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Human leukocyte antigen (HLA)-G during pregnancy part I: Correlations between maternal soluble HLA-G at midterm, at term, and umbilical cord blood soluble HLA-G at term

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ABSTRACT

Human leukocyte antigen (HLA)-G is a class Ib molecule with restricted tissue distribution expressed on trophoblast cells and has been proposed to have immunomodulatory functions during pregnancy. Soluble HLA-G1 (sHLA-G1) can be generated by the shedding of membrane-bound HLA-G molecules; however, three soluble isoforms also exist (HLA-G5 to -G6). During pregnancy, it is unknown whether there is a correlation between sHLA-G levels in maternal and fetal blood. In 246 pregnancies, we have measured the levels of sHLA-G1/-G5 in maternal blood plasma samples from gestational week 20 (GW20) and at term, as well as in umbilical cord blood samples. Soluble HLA-G levels declined by 38.4% in maternal blood from GW20 to term, and sHLA-G levels were significantly lower in maternal blood at term than in GW20 ($P < 0.001$). At term, the sHLA-G levels were significantly higher in maternal blood than in umbilical blood ($P < 0.001$). HLA-G levels in maternal blood in GW20 and at term, and in maternal blood at term and umbilical cord blood, were correlated ($P < 0.001$ and $P < 0.01$, respectively). This is the first large study simultaneously measuring sHLA-G in both maternal and umbilical cord blood. The finding that sHLA-G levels are significantly lower in fetal compared with maternal blood at term documents for the first time that sHLA-G is not freely transferred over the placental barrier. Soluble HLA-G levels in maternal and fetal blood were found to be correlated, which may be due to shared genetic factors of importance for production of sHLA-G in the mother and child, or it may support the theory that sHLA-G in the pregnant woman and the fetus is partly derived from a "shared organ", the placenta.

1. Introduction

During implantation and pregnancy, the maternal immune system comes into close contact with fetal trophoblast cells. To avoid harmful immune reactions directed against it, the maternal immune system is believed to undergo adaptations that may partly be caused by the unique HLA expression at the feto-maternal interface. Classical human leukocyte antigen (HLA) molecules except HLA-C are not expressed on trophoblast, which may protect the trophoblast from strong T cell mediated allo-reactions. Non-classical HLA proteins such as HLA-G are strongly expressed on extravillous trophoblast (EVT) in early pregnancy and these molecules may play an important role in the maintenance of pregnancy [1–3]. HLA-G exhibits a restricted tissue distribution, a unique alternative splice pattern and limited polymorphism. *In vitro* it has been shown to interact with different immune receptors, which leads to inhibition of T and NK cell proliferation and cytotoxicity, induction of regulatory T cells, inhibition of antigen

Abbreviations: APC, antigen presenting cell; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; EVT, extravillous trophoblast; GW, gestational week; HLA-G, human leukocyte antigen-G; HRP, horseradish peroxidase; MHC, major histocompatibility complex; β2m, β2-microglobulin; RM, recurrent miscarriage; RT, room temperature; sHLA-G, soluble human leukocyte antigen-G; TMB, tetramethylbenzidine; 3′UTR, 3′-untranslated region.

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presenting cell (APC) differentiation, and alterations in cytokine secretion [4–8].

The unique alternative splicing of HLA-G results in the generation of several isoforms, including four membrane-bound (HLA-G1-G4) and three soluble (HLA-G5-G7) forms [9,10]. In addition, HLA-G can be cleaved from the cell surface as soluble HLA-G1 (sHLA-G1) [11]. At the feto-maternal interface, the EVT cells have been shown to express both membrane-bound HLA-G1 and sHLA-G5/shed sHLA-G1, while expression of HLA-G in the villous cytotrophoblast and syncytiotrophoblast is still disputed [12,13]. Soluble HLA-G has been detected in blood plasma of both pregnant women, non-pregnant women and men, in follicular and amniotic fluid and in seminal plasma [14–21].

Based on the immunological interactions with immune cells and the unique expression on EVT it is hypothesized that HLA-G plays a role in inducing immunological tolerance to the trophoblast during pregnancy. Low membrane-bound HLA-G expression or low sHLA-G in plasma before or in early pregnancy seems to be associated with pregnancy complications, such as recurrent miscarriage (RM) and preeclampsia, where immunological factors are thought to play a crucial role [22,23].

The peripheral blood levels of sHLA-G vary substantially among both non-pregnant and pregnant individuals. The causes of this variation are partly unknown but genetic factors seem to be important. Of special interest is the 14 bp insertion/deletion (14 bp ins/del) polymorphism localized in the 3′-untranslated region (3′UTR) of the HLA-G gene [24]. In non-pregnant individuals the genotype 14 bp ins/14 bp ins is associated with significantly lower levels of sHLA-G1/-G5 in the blood compared with other 14 bp genotypes [20,25–28] and this polymorphism seems to be one of the most important genetic determinant of sHLA-G concentration in plasma.

If sHLA-G levels play a role for normal pregnancy and adverse pregnancy outcome it is important to know whether it is the levels in maternal or fetal blood that are associated with the complications and to know the sources of sHLA-G in the maternal and fetal circulation. It is also essential to know how sHLA-G is distributed in the maternal and fetal compartments and whether it can pass the placental barrier freely.

In non-pregnant individuals the sHLA-G is thought to consist mainly of soluble HLA-G5 produced by activated maternal monocytes or antigen-presenting cells (APCs). The sHLA-G in pregnant women is, in theory, a mixture of secreted sHLA-G5 and shed soluble HLA-G1 from the trophoblast cells, which is, therefore, fetally derived, and sHLA-G (maybe primarily sHLA-G5) molecules produced by maternal immune cells [29,30]. The sHLA-G levels in fetal blood is in theory a mixture of trophoblast-derived sHLA-G directly entering the fetal capillaries in the villi, sHLA-G secreted from fetal immune cells and sHLA-G that has passed from the maternal circulation through the placenta into the fetus. There is controversy whether it is possible to distinguish the shed sHLA-G1 and the soluble HLA-G5 isoforms by ELISA [29,31]. However, we have recently shown with the use of recombinant soluble HLA-G, HLA-G5 and soluble HLA class Ia proteins that the specificity of some of the commercial mAbs, 5A6G7 and MEM-G/9, for HLA-G5 and soluble HLA-G1/HLA-G5 seems to be high [32]. MEM-G/9 is also used as the capture mAb in the commercial ELISA used in the current study. Still, our knowledge about the origin of the sHLA-G in maternal and fetal blood during pregnancy is limited.

So far, few studies have investigated sHLA-G in pregnant women in early and late pregnancy and compared the maternal levels to those in umbilical cord blood. Initially, small studies did not find sHLA-G levels in plasma or serum different between non-pregnant and pregnant women [19,33]. Later studies, however, have found the levels significantly higher in women with normal pregnancies than in non-pregnant women with a tendency for declining levels in late pregnancy [14]. One study analyzing plasma samples found that sHLA-G levels were significantly higher during all trimesters of normal pregnancy compared with non-pregnant women [30], and two studies found significantly declining sHLA-G plasma levels from the first to the third trimester in normal pregnant women and significantly higher sHLA-G levels during pregnancy than in non-pregnant women [34,35]. In two small studies sHLA-G levels were measured in maternal and umbilical cord blood in normal pregnancy and in both studies the levels were significantly lower in cord serum [19,36]. Finally, we have in a cohort of pregnant women studied sHLA-G in pregnancies complicated with preeclampsia and without complications [37]. In this study, in-house sHLA-G assays were performed to measure sHLA-G1 and HLA-G5. Soluble HLA-G in maternal plasma was lower after gestational week (GW) 32 compared to midpregnancy (GW 16-17). Most of the sHLA-G was measured as sHLA-G1, and this was significantly lower in severe preeclampsia compared with controls. Overall, these studies seem to indicate that sHLA-G levels increase in early pregnancy and decline in the third trimester and the levels may be lower in the fetal than maternal blood at term.

We aimed at confirming or reject these findings in the largest study of mothers and offspring so far by measuring sHLA-G levels in maternal plasma in mid-pregnancy and at term, and in umbilical cord plasma at term. Furthermore, we examined the possible associations between the sHLA-G levels, maternal and fetal HLA-G 14 bp ins/del polymorphisms and placental and fetal weights since such associations may clarify which factors that determine sHLA-G levels during pregnancy and provide clues for the origin of the sHLA-G.

2. Materials and methods

2.1. Patients and samples

The study was conducted as a prospective, longitudinal cohort study with pregnant women from the Department of Obstetrics and Gynecology, Aalborg University Hospital, Denmark. A total of 246 women referred for routine antenatal care and delivery were consecutively included and prospectively followed from their first antenatal visit at the Clinic of Obstetric Ultrasound until birth in the Obstetric ward. Women with previous stillbirth or perinatal asphyxia, previous preeclampsia, previous birth of a child with birth weight $\leqslant$2500 g, or previous recurrent miscarriage, were excluded. The women should be of Caucasian ethnicity. Only singleton pregnancies achieved after conception with the husband's semen and with no suspicion of malformations or chromosomal abnormalities at routine ultrasound scans in GW12 and GW20 were included. The second set of samples from the mother and umbilical cord were only taken in case of delivery after GW37.

For most of the women, both a blood sample at GW20, at term, and an umbilical cord blood sample at delivery were obtained (n = 147). In 16 pregnancies, only two of three blood samples were obtained. In six of these pregnancies, only samples from GW20 and umbilical cord blood, in three only maternal blood from GW20 and at term, and in seven only maternal blood at term and umbilical cord blood were obtained. In 79 pregnancies only one sample at GW20 and in four pregnancies no samples at all were obtained. Individual case records were collected and data from all participants were collected in a standardized manner on pregnancy, delivery, placenta and child. Data about estimated fetal weight based on fetal biometries at the ultrasound scan in GW20 were also collected. The pregnant women were recruited during 2010 and 2011. One hundred twenty-two (49.6%) women were primiparae, and 113 (45.9%) had previously given birth at least once; in 11 cases this information was missing. One hundred ninety-one (77.6%) of the women gave birth vaginally and 27 (11.0%) by caesarean section; in 28 cases this information was missing.