Expression of membrane-bound HLA-G at the maternal–fetal interface is not associated with pregnancy maintenance among patients with idiopathic recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) has many known aetiologies. However, using current diagnostic testing, a large fraction of recurrent pregnancy losses remain unexplained. Many of these may have immune underpinnings. HLA-G is a non-classical major histocompatibility complex (MHC) class I product whose fairly restricted expression at the maternal–fetal interface suggests a role in successful embryonic implantation and/or subsequent pregnancy maintenance. The study of immune-mediated RPL should be enhanced by comparing groups of idiopathic RPL patients with normal fetal chromosomes to RPL patients with known chromosomal abnormalities in their index pregnancies. We hypothesized that if alteration of HLA-G expression at the maternal–fetal interface were associated with immune-mediated RPL, such changes might be detectable using these comparisons. HLA-G protein expression at the maternal–fetal interface in maternal and gestational age-matched women with history of idiopathic RPL and normal male fetuses were compared with expression in RPL patients with known fetal trisomy 16 in their index pregnancy. We detected no significant quantitative differences in the levels of HLA-G between these groups of RPL patients. Within the limitations of this study, we conclude that HLA-G expression is not a major immunological determinant of pregnancy maintenance among patients with idiopathic RPL.

Key words: decidua/HLA-G/MHC/placenta/recurrent pregnancy loss

Introduction

Recurrent pregnancy loss (RPL) has been defined as three or more clinically recognized pregnancies lost before 20 weeks of gestation. Using this definition, RPL occurs ~1 in 300 pregnancies (Wilcox et al., 1998). Identified causes such as uterine malformations, antiphospholipid antibodies, parental cytogenetic anomalies, endocrine disturbances, and infections account for only 20–50% of these cases (Stephenson, 1996). Aetiologies for the remainder of RPL cases have been the focus of intense research. Immunological mechanisms have been proposed to explain at least some of these cases of idiopathic RPL.

Human leukocyte antigens (HLA), major determinants of allograft rejection, have been studied intensively in the context of RPL. The first reports of a non-classical HLA gene expressed in the extravillous cytotrophoblast of the placenta (Redman et al., 1984; Ellis et al., 1986) subsequently identified as HLA-G (Ellis, 1990; Kovats et al., 1990), brought the promise of a solution to the ‘riddle of the fetal allograft’ (Medawar, 1953; Hunt and Orr, 1992; Schmidt and Orr, 1993). That promise, however, has yet to be fulfilled. HLA-G is a non-classical major histocompatibility complex (MHC) molecule (class Ib) with fairly restricted expression at the maternal–fetal interface. It is expressed constitutively, and in significant amounts, only on extravillous cytotrophoblast. Extravillous trophoblast cells are derived from villous cytotrophoblast; they differentiate at the terminal ends of anchoring villi and leave the placental villous core to invade extensively into the maternal decidua and decidual vasculature (McMaster et al., 1995). This unique expression pattern, along with HLA-G’s characteristically low level of polymorphism (van der Ven et al., 1998), has caused a great deal of attention to be focused on this molecule and its potential role in successful embryonic implantation and/or subsequent pregnancy maintenance.

Most investigators continue to believe that HLA-G may be an important modulator of immune rejection at the maternal–fetal interface. Some have hypothesized that HLA-G may alter immunological events at this site via alterations in cytokine expression patterns (Loke and King, 2000). It has also been suggested that HLA-G expression may serve merely to regulate the surface expression of another non-classical trophoblast-expressed MHC class I product—HLA-E (King et al., 2000a). The observation that all the placental cells down-regulate the expression of the classical MHC products HLA-A and -B, while invasive extravillous cytotrophoblast expresses HLA-C, -E and -G may help to inform our hypotheses concerning the role of HLA-G in reproduction.

Down-regulation of HLA-A and -B molecules in the placenta eliminates allogenic recognition of paternal classical MHC class I products by maternal cytotoxic T cells. However, protection of invasive trophoblast cells from attack by natural killer (NK) cells also may be of critical importance, since in a normal pregnancy, NK-like...
cells (decidual granular lymphocytes; DGL) represent the major lymphocyte subpopulation at the implantation site (Johnson et al., 1999). Here, the DGL closely associate with invading fetus-derived semi-allogeneic trophoblast cells (reviewed in King et al., 2000b; Loke and King, 2000). Since NK cells normally recognize and kill cells lacking MHC molecules (Ljunggren and Karre, 1990), absolute down-regulation of MHC on trophoblast cells would render them potential targets for the abundant DGL that populate sites of implantation (Johnson et al., 1999). HLA-G expression on extra-villous cytotrophoblast cells may serve to abrogate such immune targeting. To this point, the expression of HLA-G has been demonstrated to protect cells from lysis by at least some NK cells, suggesting a possible direct role for HLA-G in maternal tolerance of semi-allogeneic trophoblast cells (Pazmany et al., 1996; Rouss-Freiss et al., 1997; Yokoyama, 1997). Genetic studies also support a role for HLA-G in pregnancy maintenance (Aldrich et al., 2001; Pfeiffer et al., 2001). One recent report links particular HLA-G polymorphisms to adverse pregnancy outcomes (Pfeiffer et al., 2001).

Definition and study of immunological pregnancy loss are plagued by two major concerns. First, it is often difficult to differentiate cause and effect. Are detectable immunological alterations merely markers of the maternal response to a non-viable pregnancy, or were they the inciting event for the fetal demise? Second, we now know that the majority of first trimester pregnancy losses are related to fetal chromosomal abnormalities, many of which have arisen de novo. Therefore, without accompanying fetal chromosomal data, the study of immune mechanisms and early pregnancy loss, including RPL, is often addressed in populations with unduly heterogeneous aetiologies. We designed the present study to address both concerns. To do this, we hypothesized that, if alterations in trophoblast HLA-G protein expression at the maternal–fetal interface were associated with immune-mediated RPL, such cases might be enriched among idiopathic RPL patients with known normal fetal chromosomal content. We chose as our comparison group, couples with idiopathic RPL and known fetal trisomy 16 in the index pregnancy. Our choice of this particular numerical chromosomal abnormality was based on its known absolute lethality. We therefore compared the expression of HLA-G in decidual tissues from maternal and gestational age-matched women with histories of idiopathic RPL and index trisomy 16 (TS16) conceptuses (47XY+16 or 47XX+16) to those with index pregnancies that were determined to be chromosomally normal (46XY). Control decidual tissues were obtained from a group of maternal and gestational age-matched women having elective termination of pregnancy.

Materials and methods

Tissues

This study was conducted under the approval of the Brigham and Women’s Hospital Institutional Review Board. Decidual and trophoblast tissues were obtained from 47 women having dilation and curettage (D&C) procedures at Brigham and Women’s Hospital between 6 and 10 weeks of gestation. Of these 46 women, 30 had a history of at least three first trimester spontaneous pregnancy losses with D&C for the same. The aetiology for these first trimester losses was considered to be idiopathic after standard testing. This work-up included normal parental karyotypes and negative lupus anticoagulant and anticardiolipin antibody testing. None of the patients had abnormalities detected using intrauterine structural studies and all had negative cervical cultures. All had normal luteal phase endometrial biopsies. Fetal tissues from all evacuation procedures were analysed for numerical chromosomal content. Of the 46 women, 14 had trisomy 16 conceptuses and 16 women had normal karyotype conceptuses. The remainder of the 16 study subjects were gestational age-matched women who had undergone elective termination of pregnancy. Cytogenetic analyses and pregnancy histories were not available for patients undergoing elective termination of pregnancy.

Immunohistochemistry

Tissues were immediately fixed in 10% formaldehyde and embedded in paraffin. Sections 5 μm thick were cut, mounted on clean glass slides and dried at 55–60°C. Slides were stored at room temperature prior to immunohistochemistry. Sections were deparaffinized in Propar (Anatech, USA) and rehydrated once through descending grades of alcohol (100–95%) and twice through distilled water. Sections were blocked with normal goat serum for 30 min at room temperature and incubated with primary antibody or control at 4°C overnight in a humidified chamber. HLA-G was detected using the HLA-G alpha-1 specific mAb 4H84 (generously provided in ascites fluid from M.McMaster, University of California, San Francisco) at a dilution of 1:500. Negative controls included serial trophoblast/decidua sections processed in the absence of primary antibody (normal goat serum) or using an idiotype matched control (mouse IgG1, dilution 1:100; Chemicon International, Inc., USA) and incubated overnight at 4°C in a humidified chamber. Human tonsil sections were used as negative tissue controls.

After incubation with primary antibodies, control antibody, or serum control, tissue sections were washed with 1% phosphate-buffered saline (PBS) for 15 min and incubated for 30 min at room temperature in a humidified chamber with a link antibody, biotinylated anti-mouse immunoglobulin (Biogenex, USA). Biotinylated link antibodies were visualized using a mouse monoclonal alkaline phosphatase/anti-alkaline phosphatase detection system (Biogenex) that stains cells pink using Fast Red. Tissues were counterstained with haematoxylin (Vector Laboratories, Inc., USA) for 2 min at room temperature.

Quantification of HLA-G immunostaining

Positively stained cells were counted at ×10 magnification using a 10×10 mm reticle. Slides were analysed in three distinct ways. First, cells were counted in five distinct random fields per section for quantification of total HLA-G positivity at the decidua/villi interface. Subsequent quantification of HLA-G positivity used similar methods, but limited the areas under investigation solely to either the placental villi or the maternal decidua basalis. For both the global (decidua + villi) analysis and sub-analyses, the average number of positive cells per 4 mm² for each tissue sample was used for statistical analysis. All sections were counted by a single blinded investigator for internal consistency. Random sections were counted by a blinded independent investigator for external verification of the results.

Statistical analysis

Demographic analyses

Demographic analyses comparing maternal age and gestational age at the time of pregnancy evacuation utilized non-parametric comparisons via the Kruskall–Wallis test. Post-hoc testing of any observed differences used Fisher’s protected least-significant difference test. Since we did not have information on pregnancy history for subjects with therapeutic terminations of pregnancy, the number of prior spontaneous pregnancy losses among the remaining subject groups was compared using the Mann–Whitney U-test.

HLA-G immunohistochemistry quantification

For both total and sub-analyses, potential differences in HLA-G expression were tested using the Kruskall–Wallis non-parametric analysis of variance. A significant Kruskal–Wallis test was followed by the Dunn’s Multiple comparison test to determine group differences in HLA-G expression. For all statistical analyses, statistical significance was assumed when P < 0.05. Statistical analysis was performed on StatView 5.01 (SAS Institute, USA) and Prism 2.0b (GraphPad Software Inc., USA).

Results

Demographic analyses

Demographic data and its analyses are presented in Table I. Briefly, no statistically significant differences in gestational age at the time of pregnancy evacuation were noted among the three study groups. There
was no significant difference in the number of prior spontaneous miscarriages between the group with RPL and normal chromosomal content in the index pregnancy and those with RPL and TS16 in the index pregnancy. Pregnancy history was not known for those patients who had elective terminations of pregnancy. Maternal age at the time of pregnancy evacuation was not different for our study patients with RPL and normal chromosomal content in the index pregnancy versus those with RPL and TS16 in the index pregnancy. Patients who underwent elective termination of pregnancy were younger than those in either of the RPL groups.

### HLA-G immunohistochemistry

No immunohistochemical staining for HLA-G was noted in negative control tissues (Figure 1a), isotype controls (Figure 1b) or normal serum controls (Figure 1c). Excellent cell surface expression of HLA-G was noted within anchoring villi of placenta in all patient groups, while no expression was noted among floating villi (Figure 1d). Streaming columns of HLA-G positive cells were also documented within the maternal decidua among tissues derived from all patient groups (Figure 1e). HLA-G positive cells could be visualized within decidual arterial wall in the majority of specimens from each patient subgroup (Figure 1f).

Table II summarizes the quantification of HLA-G positive cells. The second data column relates mean values for random counting within the entire tissue section. This includes combined representative fields from both villi and decidual areas. It is possible that bias was introduced into our quantification scheme by tissue and/or sectioning variability. If detectable differences in HLA-G positivity were limited to either the maternal decidua or to the placental villi, then differences in the proportions of these distinct tissues among the study groups would be predicted to affect study conclusions. To determine whether such biases existed, we performed sub-analyses, counting HLA-G positive cells separately within the decidual tissue and the placental villi for each histological specimen. These results are also summarized in Table II, data columns 3 and 4.

Sub-analysis of those HLA-G positive cells isolated to the maternal decidua was revealing. Significantly more HLA-G positive cells were noted in the decidua of the combined group of RPL patients when compared with control patients; although significance could not be detected when either group of RPL patients was compared separately with patients with elective terminations of pregnancy. In contrast, no statistical differences were noted when HLA-G positive cells isolated within the villi of either single or combined RPL groups were compared with control patients who underwent elective pregnancy termination.

### Discussion

Although there have been many studies over the last decade describing possible roles of HLA-G at the maternal–fetal interface, its ultimate relationship with pregnancy outcome remains to be elucidated. The balance of the literature suggests that the molecule exerts an immunoprotective function at the maternal–fetal interface. The exact mechanism of these effects remains unclear. Some have hypothesized direct effects. HLA-G has been demonstrated to interact with CD8 (Sanders et al., 1991; Horuzcko et al., 1999) and to mediate transplant rejection in some experimental models (Horuzcko et al., 1997); however, HLA-G’s limited polymorphism argues against a major role for this molecule in antigen presentation. Others have proposed that HLA-G is immunoprotective via indirect mechanisms. HLA-G may be an important class I molecule in innate immune interactions, although the exact NK cell ligand for HLA-G remains controversial (Avril et al., 1999; Navarro et al., 1999; Rajagopal and Long, 1999; Lopez-Botet et al., 2000). HLA-G may promote its immunomodulatory effects via alterations in the local cytokine milieu (Loke and King, 2000) at the maternal–fetal interface. Investigations demonstrating that the signal sequence of HLA-G can promote stable cell surface expression of HLA-E in HLA-G-expressing cells are also intriguing (Braud et al., 1997; Lee et al., 1998). HLA-E is a known important NK cell ligand via the CD94/NKG2 receptor (Braud et al., 1998; Weiss et al., 1998) and it has been suggested that HLA-G may be expressed in extravillous cytotrophoblast cells merely to modulate HLA-E expression. HLA-G could thereby mediate important, albeit indirect, effects on innate immunity at the maternal–fetal interface.

Others have proposed that placental HLA-G expression correlates with trophoblast invasive capabilities (Fisher and Damsky, 1993; McMaster et al., 1995). HLA-G expression could be driving such invasiveness. Alternatively, its expression on invasive extravillous cytotrophoblast cells may merely be a non-mechanistic response to other factors related to trophoblast invasion such as hypoxia (Kilburn et al., 2000). Goldman-Wohl et al. (2000) used RNA in-situ hybridization to examine HLA-G gene expression in placenta of pre-eclamptic (PE) patients, who classically display histological evidence of limited trophoblast invasion. When compared with control pregnancies, tissue sections from the maternal–fetal interface of...
pre-eclamptic patients had decreased invasion into the maternal decidua and decreased expression of HLA-G. Others have reported similar associations with HLA-G down-regulation and pre-eclampsia (Hara et al., 1996; O'brien et al., 2001a,b). More recently, Rabreau et al. (2000) histologically examined HLA-G expression in the placentas of patients with molar pregnancy, spontaneous miscarriage, electively terminated pregnancies, and tubal ectopic pregnancies.

Table II. Histological analysis of HLA-G positive cells

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>HLA-G positive cells/4 mm²</th>
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</thead>
<tbody>
<tr>
<td>Total (decidua + villi)</td>
<td>24.8 ± 10 (102)</td>
</tr>
<tr>
<td>Decidua</td>
<td>34.9 (114-724)</td>
</tr>
<tr>
<td>Villi</td>
<td>25.7 (126-910)</td>
</tr>
<tr>
<td>RPL (normal chromosomes)</td>
<td>33.8 (120-130)</td>
</tr>
<tr>
<td>RPL (fetal trisomy 16)</td>
<td>30.5 (120-130)</td>
</tr>
<tr>
<td>RPL (combined, normal chromosomes + trisomy 16)</td>
<td>17.3 (0.0-57.3)</td>
</tr>
</tbody>
</table>

*p = 0.0113, Fisher's protected least-significant difference test. 
*p = 0.0205, Mann-Whitney U-test. 
*p = 0.0302, Mann-Whitney U-test.

RPL = recurrent pregnancy loss.

Figure 1. Representative micrographs showing HLA-G positive cells at the maternal-fetal interface. All full micrographs are ×10 magnification. (a) Negative control; human thymus stained for HLA-G using the mAb 4H84. (b) Human decidua/villi isotype control (obtained from patient with history of RPL and normal fetal chromosomes in the index pregnancy). (c) Serum negative control [tissue from patient with history of RPL and trisomy 16 (TS16) fetus in index pregnancy]. (d) Serial section from patient with history of RPL and trisomy 16 (TS16) fetus in index pregnancy). (e) Tissue from the decidua of a patient with RPL (TS16) stained with HLA-G (4H84)-positive cells within the adventitial layer of spiral arteries. Pink bar indicates the site of the maternal spiral arteries. Blue arrowhead indicates the site of the maternal spiral arteries. Yellow arrowhead indicates the site of the maternal spiral arteries. Green arrowhead indicates the site of the maternal spiral arteries. Orange arrowhead indicates the site of the maternal spiral arteries.
Idiopathic RPL is certainly a heterogeneous diagnosis and patients with pregnancy losses of immune aetiology most likely comprise a poorly defined subset within this group. In an attempt to isolate a subgroup of idiopathic RPL patients who might demonstrate an isolated immunological defect, we compared well-defined patients with idiopathic RPL who had normal fetal chromosomes in the index pregnancy with those who demonstrated fetal TS16. We chose the latter chromosomal anomaly based on its lethality, with the supposition that, in the index pregnancy, TS16 losses were of primary genetic aetiology.

Expression of HLA-G protein at the maternal–fetal interface was not statistically different when idiopathic RPL patients were segregated and compared by index fetal chromosomal status. This held true both when global (decidua + placental villi) HLA-G positive cell counts were compared and when analyses were isolated to either the maternal decidua or to the placental villi. The latter sub-analyses were performed in an effort to avoid bias arising from heterogeneity among the amounts of each histological tissue contained within a particular sample.

Based on our results, HLA-G protein expression at the maternal–fetal interface does not appear to be associated with pregnancy outcome in idiopathic RPL patients. This finding has a number of interpretations. Ours is the first to quantitatively study HLA-G expression at the protein level and HLA-G simply may not be involved in pregnancy maintenance. This contradicts a large, although still incomplete, series of related investigations. The limited expression pattern of HLA-G and its numerous reported direct and indirect immunological characteristics point to a potential role in early pregnancy maintenance. Post-hoc power analyses indicate that our RPL group sizes allow detection of differences in HLA-G gene expression if expression differs by >1.75-fold. Real, but undetected, differences in HLA-G expression may have been more subtle than those that could be revealed using our data set. Further, although an attempt was made to concentrate immune-mediated RPL into a single group, undoubtedly aetiological variation within the idiopathic RPL group with normal chromosomes remains. If alterations in HLA-G expression were responsible for pregnancy loss in only a minority of these patients, this intra-group heterogeneity may have diluted even major causative differences.

There are areas of HLA-G biology that are not or cannot be addressed by our study design. For instance, although this represents the first quantitative analysis of protein expression in idiopathic RPL groups, by default, we do not have information on maternal or paternal HLA-G DNA polymorphisms in these patients. Although limited in number and often silent at the amino acid level, these polymorphisms have been linked to pregnancy outcome in other studies in RPL patients (Aldrich et al., 2001; Pfeiffer et al., 2001). HLA-G protein is known to be expressed in a variety of spliced variant forms, including soluble/secreted forms (Rouas-Freiss et al., 1999). Soluble HLA-G can be detected in maternal sera and amniotic fluid, where it is hypothesized to be involved in tolerance induction (Athanassakis et al., 1999; Fournel et al., 1999; Rebmann et al., 1999; Rouas-Freiss et al., 1999; Hunt et al., 2000). The antibody used for our investigation was raised against the α1 domain of the HLA-G heavy chain and detects all variant forms of the molecule (McMaster et al., 1998). Therefore, our study is not able to distinguish potential isolated differences in the levels of a particular HLA-G spliced variant form, including soluble HLA-G. Further, immunohistochemical analysis of paraffin-embedded tissue sections would not represent a reliable method for detecting and quantitatively comparing levels of soluble MHC isoforms. Variability between tissue sections made analysis of the depth of invasion of HLA-G positive cells into the maternal

interface with their control samples, these investigators reported increased expression in tissues from patients with molar pregnancies (a condition associated with abnormally increased trophoblastic invasion) and decreased expression in patients who underwent spontaneous miscarriage. The control group of patients undergoing elective termination of pregnancy had HLA-G expression levels similar to those described for patients with tubal ectopic pregnancies. The study suggested that the placental expression of HLA-G was associated with increased invasion of trophoblastic cells and that such expression was both independent of place of implantation and of fetal development. Unfortunately, this study offers no statistical analyses.

To our knowledge, our study is the first to present a quantitative analysis of HLA-G protein expression at the maternal–fetal interface in normal and abnormal pregnancies. In doing so, it complements previous studies in humans examining HLA-G DNA (Aldrich et al., 2001; Pfeiffer et al., 2001) and RNA (Goldman-Wohl et al., 2000). Unlike the only other protein expression study (see above, Rabreau et al., 2000), we have quantified and statistically evaluated HLA-G expression among patients with histories of idiopathic RPL.

![Figure 2](http://molehr.oxfordjournals.org/)

**Figure 2.** Box plots of HLA-G immunohistochemistry quantification. y-Axes represent the average number of HLA-G positive cells/4 mm². Group A: patients with histories of idiopathic recurrent pregnancy loss and normal fetal chromosomes in their index pregnancies. Group B: patients with histories of idiopathic recurrent pregnancy loss and fetal trisomy 16 in their index pregnancies. Group C: patients who underwent elective termination of the index pregnancy. (a) Total HLA-G positive cells (global; decidua + villi). (b) HLA-G positive cells in the maternal decidua. (c) HLA-G positive cells in the placental villi.
decidua impossible to quantify; however, gross differences in invasion were not noted.

Our inclusion of a control group of gestational age-matched patients who underwent elective terminations of pregnancy may provide insight into some of the difficulties encountered in the study of immune causes of otherwise idiopathic RPL. We did detect statistically more HLA-G positive cells in global analyses of tissue sections from the combined group of idiopathic RPL patients (normal chromosomes + TS16) when compared with patients having elective terminations. Similar statistical significance was also reached when RPL patients with TS16 were compared with those who underwent elective terminations. Tissues from patients with RPL and TS16 had more HLA-G positive cells than those from elective terminations. Although tissues from RPL patients with normal chromosomes had greater HLA-G positivity than those from patients with elective pregnancy terminations, statistical significance was approached but not reached.

Again, these results could be interpreted in several ways. First, we collected neither fetal chromosomal nor obstetrical histories for patients with elective terminations of pregnancy. Either could have introduced undocumented biases. Second, patients with elective terminations typically differ from RPL patients in that the latter have highly desired pregnancies. Differences in social habits and prenatal medical care between these groups may introduce potential bias. Third, it is possible that HLA-G expression is truly higher among RPL patients; a negative association with pregnancy maintenance. This view is not consistent with the majority of pre-existing literature. A more parsimonious explanation rests on unavoidable differences in the human populations studied. Elective terminations have live fetal tissue and evacuations in RPL patients were of non-viable tissues. Alterations in the tissue expression of immune molecules may have been the effect of an endogenous maternal reaction to the presence of unhealthy tissue within the intrauterine environment rather than the inciting event. Sub-analyses indicate that this may indeed be the case, since statistically more HLA-G was noted within the decidua of RPL patients when compared with patients who underwent elective terminations. No differences were noted in the sub-analysis isolated to placental villi. These data are consistent with alterations in HLA-G tissue and evacuations in RPL patients being of non-viable tissues.

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It will be important, in future studies, to specifically analyse the expression of soluble HLA-G (both locally and in maternal serum) in similarly matched groups of idiopathic RPL patients. Associations between high-risk HLA-G gene alleles and expression of HLA-G gene locally and systemically may also prove informative. A larger study of HLA-G protein expression at the maternal–fetal interface would have the potential to detect more subtle alterations in HLA-G expression among our selected groups. The inherent aetiological heterogeneity within any standard idiopathic RPL population, however, would necessitate either major alterations in HLA-G expression among study subjects or prohibitively large study groups. The clinical RPL population represents a difficult study group for those interested in immune-mediated RPL, since the approach to the determination of immunological cause versus effect at the maternal–fetal interface in patients with highly desired pregnancies will continue to present a challenge. Still, these studies are clinically important. Ours represents the first such study to address quantitatively alterations in HLA-G protein expression at the maternal–fetal interface in patients with RPL. While larger studies may be necessary to detect more subtle differences, ours indicates that HLA-G expression at the surface of extravillous cytotrophoblast cells is not a major determinant of pregnancy success among patients with otherwise idiopathic RPL.

Acknowledgements

This work was supported in part by grants from the Society for Gynecological Investigation and The National Institutes of Health (HD00840 and K12HD02155) to D.J.S. The authors gratefully acknowledge the statistical assistance of Joseph Politch and the editorial assistance of Drs Linda Grazier Schust and Naimish Patel.

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Submitted on March 4, 2003; accepted on May 19, 2003

HLAG expression in recurrent pregnancy loss

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