Jpn J Human Genet 42, 181-186, 1997

BIPARENTAL ALLELES OF *HLA-G* ARE CO-DOMINANTLY EXPRESSED IN THE PLACENTA

Kazumasa HASHIMOTO,* Chihiro AZUMA, Masayasu KOYAMA, Toshikatsu NOBUNAGA, Tadashi KIMURA, Koichiro SHIMOYA, Yasue KUBOTA, Fumitaka SAJI, and Yuji MURATA

Department of Obstetrics and Gynecology, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan

Summary HLA-G is the only major histocompatibility complex molecule expressed in the human placenta and thus has been considered to be necessary for maintenance of pregnancy. We investigated whether *HLA-G* expression is regulated in a parent-of-origin allele-specific manner. Of six first trimester and three third trimester placentas, three first trimester and two third trimester placentas showed heterozygosity at the *Pst*I polymorphic site in the 3′-untranslated region. Reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed biallelic expression of *HLA-G* in all the informative cases, indicating that *HLA-G* is not imprinted during the gestational period, at least at the transcriptional level. As HLA-G has been postulated to be polymorphic not only at the DNA sequence level but also at the peptide level, co-dominant expression of the gene suggests that each parental allele is involved in the allogenic response during pregnancy.

Key Words HLA-G, genomic imprinting, placenta

INTRODUCTION

It is of interest that human embryos and fetuses, semiallografts to the maternal immune system, are not rejected during pregnancy. This is in part explicable by the lack of expression of the classical class I major histocompatibility complex (MHC) molecules (HLA-A, -B, and -C) (Lata *et al.*, 1990; Hunt and Orr, 1992) involved in graft rejection in placental trophoblasts. However, according to recent investigations, instead of classical class I HLAs, HLA-G (Geraghty *et al.*, 1987), a non-classical class I MHC is expressed in the placenta (Ellis *et al.*, 1990; Hunt and Orr, 1992; Chumbley *et al.*, 1993). Although the fundamental function of HLA-G remains unclear, it may regulate feto-maternal immunity in the tolerant condition

Received August 23, 1996; Revised version accepted December 17, 1996.

* To whom correspondence should be addressed.

during pregnancy since HLA-G is the only major histocompatibility antigen that is expressed in the human placenta. The importance of placental HLA-G is also suggested by its tissue distribution; it is expressed mainly in the placenta/fetal membranes and eyes (Shukla *et al.*, 1990), both of which are immunologically unique organs.

To explain the feto-maternal immunotolerance during pregnancy, one attractive hypothesis is that *HLA-G* expression is regulated in a parent-of-origin allele-specific manner, *i.e.*, by genomic imprinting. The state may be accomplished in the maternal body in which only one parental antigen of the fetus is exposed by repressing either parental allele. Although the mouse equivalent counterpart of HLA-G remains unidentified (Hunt, 1992), an evidence has been presented that rat basal trophoblast expresses a non-classical class I MHC antigen (Pa), which is parentally imprinted (Kanbour-Shakir *et al.*, 1990). In fact, *HLA-G* has been suggested to be a candidate of an imprinted gene (Goshen *et al.*, 1994). In man, this gene maps to the short arm of chromosome 6 (Koller *et al.*, 1989), a region syntenic to the proximal portion of mouse chromosome 17, where paternal imprinting effects have been observed (Cattanach, 1991). By analyzing cases of uniparental disomy, human chromosome 6 has been suggested to be subject to imprinting (Ledbetter and Engel, 1995). Thus, whether *HLA-G* is an imprinted gene or not is a subject of interest. Although HLA-G is classified as a non-classical class I (class Ib) MHC molecule and was initially thought to be a monomorphic antigen, several DNA sequence polymorphisms within the peptide-coding regions have been reported recently (van der Ven and Ober, 1994; Watanabe *et al.*, 1995), suggesting that HLA-G is actually a polymorphic antigen. In the present study, we investigated the allele-specific expression of *HLA-G* by utilizing RT-PCR with polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) at the 3′-untranslated region to examine whether *HLA-G* is imprinted in the human placenta.

MATERIALS AND METHODS

Establishment of PCR-RFLP in exon 8 of HLA-G. *Pst*I RFLP was established on the basis of the reported single-nucleotide polymorphisms (Tamaki *et al.*, 1993). Genomic DNA (100 ng) from 54 unrelated Japanese individuals was amplified in 50 μl PCR mixtures containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, dNTPs (200 μM each), 200 nM primer GPM-6 (5′-AATGTGTCTCTCACGGCTTGT-3′), 200 nM primer GPM-5 (5′-GAAAATAC-AATTGAAAGAGAC-3′) (Fig. 1a), and 1 unit of *Taq* DNA polymerase (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) in a thermal sequencer (Iwaki Glass, Co., Ltd., Chiba, Japan) under the following conditions: denaturation for 2 min at 94°C, then 35 cycles of 1 min at 94°C, 2 min at 57°C and 2 min at 72°C. The amplified fragments (847 bp) were digested with *Pst*I (Toyobo,

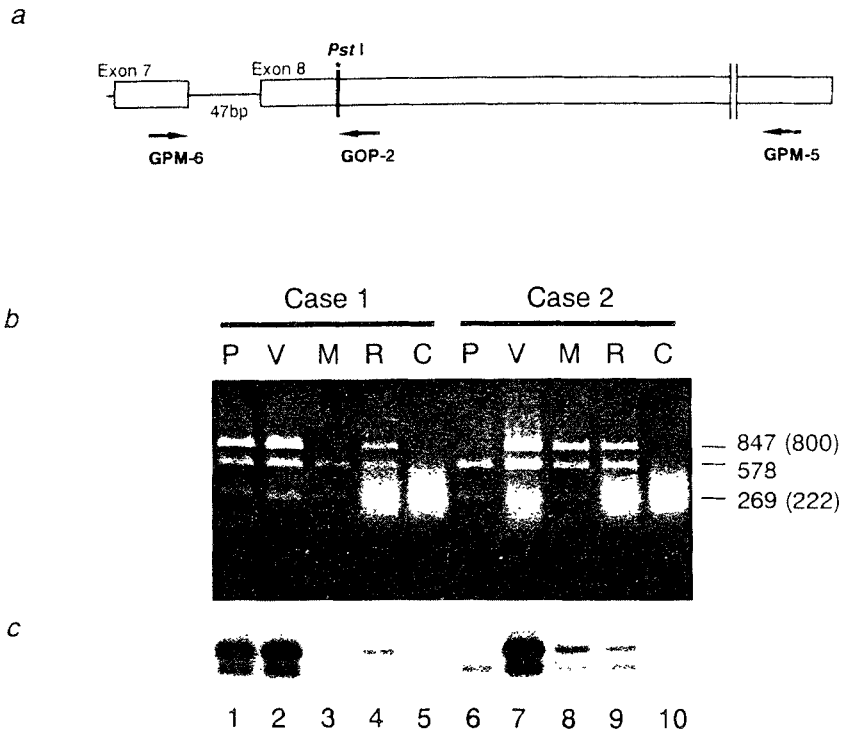

Fig. 1. Allele-specific expression of *HLA-G*. a, Restriction map showing sites of *Pst*I RFLP, oligonucleotide primers, and Southern blotting probe. b, Examination of expressed alleles by RT-PCR. Two representative cases are shown (Cases 1 and 2 are 7 and 10 weeks of gestation, respectively). The parental origin of each allele in placental DNA (V) was determined by comparison with the paternal (P) and maternal (M) blood DNA. Analysis of RT-PCR products (R) showed that *HLA-G* was biallelically expressed (lanes 4 and 9). Although the sequence between the primers contains a 47 bp intron, amplified fragments of genomic DNA (847 bp) and cDNA (800 bp) could not be discriminated by size on the gel used in this study. Thus, contamination by genomic DNA was excluded by running control samples (C) to which reverse transcriptase was not added (lanes 5 and 10). c, Southern blotting of the amplified products by the *HLA-G*-specific [^{32}P]-labeled internal oligoprobe. Lanes correspond to those in Fig. 1b.

Co., Ltd., Tokyo, Japan), followed by 1% agarose gel electrophoresis. In alleles containing a *Pst*I restriction site, digestion would yield 578 and 269 bp bands. Allele frequencies were as follows: allele A1 (without *Pst*I restriction site), 0.31; allele A2 (containing *Pst*I site), 0.69. The observed heterozygosity was 0.44.

Analysis of allele-specific expression by RT-PCR. After obtaining informed consent, 6 first trimester placentas (6–10 weeks of gestation) and 3 third trimester placentas (39–40 weeks of gestation) were collected at therapeutic termination and

at the time of delivery, respectively in addition to paternal and maternal peripheral blood samples. Genomic DNA was prepared from these samples by the standard protocol using proteinase K/SDS digestion and phenol/chloroform extraction and was amplified by PCR. From placentas showing DNA heterozygosity at the *Pst*I RFLP site, total RNA was extracted using the acid guanidium-phenol-chloroform method (Silbert and Chenchik, 1993) and RT-PCR was performed for analysis of allelic expression. cDNA was synthesized from 1 μg of total RNA, using 50 pmol of primer GPM-5 and 200 units of Moloney murine leukemia virus reverse transcriptase (BRL, Gaithersburg, MD) in a 20 μl reaction mixture for 1 hr at 37°C. The mixture was then heated for 10 min at 95°C to inactivate the reverse transcriptase. PCR and RFLP analysis were also performed similarly as described for genomic DNA. After restriction enzyme digestion, the expressed alleles were determined by comparing their parental genomic alleles (Fig. 1b).

Southern blotting. To further confirm that the amplified products were specific to *HLA-G*, the internal oligonucleotide probe GOP-2 (5′-CACAGGGG-TGGGCTGGTCTC-3′) was synthesized to correspond to the specific site. The alkaline-blotted membrane was hybridized with the [^{32}P]-labeled probe at 50°C for 1 hr in hybridization solution (Stratagene, La Jolla, CA), washed in 2×SSC containing 0.1% SDS, and exposed to Kodak XAR film (Eastern Kodak, Rochester, NY) overnight at −80°C (Fig. 1c).

RESULTS AND DISCUSSION

A total of 6 first trimester placentas and 3 third trimester placentas were examined for heterozygosity at the *Pst*I RFLP site in exon 8. Among them, 3 first trimester placentas and 2 third trimester placentas were heterozygous at this site. Since class I HLAs contain highly homologous sequences, the internal oligonucleotide probe GOP-2 specific for *HLA-G* was used to further confirm the specificity of the products (Fig. 1a). In contrast to our expectations, amplification of mRNAs by RT-PCR showed that both parental alleles were co-dominantly expressed in all the first and the third trimester placentas showing heterozygosity (two representative cases are shown in Fig. 1). These results indicated that (1) *HLA-G* is not subject to genomic imprinting at least at the transcriptional level and (2) the lack of imprinting in the placenta may be universal regardless of gestational weeks. Therefore, the possibility that imprinting is functionally established during the course of pregnancy as in human H19 gene (Jinno *et al.*, 1995) is unlikely. Also in equine trophoblasts, MHC class I molecules are biallelically coexpressed (Donaldson *et al.*, 1994), thus, regulation of allelic expression of MHC molecules in the placenta may be specific to species, although further investigations on genomic imprinting in a wide-range of mammalian species are required before a conclusion can be made.

HLA-G may encode a wide variety of peptides that can act in the allogenic

response since evidences suggesting the existence of HLA-G antigenic polymorphisms have been reported (van der Ven and Ober, 1994; Watanabe *et al.*, 1995). If so, our observation that *HLA-G* is expressed biallelically suggests an involvement of the MHC antigen derived from the paternal allele which is in contact with the maternal immune system in the maintenance of pregnancy. The paternal allele of *HLA-G* may play a major role in the development or differentiation of trophoblasts. This is further supported by the results of mouse pronuclear transfer experiments in which the paternal genome was shown to be essential for placental development (Barton *et al.*, 1984).

The expression of *HLA-G* is unique in the placenta in that its temporal and spatial expression patterns are altered during pregnancy. *HLA-G* expression is restricted to differentiated cytotrophoblasts (McMaster *et al.*, 1995) and its expression level in the placenta decreases with advance of pregnancy (Wei and Orr, 1990), while strong expression was observed in the extravillous membrane at term (Wei and Orr, 1990). The aberrant expression of *HLA-G* may be relevant to some prenatal complications that have been considered due to unexplained abnormal feto-maternal immunity. For instance, spontaneous abortion including habitual abortion or fetal growth retardation might be explained by the involvement of HLA-G. In other situations, HLA-G may play a defensive role in protecting the placenta from stressful conditions such as infections (Onno *et al.*, 1994). Thus, detection of paternal and maternal alleles of *HLA-G* at the DNA and mRNA levels by the *Pst*I RFLP described here may be a useful tool for genetic analysis of these pathological conditions.

Acknowledgments This work was supported in part by Grants-in-Aid for Scientific Research (No. 07457387, 07671780, 07671781, 08671888, and 08671889) from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

Barton SC, Surani MA, Norris ML (1984): Role of paternal and maternal genomes in mouse development. Nature **311**: 374–376

Cattanach BM (1991): Chromosome imprinting and its significance for mammalian development. Genome Anal **2**: 41–71

Chumbley G, King A, Holmes N, Loke YW (1993): *In situ* hybridization and Northern blot demonstration of HLA-G mRNA in human trophoblast populations by locus-specific oligonucleotide. Hum Immunol **37**: 17–22

Donaldson WL, Oriol JG, Pelkaus CL, Antczak DF (1994): Paternal and maternal major histocompatibility complex class I antigens are expressed co-dominantly by equine trophoblast. Placenta **15**: 123–135

Ellis SA, Palmer MS, McMichael AJ (1990): Human trophoblast and the choriocarcinoma cell line BeWo express a truncated HLA class I molecule. J Immunol **144**: 731–735

Geraghty DE, Koller BH, Orr HT (1987): A human major histocompatibility complex class I gene that encodes a protein with a shortened cytoplasmic segment. Proc Natl Acad Sci USA **84**: 9145–9149

Goshen R, Ben RZ, Gonik B, Lustig O, Tannos V, de Groot N, Hochberg AA (1994): The role

of genomic imprinting in implantation. Fertil Steril **62**: 903-910
Hunt JS (1992): Immunobiology of pregnancy. Curr Opin Immunol **4**: 591-596
Hunt JS, Orr HT (1992): HLA and maternal-fetal recognition. FASEB J **6**: 2344-2348
Jinno Y, Ikeda Y, Yun K, Maw M, Masuzaki H, Fukuda H, Inuzuka K, Fujishita A, Ohtani Y, Okimoto T, Ishimaru T, Niikawa N (1995): Establishment of functional imprinting of the H19 gene in human developing placentae. Nature Genet **10**: 318-324
Kanbour-Shakir A, Zhang X, Rouleau A, Armstrong DT, Kunz HW, Macpherson TA, Gill TJ III (1990): Gene imprinting and major histocompatibility complex class I antigen in the rat placenta. Proc Natl Acad Sci USA **87**: 444-448
Koller BH, Geraghty DE, DeMars R, Duvick L, Rich SS, Orr HT (1989): Chromosomal organization of the human major histocompatibility complex class I gene family. J Exp Med **169**: 469-480
Lata JA, Cowchock FS, Jackson LG, Smith JB (1990): Cell surface antigen expression of first trimester chorionic villus samples. Am J Reprod Immunol **22**: 18-25
Ledbetter DH, Engel E (1995): Uniparental disomy in humans: development of an imprinting map and its implications for prenatal diagnosis. Hum Mol Genet **4**: 1757-1764
McMaster MT, Librach CL, Zhou Y, Lim KH, Janatpour MJ, DeMars R, Kovats S, Damsky C, Fisher SJ (1995): Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. J Immunol **154**: 3771-3778
Onno M, Guillaudeux T, Amiot L, Renard I, Drenou B, Hirel B, Girr M, Semana G, Le BP, Fauchet R (1994): The HLA-G gene is expressed at a low mRNA level in different human cells and tissues. Hum Immunol **41**: 79-86
Shukla H, Swaroop A, Srivastava R, Weissman SM (1990): The mRNA of a human class I gene HLA G/HLA 6.0 exhibits a restricted pattern of expression. Nucleic Acids Res **18**: 2189
Silbert PD, Chenchik A (1993): Modified acid guanidium thiocyanate-phenol-chloroform RNA extraction method which greatly reduces DNA contamination. Nucleic Acids Res **21**: 2019-2020
Tamaki J, Arimura Y, Koda T, Fujimoto S, Fujino T, Wakisaka A, Kakinuma M (1993): Heterogeneity of HLA-G genes identified by polymerase chain reaction/single strand conformational polymorphism (PCR/SSCP). Microbiol Immunol **37**: 633-640
van der Ven K, Ober C (1994): HLA-G polymorphisms in African Americans. J Immunol **153**: 5628-5633
Watanabe Y, Fujii T, Tokunaga K, Tadokoro K, Taketani Y, Juji T (1995): Polymorphism of HLA-G gene in Japanese. Hum Immunol **44** (suppl 1): 13
Wei X, Orr HT (1990): Differential expression of HLA-E. HLA-F, and HLA-G transcripts in human tissue. Hum Immunol **29**: 131-142