

Soluble HLA-G circulates in maternal blood during pregnancy

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OBJECTIVE: Soluble isoforms of the HLA class Ib gene HLA-G have been identified at the maternal-fetal interface. Because soluble forms of other HLA class I antigens modulate T-cell reactivity and induce cell-activated apoptosis, our goal was to determine whether soluble HLA-G circulates in maternal or fetal blood and to identify the specific isoform.

STUDY DESIGN: Capture enzyme-linked immunosorbent assays with mouse monoclonal antibodies directed toward an epitope present on all isoforms of soluble HLA-G were constructed to identify soluble HLA-G in 44 serum samples from nonpregnant control subjects, 129 serum samples from pregnant women, and 10 samples of term cord blood. Distinguishing between soluble HLA-G1, which is composed of heavy chains complexed with light chains (β_2 -microglobulin), and soluble HLA-G2, which consists only of heavy chains, was achieved by substituting a monoclonal antibody that requires β_2 -microglobulin for binding (W6/32) in the capture phase of the enzyme-linked immunosorbent assay.

RESULTS: Capture enzyme-linked immunosorbent assays with mouse anti-soluble HLA-G showed that soluble HLA-G was present at all stages of gestation and that levels of soluble HLA-G were statistically significantly higher in serum samples from pregnant women than in serum samples from nonpregnant women. In contrast, W6/32 failed to detect soluble HLA-G in serum samples from pregnant women. Cord serum samples did not contain detectable soluble HLA-G.

CONCLUSION: Collectively, the data indicate that pregnancy is characterized by the presence of soluble HLA-G circulating in maternal blood and strongly suggest that the major isoform is soluble HLA-G2. (Am J Obstet Gynecol 2000;183:682-8.)

Key words: Pregnancy, serum, soluble HLA-G

Beginning with Medawar¹ in 1953, immunologists have been intrigued by the unexpected success of the fetal allograft. It is generally agreed that the ability of placental trophoblast cells at the human maternal-fetal interface to avoid expressing the highly polymorphic human leuko-

cyte antigens (HLA-A, -B) that would stimulate graft rejection is a major protective mechanism.²⁻⁵ Recent studies have shown that some subpopulations of trophoblast cells express class I antigens,^{6, 7} and many of these are derived from the *HLA-G* gene, which has little polymorphism and is therefore unlikely to be perceived as foreign and to stimulate rejection in mothers.⁸

The *HLA-G* gene is one of several nonclassical class Ib genes located telomeric to the *HLA-A* gene in the major histocompatibility complex on the short arm of chromosome 6.²⁻⁵ Multiple transcripts derived by alternative splicing of *HLA-G* messenger ribonucleic acid encode membrane-bound and soluble forms of the antigen.⁹ The soluble forms of HLA-G (sHLA-G) are generated from transcripts that include sequences derived from intron 4, which contains a premature stop codon that precludes generation of the transmembrane and cytoplasmic domains of the antigen.¹⁰ No genetic polymorphisms are detectable in this region.⁸ The *HLA-G* gene is highly expressed in placentas where both cell-bound and soluble forms of the protein have been identified.^{6, 7, 11, 12}

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Transcripts encoding sHLA-G have been detected in term placenta by means of reverse transcriptase polymerase chain reaction, and the protein has been localized to trophoblast cells and placental macrophages by immunohistochemistry. Trophoblastic cells and interferon γ -activated macrophages synthesize sHLA-G, but placental fibroblasts do not.

Soluble forms of HLA class Ia antigens (HLA-A, HLA-B) circulate in blood¹³⁻¹⁵ and have profound effects on immunity that include interference with cytotoxicity of both natural killer cells and T lymphocytes.¹⁶⁻¹⁹ Soluble class Ia antigens have been shown to induce apoptosis in receptor-positive lymphocytes through the *Fas-Fas* ligand pathway.¹⁷ By contrast, sHLA-G has not been reported to circulate in blood, and although it is possible that sHLA-G is also immunosuppressive, this has not been documented.

In this study we hypothesized that sHLA-G circulates in maternal blood, a positioning that would permit this molecule to promote systemic features of allograft protection. To test our hypothesis an enzyme-linked immunosorbent assay (ELISA) that detected only the soluble form of the antigen was developed and was used to test serum samples from pregnant and nonpregnant women and neonates.

Material and methods

Serum samples from nonpregnant female control subjects ($n = 44$) were obtained from the Community Blood Center of Greater Kansas City with the kind assistance of G. Tegtmeier, director of the Viral Testing Laboratory. Serum samples from pregnant women ($n = 137$) were collected between 1980 and 1985 from women with and without diabetes as described elsewhere.²⁰ The samples were continuously maintained at -80°C and were shipped to the University of Kansas Medical Center on dry ice. The 137 samples included 8 duplicates in which 2 samples had been drawn from the same woman at the same time. Seventeen women contributed samples during two different trimesters, and 5 women contributed samples during all 3 trimesters of pregnancy. Thus 102 women were represented by the 137 samples tested in this study. Ten serum samples from term cord blood were obtained from the blood bank of the University of Kansas Hospital with the kind assistance of P. Brown, the director, under a protocol for discarded tissues approved by the Human Subjects Committee of the University of Kansas Medical Center, and 1 cord serum sample was included in the group of samples obtained from the University of Chicago. S14/8 cells, a mouse fibroblast cell line transfected with 6.0 kilobases of genomic *HLA-G*,^{12, 13} were cultured in Dulbecco minimum essential medium containing 2-mmol/L glutamine, antibiotics, 1-mmol/L sodium pyruvate, 10% fetal bovine serum, and 200 $\mu\text{g}/\text{mL}$ geneticin (Sigma, St Louis, Mo). Culture supernatant was acquired when the cells had reached $>80\%$

Table I. Comparison of binding of sHLA-G by 2 monoclonal antibodies, 16A1 and 16G1, used in capture ELISAs

Sample	16A1	16G1
Pregnancy serum 1	0.09 \pm 0.01	0.08 \pm 0.01
Pregnancy serum 2	0.14 \pm 0.01	0.14 \pm 0.01
Negative control (nonpregnancy serum 1)	0.04 \pm 0.01	0.03 \pm 0.01
Negative control (nonpregnancy serum 2)	0.06 \pm 0.01	0.04 \pm 0.01
Positive control (S14/8 supernatant)	0.17 \pm 0.01	0.13 \pm 0.01

Monoclonal antibodies 16A1 and 16G1 were used to coat replicates of 6 microwells per sample. Values shown were obtained by subtracting the mean absorbance at 405 nm obtained for the 3 wells in which preimmune rabbit serum was used to detect binding of sHLA-G from the mean absorbance at 405 nm obtained for the 3 wells in which rabbit anti-sHLA-G was used to detect binding.

confluence. All of the serum samples and the culture supernatants were stored at -80°C until used.

Monoclonal and polyclonal anti-sHLA-G antibodies were derived by immunizing animals with peptides unique to intron 4 of the *HLA-G* gene. The mouse monoclonal antibodies 16A1 and 16G1 have been previously characterized: 16G1 has been reported to identify an approximately 37-kd protein in immunoblots.^{2, 21, 22} In the capture ELISA tests described here the 2 monoclonal antibodies yielded essentially identical results (Table I). The polyclonal rabbit antibody was generated to a 10-amino acid peptide (cysteine-aspartic acid-glycine-isoleucine-methionine-serine-valine-lysine-glutamic acid-serine) with an *N*-terminal cysteine added to aid in preparation of an immunoaffinity column. Lyophilized peptide synthesized in the institutional Biotechnology Support Facility was used by Research Genetics, Huntsville, Ala, to produce antibody in two New Zealand White rabbits. Preimmune IgG was purified from preimmune serum by immunoaffinity chromatography with an ImmunoPure (A/G) IgG Purification Kit from Pierce Chemical Company, Rockford, Ill. Pooled IgG fractions were desalted and concentrated with Ultrafree-15 centrifugal filters (Millipore Corporation, Bedford, Mass). For antibody purification an immunoaffinity column was prepared by binding peptide used as an immunogen through its *N*-terminal cysteine to SulfoLink Coupling Gel (Pierce Chemical) according to the manufacturer's protocol. Peptide-specific antibody was isolated from immune serum according to standard immunoaffinity protocols. Antibody-containing fractions were pooled, desalted, and concentrated as for preimmune IgG. Recombinant sHLA-G was generated as previously described.^{23, 24}

Mouse anti-sHLA-G (16A1 and 16G1) antibody concentrations were adjusted to 10 $\mu\text{g}/\text{mL}$ in sodium carbonate buffer (pH 9.6) and 100 μL was added to each

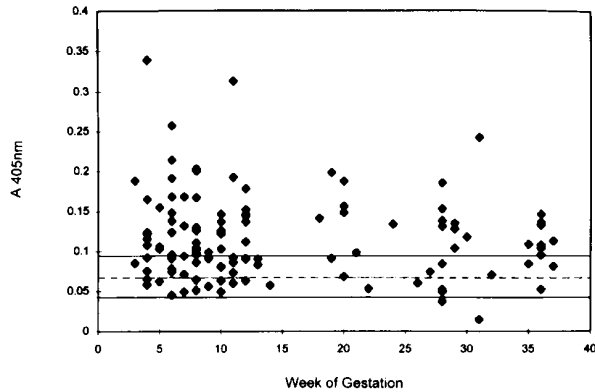


Fig 1. Identification of sHLA-G in serum samples from pregnant women by capture ELISA. sHLA-G in 1:4 dilutions of serum samples from pregnant women was captured with monoclonal antibody to sHLA-G, and bound sHLA-G was detected using a rabbit antibody. Mean absorbance at 405 nm for serum samples from nonpregnant control subjects ($n = 44$) is shown with dotted line, and SD of these samples is shown with solid lines. Points on figure represent absorbance at 405 nm obtained for 129 serum samples from pregnant women.

well of a 96-well polystyrene plate. The plates were incubated overnight at 4°C in a moist, sealed container. The wells were washed 4 times with phosphate-buffered sodium chloride solution (PBS, pH 7.0) containing 0.05% Tween 20 (PBS/Tween), and the uncoupled sites were blocked by incubating with 200 μ L 0.4% bovine serum albumin (BSA) (Sigma) in PBS/Tween for 3 hours at room temperature (RT). After removal of the blocking solution 100 μ L/well of an appropriate dilution of control or test sample was added and the plates were incubated at 4°C overnight. The samples were removed, the wells were washed 4 times with PBS/Tween, and then either preimmune or immune rabbit antibody to sHLA-G (50 μ L/well, 5 μ g/mL in 2% BSA/PBS) was added. The plates were incubated for 3 hours at RT. The plates were washed 4 times with PBS/Tween, and then 50 μ L/well of goat antirabbit IgG (H+L) at 5 μ g/mL (Vector Laboratories, Inc, Burlingame, Calif) was added and incubation was continued for 3 hours at RT. The wells were washed 4 times with PBS/Tween, and then 50 μ L ABC reagent was added (Vector Laboratories). The plates were incubated for 30 minutes at RT. The wells were washed again 4 times with PBS/Tween, then 100 μ L of 1-mg/mL P-NPP substrate (Southern Biotechnology Associates, Inc, Birmingham, Ala) made in 10% diethanolamine buffer (pH 9.8) was added to each well and the plates were incubated at RT for 1 hour. The reaction was stopped by adding 50 μ L of 0.5-mol/L sodium hydroxide to each well, and color reactions were read at 405 nm with an EL_X 808 (Bio-Tek Instruments, Inc, Winooski, Vt) microplate reader. The same procedure was used when the W6/32 monoclonal antibody was substituted for anti-sHLA-G, antibody, except that incubation with substrate was con-

cluded after 10 minutes. In the capture phase of the ELISA, replicate samples were plated into 6 wells containing bound monoclonal anti-sHLA-G or W6/32 antibody. In the detection phase 3 of the wells were tested with preimmune rabbit IgG and 3 were tested with immune rabbit anti-sHLA-G antibody. Mean values obtained for preimmune IgG (absorbance at 405 nm) were subtracted from mean values obtained with immune serum (absorbance at 405 nm) to give mean specific binding for each sample. All tests were performed with the experimenter blinded as to stage of gestation and pregnancy outcome.

Radioimmunoassays were used to detect human chorionic gonadotropin β subunit (β -hCG), progesterone, and 17 β -estradiol in serum samples. All of the serum samples obtained from Community Blood Center were tested in these assays according to the manufacturer's instructions (Diagnostic Products Corp, Los Angeles, Calif).

Results

The capture ELISA that used monoclonal and polyclonal antibodies directed toward an amino acid sequence derived from intron 4 specifically detected sHLA-G in serum, as determined by inclusion of positive and negative control preparations in each experiment. The positive control preparation consisted of undiluted supernatant culture medium from an HLA-G-transfected mouse fibroblast cell line, the S14/8 cells. The mean (\pm SEM) specific binding for S14/8 supernatants in 23 separate experiments was 0.14 ± 0.04 .

Serum samples from 44 female blood donors were used to establish baseline values for nonpregnant women. The mean specific binding was 0.064 ± 0.011 . Two serum samples had high values in ELISAs for soluble HLA-G. To evaluate the possibility that these reflected unidentified pregnancy, the 2 samples were tested for β -hCG, progesterone, and 17 β -estradiol. Normal values were obtained.

Fig 1 shows the results of testing 129 serum samples from pregnant women. Eighty percent of the serum samples from pregnant women yielded specific binding values that were above the mean specific binding value of serum samples from nonpregnant women, and 20% yielded values that were below this mean. The total number of samples tested in the ELISA was 137, which included duplicate samples from 8 women. Absolute values for the 2 samples differed slightly, but all pairs matched in terms of relationships with values from serum samples from nonpregnant control subjects. Seven pairs were above the mean for serum samples from nonpregnant women and 1 pair was below this mean. Because absolute values obtained in the ELISAs varied as a result of environmental conditions, lot numbers of developing reagents, and other nonspecific factors, further analyses were conducted after the values were normalized to the mean of the serum samples from nonpregnant women.

Table II. Longitudinal analysis of soluble HLA-G in 22 serum samples from pregnant women

Sample No.	First trimester (%)	Second trimester (%)	Third trimester (%)
223	174	142	
262	143	220	
284	119	209	
213		244	199
240		291	170
12	293		376
15	163		184
89	196		204
131	98		228
136	315		307
153	242		211
163	77		115
190	374		289
227	193		111
234	168		169
263	195		76
271	132		104
54	133	105	93
174	110	130	131
286	188	231	197
292	80	111	82
296	311	308	143
Mean ± SEM	185 ± 83	199 ± 73	178 ± 81

Data are shown as percentages of mean absorbance at 405 nm ELISA value for negative control preparations (n = 44 samples from nonpregnant women) to normalize the data obtained in different experiments.

In Fig 2 the values obtained for 129 serum samples from pregnant women are normalized against the mean value for serum samples from nonpregnant women (0.064 ± 0.011) and sorted according to trimester of pregnancy. For patients with duplicate samples only the sample with the lower sample number (the sample drawn first) was used. sHLA-G was detectable at all stages of gestation and was prominent very early in pregnancy. Values in 2 samples from week 3 of gestation averaged 189% of values in serum samples from nonpregnant control subjects (127% and 251%), and 11 samples from week 4 of gestation averaged 173% of values in serum samples from nonpregnant control subjects (range, 87%-365%). Values for samples taken during pregnancy were approximately doubled with respect to the values for samples taken from nonpregnant women. The mean and SEM for each trimester were as follows: first trimester, 0.121 ± 0.0087; second trimester, 0.138 ± 0.018; and third trimester, 0.128 ± 0.012.

Because the 129 serum samples included more than one sample from each of 22 women that had been acquired during the course of the pregnancy, statistical analyses of the data shown in Fig 2 were conducted with both classical analysis of variance (ANOVA) and a linear mixed model, which mirrors ANOVA but accounts for the correlation associated with multiple observations. ANOVA yielded statistically significant differences between first-trimester and nonpregnant values ($P < .001$),

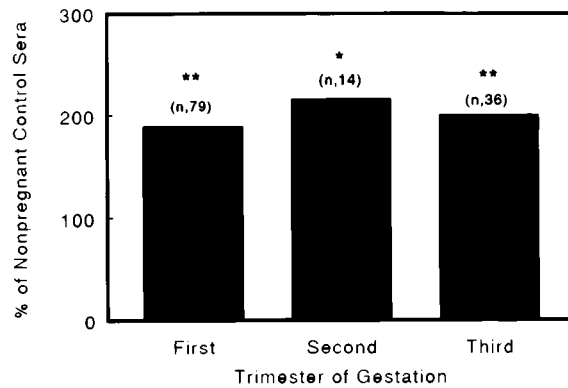


Fig 2. Identification of sHLA-G in serum samples from pregnant women during first, second, and third trimesters by capture ELISA. sHLA-G in 1:4 dilutions of serum samples from pregnant women was captured with monoclonal antibody to sHLA-G, and bound sHLA-G was detected using a rabbit antibody. Data are shown as a percentage of the mean absorbance at 405 nm for serum samples from nonpregnant control subjects (n = 44) to normalize results of different experiments. 1 Asterisk, $P < .004$; 2 asterisks, $P < .0001$.

between second-trimester and nonpregnant values ($P < .05$), and between third-trimester and nonpregnant values ($P < .001$). The overall F test for the model that looked for general differences among the means was highly significant ($P < .0001$), and the P values for the individual comparisons after adjustment for multiple comparisons were all highly significant ($P < .0001$, comparisons of nonpregnant women with women in the first and third trimesters of pregnancy; $P = .004$, comparison of nonpregnant women with women in the second trimester of pregnancy).

The acquisition of repeated samples from 22 women permitted longitudinal analysis. Consistent with the overall findings shown in Fig 2, when these serum samples were grouped by trimester and compared with one another, no statistically significant temporal changes were observed. Table II shows that, although there were fluctuations, there were no general trends toward changes in sHLA-G levels when each mother was considered individually.

Forty-nine of the serum samples from pregnant women tested in this assay were acquired from women with diabetes. Comparisons were made between the binding values of pregnant women with and without diabetes, because concentrations of proinflammatory cytokines that enhance expression of HLA class I antigens, such as tumor necrosis factor α , are elevated in the uterus in women with diabetes. There were no statistically significant differences between the values obtained for pregnant women with and without diabetes, although in each trimester values for women with diabetes (first trimester, 13%; second trimester, 9%; third trimester, 14%) were slightly higher than values for women without diabetes. The data are shown in Fig 3.

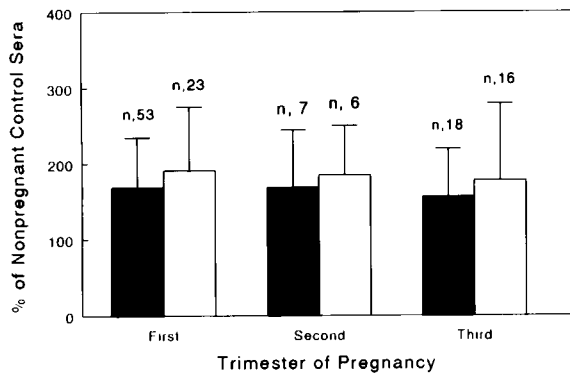


Fig 3. Comparison of specific binding values for sHLA-G in serum samples from pregnant women with and without diabetes. Capture ELISA was performed as outlined in Material and Methods section.

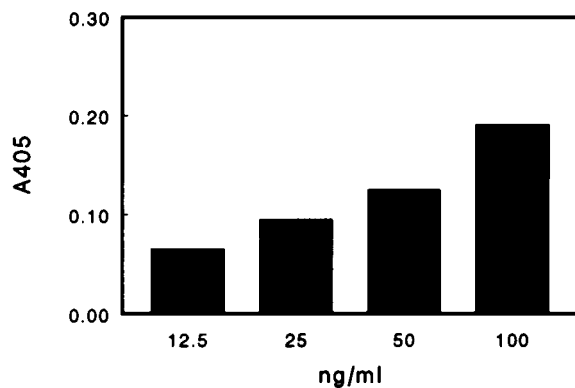


Fig 4. Identification of recombinant sHLA-G in capture ELISA. Recombinant sHLA-G was diluted in sodium chloride solution and tested in duplicate in capture ELISA with 16G1 and rabbit anti-sHLA-G. Average absorbance at 405 nm reading is shown for each concentration of recombinant sHLA-G.

Experiments were then done to establish which isoforms of HLA-G were present in maternal serum samples. First, the ability of the capture ELISA to identify free HLA-G heavy chain was documented. Recombinant sHLA-G was diluted in isotonic sodium chloride solution to avoid soluble antigens in serum, and values obtained for duplicate samples were averaged after subtraction of sodium chloride solution control values. Fig 4 shows concentration-dependent detection of sHLA-G free heavy chains in the capture ELISA. From 25 to 50 ng of recombinant sHLA-G yielded approximately the same absorbance value at 405 nm as did the supernatants of S14/8 cells used as positive control preparations (absorbance [mean \pm SEM] at 405 nm, 0.14 ± 0.04).

Subsequent studies indicated that the sHLA-G2 isoform predominated in maternal serum samples. The sHLA-G1 isoform contains α_1 , α_2 , and α_3 domains and forms noncovalent associations with the light chain, β_2 -microglobulin (β_2m). In contrast, the sHLA-G2 isoform

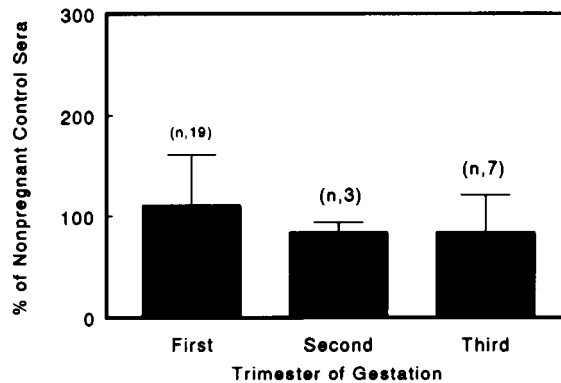


Fig 5. Use of W6/32 to distinguish sHLA-G1 and sHLA-G2 isoforms in serum samples from pregnant women. W6/32, which requires heavy chain–light chain association, was substituted for monoclonal antibody to sHLA-G in the capture phase of ELISA. Binding was detected as before. Data are shown as percentages of the mean absorbance at 405 nm for serum samples from nonpregnant control subjects ($n = 44$) to normalize the results of different experiments.

lacks the α_2 domain and does not associate with β_2m but instead forms homodimers. Association with β_2m was therefore used to distinguish between the two isoforms. Anti-sHLA-G monoclonal antibodies were replaced with the monoclonal antibody W6/32, which requires heavy chain– β_2m association for recognition. Fig 5 demonstrates that W6/32 failed to capture sHLA-G in serum samples from pregnant women, yielding values in samples from first, second, and third trimesters that were little different from the values in serum samples from nonpregnant female control subjects.

Having determined that sHLA-G circulates in the mother, we then investigated the possibility that sHLA-G circulates in the baby at the time of delivery. Ten cord serum samples taken from term deliveries were tested, and these yielded a mean specific binding (absorbance at 405 nm) of 0.04 ± 0.01 , which was $90\% \pm 10\%$ of the mean of 3 serum samples from nonpregnant female control subjects included in the same assay. One cord serum sample was included in the group shipped from Chicago, and this also contained no specific binding activity. Thus sHLA-G concentration was not elevated in neonatal serum at term.

Comment

The data presented in this report show for the first time that sHLA-G circulates in mothers during pregnancy. Multiple control preparations were used to verify the specificity and sensitivity of the capture ELISA. Supernatant culture media of the HLA-G–transfected mouse fibroblast cell line S14/8, which has been shown previously to transcribe messenger ribonucleic acid encoding for sHLA-G,¹² was used as a positive control preparation. Values in supernatants were consistently 2-fold

greater than values obtained with serum samples from nonpregnant control subjects, which were obtained from a local blood bank. Most of these samples from nonpregnant women yielded predictably low values. The fact that some sHLA-G appeared to be present in the sera of nonpregnant women is of considerable interest, however, and warrants further investigation. Two of the 44 samples gave high readings. We obtained no evidence of unsuspected pregnancy by testing all the samples for β -hCG, progesterone, and estrogen. Possibly in the two samples with high values inflammatory conditions gave rise to increases in sHLA-G concentration, as is the case for other HLA class I antigens.²⁰ In support of this idea, activation of macrophages with the proinflammatory cytokine interferon γ gives rise to production of sHLA-G.^{12, 13} Further studies showed that the capture assay detected recombinant sHLA-G free heavy chains in a dose-dependent manner, thus verifying the specificity of the assay. Because it is not known whether sHLA-G in serum exists in free heavy chains, as homodimers or heterodimers, or in combination with light chains, recombinant sHLA-G was not an appropriate standard for quantification of sHLA-G in serum samples.

sHLA-G levels rose during early gestation and persisted throughout pregnancy. Even though HLA-G in placentas is most prominent during the first trimester,⁷ we observed no statistically significant differences among values obtained during the first, second, and third trimesters of pregnancy. However, there was a tendency for values to decline modestly as pregnancy progressed toward termination. The failure to observe statistically significant temporal changes may have been caused by the comparatively wide range of values in each trimester; alternatively, the HLA-G in early placentas may comprise mainly membrane-bound rather than soluble antigen. In any event, the results obtained in longitudinal analyses tended to support the idea that sHLA-G levels fluctuate but that this is caused by factors other than stage of gestation, such as the kinetics of production and use or genetic predisposition toward production of sHLA-G. In support of the latter idea, although the data shown in Table II were not analyzed because too few patients were tested longitudinally, there did appear to be an emerging pattern for patient-specific levels; that is, some women (eg, patients 54, 174, and 292) had fairly low levels throughout pregnancy, whereas others (eg, patients 286 and 296) had consistently high levels throughout pregnancy.

It will be of considerable interest to learn whether serum levels of sHLA-G are different among women who are infertile or have difficulty in maintaining pregnancy. In this study we investigated only diabetes as a condition that complicates pregnancy. Diabetic pregnancy is characterized by high levels of tumor necrosis factor α , a proinflammatory cytokine that might alter production of sHLA-G. Although no statistically significant differ-

ences were observed at any stage of gestation when levels of sHLA-G in women with and without diabetes were compared, there was a trend for samples from women with diabetes to demonstrate higher specific binding than those from women without diabetes. This did not affect pregnancy outcome, because all the mothers in this study carried the conceptus to term. It is possible that higher levels of sHLA-G in maternal serum samples simply reflected the generally larger size of placentas in women with diabetes.

We present evidence in support of the idea that the major form of sHLA-G in the serum during pregnancy is likely to be the sHLA-G2 isoform, which does not bind β 2m. The specific isoform of sHLA-G in the serum during pregnancy is clearly of interest, because the 2 isoforms that have been identified may differ functionally as a consequence of biochemical structure. The sHLA-G1 isoform includes the α_2 domain required for complexing with β 2m and binding peptide in the light chain-heavy chain groove, whereas the sHLA-G2 isoform lacks α_2 and associates into HLA class II-like homodimers that do not recognize β 2m but are capable of binding peptides.^{3, 5, 9, 23, 24} A monoclonal antibody that requires heavy chain- β 2m associations, W6/32, failed to capture sHLA-G in serum samples from pregnant women, which indicates that the sHLA-G2 isoform probably predominates. No isoform-specific antisera that might answer this question definitively have been reported as yet. One possible functional implication of this finding is that perhaps maternal cells programmed to recognize HLA class II plus peptide bind circulating sHLA-G2 and initiate a peptide-specific immune response. A second possibility is that lymphocytes bearing HLA-G-specific T-cell receptors might recognize circulating sHLA-G2 and be induced to "commit suicide" through the Fas-Fas ligand apoptosis pathway.¹⁸ This is an attractive hypothesis that could account for the lack of circulating maternal antifetal T lymphocytes. Third, sHLA-G2 might interact with killer inhibitory receptors on natural killer cells at the maternal-fetal interface and prevent attack on the placenta. Further experiments are required to learn whether any of these events, which could have important impacts on the maternal immune system, do indeed occur.

Despite the fact that concentrations of sHLA class I antigens are known to be high in cord blood,¹⁶ little if any sHLA-G was identified in cord serum samples from term deliveries. Cord serum samples from early gestation were not available for testing, so the possibility remains that sHLA-G is present early but declines to undetectable levels at pregnancy termination. Alternatively, mechanisms may be in place for prevention of placental sHLA-G from leaking into the fetal blood circulation where it could influence the developing fetal immune system.

In summary, we have demonstrated that sHLA-G circulates in mothers and that the predominant isoform is likely to be sHLA-G2. The predominance of the sHLA-G2 isoform may account for our recent and surprising finding that fetal survival is not compromised by absence of the HLA-G1 isoform; we identified subjects who were homozygous for a mutation that precludes synthesis of the HLA-G1 isoforms.²⁵ Collectively the data strongly suggest that the sHLA-G2 isoform may have considerable functional importance, a hypothesis that is under evaluation in our laboratories.

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