnancy. Group One consisted of both women who underwent elective cesearean sections without labor and women on whom cesarean sections were performed after the spontaneous beginning of labor. Group Two comprised both women who underwent cesarean sections during advanced labor and women who experienced spontaneous, uncomplicated vaginal delivery at term. Group Three consisted of women with preterm delivery.

and then declines as term approaches. Similar changes in expression throughout pregnancy have been noted for a membrane-bound HLA-G isoform [22].

Expression of HLA-G at the feto-maternal interface is thought to protect the semiallogenic fetus from being rejected by acting as a barrier to maternal allo-immune competent cells. Membrane-bound HLA-G isoforms inhibit uterine natural killer (NK) cell function while soluble proteins modulate cytokine production by blood cells [14,15] and affect cytotoxic T lymphocytes by diverting their cycle to a programmed death [5,8,16,17]. In contrast, T helper lymphocytes are programmed into tolerant ones [12]. It has been suggested, based on the studies of embryo cultures, that HLA-G secretion is necessary for successful implantation [50]. Impaired expression of the HLA-G antigen by trophoblast cells as well as its soluble isoform is associated with obsteric complications. Decreased expression of HLA-G has been noted in recurrent pregnancy losses [51]. Moreover, compared to the levels found in normal pregnancy, the plasma levels of sHLA-G in cases of placental abruption and preeclampsia are low. [24–26, 52,53]. Furthermore, a lower level early in the course of pregnancy may predict the occurence of preeclampsia later on [53]. In our study, the highest maternal blood serum sHLA-G concentration level was observed in patients with preterm labor. These results correlate well with the findings of Steinborn *et al.* who have reported that the level of sHLA-G in women with uncontrollable preterm labor as well as in the patients with HELLP syndrome is significantly increased [24,25]. The latter syndrome is also associated with a higher concentration level of sHLA-DR antigens in maternal circulation [54] than had previously been documented for the state of acute graft rejection after transplantation. The authors have therefore suggested that the patomechanism of these complications may resemble that of acute rejection where excessive allogenic reaction is key.

Despite reports about how HLA-G isoforms function throughout pregnancy and in pregnancy-related disorders, they have yet to be evaluated during the course of labor. In one of the studies, Steinborn *et al.* evaluated pregnancies at term, and a significant difference between the mode of delivery and the level of sHLA-G in maternal circulation was not found. This would suggest that the status of labor has no influence on the content of sHLA-G in the maternal plasma; however, the authors mentioned did not evalute sHLA-G according to the particular stage of labor [24].

So far it has been known that the beginning of parturition is dependent upon signals from the fetal adrenal glands; more recently, a change in the pattern of cytokine concentration (IL-6, Il-8) [33] as well as an increase in the cytotoxic activity of the immune cells has also been identified [30,31,35]. Our previous reports have shown that parturition is a dynamic process accompanied by particular immunological alterations; for example, alterations in the infiltration and activity of CD3+ and CD56+ cells in decidua, in immunoreactivity, and in the concentration of inhibitory proteins [27,28,32,35,42].

Steinborn *et al.* have observed that spontaneous labor at term is accompanied by a significantly elevated release of IL-6 from fetal monocytes that has not been found in cases of induced term or preterm delivery. It has been suggested that the fetal phagocyte activation was due to the presence of the maternal lymphocyte-T alloantigen stimulation that crosses the placental barrier at the end of pregnancy [29].

The serum level of sHLA-G is higher in pregnant women than in non-pregnant women [22,23]. Soluble HLA-G concentration peaks in the first trimester and then decreases until term. As has been shown to be the case with the level of sHLA-G concentration in maternal blood during pregnancy, sHLA-G in amniotic fluid significantly decreases from the second trimester until term [22]. Moreover, while a significant correlation between the sHLA-G concentration levels in maternal and fetal blood has been found, no such correlation between the levels of concentration in the maternal blood and amniotic fluid was observed [22]. The varied distribution of sHLA-G in body fluids during pregnancy as well as the changes in the protein concentration over the course of labor may suggest that the protein has a different origin. For this reason we can speculate that during delivery sHLA-G may be derived not only from trophoblasts, but also from the activated immune cells of both mother and fetus. Alegre *et al.* have discovered that maternal monocytes as well as DCs express sHLA-G in pregnancy. This suggests that the contribution of maternal APCs to the circulating pool of HLA-G protein probably becomes more important as pregnancy advances [55. Furthermore, the secretion of sHLA-G was increased by IFN-gamma [55]; this finding agreed with previous reports where the role of cytokines in regulating such antigen expression has been documented [56–58]. Moreover, IL-10, which is also produced by cytotrophoblast cells, was seen to increase HLA-G expression. This cytokine plays an important role in maintaining the immune tolerance level crucial for a successful pregnancy. Additionally, it has been observed that the level of IL-10 was lower in women suffering from recurrent pregnancy losses [59]; this is similar to what has been reported for HLA-G [51]

In our recent study, we have seen that this growth of maternal immune cell activity at the beginning of labor is associated with the subsequent growth of the expression of a decidual protein, such as RCAS1, and that its soluble form in maternal blood serum inhibits maternal cytotoxic cell activity [28,32,42,45,60–65]. In the present study we have found that sHLA-G concentration rises with the progression of labor in a way comparable to the increase in the blood serum level of RCAS1 that takes place as labor advances. Moreover, the sHLA-G level was significantly higher in advanced labor than at the beginning of delivery. The changes in sHLA-G concentration levels observed during the various stages of labor may indicate that it participates in maintaining reproductive tract homeostasis.

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