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HLA-G in human reproduction: aspects of genetics, function and pregnancy complications

Thomas Vauvert F.Hviid

Department of Clinical Biochemistry, Copenhagen University Hospital, Rigshospitalet and H:S Hvidovre Hospital, Copenhagen, Denmark

To whom correspondence should be addressed at: Department of Clinical Biochemistry, Roskilde University Hospital, 7–13 Køgevej, DK-4000 Roskilde, Denmark. E-mail: hviid@dadlnet.dk

The non-classical *human leukocyte antigen (HLA) class Ib* genes, *HLA-E*, *-G* and *-F*, are located on chromosome 6 in the human major histocompatibility complex (MHC). HLA class Ib antigens resemble the HLA class Ia antigens in many ways, but several major differences have been described. This review will, in particular, discuss HLA-G and its role in human reproduction and in the human MHC. HLA-G seems to be important in the modulation of the maternal immune system during pregnancy and thereby the maternal acceptance of the semiallogenic fetus. Recent findings regarding aspects of *HLA-G* polymorphism, the possible significance of this polymorphism in respect to HLA-G function and certain complications of pregnancy (such as pre-eclampsia and recurrent spontaneous abortions (RSA)) are discussed together with possible importance to IVF. Finally, aspects of a possible role of HLA-G in organ transplantation and in inflammatory or autoimmune disease, and of HLA-G in an evolutionary context, are also briefly examined.

Key words: gene expression/immunology/implantation/pregnancy/trophoblasts

Introduction

During pregnancy, the maternal immune system is in close contact with cells and tissue from the semiallogenic fetus. Therefore, specific mechanisms must exist to modulate and moderate the maternal immune system, so that the pregnant woman does not reject her own fetus. Additionally, aberrations in these mechanisms may in theory lead to complications in pregnancy. The so-called non-classical human leukocyte antigen (HLA) class Ib molecules may be involved in these mechanisms, as they are expressed on trophoblast cells in the placenta (Kovats *et al*., 1990; Ishitani *et al*., 2003). The most extensively studied *class Ib* gene in this respect is *HLA-G*. The trophoblast cells, which originate from the fetus, do not express classical HLA class Ia and II antigens, except for a possible weak expression of HLA-C (Redman *et al*., 1984; Hunt *et al*., 1987; Lata *et al*., 1990; King *et al*., 1996, 2000). HLA-G seems to be important for the modulation of the maternal immune system during pregnancy and, thereby, for the maternal acceptance of the semiallogenic fetus. Although there is some controversy, HLA-G can already be detected in at least some blastocysts and may play a role in implantation as well (Jurisicova *et al*., 1996; Fuzzi *et al*., 2002; Sher *et al*., 2004; Yie *et al*., 2005). HLA-G is almost monomorphic and has a restrictive pattern of expression (Ellis *et al*., 1990; Kovats *et al*., 1990; Yamashita *et al*., 1996; Blaschitz *et al*., 1997; Hviid *et al*., 1997; Ishitani *et al*., 1999, 2003; Le Bouteiller and Blaschitz, 1999). However, polymorphisms have been described in regulatory regions of the *HLA-G* gene, which may have functional significance for HLA-G expression (Harrison *et al*., 1993; Fujii *et al*., 1994; Rebmann *et al*., 2001; Hviid *et al*., 2003, 2004b). A very strong expression of HLA-G is observed in the invasive trophoblast cells of the placenta. Furthermore, HLA-G may have a role in certain complications of pregnancy that may involve immunological malfunction, such as pre-eclampsia and certain cases of recurrent spontaneous abortions (RSA) and genetic predisposition to these (Hara *et al*., 1996; Goldman-Wohl *et al*., 2000; Aldrich *et al*., 2001; O'Brien *et al*., 2001; Pfeiffer *et al*., 2001; Hviid *et al*., 2002; Hylenius *et al*., 2004; Yie *et al*., 2004).

This review presents and critically discusses recent findings in primary studies of HLA-G polymorphism, genetics, expression and function. Furthermore, the review will focus on studies that examine a possible role for *HLA-G* genetics and HLA-G expression in the pathogenesis of pre-eclampsia and certain cases of RSA. Evidence for the importance of HLA-G in blastocyst implantation and the success of IVF will also be discussed. Finally, aspects of HLA-G in organ transplantation and in autoimmune disease will briefly be presented, together with a brief discussion of broader perspectives on HLA-G and the major histocompatibility complex (MHC) in reproduction.

The MHC in humans

The human MHC is located on the short arm of chromosome 6. It spans ~4 Mb and encodes at least ~130 functional genes. The best

described are the classical *HLA class Ia* and *II* genes (*HLA-A*, *-B*, *-C*, *-DR*, *-DQ* and *-DP*) (The MHC Sequencing Consortium, 1999). These are well known for their role in organ transplantation and antigen-peptide presentation and for their association with a range of autoimmune diseases (Doherty and Zinkernagel, 1975; Svejgaard *et al*., 1983). However, since the late 1980s, the existence of another interesting group of so-called non-classical *HLA class Ib* genes has become evident (Redman *et al*., 1984; Geraghty *et al*., 1987; Ellis *et al*., 1990; Schmidt and Orr, 1991).

The non-classical *HLA class Ib* genes

The HLA class Ib antigens, HLA-E, -F and -G, share some characteristics with the class Ia antigens, but also differ from them in a range of ways. There seems to be growing evidence to support the viewpoint that HLA class Ib molecules, at least in respect to HLA-G, may play a role in the suppression of immune responses and contribute to long-term immune escape or tolerance (Carosella *et al*., 1999; Ishitani *et al*., 2003; LeMaoult *et al*., 2004). As will be described in more detail later, HLA-G seems to be able to inhibit both a cytotoxic T-lymphocyte (CTL) response and natural killer (NK) functions (Le Gal *et al*., 1999). Furthermore, antigen-presenting cells (APC), which have been transfected with HLA-G, can prevent the proliferation of CD4+ T cells and apparently direct these cells towards immunosuppression (LeMaoult *et al*., 2004). Finally, soluble HLA-G (sHLA-G) expression seems to be able to induce CD8+ T-cell apoptosis through the Fas/FasL pathway (Fournel *et al*., 2000a; Contini *et al*., 2003). However, exactly how HLA-G seems to elicit tolerance still remains to be elucidated.

The *HLA-G* gene is located on chromosome 6 close to *HLA-A*, the classical *HLA class Ia* gene with which it seems to be in closest homology (Messer *et al*., 1992) (Figure 1). The *HLA-E* gene is localized between *HLA-C* and *-A*, whereas *HLA-F* is located close to *HLA-G* and *-A* (The MHC Sequencing Consortium, 1999) (Figure 1). The gene structure of the *HLA class Ib* genes is very similar to that of *HLA class Ia* genes. However, differences exist, especially regarding the 3′-end (the cytoplasmic tail) (Geraghty *et al*., 1987; Heinrichs and Orr, 1990). The short cytoplasmic tail of HLA-G seems to be important for the highly reduced spontaneous endocytosis of HLA-G (Davis *et al*., 1997). HLA-E is expressed on many different types of cells and tissues as the class Ia antigens (Lee *et al*., 1998). The HLA-E-binding groove has a great affinity for the HLA-G signal peptide, and this binding is important for the expression of HLA-E on the trophoblast cell surface. The intracellular transport and expression on the cell surface of HLA class I proteins are dependent on peptides within the endoplasmic reticulum.

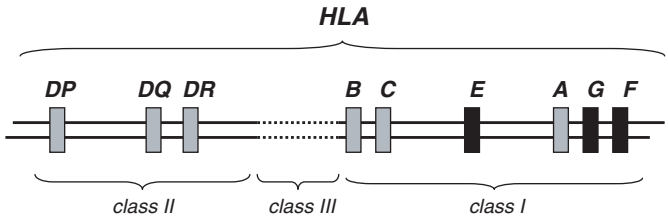

Figure 1. The non-classical *human leukocyte antigen (HLA) class Ib* genes, *HLA-E*, *-F* and *-G* are located in the same region as the classical *HLA class Ia* genes on the short arm of chromosome 6.

It seems that most peptides bound to HLA-E are nonamers derived from HLA class I signal sequences and that the binding of heavy chains to these peptides is dependent on a functional transporter associated with antigen processing. The HLA-E molecule has unique amino acid substitutions in the peptide-binding cleft, probably facilitating the binding of class I signal sequence-derived peptides. HLA-E interacts with the CD94/NKG2C receptor, and HLA-E is probably the most important ligand for the inhibition of NK cells (Llano *et al*., 1998). It is possible that HLA-G interacts with a killer inhibitory receptor (KIR) and balances the activation signal from HLA-E/the G-nonamer, resulting in the inhibition of lysis by a possible activation of other pathways (e.g. secretion of cytokines) (Ishitani *et al*., 2003).

The function of HLA-F is not known at present. HLA-F expression has only been detected on invasive cytotrophoblast cells in the placenta, and these are the only cells described so far that express all three HLA class Ib molecules. In this regard, the three class Ib molecules may act in synergy in the placenta and may to some extent functionally be able to substitute for each other (Ishitani *et al*., 2003).

The gene polymorphism of the *HLA class Ib* genes is very sparse in contrast to the highly polymorphic *HLA class Ia* and *II* genes. Regarding HLA-E, there is only consensus for three alleles at the protein level and for HLA-F only two; however, the difference is related to a shortened cytoplasmic tail (He *et al*., 2004) (HLA Informatics Group, http://www.anthonynolan.org.uk/HIG/lists).

The *HLA-G* gene

Figure 2 shows the gene structure of *HLA-G*. The external part of the HLA-G molecule consists of three parts: the α1, α2 and α3 domains (exons 2–4) (Figure 2); α1 and α2 contribute to the peptide-binding cleft. The crystal structure of HLA-G has recently been determined (Clements *et al*., 2005). Interestingly, the candidate-binding site for leukocyte Ig-like receptor 1 (LIR-1 or ILT2) and LIR-2 (or ILT4) inhibitory receptors is on the α3 domain of HLA-G, and this domain is structurally distinct from the α3 domain of classical MHC class I molecules. HLA-G can bind peptides. In HLA-G transfected cells (B lymphoblastoid cell line 721.221), HLA-G molecules are associated with peptides derived from a variety of intracellular proteins. However, the complexity of the bound peptides may be lower than that of some HLA class Ia molecules (Lee *et al*., 1995; Diehl *et al*., 1996). *In vivo*, in the placenta, a single peptide derived from a cytokine-related protein accounted for 15% of the molar ratio of HLA-G bound peptide; the significance of this is not known. Furthermore, the complexity of peptides bound to HLA-G molecules in placenta seems to be lower than that of peptides bound to HLA-G in transfected cells (Ishitani *et al*., 2003). Peptide binding in HLA-G seems to be a constrained mode of binding reminiscent of that of the HLA-E molecule (Clements *et al*., 2005). The HLA-G full-length membrane protein is anchored in the cell membrane by the transmembrane region (exon 5). Among the HLA class I antigens, HLA-G is special because it is expressed in several different membrane and soluble isoforms generated by alternative splicing of *HLA-G* mRNA (Ishitani and Geraghty, 1992; Fujii *et al*., 1994; Kirszenbaum *et al*., 1994; Hviid *et al*., 1998; Hiby *et al*., 1999) (see Figure 2 and section on HLA-G mRNA alternative splicing-membrane-bound and soluble isoforms).

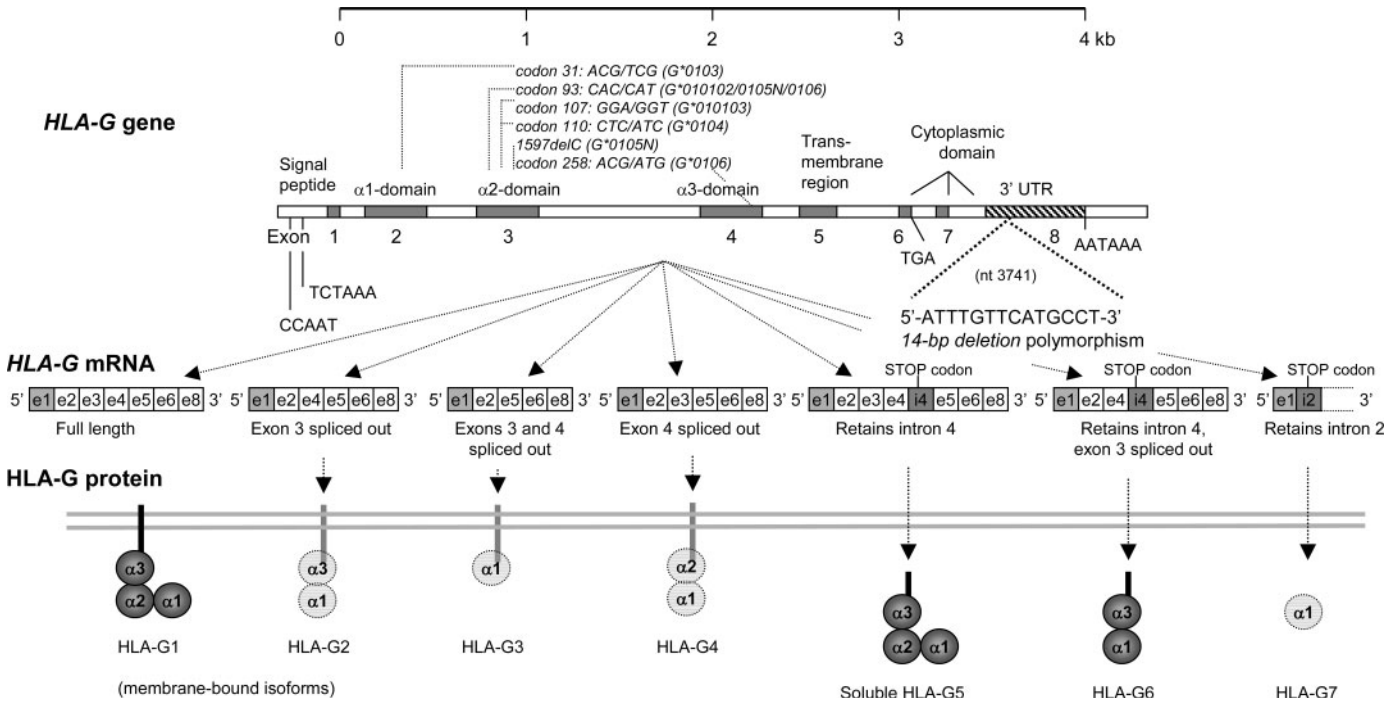

Figure 2. The *human leukocyte antigen-G (HLA-G)* gene and expression. The *HLA-G* gene has almost the same structure as the classical *HLA class Ia* genes. However, HLA-G has a shortened cytoplasmic domain, and the polymorphism in the coding parts is very sparse. In the 3′-untranslated region (3′UTR) of the *HLA-G* gene/transcript, an insertion/deletion polymorphism of 14 bp is localized. Altogether, seven HLA-G mRNA and protein isoforms are generated by alternative splicing of mRNA. Furthermore, soluble HLA-G (sHLA-G) isoforms exist. In these, intron 4 or intron 2 with a stop codon is retained. These soluble isoforms (HLA-G5, -G6, -G7) can be secreted by the cell. It is controversial whether the shortened membrane-bound isoforms (HLA-G2, -G3, -G4) are expressed on the cell surface. (Based on Geraghty *et al*., 1987; Ishitani and Geraghty, 1992; Fujii *et al*., 1994; Kirszenbaum *et al*., 1995; Hviid *et al*., 1998; Paul *et al*., 2000).

HLA-G polymorphism

The HLA-G proteins are nearly monomorphic, with only four single amino acid polymorphisms described in the literature so far. This stands in marked contrast to the very polymorphic HLA class Ia and II antigens, which exhibit some of the largest genetic polymorphism in the human genome. Regarding *HLA-G*, polymorphism has also been reported in the 5′-upstream regulatory region (5′URR)/the promotor region and in the 3′-untranslated region (3′UTR) of the gene (Harrison *et al*., 1993; Hviid *et al*., 1999, 2003).

HLA-G polymorphism in coding regions

HLA-G polymorphism has by now been studied extensively. The first reports on Caucasian populations showed a very limited polymorphism (Morales *et al*., 1993; Ober *et al*., 1996; Yamashita *et al*., 1996; Hviid *et al*., 1997); in marked contrast to this, an early study of African Americans showed a rather high polymorphism in exon 3 (the α2 domain) (van der Ven and Ober, 1994). However, these findings have never been reproduced, and different African populations have shown the same limited polymorphism as that described in Caucasian and Japanese populations (Bainbridge *et al*., 1999; Ishitani *et al*., 1999; Matte *et al*., 2000, 2002). Fifteen *HLA-G* alleles at the nucleotide level have been acknowledged by the WHO Nomenclature Committee for Factors of the HLA System (Marsh *et al*., 2002; http://www.anthonynolan.org.uk/HIG/). However, more *HLA-G* alleles at the DNA level have been described in several publications (Ober *et al*., 1996; Hviid *et al*., 1997, 2002; Kirszenbaum *et al*., 1999) (Table I). Regarding the HLA-G protein, only five HLA-G alleles with (single) amino acid substitutions have been described in the literature (Table I). These polymorphisms are located outside the binding groove. Two amino acid substitutions have been found in exon 2 (defining the alleles G*0102 and G*0103), one in exon 3 (defining the G*01040x alleles) and one in exon 4 (the G*0106 allele) (Hviid *et al*., 2001, 2002). So single-nucleotide polymorphisms (SNPs) that change the amino acid sequence of the HLA-G protein define the major groups of *HLA-G* alleles; silent nucleotide variation within these groups defines further specific allelic variants. However, polymorphism in the non-coding regions of the *HLA-G* gene (see section on HLA-G polymorphism in non-coding regions) is not included in the definition of the WHO-acknowledged *HLA-G* alleles. HLA-G*0105N is a null allele; it includes a deletion of the first base of codon 130 or the third of codon 129, which results in a frameshift (Ober *et al*., 1996; Hviid *et al*., 1997; Suarez *et al*., 1997). Table II lists the *HLA-G* allele distributions in different ethnic populations. An interesting observation is that the *G*01010x* group of alleles, except *G*010102*, are the predominant alleles, having a frequency up to approximately 80% in certain African populations, whereas these alleles only constitute approximately 50–60% in Caucasian and Japanese populations. The *G*010102* allele, however, which includes a 14 bp sequence in the 3′UTR of the gene, is sparsely represented in the African populations, but has a frequency of

Table I. DNA polymorphisms defining the *human leukocyte antigen-G (HLA-G)* alleles

HLA-G alleles	5′URR/ promotor*		Exon 2					Exon 3					Exon 4				3′UTR* (nt 3741)
	–725	–201	31	35	54	57	69	93	100	107	110	130	188	236	241	258	
*G*010101*	C or G	G	ACG	CGG	CAG	CCG	GCC	CAC	GGC	GGA	CTC	CTG	CAC	GCA	TTC	ACG	–
*G*010102*	C	A	---	---	---	--A	---	--T	---	---	---	---	---	---	---	---	+14 bp
*G*010103*	C	A	---	---	---	--A	---	---	---	--T	---	---	---	---	-C-	---	+14 bp
*G*010104*	C	G	---	---	---	---	--T	---	---	---	---	---	nd	nd	nd	nd	nd
*G*010105*	nd	nd	---	---	---	---	---	---	---	--T	---	---	---	---	---	---	–
*G*010106*	nd	nd	---	---	---	---	---	---	---	---	---	---	--T	---	---	---	nd
*G*010107*	nd	nd	---	---	---	--A	---	--T	---	--T	---	---	---	---	---	---	nd
*G*010108*	C	G	---	---	---	--A	---	---	---	---	---	---	---	---	---	---	–
*G*0102*	nd	nd	---	---	***-G-***	---	---	---	---	---	---	---	---	--C	---	---	nd
*G*0103*	C (or T)	G	***T--***	---	---	---	---	---	---	---	---	---	--T	nd	nd	---	+14 bp
*G*010401*	C	A	---	---	---	--A	---	---	---	---	***A--***	---	---	---	---	---	–
*G*010402*	nd	nd	---	---	---	--C	---	---	---	---	***A--***	---	--T	---	---	---	nd
*G*010403*	nd	nd	---	---	---	---	---	---	---	---	***A--***	---	---	---	---	---	–
*G*0105N*	C	A	---	---	---	--A	---	--T	---	---	---	***•TG***	---	nd	nd	---	+14 bp
*G*0106*	C	A	---	---	---	--A	---	--T	---	---	---	---	---	---	---	***-T-***	+14 bp
*G*0101g*†	nd	nd	---	--A	---	---	---	---	---	---	---	---	---	nd	nd	---	+14 bp
*G*G3d5*†	nd	nd	---	---	---	--A	---	--T	--T	---	---	---	---	nd	nd	---	+14 bp

Data regarding exons 2–4 are from http://www.anthonynolan.org.uk/HIG/ and from the following publications: Ober *et al*. (1996); Yamashita *et al*. (1996); Hviid *et al*. (1997, 2002); Suarez *et al*. (1997); van der Ven *et al*. (1998); Ishitani *et al*. (1999); Matte *et al*. (2000); Hylenius *et al*. (2004). Data regarding the 5′-upstream regulatory region (5′URR) and 3′-untranslated region (3′UTR) parts of the *HLA-G* gene are from Hviid *et al*. (1999, 2002; 2004b); Ober *et al*. (2003); Hylenius *et al*. (2004). The four codons with amino acid polymorphisms (shown in bold italics) are codon 31 (ACG/Thr to TCG/Ser), codon 54 (CAG/Gln to CGG/Arg), codon 110 (CTC/Leu to ATC/Ile) and codon 258 (ACG/Thr to ATG/Met). The deletion of C from codon 130 results in a frameshift in the *G*0105N* allele. nd, not determined.

*More polymorphisms have been reported in these regions of the *HLA-G* gene in several independent studies; the three polymorphisms listed are discussed in the text.

†These two *HLA-G* alleles have not been acknowledged by the official WHO Nomenclature Committee for Factors of the HLA System, but they have been reported in several independent studies (Ober *et al*., 1996; Hviid *et al*., 1997, 2002; Hylenius *et al*., 2004).

Table II. *Human leukocyte antigen-G (HLA-G)* allele frequencies in different ethnic populations

HLA-G alleles	African American (%)	African Ghanaian (%)	African Shona (%)	Danish (%)	Danish (%)*	Finnish (%)	German/ Croatian (%)	Hutterite (%)	North Indian (%)†	Japanese (%)
*G*010101*	70	83	39.3	62	56	58	32	46	10	33
*G*010102*	6	2.4	14.4	27	25	38	36	20	16	16
*G*010103*	2.4	0	0	5.1	5	4.8	6.8	2	5	6
*G*010104*	–	–	0	–	0	–	–	4.4	8	–
*G*010105*	–	–	0	–	0	–	–	–	0	–
*G*010106*	–	–	0	–	–	–	–	–	–	–
*G*010107*	–	–	0	–	0	–	1.9	–	0	–
*G*010108*	–	–	14.4	–	1	–	9.1	–	0	–
*G*0102*	–	–	0	–	0	–	–	–	1	–
*G*0103*	–	–	0	–	4	–	2.3	2.7	24	–
*G*010401*	13	9.5	20.4	4.5	9	–	6.1	13	18	45
*G*010402*	–	–	–	–	–	–	–	–	–	–
*G*010403*	–	–	0.4	–	0	–	–	–	–	–
*G*0105N*	8.3	4.8	11.1	0.6	1	–	2.3	–	15	–
*G*0106*	–	–	–	–	–‡	–	–	–	3	–
Others	–	–	–	–	–	–	3.1	12	–	–
n	84	84	216	144	104	104	264	160	240	108
Reference	Ishitani *et al*. (1999)	Ishitani *et al*. (1999)	Matte *et al*. (2000)	Hviid *et al*. (1997)	Hviid (unpublished data)	Karhukorpi *et al*. (1996)	van der Ven *et al*. (1998)	Ober *et al*. (1996)	Abbas *et al*. (2004)	Yamashita *et al*. (1996)

n, numbers of chromosomes tested.

*Based on sequencing of exons 2 and 3.

†This represents a female control population used in a recurrent spontaneous abortions (RSA) study (at least three live births).

‡The *G*0106* allele has a frequency of 4.0% in the Danish population (Hviid *et al*., 2001).

approximately 30% in the other populations (Table II). This is curious, because the *G*010102* allele might be an older allele than the others; all studied *MHC-G* genes in primates (chimpanzee, gorilla, orangutan) include the 14 bp sequence (Castro *et al*., 2000a).

HLA-G *polymorphism in non-coding regions*

Several studies have identified DNA sequence variation in the 5′URR in introns and in the 3′UTR of the *HLA-G* gene (Harrison *et al*., 1993; Tamaki *et al*., 1993; Hiby *et al*., 1999; Hviid *et al*., 1999, 2004b; Yamashita *et al*., 1999; Ober *et al*., 2003). It is beyond the scope of this review to list and discuss sequence variation in introns of the *HLA-G* gene. Efforts have been made to elucidate the polymorphism in the 5′URR and in the 3′UTR parts of the *HLA-G* gene. Table I lists some of these polymorphisms. In brief, sequences and polymorphism in both the 5′URR and 3′UTR parts of the *HLA-G* gene may be of importance in the regulation of HLA-G expression. Based on studies of both HLA-G transgenic mice and the binding of nuclear factors, a region between 1.1 and 1.4 kb from the start site of transcription of the *HLA-G* gene has been proposed to contain an important regulatory element (Schmidt *et al*., 1993; Moreau *et al*., 1997). Sequence polymorphism has also been reported in this region (Hviid *et al*., 1999, 2004b; Ober *et al*., 2003). However, the functional links and importance of this polymorphism are still uncertain. A polymorphism at position –725 might be associated with SA (Ober *et al*., 2003) (Table I), and there might be an association with the status of the methylation and expression of sHLA-G; however, further studies are clearly needed (Ober *et al*., 2003; Hviid *et al*., 2004b). In the promotor region of *HLA-G*, an interesting polymorphism at position –201 has been reported (Hviid *et al*., 1999) (Table I). A ‘G’ in *G*010101* is changed to an ‘A’ in *G*010102*, *G*010103*, *G*0104* and *G*0105N*. The polymorphism is located in the NF-κB2 element (enhancer A) and reverts the regulatory sequence to that of *HLA-A2*. Still, further studies are needed to elucidate whether this promotor polymorphism has any effect on the regulation of expression of certain *HLA-G* alleles by allowing the binding of NF-κB2 factors to the *HLA-G* enhancer A (Hviid *et al*., 1999; Solier *et al*., 2001a). Harrison *et al*. (1993) was the first to describe a *14 bp* deletion/insertion polymorphism (5′-ATTTGTTCATGCCT-3′) in the 3′UTR of the *HLA-G* gene located at position 3741 in exon 8 (according to the reference sequence of Geraghty *et al*., 1987) (Figure 2). In Caucasian populations, the frequencies of these two *HLA-G* polymorphisms are nearly equal (frequency of alleles including the 14 bp sequence: ~45%; alleles with the 14 bp sequence deleted: ~55%); however, in African populations the allele with the deleted 14 bp sequence may dominate when the linkage disequilibrium between this polymorphism and the WHO-acknowledged *HLA-G* alleles is considered (Table II), although no direct study of this matter has yet been published.

In conclusion, there are no convincing data that the few polymorphisms in the coding regions of the *HLA-G* gene affect HLA-G function, except for the deletion in the HLA-G-null allele *G*0105N*. Polymorphism in the 3′UTR and 5′URR parts of the *HLA-G* gene has been found to be associated with differences in HLA-G expression, which may affect HLA-G function and may be of importance in certain complications of pregnancy. These aspects will be outlined in detail in later sections of this review.

HLA-G expression and function

HLA-G expression

The expression of HLA-G is restrictive. Although *HLA-G* mRNA has been detected in many different tissues, HLA-G protein expression has been described repeatedly only on and by the trophoblast cells in placenta, on and by certain immune cells (in most cases monocytes) and in the thymus (Kovats *et al*., 1990; Crisa *et al*., 1997; Lila *et al*., 2001; Ishitani *et al*., 2003; Rebmann *et al*., 2003). However, HLA-G protein expression can sometimes be observed in muscle fibres and in liver biliary and renal tubular epithelial cells (Wiendl *et al*., 2000; Lila *et al*., 2002; Creput *et al*., 2003a). HLA-G can be detected in serum/plasma from both women and men, but there is controversy in various published studies to whether sHLA-G can be detected in the blood of *all* men and non-pregnant women under normal conditions (Rebmann *et al*., 2001; Lila *et al*., 2002; Hviid *et al*., 2004b). Reviewing the literature, it seems that sHLA-G can be detected in all plasma samples from pregnant and non-pregnant women, whereas sHLA-G can only be detected in some serum samples, at least from non-pregnant women (and from men); however, one study using a unique sHLA-G assay seemed to detect sHLA-G in all serum samples from pregnant women (Yie *et al*., 2004). It can be speculated that in blood containing small amounts of sHLA-G, the sHLA-G can be lost in some way in the subsequent generation of the serum samples. In future sHLA-G studies, plasma samples may be preferred or serum samples should only be compared with other serum samples in, e.g., case–control studies. Furthermore, no consensus exists regarding the sHLA-G assays used in various publications. So far, they have all been ‘in-house’ assays with different antibodies and sHLA-G standards, so evaluation and standardization of the different assays are thus greatly needed. The main source of HLA-G5 (sHLA-G) in the blood of non-pregnant women and in men is most likely monocytes; though CD4+ and CD8+ T cells and B cells seem to be able to secrete HLA-G5 as well, though to a lesser extent (Rebmann *et al*., 2003). Studies so far indicate that the presence, or the level, of sHLA-G in serum/plasma samples is associated with *HLA-G* polymorphism (Rebmann *et al*., 2001; Hviid *et al*., 2004b; Rizzo *et al*., 2005a).

HLA-G mRNA alternative splicing—membrane-bound and soluble isoforms

HLA-G potentially exists in four membrane-bound isoforms, HLA-G1 to -G4, and three soluble isoforms, HLA-G5 to -G7 (Ishitani and Geraghty, 1992; Fujii *et al*., 1994; Kirszenbaum *et al*., 1994; Hviid *et al*., 1998; Paul *et al*., 2000; LeMaoult *et al*., 2003) (Figure 2). The possible expression on the cell surface of the shortened isoforms HLA-G2–G4 is controversial (Menier *et al*., 2000; Bainbridge *et al*., 2000; Mallet *et al*., 2000; Riteau *et al*., 2001b; Morales *et al*., 2003; Ulbrecht *et al*., 2004). The sHLA-G isoforms are generated by alternative splicing of the *HLA-G* transcript. Intron 4 includes a stop codon, and this intron is retained in the *HLA-G* mRNAs which are translated into the sHLA-G

isoforms (Fujii *et al*., 1994; Hviid *et al*., 1998). sHLA-G, like HLA class Ia soluble molecules, is probably also generated by the shedding of membrane-bound HLA-G molecules (Park *et al*., 2004). The expression of soluble HLA-G6 with exon 3 spliced out has been reported; however, more independent studies are needed to establish the secretion and functionality of this HLA-G isoform beyond any doubt.

Associations between HLA-G *polymorphisms and HLA-G expression*

The *14 bp* deletion/insertion polymorphism in exon 8 of the 3′UTR is present in both the *HLA-G* gene and transcript (Harrison *et al*., 1993) (Figure 2). HLA-G was previously found to be co-dominantly expressed (Hviid *et al*., 1998); however, *HLA-G* alleles containing the 14 bp sequence have been found to be associated with a lower *HLA-G* mRNA level for most isoforms in heterozygous (first trimester) trophoblast samples (Fujii *et al*., 1994; Hiby *et al*., 1999; Hviid *et al*., 2003) (Figure 3). Hviid *et al*. (2003) have investigated villous and extravillous trophoblast cell fractions as well as whole biopsy samples from late first-trimester placentas for *HLA-G* mRNA expression. The *14 bp* sequence polymorphism in the 3′UTR of the *HLA-G* gene made it possible to study the two allelic *HLA-G* mRNA expression levels and alternative splicing patterns in heterozygous trophoblast samples. Overall, when the expression of different *HLA-G*010102* mRNA isoforms including the 14 bp sequence were compared with that of *G*010101*, where the 14 bp sequence is deleted, the expression levels were significantly reduced. These more detailed results were in accordance with the data of O'Brien *et al*. (2001). In the cases of transcription and alternative splicing that involved the *G*010102* allele, additional mRNA isoforms were observed to be lacking the first 92 bp of exon 8, these *HLA-G* mRNAs were variants of *HLA-G1* and *HLA-G5/G6*. These *HLA-G* mRNA isoforms were also found in the *G*010103* allele, and here unique *G2* (–92 bp), and possibly *G4* (–92 bp), isoforms were observed. On the other hand, regarding the levels of HLA-G mRNA expression, the *G*010103* allele more closely resembled the *G*010101* allele. In conclusion, it can be speculated whether the presence of the 14 bp sequence at the beginning of exon 8 functions as a cryptic branch-point sequence for *HLA-G* mRNA splicing. Interestingly, *HLA-G* transcripts with the 92 bp deletion have been reported to be more stable than the complete mRNA forms (Rousseau *et al*., 2003). However, at the moment, it has not been determined whether the *14 bp* polymorphism is directly involved in the observed differences in *HLA-G* mRNA expression or whether it is polymorphism in the *HLA-G* 5′URR in linkage disequilibrium with the *14 bp* polymorphism. Furthermore, several studies have been unable to detect sHLA-G in serum samples from individuals homozygous for the presence of the 14 bp sequence. These samples originated from both donors and couples attending IVF clinics (Hviid *et al*., 2004b; Rizzo *et al*., 2005a). sHLA-G was measured in serum by enzyme-linked immunosorbent assay (ELISA), which detects the HLA-G molecule in a β_2-microglobulin-associated form, so the assay in theory detects both sHLA-G1 that is shed by membrane-bound HLA-G1 and the soluble HLA-G5 isoform. In only 15.4% (23/149) of all serum samples could HLA-G5/sHLA-G1 be detected with a mean concentration of 11.7 ± 2.5 ng/ml (±SEM) for the positive samples; no differences being observed between males and females. No HLA-G5/sHLA-G1 was detected in any of the *+14/+14 bp* samples (P = 0.011). These findings are somewhat in agreement with a study of serum samples from heart transplant patients, 18% of whom were sHLA-G positive; but this is, naturally, an abnormal condition (Lila *et al*., 2002). Rebmann *et al*. (2001) have also studied associations between *HLA-G* alleles and sHLA-G plasma levels; however, no HLA-G-specific monoclonal antibodies (mAbs) were included in the ELISA used. The main results were a split between 'low-' and 'high-'secretor *HLA-G* alleles, *G*01013* and *G*0105N* being low secretors, *G*0104* a high secretor and *G*01011* and *G*01012* being between the two levels. When families were studied, the low and high sHLA-G plasma levels did not segregate with *HLA* haplotypes, so it was concluded that additional mechanisms might be involved in the regulation of the individual sHLA-G levels. These results are only partly in agreement with those of studies of the *14 bp* polymorphism and HLA-G5/sHLA-G1 serum levels. Further study is clearly needed in this area. Discrepancies between results may well depend on the specific anti-HLA-G antibodies used and whether they bind to β_2-microglobulin-associated HLA-G or intron 4-retaining sHLA-G isoforms (Fournel *et al*., 2000b). There might even be some differences related to whether the analysis is of serum or plasma samples. Shed HLA-G molecules may also blur associations between *HLA-G* polymorphism and sHLA-G protein expression. Finally, preparations of HLA-G protein standards may also vary and influence comparisons between different sHLA-G assays.

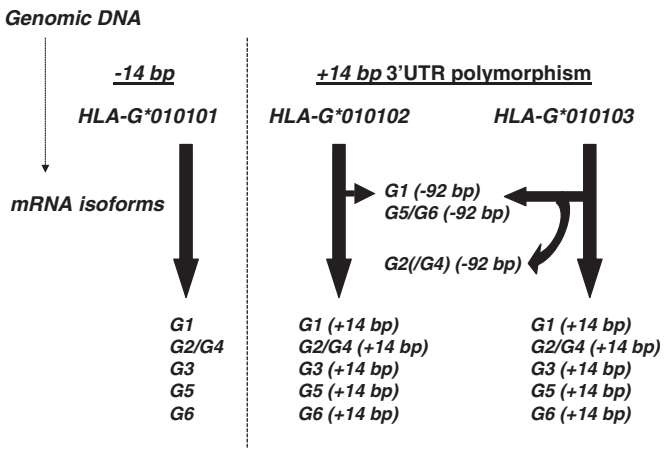

Figure 3. Expression profiles of *human leukocyte antigen-G (HLA-G)* mRNA isoforms in relation to *HLA-G* polymorphisms. The *HLA-G* mRNA isoforms lacking the first 78 bp of exon 8 are only observed in the *+14 bp HLA-G* alleles [*G1* (–92 bp), *G5/G6* (–92 bp), *G2/(G4)* (–92 bp)] (Hviid *et al*., 2003).

HLA-G function

The first and also the most comprehensive studies of the functions of HLA-G molecules relate to a range of *in vitro* experiments, which have shown that HLA-G can inhibit NK and T-cell-mediated cell lysis, both through direct interaction with the receptors ILT2 and ILT4 and with the killer Ig-like receptor 2 DL4 (KIR2DL4 receptor) (Navarro *et al*., 1999; Ponte *et al*., 1999; Rajagopalan and Long, 1999; Riteau *et al*., 2001a; Menier *et al*.,

2002) (Figure 4). Regarding the KIR2DL4 receptor, different responses are elicited depending on the activation of the effector cells (Rajagopalan and Long, 1999; Hofmeister and Weiss, 2003). The HLA-G α1 domain may be important in the inhibition of NK cell activity, and Met76 and Gln79 residues in the α1 domain may be involved in KIR2DL4 recognition (Rouas-Freiss *et al*., 1997; Yan and Fan, 2005). This is interesting in relation to preserved functions of HLA-G isoforms lacking exon 3 (the α2 domain).

On the trophoblast cells of the placenta, which originate from the fetus, no classical HLA class Ia and II antigens are expressed, except for a possible weak expression of HLA-C (Redman *et al*., 1984; Hunt *et al*., 1987; Lata *et al*., 1990; King *et al*., 1996, 2000). In this way, the HLA-semiallogenic fetal cells will not come in direct contact with the maternal immune system. However, cells that do not express MHC/HLA on the surface will undergo NK-mediated cell lysis. The strong expression of the nearly monomorphic HLA-G molecule on and by the invasive cytotrophoblast cells, together with the expression of HLA-E (and -F) in the placenta, will prevent this. As mentioned earlier, the function of HLA-F is not known. However, HLA-F expression has so far only been detected on invasive trophoblast cells. Actually, these cells are the only cells that express all three HLA class Ib molecules, which is an interesting observation regarding these non-classical HLA antigens' probable importance in the pregnant woman's acceptance of the semiallogenic fetus. It can be hypothesized that the products of the three genes may act in synergy and may even be able to substitute for each other (Ishitani *et al*., 2003).

A so-called T-helper 1 (Th1) response is mediated by a subset of CD4+ T cells that are primarily characterized by the cytokines [e.g. interferon-γ (IFN-γ), interleukin-2 (IL-2) and tumour necrosis factor-α (TNF-α)] they produce. A Th1 response is also called a (pro)inflammatory response. A Th2 response is mediated by another subset of CD4+ T cells that produce cytokines, such as IL-10, IL-3 and IL-4. Th2 cells are mainly involved in stimulating B cells to produce antibodies (humoral immune response). A successful pregnancy has been called a 'Th2 phenomenon' characterized by a Th2 cytokine profile, whereas certain complications of pregnancy, such as RSA and pre-eclampsia, have been associated with a Th1 response (Chaouat *et al*., 2004).

IL-10 has been shown to be able to activate HLA-G expression (Moreau *et al*., 1999). On the other hand, the presence of both membrane-bound and sHLA-G seems to induce changes in the secretion of cytokines from allo-CTL-activated peripheral blood mononuclear cells (PBMCs) (Maejima *et al*., 1997; Kapasi *et al*., 2000). However, the exact nature of the induced changes in cytokine profiles is controversial. Comparisons of various *in vitro*

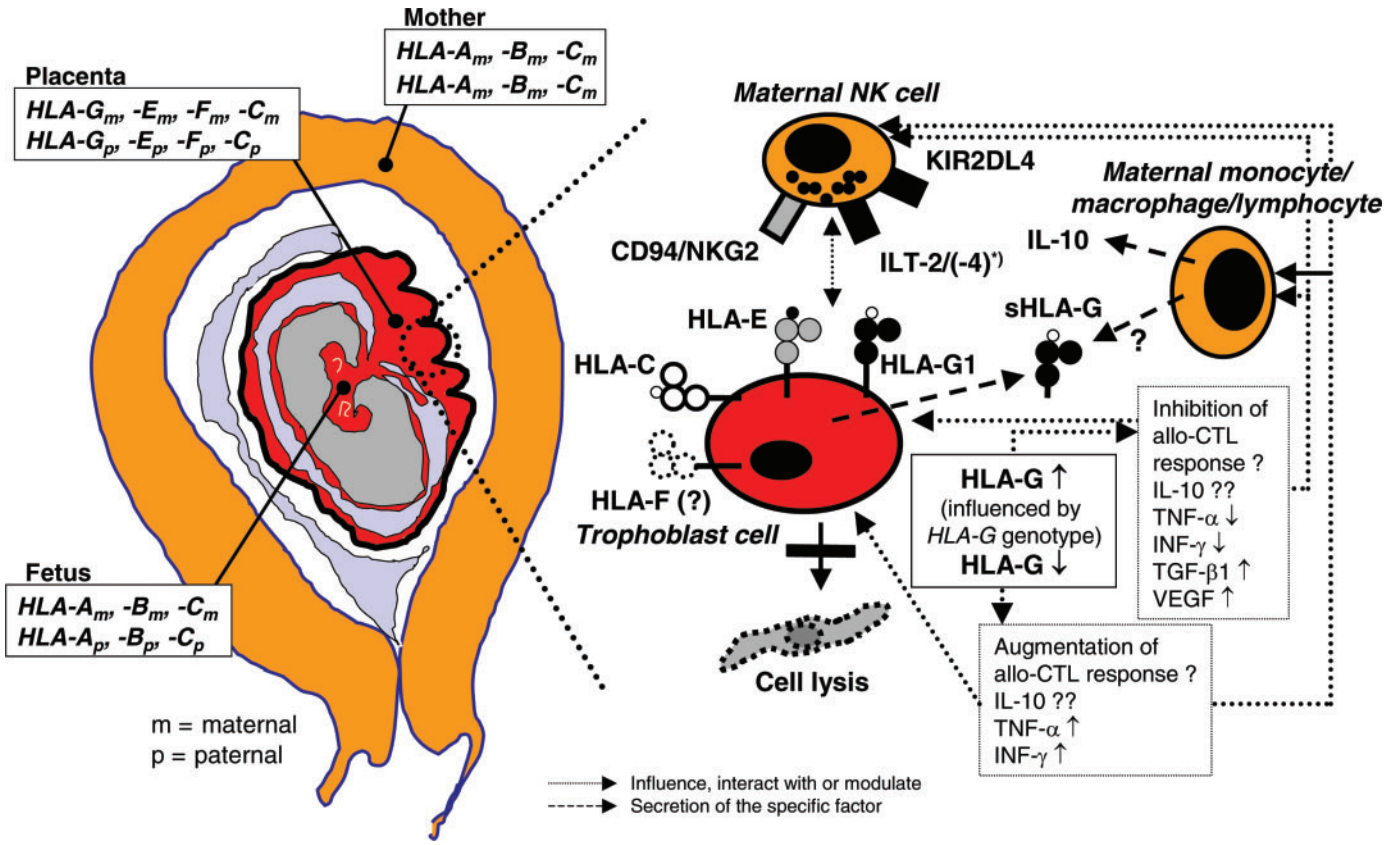

Figure 4. Expression of human leukocyte antigen (HLA) molecules during pregnancy and interactions between HLA class Ib molecules, natural killer (NK) receptors and cytokines at the feto–placental interface. The fetus inherits one *HLA* haplotype from the mother and one from the father and is thereby semiallogenic for the mother. However, the extreme polymorphic classical HLA class Ia and II antigens, HLA-A, -B and –DR, are not expressed by the trophoblast cells in the placenta. Instead, the nearly monomorphic non-classical HLA class Ib antigens, especially HLA-G, are expressed on the invasive cytotrophoblast cells. In this way, the trophoblast cells escape NK-cell-mediated lysis. Membrane-bound and soluble HLA-G (sHLA-G) can influence cytokine secretion and an allo-cytotoxic T-lymphocyte (CTL) response as described in detail in the text. *, ILT-4 is expressed on monocytes, macrophages and dendritic cells (Hofmeister and Weiss, 2003).

studies are complicated by differences in the sources of HLA-G used and in the cytokine-secreting cells studied; therefore, it is also difficult to judge which results may be the most credible. The studies can be divided into four categories.

Cell lines transfected with membrane-bound HLA-G1 in co-culture with PBMC

A range of studies in which cell lines (the B-lymphoblast cell line 721.221 or K-562 leukaemia cells) have been transfected with the membrane-bound *HLA-G1* (*mHLA-G1*) gene and co-cultured with PBMC or uterine mononuclear cells (UMC) or perhaps specific subsets of these, has been performed (Maejima *et al*., 1997; Kanai *et al*., 2001a,b, 2003; Rieger *et al*., 2002; van der Meer *et al*., 2004). Regarding the PBMC response to mHLA-G1-transfected cells, there is consensus concerning the four most-studied cytokines. The secretion of TNF-α and IFN-γ, both considered Th1 cytokines, decreased, whereas IL-10 and IL-4, both Th2 cytokines, increased (Maejima *et al*., 1997; Kanai *et al*., 2001a,b). IL-10 is an important anti-inflammatory cytokine, and elevated levels of IL-10 in pregnancy may be a mechanism for inducing tolerance by generating tolerance-inducing T cells (Huizinga *et al*., 1999). However, this interpretation may have to be modified and will be discussed later. Maejima *et al*. (1997) found a decrease in IL-3, while Kanai *et al*. (2001a) found an increase in this cytokine.

Cell lines transfected with membrane-bound HLA-G1 in co-culture with uterine or decidual mononuclear cells

Regarding the responses of UMC from non-pregnant women on day 7 after the LH surge, large granular lymphocytes (LGL) and decidual mononuclear cells (DMC), both from human deciduas to mHLA-G1 transfected cells, there is almost consensus (Kanai *et al*., 2001b, 2003; Rieger *et al*., 2002; van der Meer *et al*., 2004). Again, TNF-α and IFN-γ decreased; however, the level of IL-10 decreased or was unchanged compared with that of controls, and IL-4 was unchanged. One study has investigated the response of uterine NK cells from non-pregnant women (van der Meer *et al*., 2004), finding that IFN-γ increased in these cells. Interestingly, in the same study, vascular endothelial growth factor (VEGF) was found to increase in both UMC and uterine NK cells in comparison with the controls.

In conclusion, these studies of the effect of mHLA-G1-transfected cells show a diminishing of Th1-like cytokines, and especially in the studies with PBMC, a Th2-like response. A Th2 response in mononuclear cells from the uterus is, however, not that evident. Importantly, the effect of mHLA-G1 on IL-10 secretion seems modest (Kanai *et al*., 2001a; Rieger *et al*., 2002). These *in vitro* experiments can be difficult to interpret in a physiological context. The most relevant studies in relation to pregnancy are the ones that include UMC. Here, the HLA-G transfected cells can be viewed as the trophoblast part. The traditional concept of an up-regulation of Th2 and down-regulation of Th1 in an uncomplicated pregnancy is somewhat challenged in relation to the expression of membrane-bound HLA-G in these experiments; this will be further addressed later in this review. In relation to the studies with PBMC, it is important to note that these cells originated from males or nulligravidae women. As a whole, the studies indicate that the cytokine response to mHLA-G-expressing cells may differ between a non-pregnant and a pregnant state, or at least the response may differ between cells from peripheral blood and local uterine cells. However, it must be remembered that the content of specific mononuclear cells in PBMC and UMC populations is different, and the UMC samples may be contaminated with other cell populations.

Cytokine secretion from model systems with immune cells and addition of sHLA-G

A range of studies of the effect of sHLA-G on cytokine secretion from immune cells has been conducted. In mixed lymphocyte cultures (MLC), Kapasi *et al*. (2000) found biphasic responses regarding TNF-α, IFN-γ and IL-10 when HLA-G from placentas was added in the 0–1000 ng/ml range. At HLA-G concentrations of ≤50 ng/ml, secretion of TNF-α and IFN-γ increased and that of IL-10 was decreased compared with that of control cultures; that is, the IL-10 concentrations *rose* as sHLA-G levels diminished to zero. At an HLA-G concentration of 1000 ng/ml, the cytokine levels were inverted, as in a Th2 response. These results are partly in accordance with a study of recombinant sHLA-G1 (rsHLA-G1) added to DMC, in which rsHLA-G1 concentrations of 250 and 500 ng/ml were found to decrease levels of TNF-α and IFN-γ; however, when rsHLA-G1 was added to PBMC at a concentration of 1000 ng/ml, the levels of these two cytokines increased (Kanai *et al*., 2001a, 2003). Two more studies have measured IL-10 levels after the addition of sHLA-G. At a concentration of 1000 ng/ml, rsHLA-G1 significantly increased IL-10 levels in PBMC/.221 cell cultures, quite in accordance with studies by Kapasi *et al*. (2000) and McIntire *et al*. (2004) in which phorbol 12-myristate 13-acetate (PMA)/IFN-γ-treated U937 myelomonocytic cells increased IL-10 levels after the addition of 10 nM (~250 ng/ml, according to McIntire *et al*., 2004) of rHLA-G5 (Kanai *et al*., 2001a; McIntire *et al*., 2004). However, in this last study, rHLA-G5 and -G6 at concentrations of 1000 nM (~25 000 ng/ml) were found to suppress IL-10 secretion, while rHLA-G5/-G6 at concentrations of only 1 nM (~25 ng/ml) did not affect IL-10 levels. IL-10 has been shown to stimulate HLA-G expression (Moreau *et al*., 1999). In *in vitro* time-course experiments, IL-10 secretion preceded HLAG5/sHLA-G1 expression in lipopolysaccharide (LPS)-activated PBMC cultures. Addition of anti-IL-10 mAb to the LPS-activated cultures also blocked the HLA-G5/sHLA-G1 expression (Rizzo *et al*., 2005a). In PBMC cultures with the *+14/+14 bp HLA-G* genotype, however, a significantly higher concentration of IL-10 was observed together with the same basic level of HLA-G5/sHLA-G1, indicating that in some individual PBMC samples a higher level of IL-10 is needed to induce sHLA-G expression. Furthermore, in a study of donor serum samples, a trend towards an inverse relationship between HLA-G5/sHLA-G1 and IL-10 levels was observed (Rizzo *et al*., 2005a). Furthermore, LPS activation of PBMCs resulted in a rough trend towards decreasing levels of IL-10 with increasing concentrations of HLA-G5/sHLA-G1 in the cultures (Rizzo *et al*., 2005a). These results are somewhat in accordance with those of other studies of IL-10 and sHLA-G (Kapasi *et al*., 2000; McIntire *et al*., 2004), although not with those of Kanai *et al*. (2001a), which examined unstimulated PBMCs. Finally, McIntire *et al*. (2004) found that rHLA-G5 at 10 nM (~250 ng/ml) and 1000 nM (~25 000 ng/ml) augments transforming growth factor (TGF)-β1 secretion by the human myelomonocytic cell line. TGF-β1 may promote placental cell growth and differentiation (Ingman and Robertson, 2002). It seems that rHLA-G5 and -G6 mediate their effects through interaction with ILT4 and ILT2 receptors. IL-4 and IL-6 secretion seem not to be

affected by rsHLA-G1, rHLA-G5 or rHLA-G6 (Kanai *et al*., 2001a, 2003; McIntire *et al*., 2004). In conclusion, it is rather difficult fully to compare the cited studies of sHLA-G added to or spontaneously secreted by different mononuclear cells. Some studies investigate MLC, others PBMC/DMC or cell lines stimulated in different ways. It seems that for IL-10, TNF-α and IFN-γ secretion, the levels secreted are related to sHLA-G concentration. A trend found is that at increasing levels of sHLA-G, both TNF-α and IFN-γ secretion seems to decrease, at least in the MLC and DMC experiments. For IL-10, decreasing amounts of sHLA-G may result in increased secretion of IL-10 from activated mononuclear cells.

Cytokine secretion in a model system with both mHLA-G1-expressing cells and addition of sHLA-G1

One unique study examined the effect of rsHLA-G1 added to PBMC/HLA-G1.221 cells at concentrations of 250–1000 ng/ml (Kanai *et al*., 2001a). The results, in comparison with control cultures, showed a significant *increase* in TNF-α, IFN-γ and IL-10 and unchanged levels of IL-4. However, a similar study of DMC/HLA-G1.221 cells found a significant *decrease* in TNF-α and IFN-γ levels, whereas IL-4 levels were unaltered, at rsHLA-G1 concentrations of both 250 and 500 ng/ml; IL-10 was not considered in this study (Kanai *et al*., 2003). This model system with both mHLA-G and sHLA-G in the cultures is very relevant to the *in vivo* situation in the placenta.

Several of the studies discussed above point out important differences between the cytokine response in PBMC and in uterine/DMC populations to HLA-G expression. Also, the expression levels of HLA-G in the placenta are probably of critical importance for the exact cytokine response. The sHLA-G concentrations in plasma/sera of pregnant women are approximately 5–100 ng/ml (Pfeiffer *et al*., 2000; Steinborn *et al*., 2003; Yie *et al*., 2004), although one study has reported finding several serum concentrations in the third trimester as high as 3000–4000 ng/ml; however, local concentrations of sHLA-G may be much higher. Unfortunately, not many data regarding HLA-G local concentrations in the placenta are available. Yie *et al*. (2004) have studied the HLA-G levels in 14 placental lysates (gestational weeks 38–42) and found a median concentration of 88 ng/mg protein (range 6.5–200 ng/mg protein). However, the most interesting finding in relation to the *in vitro* experiments would be sHLA-G concentrations in uterine blood samples, which to the best of the author's knowledge have not been published. Altogether, the *in vivo* data might indicate whether the sHLA-G concentrations used in the *in vitro* experiments are physiologically relevant. One problem in drawing a general conclusion from these studies is that both the sHLA-G preparations and sHLA-G assays used need to be standardized, so direct comparisons might be problematic.

So, in conclusion, a possible function of HLA-G in uncomplicated pregnancy could be to shift a proinflammatory Th1 cell-mediated response towards a Th2 response, although this concept might have to be revised, especially as regards the local situation at the feto–maternal interface. It is important to note that the concept of Th1/Th2 responses may be far too simplistic as discussed (Chaouat *et al*., 2004). Also, HLA-G seems able to inhibit an allo-CTL response *in vitro*, but it seems that the response is biphasic, so a moderate amount of HLA-G added to the cell culture system increases the response (Kapasi *et al*., 2000). Further investigation is needed to elucidate the relationships between HLA-G expression and cytokine secretion.

Regarding allograft tolerance, this can be transferred between recipients by regulatory T cells: suppression can be actively mediated by regulatory T cells, or tolerance can be maintained by the deletion or functional non-responsiveness (anergy) of alloreactive T cells (Wekerle *et al*., 2002). In primary mixed lymphocyte reactions (MLR), responder T cells (both CD4+ and CD8+) could express both sHLA-G1 and HLA-G5, and this suppressed the allo-proliferative response of T cells (Lila *et al*., 2001; Le Rond *et al*., 2004). No HLA-G expression could be detected in autologous MLR combinations. However, HLA-G expression was not detected in all allogenic combinations, and differences existed in the HLA-G isoform profile. Interestingly, HLA-G expression was influenced by the specific MLR combinations.

A recent study found that HLA-G1 transfected antigen-presenting cells can inhibit the proliferation of CD4+ T cells, shed HLA-G1 molecules, induce CD4+ T cell anergy or at least long-term unresponsiveness and cause the differentiation of CD4+ T cells into suppressive cells (LeMaoult *et al*., 2004). So HLA-G-positive antigen-presenting cells may be directly involved in the suppression of immune responses and contribute to long-term immune escape or tolerance (LeMaoult *et al*., 2004).

HLA-G can bind peptides, as noted above, and a single study of HLA-G transgenetic mice has rendered it probable that HLA-G may be able to present peptides to the T-cell receptor, though this finding is controversial (Lenfant *et al*., 2003). Finally, HLA-G may also be implicated in cellular aggregation and cell adhesion (Odum *et al*., 1991).

HLA-G in human reproduction

HLA-G expression and function during implantation and pregnancy

Some studies have found HLA-G expression in blastocysts and maybe even in preimplantation blastocysts; however, this issue is still rather controversial (Jurisicova *et al*., 1996; Hiby *et al*., 1999; Fuzzi *et al*., 2002; van Lierop *et al*., 2002; Sher *et al*., 2004; Noci *et al*., 2005; Yie *et al*., 2005). One study detected HLA-G mRNA and protein expression in 40% of 2- to 16-cell stage preimplantation embryos with a total of 148 blastocysts investigated (Jurisicova *et al*., 1996). Expression of the *HLA-G* transcript was associated with an increased cleavage rate when compared with embryos lacking *HLA-G* mRNA. Another study of *HLA-G* mRNA in 108, day-3 preimplantation embryos from 25 couples, found that 44% were positive (Cao *et al*., 1999). On the other hand, Hiby *et al*. (1999) could not detect *HLA-G* mRNA in embryos from the 2-cell to the blastocyst stage; however, only 11 embryos were investigated. These findings should be considered in relation to more general studies, which find that the embryonic genome becomes activated by the 2-cell stage in mammals, and perhaps by the 4-cell stage in humans (Braude *et al*., 1988; Zeng and Schultz, 2005). In another study of human embryo culture supernatants from embryos at the 8-cell morula or beyond the blastocyst stage, no sHLA-G could be detected using three different ELISA set ups (van Lierop *et al*., 2002). However, it is not quite clear how many embryos were tested, and only pooled supernatants were studied. In line with the finding that only a fraction of preimplantation

embryos may express HLA-G, three independent groups have detected sHLA-G expression in the culture media of these in relation to IVF procedures after 46–72 h of culture before transfer. Noci *et al*. (2005) recorded the presence of sHLA-G in 36.2% of single-embryo cultures (N = 318) after 72 h of culture, while only 8.9% of the cultures were sHLA-G-positive after 48 h. In another study of 594 embryos from 201 women, the exact frequency of sHLA-G-'positive' single-embryo cultures was not specified; however, in 65% of these women at least one of the transferred embryos (mean transferred 3.0) was 'positive' (Sher *et al*., 2004). Caution should be exercised, because in the sHLA-G ELISA used in this study, an mAb MEM-G1 was directed against denaturated HLA-G. Noci *et al*. (2005) used an mAb against HLA-G in a β_2-microglobulin-associated form (HLA-G5/sHLA-G1). The frequencies of HLA-G-positive preimplantation embryos found by the two independent studies of Jurisicova *et al*. (1996) and Noci *et al*. (2005) are in agreement. Interestingly, the detection of sHLA-G in the culture media is associated with a higher success in IVF treatment, defined as the obtaining of a clinical pregnancy. But the expression of HLA-G is no guarantee of implantation, in that a large fraction of sHLA-G-positive IVF treatments fail. On the other hand, these studies found that all IVF treatments with sHLA-G-negative preimplantation embryo cultures ended unsuccessfully with no signs of implantation (Fuzzi *et al*., 2002; Sher *et al*., 2004; Noci *et al*., 2005). A recent study by Yie *et al*. (2005) further supports these findings. Of 386 embryo culture supernatants (with 2.9 embryos per culture), 69.9% were positive for sHLA-G after 72 h of culture. The embryos originated from 137 IVF cycles (corresponding to the same number of couples). Again, the live birth rate in women who had HLA-G-positive embryos transferred was significantly higher than that in women who had only HLA-G-negative embryos transferred (48.4 versus 17.1%; P = 0.0026). Furthermore, a significantly higher mean embryo cleavage rate was found in the HLA-G-positive group than in the HLA-G-negative group, in agreement with Jurisicova *et al*. (1996). Interestingly, in respect to the further discussion of HLA-G expression presented below, Yie *et al*. (2005) did observe pregnancy and live births in six of 37 HLA-G-negative IVF cycles studied. However, the rate of SA was higher in the HLA-G-negative group (25%) than in the HLA-G-positive group (11%). Finally, it is important to remember that the allocation of samples as either HLA-G 'positive' or HLA-G 'negative' may, in part, be influenced by the sensitivity of the specific sHLA-G assay. Another recent study by Sher *et al*. (2005) also found that when up to three sHLA-G-negative embryos were transferred, viable pregnancies are observed. However, this study only examined ICSI-derived embryos (n = 1404 from 482 women), finding a significant increase in the pregnancy rate (PR, a viable intrauterine pregnancy confirmed by ultrasound performed after the seventh week of gestation) and the implantation rate (IR, the number of viable clinical pregnancies as defined above divided by the number of embryos transferred) with the addition of each sHLA-G-positive embryo, regardless of the woman's age. When all of the embryos transferred were sHLA-G-negative, the PR and IR were 25 and 13% for women ≤38 years, respectively; however, when at least two embryos were sHLA-G-positive, PR and IR increased to 69 and 36%. In conclusion, the studies of IVF embryo cultures suggest that it may be necessary to monitor sHLA-G at 72 h after fertilization, for use in selecting optimal embryos for transfer. This may be in line with the fact that the activation of the human embryonic genome starts between the 4- and 8-cell stages, approximately 70 h after oocyte insemination (Tesarik *et al*., 1986; Braude *et al*., 1988).

It could be argued that the sHLA-G found in the culture media might originate from the follicular fluid (and thus from the woman), sticking to the oocyte and being released into the culture media. However, van Lierop *et al*. (2002) found follicular fluid to be negative for sHLA-G. More research is clearly needed to clarify this matter; however, irrespective of the origin of the sHLA-G, the clinical potential for improving IVF treatment and reducing multiple pregnancies is intriguing.

These studies of HLA-G expression in and by blastocysts should also be considered in relation to the fact that expression of the full-length HLA-G1 and -G5 isoforms may not be necessary for implantation and a successful pregnancy. Homozygous individuals for the frame-shift mutation in exon 3 of the *HLA-G* gene (the *G*0105N* allele) have been described (Ober *et al*., 1998; Casro *et al*., 2000). However, these individuals will be able to express shortened HLA-G isoforms in which exon 3/the α2 domain is spliced out. The problem is that, as outlined previously, different studies are contradictory regarding the functionality and expression on the cell surface of these isoforms. However, HLA-G2 may be secreted, and there is some evidence for functionality linked to this isoform, possibly through the α1 domain (Hunt *et al*., 2000; Yan and Fan, 2005). Interestingly, the HLA-G*0105N allele is also able to induce cell surface expression of HLA-E molecules capable of interacting with CD94/NKG2A and keeping up this important function (Sala *et al*., 2004). Finally, a recent study by Le Discorde *et al*. (2005) claimed that cloned genomic *G*0105N* DNA transfected into an HLA-class I-positive human cell line protected the cells against NK cell lysis. HLA-G proteins were also detected in the cells.

A wide range of studies has shown that HLA-G is expressed on trophoblast cells from early in the first trimester and throughout the rest of the pregnancy; HLA-G may thus already be expressed by the blastocyst stage, according to the discussion above. There have been some divergent results regarding the pattern of HLA-G expression in different subtypes of trophoblast cells. However, the consensus seems to be that the invasive, extravillous cytotrophoblast cells have the strongest expression of membrane-bound HLA-G (Kovats *et al*., 1990; Le Bouteiller and Blaschitz, 1999; Morales *et al*., 2003). The endovascular trophoblast cells also express membrane-bound HLA-G (Proll *et al*., 1999). The syncytiotrophoblast only expresses sHLA-G, and it seems that all other trophoblast subtypes also express sHLA-G (Kovats *et al*., 1990; Ishitani *et al*., 2003).

In the light of the described functions of HLA-G, adequate expression of HLA-G at the feto–placental contact zone is believed to be important for the pregnant woman's acceptance of the semiallogenic fetus. However, given the existence of homozygous individuals for the HLA-G-null mutation, it is interesting to consider which of the membrane-bound or soluble isoforms of HLA-G may be most functionally important. There are no clear answers to this question yet; furthermore, the three non-classical HLA class Ib genes may be able to substitute for each other functionally during human reproduction.

HLA-G genetics and expression in RSA and assisted human reproduction

The level of sHLA-G in maternal blood was, in a study of IVF pregnancies, associated with a risk of SA during the first trimester. sHLA-G levels in the serum samples of 20 women experiencing early SAs were significantly reduced during the first 9 weeks of gestation, compared with those of 37 women with intact pregnancies (Pfeiffer *et al*., 2000). Even the mean pre-ovulatory serum sHLA-G concentration in the 20 women experiencing early SAs (25.9 ± 3.9 SEM ng/ml) was significantly lower than the mean level in women with successful pregnancies (35.9 ± 3.3 ng/ml).

Later in pregnancy during the third trimester, the sHLA-G concentrations in women with placental abruption have been reported to be more than three times lower than in women with uncomplicated pregnancies. Women with sHLA-G levels below 9.95 ng/ml have a relative risk of 7.1 for the development of placental abruption during the further course of pregnancy (Steinborn *et al*., 2003). Interestingly, maternal sHLA-G plasma levels do not change substantially during the normal course of pregnancy. Furthermore, the sHLA-G levels of non-pregnant and pregnant women seem to be very similar, even taking different sHLA-G assays into account (Puppo *et al*., 1999; Rebmann *et al*., 1999; Pfeiffer *et al*., 2000; Hviid *et al*., 2004b). Therefore, it does not appear that much sHLA-G from trophoblast cells in the placenta reach the maternal blood. Moreover, a substantial part of the sHLA-G detected in maternal circulation may be produced by immunocompetent cells of the mother (Steinborn *et al*., 2003). However, not much is known about changes in HLA-G expression on the part of the immune cells of the mother during pregnancy.

RSA, defined as ≥3 SA, have been shown to be associated with the woman being homozygous for the +14 bp sequence in the 3′UTR end of the *HLA-G* gene in a study of 61 Danish RSA women and 93 control women with ≥2 uncomplicated pregnancies [odds ratio (OR) = 2.7; 95% confidence interval (CI) = 1.1–6.5; $P = 0.03$] (Hviid *et al*., 2002, 2004a). Interestingly, in a pilot study of 14 women with no pregnancy after ≥3 IVF treatments (group A) compared with 15 women with a successful twin pregnancy after the first IVF (group B), four women had a *+14/+14 bp HLA-G* genotype in group A compared with none in group B (Hviid *et al*., 2004a). However, a study of 120 Indian RSA (≥3 SA) and 120 control women (≥3 live births) found a significant increase in the number of heterozygotes for the *14 bp HLA-G* polymorphism in the RSA women versus the control women (Abbas *et al*., 2004; Tripathi *et al*., 2004). So far, there is no clear explanation of this discrepancy between the two studies. However, in Tripathi *et al*. (2004), 300 women were initially selected for inclusion, but only 120 of them gave informed consent to participate, and it is not clear whether these 120 women were truly representative of the study population as a whole. There is no obvious functional explanation to why *HLA-G 14 bp* heterozygosity should be associated with RSA. In contrast, HLA-G5/sHLA-G1 have not been detected in serum form *+14/+14 bp* homozygotes in two studies (Hviid *et al*., 2004b; Rizzo *et al*., 2005a).

Several other studies of *HLA-G* polymorphism in RSA have been performed (Tables III and IV). This is also interesting in relation to the study of IVF pregnancies, where the pre-ovulatory serum sHLA-G concentration in women with early abortion was significantly reduced compared with women with intact pregnancy. Two studies have investigated a hypothesis of HLA-G histo-incompatibility or sharing of alleles between the woman and her partner (and thus the fetus) but found no support for this (Karhukorpi *et al*., 1997; Hviid *et al*., 2002). Yamashita *et al*. (1999) studied an *HLA-G* polymorphism in intron 4 in a small study of 20 RSA couples but found no association. A study of 18 SNPs in the 5′URR of the *HLA-G* gene in a cohort of 42 Hutterite couples, found an increased risk of abortion in couples in which both members carried a *–725G* allele (OR = 2.8; 95% CI = 1.1–7.1) (Ober *et al*., 2003). This polymorphism may be associated with low levels of sHLA-G or undetectable HLA-G5/sHLA-G1; however, further study is needed (Hviid *et al*., 2004b). One study has reported the *HLA-G*010103* and *G*0105N* alleles to be associated with RSA (Pfeiffer *et al*., 2001), while another study found *G*0104* and *G*0105N* to be associated with an increased risk of abortion (Aldrich *et al*., 2001). Another study by Hviid *et al*. (2002) of 61 RSA couples (≥3 SA) and 47 fertile control couples, investigating polymorphisms in exons 2–4 (and 8), found no statistically significant differences in the distribution of *HLA-G* alleles between the two groups. However, 15% of the RSA women carried the *HLA-G*0106* allele compared with 2% of the control women.

Thus, it is not possible to discern any real consensus regarding the importance of *HLA-G* polymorphisms in RSA, according to published case–control studies (Table III). However, some studies suggest that *HLA-G* alleles, including the 14 bp sequence in the 3′UTR part, might be associated with a risk of RSA. The few studies finding decreased levels of sHLA-G in women with SA, as well as the described associations between *HLA-G* polymorphism and blood levels of sHLA-G, may support this possibility. So, a multicenter study using the same *HLA-G* genotyping methods and several sHLA-G assays, together with identical design and inclusion criteria and consideration of ethnic differences, would be most welcome.

Two studies of HLA-G expression applying immunohistochemistry to tissue sections from abortions have also reached opposite conclusions (Table IV). One found decreased expression of HLA-G in RSA tissue (Emmer *et al*., 2002), while another found no differences between decidual/trophoblast tissue sections from RSA cases with normal chromosomes compared with those with fetal trisomy 16 (Patel *et al*., 2003). However, the choice of control group in the latter study seems not quite appropriate; in such studies of aborted tissue, it is also important to consider whether the changes are of aetiological importance or are a mere consequence of some primary abnormalities.

HLA-G genetics and expression in pre-eclampsia

Pre-eclampsia is a disorder that evolves in the second half of pregnancy, affecting 2–7% of all pregnancies with varying severity. The pregnant woman develops hypertension, proteinuria and often oedema, whereas the fetus' condition may also be compromised by intrauterine growth retardation and reduced placental blood flow. The aetiology probably involves a combination of genetic predisposition with maternal and fetal contributions and environmental factors. One prominent hypothesis suggests that immune maladaptation is involved in pre-eclampsia.

Two independent studies have reported a significantly increased risk of pre-eclampsia in primiparas, when the fetus carries a *+14/+14 HLA-G* genotype (O'Brien *et al*., 2001; Hylenius *et al*., 2004)

Table III. Published studies of *human leukocyte antigen-G (HLA-G)* polymorphism in recurrent spontaneous abortions (RSA)

Study (listed by type and chronology)	Type of study	Technical comments	Cases	Controls	Results
Penzes *et al*. (1999)	Case–control	PCR–RFLP of exons 2 and 3; low resolution of alleles	21 RSA couples	72 healthy, unrelated individuals	Negative. However, a trend towards a higher frequency of the *G*01012* allele in the controls
Yamashita *et al*. (1999)	Case–control	PCR–SSCP of exons 2, 3 and 4. DNA sequencing of intron 4	20 RSA couples	54 healthy fertile controls (27 females/ 27 males)	Negative; no implication of polymorphism in intron 4
Pfeiffer *et al*. (2001)	Case–control	DNA sequencing of exons 2 and 3	78 RSA women (≥3 abortions; 28% secondary aborters)	52 women (≥1 successful pregnancies)	*G*010103* and *G*0105N* associated with RSA
Aldrich *et al*. (2001)	Cohort	PCR–SSOP, exons 2 and 3	113 couples (≥3 abortions; one live born child)		*G*0104* or *G*0105N* in either partner associated with increased risk for abortion
Ober *et al*. (2003)	Cohort (15 years)	DNA sequencing of 5′URR; 18 SNPs	42 Hutterite non-RSA women		Increased risk for abortion in couples both members of which carry a *–725G* allele (OR = 2.7; 95% CI = 1.1–7.1)
Hviid *et al*. (2002, 2004a)	Case–control	DNA sequencing of exons 2 and 3, polymorphisms in exons 4 and 8	61 RSA couples (≥3 abortions; 38% secondary aborters)	47 fertile couples/93 fertile women (≥2 normal pregnancies)	*+14/+14 bp HLA-G* genotype of female associated with RSA (OR = 2.7; 95%CI = 1.1–6.5). More RSA women carried the *G*0106* allele (15%) compared with controls (2%) (ns)
Tripathi *et al*. (2004)	Case–control	ACLA/lupus anticoagulant positive RSA women? 300 included; 180 lost? PCR–SSOP analysis of exons 2 and 3; typing of *G*0106*?	120 RSA women (≥3 abortions; primary aborters)	120 fertile women (≥3 live births)	*–14/+14 bp HLA-G* genotype of female associated with RSA
Abbas *et al*. (2004)	Case–control				Higher frequency of *G*010103* in RSA women (ns)

5′URR, 5′-upstream regulatory region; ACLA, anticardiolipin antibodies; CI, confidence interval; ns, not significant; OR, odds ratio; PCR–RFLP, PCR–restriction fragment length polymorphism; PCR–SSCP, PCR–single-strand conformation polymorphism; PCR–SSOP, PCR–sequence specific oligonucleotide probe; SNPs, single-nucleotide polymorphisms.

(Tables V and VI). The largest study by Hylenius *et al.* (2004) included 57 family triads (offspring and parents) with severe pre-eclampsia/eclampsia and 98 control family triads. Polymorphisms in exons 2–4 and 8 were investigated in the 465 samples. No differences were observed when the distributions of the *HLA-G* alleles in the pre-eclampsia and control triads were compared across the entire data set. However, a significant overrepresentation of the *+14/+14 bp HLA-G* genotype was found in the pre-eclamptic offspring in primiparas (40 with pre-eclampsia and 70 controls) with an OR of 5.6 (95% CI = 1.8–17.3; $P = 0.002$). For the combined data regarding both primiparas and multiparas, this was only of borderline significance. Furthermore, the results indicated that combined mother–child *HLA-G* genotypes might influence the risk of developing pre-eclampsia, because a trend towards more *+14 bp HLA-G* alleles was observed in combined mother–child genotypes in pre-eclamptic cases; however, no significant differences in the *14 bp* genotype distributions were found between women with pre-eclampsia and control women, as some studies have observed in cases of RSA. The *–14 bp polymorphism HLA-G* allele was also more often inherited from the father in heterozygotic fetuses in pregnancies not complicated with pre-eclampsia, and the opposite was observed for the *+14-HLA-G* allele in the pre-eclamptic cases (Hylenius *et al*., 2004). In the family triad case–control study, no evidence was found of HLA-G antigen incompatibility between the pregnant woman and her fetus in pre-eclampsia (Hylenius *et al*., 2004). Previous studies had reached other conclusions regarding the *14 bp HLA-G* polymorphism and pre-eclampsia. However, one study examined only 13 individuals for the effect of fetally expressed HLA-G on pre-eclampsia susceptibility with no well-defined control group and found no association (Humphrey *et al*., 1995). Another study of 65 pre-eclampsia and 74 control offspring from primigravidae found a

Table IV. Published studies of human leukocyte antigen-G (HLA-G) histoincompatibility/sharing in couples experiencing recurrent spontaneous abortions (RSA), of HLA-G expression in spontaneous abortions and of *HLA-G* polymorphism in IVF women

Study (listed by type and chronology)	Type of study	Technical comments	Cases	Controls	Results
HLA-G polymorphism and HLA-G histo-incompatibility/ sharing					
Karhukorpi *et al*. (1997)	Case–control	PCR–RFLP; low resolution of alleles	38 RSA couples	26 random couples	No support for parental *HLA-G* sharing
Hviid *et al*. (2002)	Case–control	DNA sequencing of exons 2 and 3, polymorphisms in exons 4 and 8	61 RSA couples (≥3 abortions)	47 fertile couples (≥2 normal pregnancies)	No support for parental HLA-G histo-incompatibility
HLA-G expression					
Hamai *et al*. (1998)	Cytokine levels in PBMC co-cultured with HLA-G-transfected cells		PBMC from 11 RSA women (≥3 abortions)	PBMC from seven nulligravidae and six fertile women	IL-1β increased in RSA and decreased in controls. IL-3 unchanged in RSA and increased in controls
Emmer *et al*. (2002)	Case–control	Immunohistochemistry (mAb 4H84)	Trophoblastic tissue samples from nine RSA women	Decidual biopsies from nine women (part of routine procedure of chorionic villus biopsy)	Decreased expression of HLA-G in RSA tissue
Patel *et al*. (2003)	Case–control	Immunohistochemistry (mAb 4H84)	Decidual/trophoblast tissue sections from 14 RSA women (≥3 abortions) and normal chromosomes	Decidual/trophoblast tissue sections from 14 RSA women (≥3 abortions) and fetal trisomy 16?	No significant quantitative differences in levels of HLA-G between the two groups
HLA-G polymorphism and IVF					
Hviid *et al*. (2004a)	Case–control	*14 bp* polymorphism in exon 8 of the *HLA-G* gene	14 IVF women; no pregnancy after ≥3 IVF treatments	15 IVF women; successful twin pregnancy after first IVF	*+14/+14 bp HLA-G* genotype associated with unsuccessful IVF

IL, interleukin; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; PCR–RFLP, PCR–restriction fragment length polymorphism.

high frequency of the *–14/+14 bp HLA-G* genotype in pre-eclamptic offspring; however, this was not significant when compared with the distribution in the controls (Bermingham *et al*., 2000). Further studies of the *14 bp* polymorphism in pre-eclampsia are clearly needed. Aldrich *et al*. (2000) found no association between the *HLA-G*0105N* allele and pre-eclampsia in African Americans, and this was confirmed in Caucasians by Hylenius *et al*. (2004). Several studies have found an aberrant or reduced expression of both *HLA-G* mRNA and protein in pre-eclamptic compared with control placentas (Hara *et al*., 1996; Lim *et al*., 1997; Goldman-Wohl *et al*., 2000; O'Brien *et al*., 2001; Yie *et al*., 2004). Again, it is important to consider cause and effect. The abnormal HLA-G expression in pre-eclampsia may just be a consequence of some more profound and general pathology. However, hypoxia, e.g., did not seem to reduce HLA-G expression on extravillous cytotrophoblasts in one experimental set up (Nagamatsu *et al*., 2004). The associations between *HLA-G* genetics and pre-eclampsia may also support a pathogenetic role of HLA-G in pre-eclampsia. One study of 20 women with pre-eclampsia has also found significantly reduced levels of sHLA-G in maternal serum compared with that of 14 controls (Yie *et al*., 2004). However, another study of 55 pre-eclamptic cases and 66 controls found no difference in sHLA-G blood concentrations in the third trimester; however, this was partly contradicted in the same report, by a finding of five cases of pre-eclampsia in 54 pregnant women with abnormal Doppler findings, all of whom had low sHLA-G levels in the second trimester (Steinborn *et al*., 2003). One study has found a significant increase in the *HLA-G5* mRNA isoform in 12 pre-eclamptic placentas compared with that in placentas from 15 uncomplicated pregnancies using a semiquantitative RT–PCR analysis (Emmer *et al*., 2004). The findings of this study seem somewhat to contradict those of studies of sHLA-G protein concentrations.

It can be speculated that the *+14/+14 bp HLA-G* genotype may predispose to pre-eclampsia because of an aberrant, possibly reduced HLA-G expression in the placenta (O'Brien *et al*., 2001; Hviid *et al*., 2003; Hylenius *et al*., 2004). It is believed that the pathogenesis of pre-eclampsia is characterized by an excessive maternal inflammatory response to pregnancy (Redman *et al*., 1999) and that pre-eclampsia is characterized by a Th1 response, in contrast to an uncomplicated pregnancy, which is more likely to display a Th2 response (Darmochwal-Kolarz *et al*., 1999). However, whether the changes in cytokine expression are a direct aetiological factor or just a consequence of some primary pathological changes is not known. Furthermore, the concept of Th1/Th2

Table V. Published studies of human leukocyte antigen-G (HLA-G) expression in placenta and in serum in pre-eclampsia

Study (listed by type and chronology)	Type of study	Technical comments	Cases	Controls	Results
HLA-G mRNA expression in placenta					
Colbern *et al*. (1994)	Case–control. mRNA/ ribonuclease protection assay		23 term placental samples (16 primiparas, 7 multiparas, 1 chronic hypertension)	18 term placental samples from normal pregnancies	To some extent inconclusive. Reduced *HLA-G* mRNA in pre-eclampsia; however, the decrease appeared to be related to reduced numbers of trophoblast in pre-eclamptic placentas from primiparas
Lim *et al*. (1997)	Case–control. *In vitro* trophoblast cultures, mRNA/protein		9 (24–38 GW)	8 (22–38 GW)	Trophoblast cells from pre-eclamptic placentas failed to up-regulate HLA-G protein and mRNA
Goldman-Wohl *et al*. (2000)	Case–control. *In situ* hybridization		10 placental samples (3rd trimester)	12 placental samples (3rd trimester)	In 9/10 pre-eclamptic placentae HLA-G expression was absent or reduced
Emmer *et al*. (2004)	Case–control. mRNA/ RT–PCR	Semiquantitative RT–PCR	12 placental samples	15 placental samples	Increased frequency of *HLA-G5* mRNA in pre-eclamptic placentas
Hviid *et al*. (2004c)	Case–control. RNA/ microarray (immuno-histochemistry)		Nine placental samples (in three pools) (3rd trimester)	Nine placental samples (in three pools) (3rd trimester)	Trend towards decreased *HLA-G* mRNA expression in pre-eclamptic placentas. This study also addressed HLA-G expression in placenta by immunohistochemistry in relation to HLA-G genotype but was inconclusive
HLA-G protein expression in placenta					
Hara *et al*. (1996)	Case–control	Immunohistochemistry mAb: 87G (and CAM5.2, anti-cytokeratine)	Five placental samples	Seven placental samples	Extravillous trophoblast insularly derived of HLA-G in pre-eclamptic samples
Soluble HLA-G expression in serum					
Steinborn *et al*. (2003)	Case–control	sHLA-G assay: no HLA-G mAb	55 (3rd trimester), 5 (2nd trimester; abnormal Doppler findings)	66 (3rd trimester), 49 (2nd trimester; abnormal Doppler findings)	No difference in serum sHLA-G in 3rd trimester. Possible difference in 2nd trimester
Yie *et al*. (2004)	Case–control. Both sHLA-G in serum and placenta	sHLA-G assay: mAb 4H84 and mAb 3C/64	20 (3rd trimester)	14 (3rd trimester)	Significant reduced levels of sHLA-G in serum and placenta in pre-eclampsia

GW, weeks of gestation; mAb, monoclonal antibody.

responses in pregnancy may itself be questioned (Chaouat *et al*., 2004).

The possible implication of *HLA-G* polymorphism and aberrant HLA-G expression has been described in both pre-eclampsia and RSA (Tables IV–VI). In relation to this, hypotheses involving possible links between risks of SA, pre-eclampsia and intrauterine growth retardation have been put forward. It has been proposed that some kind of immunological malfunction may be involved in at least some groups of patients with these complications (Labarrere and Althabe, 1986; Christiansen *et al*., 1990a; Yasuda *et al*., 1993). From an epidemiological viewpoint it is interesting that a recent large study of almost 45 000 pregnancies/births included in the Danish National Birth Cohort showed that subfecundity is a correlate of pre-eclampsia (Basso *et al*., 2003). Fecundity was measured as the number of cycles required for a couple to conceive from the start of unprotected intercourse. Again, this result indicates possible unifying pathological mechanisms in at least some cases of these two complications of human reproduction. In addition to an association between HLA-G and the two conditions, associations between *HLA class II* genes and RSA and pre-eclampsia have also been observed. However, more studies are needed further to elucidate whether *HLA class Ib* genes, and

Table VI. Published studies of *human leukocyte antigen-G (HLA-G)* polymorphism in pre-eclampsia

Study (listed by type and chronology)	Type of study	Technical comments	Cases	Controls	Results
Humphrey *et al*. (1995)	Case–control/ family study	*14 bp* polymorphism in exon 8	13 individuals for study of fetally expressed HLA-G	Not well defined	No association (*14 bp*)
Bermingham *et al*. (2000)	Case–control	*14 bp* polymorphism in exon 8, SNP codon 93	65 offspring	74 offspring	No association (*14 bp/SNP codon 93*)
Aldrich *et al*. (2000)	Case–control	*G*0105N*	57 placental samples	36 placental samples	No association (*G*0105N*)
Hviid *et al*. (2001)	Case–control	Codon 258 polymorphism defining *G*0106*	31 women with a history of pre-eclampsia and their partners	22 fertile women with uncomplicated pregnancies, 22 fertile men	No association (*G*0106*)
O'Brien *et al*. (2001)	Case–control	*14 bp* polymorphism, SNP codon 93. *HLA-G* mRNA expression	Seven placental samples	11 placental samples	*+14/+14 bp HLA-G* genotype associated with pre-eclampsia. Reduced *HLA-G* mRNA expression in pre-eclamptic placentas. Deficit of *HLA-G3* mRNA in pre-eclampsia
Carreiras *et al*. (2002)	Case–control	PCR–SSOP	25 mother/child pairs	22 mother/child pairs	*G*0104, HCMV* sequences
Hylenius *et al*. (2004)	Case–control triad, family triads	DNA sequencing of exons 2 and 3, polymorphisms in exons 4 and 8	40 primipara, 17 multipara	70 primiparas, 28 multiparas	Primipara: (i) association between *+14/+14 bp HLA-G* genotype in offspring and pre-eclampsia (OR = 5.6; 95% CI = 1.8–17.3); (ii) significant differences in transmission of *14 bp* alleles from father to offspring; (iii) no associations with *G*0105N* or *G*0104*; (iv) no HLA-G histo-incompatibility between mother and fetus

CI, confidence interval; HCMV, human cytomegalovirus; OR, odds ratio; PCR–SSOP, PCR–sequence specific oligonucleotide probe; SNP, single-nucleotide polymorphism.

possibly other *MHC* genes, are involved in the aetiology and pathogenesis of RSA, pre-eclampsia and associated intrauterine growth retardation.

In conclusion, it is of specific clinical relevance that several studies may indicate that measurements of sHLA-G in maternal plasma/serum samples may be of value in monitoring pregnancies with an increased risk of complications, such as early SA, pre-eclampsia and placental abruption. However, more evidence is clearly needed and should be obtained through large, systematic and preferably prospective clinical studies.

HLA-G *polymorphism and variation in birth and placental weight*

HLA-G is expressed extensively in the placenta; there seem to be associations between HLA-G expression and cytokine secretion, and HLA-G expression is associated with HLA-G polymorphism. In this context, it is interesting that cytokines have been shown to influence placental development, growth and invasiveness (Roth and Fisher, 1999; Ingman and Robertson, 2002). In that case, it can be speculated that HLA-G polymorphism may be associated with clinical parameters of pregnancy, such as placental and fetal weight. However, so far only one study has investigated a possible association between HLA-G polymorphism and placental and fetal weight. Hviid (2004) investigated fetal and placental weight in 47 pregnancies complicated by severe pre-eclampsia and 87 pregnancies without this complication; fetal weight was considered in relation to the mean expected weight at the time of delivery. For the analysis of data regarding placental weight, only pregnancies terminated after 38 weeks of gestation were included. It was found that the *HLA-G* genotype homozygous for the presence of the *14 bp* sequence polymorphism was significantly associated with increased birth weight in relation to gestational age and with increased placental weight at birth (>38 weeks of gestation). There was also a slightly higher placental ratio (placental weight-to-birth weight ratio) in offspring with the *+14/+14 bp* genotype, though not significantly so (Hviid, 2004). An increased placental ratio has been proposed to represent a disproportionately heavier placenta and adaptive placental hypertrophy in response to fetal malnutrition and growth restriction. That *HLA-G* polymorphism is associated with fetal and placental weight fits well with reports concerning the mouse Qa-2 MHC class Ib protein, which may be an HLA-G homolog; Qa-2 expression is also associated with birth weight (Comiskey *et al*., 2003) (Table VII). These at first unexpected findings regarding HLA-G must, however, be reproduced in independent and larger studies. If HLA-G expression is important for placental and fetal development, then it would be expected that *reduced* fetal and placental weight would be associated with the *+14/+14 bp HLA-G* genotype. The +14 bp sequence has been associated with greater unstability of the

Table VII. Comparisons of different characteristics of *major histocompatibility complex (MHC) class Ib* genes expressed in the placenta in rodents, monkeys and humans

Species	Mouse	Rat	Rhesus monkey	Baboon	Human
Characteristic	Qa-2	RT1-E MHC class Ib	Mamu-AG	Paan-AG MHC class Ib	HLA-G
Alternative mRNA splicing	Yes	Yes	Yes	Yes	Yes
Limited polymorphism	?	?	Yes	Yes	Yes
Membrane-bound isoform	Yes	Yes	Yes	Yes	Yes
Soluble isoform	Yes	Yes	Yes	Yes	Yes
Short tail	Yes	No	Yes	Yes	Yes
Peptide binding	Yes	?	?	?	Yes
Expression by preimplantation embryos	Yes	?	?	?	Yes*
Expression in placenta	Yes	Yes	Yes	Yes	Yes
Inhibition of natural killer cell-mediated lysis	?	Possible	?	?	Yes
Influences cytokine production	?	?	?	?	Yes
Increases preimplantation growth rate/division	Yes	?	?	?	Yes*
Enhances fetal survival	Yes	?	?	?	?
Birth weight	Yes	?	?	?	Yes*
Placental weight	?	?	?	?	Yes*

References: Arnaiz-Villena *et al*. (1999); Boyson *et al*. (1999); Slukvin *et al*. (1999, 2000); Castro *et al*. (2000b); Solier *et al*. (2001b); Langat *et al*. (2002); Langat and Hunt, (2002); Ryan *et al*. (2002); Comiskey *et al*. (2003).
*Regarding human leukocyte antigen-G (HLA-G): controversial issue or only reported in one study so far.

transcripts and unique *HLA-G* mRNA isoforms (Hviid *et al*., 2003; Rousseau *et al*., 2003), and *+14/+14 bp* homozygocity of the fetus is associated with the risk of pre-eclampsia (O'Brien *et al*., 2001; Hylenius *et al*., 2004). However, the matter may be more varied. First, *MHC-G* genes of other primates include the 14 bp sequence in exon 8, so that the *+14 bp HLA-G* allele is probably the oldest allele (Castro *et al*., 2000a); on the other hand, the *G*0101* alleles without the 14 bp sequence are the most common *HLA-G* alleles (Table II). This indicates some functional significance or advantage associated with this human-specific polymorphism (or other polymorphisms in linkage disequilibrium with it). Second, sMHC-G isoforms have so far only been reported in humans (Slukvin *et al*., 2000). Therefore, any functional importance of the *14 bp deletion/insertion HLA-G* polymorphism may be related to differences in sHLA-G expression. This may be accomplished by differences in cytokine profiles related to sHLA-G expression, as discussed previously. In a model system of LPS-activated PBMC, the *+14/+14 bp HLA-G* genotype was associated with significantly higher levels of IL-10 secretion than the two other genotypes were (Rizzo *et al*., 2005a). Interestingly, in relation to pre-eclampsia, this may be in line with investigations of IL-10 as an autocrine inhibitor of human placental cytotrophoblast matrix metalloproteinase-9 (MMP-9) production and invasion (Roth and Fisher, 1999). Although some reports are contradictory, it seems that IL-10 levels can be markedly elevated in severe pre-eclampsia *in vivo*, and in this condition abnormally shallow cytotrophoblast invasion of the uterine wall is often seen (Roth and Fisher, 1999). The literature is contradictory regarding IL-10 expression during pre-eclampsia. Some studies have found a reduced amount of IL-10 in the placentas and sera of pre-eclamptic pregnancies compared with those of controls, in line with the rather simplistic concept of a Th2/Th1 bias (Hennessy *et al*., 1999; Darmochwal-Kolarz *et al*., 2002; Rein *et al*., 2003). However, a substantial portion of the published studies find no differences in IL-10 expression between pre-eclamptic cases and controls (Eneroth *et al*., 1998; Gratacos *et al*., 1998) or they find increased concentrations of IL-10 in the pre-eclamptic samples (Rinehart *et al*., 1999; Benian *et al*., 2002). Actually, the studies in which IL-10 expression was reported to decrease in pre-eclampsia were mostly based on immunohistochemistry and flow cytometry, which are generally semiquantitative techniques. Interestingly, a recent study also found that an IL-10 promotor polymorphism associated with *reduced* IL-10 production protects against development of pre-eclampsia (de Groot *et al*., 2004). In support of this, another recent study used DNA microarray technique to examine gene expression in placental tissue from cases of severe early onset pre-eclampsia; it found up-regulation of both Th1 cytokines and IL-10, and the authors hypothesized that there might be secretion of Th2-type cytokines in pre-eclamptic placentas, which may down-regulate harmful Th1 cytokines (Heikkilä *et al*., 2005). It can be hypothesized that the *+14 bp HLA-G* allele is associated with increased fetal and placental weight, partly because of locally elevated levels of IL-10, a cytokine believed to support pregnancy. However, it can furthermore be hypothesized that because of a possible initially aberrant HLA-G (or sHLA-G) expression, the *+14/+14 bp HLA-G* genotype is also associated with an increased risk of pre-eclampsia. There could be several such mechanisms, which would explain the relatively high incidence of pre-eclampsia despite its generally negative impact on both maternal and fetal health. In this regard, it is interesting that there is a significant difference between the inheritance of the *14 bp* polymorphism from the father to the fetus in cases of primiparas with severe pre-eclampsia and in controls (Hylenius *et al*., 2004). This observation may seem somewhat in line with a hypothesized conflict between fetal/paternal and maternal interests/genes, as proposed by Moore and Haig (1991); however, this would not involve genomic imprinting. A further discussion

of this matter follows later. In conclusion, most published studies support a reduced or aberrant expression of HLA-G in the placenta and in maternal serum during pre-eclampsia. *HLA-G* polymorphism may be associated with these observed differences in HLA-G expression between controls and cases of pre-eclampsia.

HLA-G and the MHC—broad perspectives

Linkage between HLA class II *genes and* HLA-G

Strong linkage disequilibrium between different gene loci in the *HLA* region has been extensively documented. Regarding the *HLA-G* locus, several studies have reported associations between specific *HLA-G* and *HLA-A* alleles (Morales *et al*., 1993; Karhukorpi *et al*., 1996; Ober *et al*., 1996; Yamashita *et al*., 1996; Suarez *et al*., 1997). A recent analysis by Hviid and Christiansen (2005) of *HLA class II* and *HLA-G* genotypes in couples with RSA and in normal fertile couples showed that the alleles in the haplotype *HLA-DRB1*03.DQA1*05.DQB1*02.G*010102* were in strong linkage disequilibrium. The linkage disequilibrium between the *HLA-DR* and the *-DQ* alleles is well known; however, the linkage with the *HLA-G*010102* allele is interesting because this *HLA-G* allele includes the 14 bp sequence. It also seems that the *HLA-DR3.G*010102* haplotype, and the *HLA-DR1.G*010102* and *HLA-DR3.G*010102* haplotypes analysed in combination, are stronger risk factors for RSA than are either the *HLA-DR3* and *-DR1* alleles or the *HLA-G*010102* allele/*14 bp HLA-G* polymorphism independently (Hviid and Christiansen, 2005). It can be speculated that a candidate gene for susceptibility to RSA may be in linkage disequilibrium with this *HLA* haplotype.

A role for HLA-G in organ transplantation, autoimmune disease and inflammatory responses?

Recently, the expression of sHLA-G in blood and in heart and liver/kidney grafts has been associated with significantly better prognosis and fewer rejection episodes (Lila *et al*., 2002; Creput *et al*., 2003b). So after all, perhaps some of the same immunological mechanisms are of significance in generating tolerance in both pregnancy and organ transplantation. Further investigation of HLA-G as a possible clinical marker for the prognosis of certain organ transplantations, and possibly even as an immunosuppressive or tolerance-inducing treatment, would be very interesting. Notably, one study has found that the severity of liver transplantation rejection is associated with a recipient *HLA* haplotype, including *HLA-A1*, *-B8*, *-DR3* and *-DQ2* (*DQA1*0502.DQB1*0201*). The *HLA-G*010102* allele is in linkage disequilibrium with this haplotype, as has been described (Hviid and Christiansen, 2005), and HLA-G expression during transplantation may be partly genetically determined; however, further research must be awaited to confirm this possibility.

HLA is associated with autoimmune disease. The described *HLA* haplotype, which includes *HLA-A1*, *-B8*, *DR3* and *DQ2* (*DQA1*0502.DQB1*0201*), is associated with a range of immunopathological diseases, such as type I diabetes, systemic lupus erythematosus (SLE), dermatitis herpetiformis and myastenia gravis (Svejgaard *et al*., 1983). Certain autoimmune diseases of women often remit during pregnancy, while still others deteriorate. Women with rheumatoid arthritis (RA) often see their symptoms improve during pregnancy (Nelson *et al*., 1993). Further study of HLA-G about these phenomena could be useful, as HLA-G is a molecule potentially involved in immune modulation and suppression (LeMaoult *et al*., 2004).

During uncomplicated pregnancy, the immune system seems to be dominated by a Th2 response. Interestingly, RA often remits during pregnancy, as described above, and RA is an autoimmune disease involving a Th1 response (Ostensen and Villiger, 2002). However, RA patients have an increased risk of developing pre-eclampsia (Wolfberg *et al*., 2004). HLA-G could be involved in the changes in cytokine profiles, as shown in *in vitro* experiments. On the other hand, a Th2-mediated autoimmune disease such as SLE often remains unaltered or even deteriorates during pregnancy (Buyon, 1998). IL-10 has been suggested as a key cytokine in these changes (Huizinga *et al*., 1999). Preliminary clinical trials have shown that administration of IL-10 reduces disease activity in RA, and increased levels of IL-10 have been found in SLE patients. These observations also support the finding of high levels of IL-10, at least locally at the feto–maternal interface as discussed earlier.

Finally, and very notably, a recent candidate study has identified HLA-G as a possible asthma-susceptibility gene (Nicolae *et al*., 2005; Rizzo *et al*., 2005b).

Non-classical MHC class Ib *genes from an evolutionary perspective*

In mice, a non-classical class Ib MHC protein, Qa-2, the so-called Ped phenotype, has been described and may be a functional homolog of HLA-G (Table VII). Qa-2-positive and -negative mice exist. Apparently, two genes, *Q7* and *Q9*, have been identified as contributing to the Ped phenotype (Cao *et al*., 1999; Comiskey *et al*., 2003). However, the genetics underlying the Qa-2 protein is not fully elucidated. There seems to be a reproductive advantage for Qa-2-positive mice (Comiskey *et al*., 2003) (Table VII). Furthermore, in rats, transcripts for a soluble form of the RT1-E MHC class Ib molecule have been detected in placenta, and this molecule could, like HLA-E and -G, play a regulatory role in NK cell function (Solier *et al*., 2001b).

In non-human primates, expression of a *Mamu-AG* gene with a soluble isoform in the placenta of the rhesus monkey has been described (Slukvin *et al*., 1999; Ryan *et al*., 2002), and in the baboon placental expression of soluble and membrane-bound Paan-AG MHC class Ib proteins has been reported (Langat *et al*., 2002). These observations in rodents, monkeys and humans support the concept of a conserved function of MHC class Ib molecules in the placenta and during pregnancy (Table VII).

Possible importance of HLA-G polymorphism in human MHC diversity?

Studies in both mice and humans indicate that MHC polymorphism may be important in mating preferences (Potts *et al*., 1991; Wedekind *et al*., 1995; Wedekind and Penn, 2000; Jacob *et al*., 2002). A preference for a mate with a different MHC haplotype from one's own allegedly exists. The mechanism of how one individual senses the MHC molecules of another individual is still not fully understood, but HLA-linked olfactory receptors and small MHC peptides may in some way be involved (Wedekind and Penn, 2000; Leinders-Zufall *et al*., 2004). One argument is that

this mechanism confers an evolutionary advantage, because greater MHC polymorphism in individuals makes the species as a whole more resistant (Apanius *et al*., 1997; Penn *et al*., 2002). Furthermore, it might also serve as a mechanism to prevent inbreeding (Apanius *et al*., 1997). In support of this are observations of MHC homozygote deficiency in small isolated populations, and the fact that the frequency of MHC heterozygotes in human populations are typically higher than would be expected by chance (Hedrick and Thomson, 1983; Kostyu *et al*., 1993; Markow and Martin, 1993). The origin of MHC allelic diversity has been the subject of much speculation. As described above, its origin might lie in pathogen defence; however, it may also have originated as a mechanism for kin recognition. Another theory is that MHC originally functioned as a genetic incompatibility system that only later became functional in immune recognition and pathogen defence (Monroy and Rosati, 1979; Tregenza and Wedell, 2000).

One study has observed significant differences in *HLA class II* allele frequencies between males with impaired versus normal spermatogenesis (van der Ven *et al*., 2000). However, no functional mechanism underlying these observations has yet been presented, and the above-cited study could not elucidate whether an impairment of gamete interaction or a defect in sperm physiology might be the basis of an MHC effect on the process of fertilization. Expression both of *HLA* and of *HLA class Ib* genes has been reported during spermatogenesis (Martin-Villa *et al*., 1996; Fiszer *et al*., 1997).

The studies of the relationship between HLA-G expression and implantation success in IVF have already been discussed. However, what is the mechanism underlying the fact that only some 4- to 8-cell stage embryos express HLA-G? If this is—at least partly—genetically determined, then there is one connection between early human reproductive success and MHC genetics.

MHC-linked genes in humans may affect fetal growth and birth weight. Interestingly, one study has found that the birth weights of infants of both the sisters and brothers of RSA women decreased with increasing *HLA* haplotype identity between the siblings and the woman experiencing RSA. Actually, the mean birth weight of the offspring of siblings sharing both parental *HLA* haplotypes with the RSA woman was significantly lower (~300 g) than the mean birth weight in the relevant population as a whole. The birth weight of infants of siblings being *HLA* distinct from the probands seemed almost identical to the expected birth weight (Christiansen *et al*., 1990b). In mice, the expression of the non-classical class Ib MHC protein, Qa-2, during fetal development has been shown, as described above, to affect the preimplantation growth rate, fetal survival and birth weight (Comiskey *et al*., 2003) (Table VII). As described previously, HLA-G also seems to be associated with differences in fetal and placental weight (Hviid, 2004) (Table VII). These results may suggest that *HLA-G* polymorphism, or any other genetic variation in linkage disequilibrium in the *MHC*, may affect feto–placental growth in a way as proposed by Haig and Moore (Moore and Haig, 1991; Haig, 1993); i.e. a 'genetic conflict' is postulated to exist between maternal and fetal genes. Fetal genes will be selected so as to increase the transfer of nutrients to their fetus, and maternal genes will be selected to limit transfers in excess of some maternal optimum. The theories of Moore and Haig primarily deal with genomic imprinting. HLA-G is co-dominantly expressed in the first-trimester placenta, so there is no evidence of genomic imprinting in the case of the *HLA-G* gene (Hviid *et al*., 1998; Hiby *et al*., 1999). However, certain *HLA-G/MHC* polymorphisms may be associated with more aggressive feto–placental growth, but this may be accompanied by a greater risk of certain complications, such as pre-eclampsia (Hylenius *et al*., 2004; Rizzo *et al*., 2005a). Thus, some *HLA-G* polymorphism may in some respects favour fetal/paternal interests, while other *HLA-G* polymorphism may favour maternal interests during pregnancy. On a higher level, this may create balance and favour heterozygosity at the MHC/HLA locus. However, this is, naturally, pure speculation. Both the *HLA-G* genotype of and HLA-G expression by the fetus/placenta and the mother may be of importance in this regard (Figure 5). *HLA-G* polymorphism in connection with cyto-megalo virus (CMV) infection may also be of importance for the risk of developing pre-eclampsia (Carreiras *et al*., 2002). This possibility eventually leads to speculation to whether *HLA-G* polymorphisms associated with differences in HLA-G expression may be of importance in the risk of maternal–fetal transmission of pathogens during pregnancy? Altogether, it can be hypothesized that the various functional aspects of HLA-G may eventually be found to play roles in directing human MHC diversity.

In conclusion, the human MHC seems to affect human reproduction before conception through mating preferences. It may even influence fertilization and the morula/blastocyst cleavage rate, while implantation and PRs are influenced through HLA-G. Finally, some evidence supports the possibility that the MHC region might influence fetal growth and survival as well as feto–maternal immune adaptation (Figure 5).

Conclusions and perspectives

Although there have been some controversies, the overall picture emerging from a range of very different studies published in recent years is that diminished or aberrant HLA-G expression seems to be associated with certain complications of pregnancy, such as implantation failure in IVF, pre-eclampsia and possibly the risk of abortion, and that this may be further linked to *HLA-G* polymorphism.

The various studies of pre-eclampsia, RSA and blastocyst implantation/IVF discussed here suggest it is probable that HLA-G expression and *HLA-G* genetics in both the woman and the embryo/fetus may be important for pregnancy and its outcome. It is also intuitively apparent that a gene with putative immunosuppressive and immunotolerant potential might be functional in both the mother and the embryo/fetus/placenta. In this regard, the mother, placenta and the fetus may form a synthesis. Therefore, future studies should address HLA-G expression and genetics in both the mother and the fetus.

Insight into the functional significance of HLA-G expression is growing, but still more basic research is needed so as further to clarify the molecular mechanisms and further identify the specific cellular networks involved in HLA-G function, both during its expression in pregnancy and in adult life. Clinical perspectives on the HLA-G molecule and the pathways involved in its expression will be interesting to elucidate. The possibility of exploiting sHLA-G in preimplantation embryo culture media as a biochemical marker for optimizing success in IVF treatments is promising. However, the potential consequences of selecting embryos partly from HLA-G expression must be elucidated. Further research must investigate why only some embryos express detectable

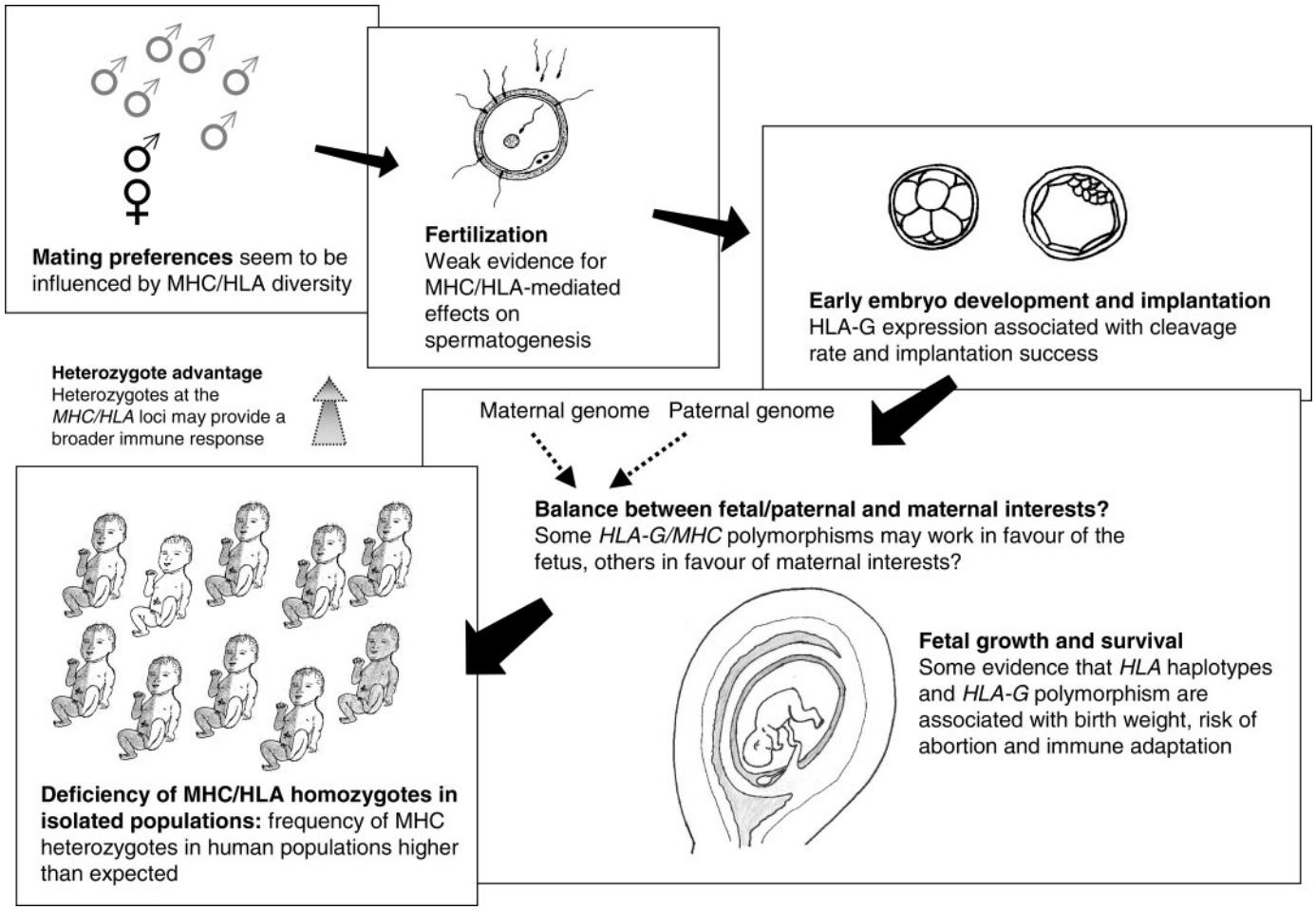

Figure 5. Proposed and speculated roles of major histocompatibility complex (MHC)/human leukocyte antigen (HLA), including HLA-G, during human reproduction in relation to MHC diversity. MHC/HLA may be involved in mechanisms both before and during pregnancy, in the interest of sustaining MHC diversity and avoiding inbreeding through kin recognition.

HLA-G. sHLA-G, and possibly *HLA-G* gene polymorphism, may be helpful markers for the prognosis of certain organ transplantations, and more pioneer studies are especially needed in this area. Further elucidation of the molecular mechanisms associated with HLA-G expression in pre-eclampsia and RSA would also be interesting. Moreover, recent findings of the possible involvement of HLA-G in inflammatory and autoimmune diseases deserve further study, and the results would build our understanding of the functional significance of HLA-G expression in adult life. Finally, further investigation of the roles of the other human non-classical HLA class Ib molecules, HLA-E and -F, especially during pregnancy, would be important.

The MHC and HLA have traditionally been regarded as very aggressive parts of the immune system: they are active in presenting antigen peptides to T cells and in initiating specific immune responses, are associated with *self*-reactivity concerning autoimmune disease and are involved in pregnancy and in the risk of rejection in organ transplantation. However, the MHC may also harbour a balancing function, in the form of an apparent immunoregulatory and tolerance-inducible HLA-G molecule (possibly together with HLA-E and -F). The question whether HLA-G turns out to be a key factor in the regulation of the human immune system awaits further investigation; however, there is already substantial evidence supporting a role for HLA-G in implantation and pregnancy.

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References

Abbas A, Tripathi P, Naik S and Agrawal S (2004) Analysis of human leukocyte antigen (HLA)-G polymorphism in normal women and in women with recurrent spontaneous abortions. Eur J Immunogenet 31,275–278.

Aldrich C, Verp MS, Walker MA and Ober C (2000) A null mutation in HLA-G is not associated with preeclampsia or intrauterine growth retardation. J Reprod Immunol 47,41–48.

Aldrich CL, Stephenson MD, Karrison T, Odem RR, Branch DW, Scott JR, Schreiber JR and Ober C (2001) HLA-G genotypes and pregnancy outcome in couples with unexplained recurrent miscarriage. Mol Hum Reprod 7,1167–1172.

Apanius V, Penn D, Slev PR, Ruff LR and Potts WK (1997) The nature of selection on the major histocompatibility complex. Crit Rev Immunol 17,179–224.

Arnaiz-Villena A, Morales P, Gomez-Casado E, Castro MJ, Varela P, Rojo-Amigo R and Martinez-Laso J (1999) Evolution of MHC-G in primates: a different kind of molecule for each group of species. J Reprod Immunol 43,111–125.

Bainbridge DR, Ellis SA and Sargent IL (1999) Little evidence of HLA-G mRNA polymorphism in Caucasian or Afro-Caribbean populations. J Immunol 163,2023–2027.

Bainbridge DR, Ellis SA and Sargent IL (2000) The short forms of HLA-G are unlikely to play a role in pregnancy because they are not expressed at the cell surface. J Reprod Immunol 47,1–16.

Basso O, Weinberg CR, Baird DD, Wilcox AJ and Olsen J (2003) Subfecundity as a correlate of preeclampsia: a study within the Danish National Birth Cohort. Am J Epidemiol 157,195–202.

Benian A, Madazli R, Aksu F, Uzun H and Aydin S (2002) Plasma and placental levels of interleukin-10, transforming growth factor-beta1, and epithelial-cadherin in preeclampsia. Obstet Gynecol 100,327–331.

Bermingham J, Jenkins D, McCarthy T and O'Brien M (2000) Genetic analysis of insulin-like growth factor II and HLA-G in pre-eclampsia. Biochem Soc Trans 28,215–219.

Blaschitz A, Lenfant F, Mallet V, Hartmann M, Bensussan A, Geraghty DE, Le Bouteiller P and Dohr G (1997) Endothelial cells in chorionic fetal vessels of first trimester placenta express HLA-G. Eur J Immunol 27,3380–3388.

Boyson JE, Iwanaga KK, Urvater JA, Hughes AL, Golos TG and Watkins DI (1999) Evolution of a new nonclassical MHC class I locus in two old world primate species. Immunogenetics 49,86–98.

Braude P, Bolton V and Moore S (1988) Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 332,459–491.

Buyon JP (1998) The effects of pregnancy on autoimmune diseases. J Leukoc Biol 63,281–287.

Cao W, Brenner CA, Alikani M, Cohen J and Warner CM (1999) Search for a human homologue of the mouse Ped gene. Mol Hum Reprod 5,541–547.

Carosella ED, Dausset J and Rouas-Freiss N (1999) Immunotolerant functions of HLA-G. Cell Mol Life Sci 55,327–333.

Carreiras M, Montagnani S and Layrisse Z (2002) Preeclampsia: a multifactorial disease resulting from the interaction of the feto-maternal HLA genotype and HCMV infection. Am J Reprod Immunol 48,176–183.

Casro MJ, Morales P, Rojo-Amigo R, Martinez-Laso J, Allende L, Varela P, Garcia-Berciano M, Guillen-Perales J and Arnaiz-Villena A (2000) Homozygous HLA-G*0105N healthy individuals indicate that membrane-anchored HLA-G1 molecule is not necessary for survival. Tissue Antigens 56,232–239.

Castro MJ, Morales P, Martinez-Laso J, Allende L, Rojo-Amigo R, Gonzalez-Hevilla M, Varela P, Moreno A, Garcia-Berciano M and Arnaiz-Villena A (2000a) Evolution of MHC-G in humans and primates based on three new 3'UT polymorphisms. Hum Immunol 61,1157–1163.

Castro MJ, Morales P, Martinez-Laso J, Allende L, Rojo-Amigo R, Gonzalez-Hevilla M, Varela P, Moscoso J, Garcia-Berciano M and Arnaiz-Villena A (2000b) Lack of MHC-G4 and soluble (G5, G6) isoforms in the higher primates, Pongidae. Hum Immunol 61,1164–1168.

Chaouat G, Ledee-Bataille N, Dubanchet S, Zourbas S, Sandra O and Martal J (2004) TH1/TH2 paradigm in pregnancy: paradigm lost? Cytokines in pregnancy/early abortion: reexamining the TH1/TH2 paradigm. Int Arch Allergy Immunol 134,93–119.

Christiansen OB, Mathiesen O, Grunnet N, Jersild C and Lauritsen JG (1990a) Is there a common genetic background for pre-eclampsia and recurrent spontaneous abortions? Lancet 335,361–362.

Christiansen OB, Mathiesen O, Riisom K, Lauritsen JG, Grunnet N and Jersild C (1990b) HLA or HLA-linked genes reduce birthweight in families affected by idiopathic recurrent abortion. Tissue Antigens 36,156–163.

Clements CS, Kjer-Nielsen L, Kostenko L, Hoare HL, Dunstone MA, Moses E, Freed K, Brooks AG, Rossjohn J and McCluskey J (2005) Crystal structure of HLA-G: a nonclassical MHC class I molecule expressed at the fetal-maternal interface. Proc Natl Acad Sci USA 102,3360–3365.

Colbern GT, Chiang MH and Main EK (1994) Expression of the nonclassic histocompatibility antigen HLA-G by preeclamptic placenta. Am J Obstet Gynecol 170,1244–1250.

Comiskey M, Goldstein CY, De Fazio SR, Mammolenti M, Newmark JA and Warner CM (2003) Evidence that HLA-G is the functional homolog of mouse Qa-2, the Ped gene product. Hum Immunol 64,999–1004.

Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S and Puppo F (2003) Soluble HLA-A-B-C and -G molecules induce apoptosis in T and NK CD8+ cells and inhibit cytotoxic T cell activity through CD8 ligation. Eur J Immunol 33,125–134.

Creput C, Durrbach A, Menier C, Guettier C, Samuel D, Dausset J, Charpentier B, Carosella ED and Rouas-Freiss N (2003a) Human leukocyte antigen-G (HLA-G) expression in biliary epithelial cells is associated with allograft acceptance in liver-kidney transplantation. J Hepatol 39, 587–594.

Creput C, Le Friec G, Bahri R, Amiot L, Charpentier B, Carosella E, Rouas-Freiss N and Durrbach A (2003b) Detection of HLA-G in serum and graft biopsy associated with fewer acute rejections following combined liver-kidney transplantation: possible implications for monitoring patients. Hum Immunol 64,1033–1038.

Crisa L, McMaster MT, Ishii JK, Fisher SJ and Salomon DR (1997) Identification of a thymic epithelial cell subset sharing expression of the class Ib HLA-G molecule with fetal trophoblasts. J Exp Med 186,289–298.

Darmochwal-Kolarz D, Leszczynska-Gorzelak B, Rolinski J and Oleszczuk J (1999) T helper 1- and T helper 2-type cytokine imbalance in pregnant women with pre-eclampsia. Eur J Obstet Gynecol Reprod Biol 86,165–170.

Darmochwal-Kolarz D, Rolinski J, Leszczynska-Goarzelak B and Oleszczuk J (2002) The expressions of intracellular cytokines in the lymphocytes of preeclamptic patients. Am J Reprod Immunol 48,381–386.

Davis DM, Reyburn HT, Pazmany L, Chiu I, Mandelboim O and Strominger JL (1997) Impaired spontaneous endocytosis of HLA-G. Eur J Immunol 27,2714–2719.

de Groot CJ, Jansen MW, Bertina RM, Schonkeren JJ, Helmerhorst FM and Huizinga TW (2004) Interleukin 10-2849AA genotype protects against pre-eclampsia. Genes Immun 5,313–314.

Diehl M, Munz C, Keilholz W, Stevanovic S, Holmes N, Loke YW and Rammensee HG (1996) Nonclassical HLA-G molecules are classical peptide presenters. Curr Biol 6,305–314.

Doherty PC and Zinkernagel RM (1975) A biological role for the major histocompatibility antigens. Lancet 1,1406–1409.

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

Emmer PM, Steegers EA, Kerstens HM, Bulten J, Nelen WL, Boer K and Joosten I (2002) Altered phenotype of HLA-G expressing trophoblast and decidual natural killer cells in pathological pregnancies. Hum Reprod 17,1072–1080.

Emmer PM, Joosten I, Schut MH, Zusterzeel PL, Hendriks JC and Steegers EA (2004) Shift in expression of HLA-G mRNA spliceforms in pregnancies complicated by preeclampsia. J Soc Gynecol Investig 11,220–226.

Eneroth E, Remberger M, Vahlne A and Ringden O (1998) Increased serum concentrations of interleukin-2 receptor in the first trimester in women who later developed severe preeclampsia. Acta Obstet Gynecol Scand 77,591–593.

Fiszer D, Ulbrecht M, Fernandez N, Johnson JP, Weiss EH and Kurpisz M (1997) Analysis if HLA class Ib gene expression in male gametogenic cells. Eur J Immunol 27,1691–1695.

Fournel S, Aguerre-Girr M, Huc X, Lenfant F, Alan A, Toubert A, Bensussan A and Le Bouteiller P (2000a) Soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8+ cells by interacting with CD8. J Immunol 164,6100–6104.

Fournel S, Huc X, Aguerre-Girr M, Solier C, Legros M, Praud-Brethenou C, Moussa M, Chaouat G, Berrebi A, Bensussan A et al. (2000b) Comparative reactivity of different HLA-G monoclonal antibodies to soluble HLA-G molecules. Tissue Antigens 55,510–518.

Fujii T, Ishitani A and Geraghty DE (1994) A soluble form of the HLA-G antigen is encoded by a messenger ribonucleic acid containing intron 4. J Immunol 153,5516–5524.

Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, Bencini E, Menicucci A and Baricordi OR (2002) HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. Eur J Immunol 32,311–315.

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

Goldman-Wohl DS, Ariel I, Greenfield C, Hochner-Celnikier D, Cross J, Fisher S and Yagel S (2000) Lack of human leukocyte antigen-G expression in extravillous trophoblasts is associated with pre-eclampsia. Mol Hum Reprod 6,88–95.

Gratacos E, Filella X, Palacio M, Cararach V, Alonso PL and Fortuny A (1998) Interleukin-4, interleukin-10, and granulocyte-macrophage colony stimulating factor in second-trimester serum from women with preeclampsia. Obstet Gynecol 92,849–853.

Haig D (1993) Genetic conflicts in human pregnancy. Q Rev Biol 68,495–532.

Hamai Y, Fujii T, Yamashita T, Miki A, Kozuma S, Geraghty DE and Taketani Y (1998) Peripheral blood mononuclear cells from women with recurrent abortion exhibit an aberrant reaction to release cytokines upon the direct contact of human leukocyte antigen-G-expressing cells. Am J Reprod Immunol 40,408–413.

Hara N, Fujii T, Yamashita T, Kozuma S, Okai T and Taketani Y (1996) Altered expression of human leukocyte antigen G (HLA-G) on extravillous trophoblasts in preeclampsia: immunohistological demonstration with anti-HLA-G specific antibody "87G" and anti-cytokeratin antibody "CAM5.2". Am J Reprod Immunol 36,349–358.

Harrison GA, Humphrey KE, Jakobsen IB and Cooper DW (1993) A 14 bp deletion polymorphism in the HLA-G gene. Hum Mol Genet 2,2200.

He X, Xu L, Liu Y and Zeng Y (2004) Identification of a novel HLA-F allele – HLA-F*010102. Tissue Antigens 63,181–183.

Hedrick PW and Thomson G (1983) Evidence for balancing selection at HLA. Genetics 104,449–456.

Heikkilä A, Tuomisto T, Häkkinen S-K, Keshi-Nisula L, Heinonen S and Ylä-Herttuala S (2005) Tumor suppressor and growth regulatory genes are overexpressed in severe early-onset preeclampsia – an array study on case-specific human preeclamptic placental tissue. Acta Obstet Gynecol Scand 84,679–689.

Heinrichs H and Orr HT (1990) HLA non-A,B,C class I genes: their structure and expression. Immunol Res 9,265–274.

Hennessy A, Pilmore HL, Simmons LA and Painter DM (1999) A deficiency of placental IL-10 in preeclampsia. J Immunol 163,3491–3495.

Hiby SE, King A, Sharkey A and Loke YW (1999) Molecular studies of trophoblast HLA-G: polymorphism, isoforms, imprinting and expression in preimplantation embryo. Tissue Antigens 53,1–13.

Hofmeister V and Weiss EH (2003) HLA-G modulates immune responses by diverse receptor interactions. Semin Cancer Biol 13,317–323.

Huizinga TW, van der Linden MW, Deneys-Laporte V and Breedveld FC (1999) Interleukin-10 as an explanation for pregnancy-induced flare in systemic lupus erythematosus and remission in rheumatoid arthritis. Rheumatology (Oxford) 38,496–498.

Humphrey KE, Harrison GA, Cooper DW, Wilton AN, Brennecke SP and Trudinger BJ (1995) HLA-G deletion polymorphism and pre-eclampsia/eclampsia. Br J Obstet Gynaecol 102,707–710.

Hunt JS, Andrews GK and Wood GW (1987) Normal trophoblasts resist induction of class I HLA. J Immunol 138,2481–2487.

Hunt JS, Jadhav L, Chu W, Geraghty D and Ober C (2000) Soluble HLA-G circulates in maternal blood during pregnancy. Am J Obstet Gynecol 183,682–688.

Hviid TV (2004) HLA-G genotype is associated with fetoplacental growth. Hum Immunol 65,586–593.

Hviid TV and Christiansen OB (2005) Linkage disequilibrium between human leukocyte antigen (HLA) class II and HLA-G-possible implications for human reproduction and autoimmune disease. Hum Immunol 66,688–699.

Hviid TV, Meldgaard M, Sørensen S and Morling N (1997) Polymorphism of exon 3 of the HLA-G gene. J Reprod Immunol 35,31–42.

Hviid TV, Møller C, Sørensen S and Morling N (1998) Co-dominant expression of the HLA-G gene and various forms of alternatively spliced HLA-G mRNA in human first trimester trophoblast. Hum Immunol 59,87–98.

Hviid TV, Sørensen S and Morling N (1999) Polymorphism in the regulatory region located more than 1.1 kilobases 5' to the start site of transcription, the promoter region, and exon 1 of the HLA-G gene. Hum Immunol 60,1237–1244.

Hviid TV, Christiansen OB, Johansen JK, Hviid UR, Lundegaard C, Møller C and Morling N (2001) Characterization of a new HLA-G allele encoding a nonconservative amino acid substitution in the alpha3 domain (exon 4) and its relevance to certain complications in pregnancy. Immunogenetics 53,48–53.

Hviid TV, Hylenius S, Høgh AM, Kruse C and Christiansen OB (2002) HLA-G polymorphisms in couples with recurrent spontaneous abortions. Tissue Antigens 60,122–132.

Hviid TV, Hylenius S, Rørbye C and Nielsen LG (2003) HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics 55,63–79.

Hviid TV, Hylenius S, Lindhard A and Christiansen OB (2004a) Association between human leukocyte antigen-G genotype and success of in vitro fertilization and pregnancy outcome. Tissue Antigens 64,66–69.

Hviid TV, Rizzo R, Christiansen OB, Melchiorri L, Lindhard A and Baricordi OR (2004b) HLA-G and IL-10 in serum in relation to HLA-G genotype and polymorphisms. Immunogenetics 56,135–141.

Hviid TV, Larsen LG, Høgh AM and Bzorek M (2004c) HLA-G expression in placenta in relation to HLA-G genotype and polymorphisms. Am J Reprod Immunol 52,212–217.

Hylenius S, Nybo Andersen AM, Melbye M and Hviid TV (2004) Association between HLA-G genotype and risk of pre-eclampsia: a case-control study using family triads. Mol Hum Reprod 10,237–246.

Ingman WV and Robertson SA (2002) Defining the actions of transforming growth factor beta in reproduction. Bioessays 24,904–914.

Ishitani A and Geraghty DE (1992) Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens. Proc Natl Acad Sci USA 89,3947–3951.

Ishitani A, Kishida M, Sageshima N, Yashiki S, Sonoda S, Hayami M, Smith AG and Hatake K (1999) Re-examination of HLA-G polymorphism in African Americans. Immunogenetics 49,808–811.

Ishitani A, Sageshima N, Lee N, Dorofeeva N, Hatake K, Marquardt H and Geraghty DE (2003) Protein expression and peptide binding suggest unique and interacting functional roles for HLA-E, F, and G in maternal-placental immune recognition. J Immunol 171,1376–1384.

Jacob S, McClintock MK, Zelano B and Ober C (2002) Paternally inherited HLA alleles are associated with women's choice of male odor. Nat Genet 30,175–179.

Jurisicova A, Casper RF, MacLusky NJ, Mills GB and Librach CL (1996) HLA-G expression during preimplantation human embryo development. Proc Natl Acad Sci USA 93,161–165.

Kanai T, Fujii T, Kozuma S, Yamashita T, Miki A, Kikuchi A and Taketani Y (2001a) Soluble HLA-G influences the release of cytokines from allogeneic peripheral blood mononuclear cells in culture. Mol Hum Reprod 7,195–200.

Kanai T, Fujii T, Unno N, Yamashita T, Hyodo H, Miki A, Hamai Y, Kozuma S and Taketani Y (2001b) Human leukocyte antigen-G-expressing cells differently modulate the release of cytokines from mononuclear cells present in the decidua versus peripheral blood. Am J Reprod Immunol 45,94–99.

Kanai T, Fujii T, Kozuma S, Miki A, Yamashita T, Hyodo H, Unno N, Yoshida S and Taketani Y (2003) A subclass of soluble HLA-G1 modulates the release of cytokines from mononuclear cells present in the decidua additively to membrane-bound HLA-G1. J Reprod Immunol 60,85–96.

Kapasi K, Albert SE, Yie S, Zavazava N and Librach CL (2000) HLA-G has a concentration-dependent effect on the generation of an allo-CTL response. Immunology 101,191–200.

Karhukorpi J, Ikaheimo I, Silvennoinen-Kassinen S and Tiilikainen A (1996) HLA-G polymorphism and allelic association with HLA-A in a Finnish population. Eur J Immunogenet 23,153–155.

Karhukorpi J, Laitinen T and Tiilikainen AS (1997) HLA-G polymorphism in Finnish couples with recurrent spontaneous miscarriage. Br J Obstet Gynaecol 104,1212–1214.

King A, Boocock C, Sharkey AM, Gardner L, Beretta A, Siccardi AG and Loke YW (1996) Evidence for the expression of HLAA-C class I mRNA and protein by human first trimester trophoblast. J Immunol 156,2068–2076.

King A, Burrows TD, Hiby SE, Bowen JM, Joseph S, Verma S, Lim PB, Gardner L, Le Bouteiller P, Ziegler A et al. (2000) Surface expression of HLA-C antigen by human extravillous trophoblast. Placenta 21,376–387.

Kirszenbaum M, Moreau P, Gluckman E, Dausset J and Carosella E (1994) An alternatively spliced form of HLA-G mRNA in human trophoblasts and evidence for the presence of HLA-G transcript in adult lymphocytes. Proc Natl Acad Sci USA 91,4209–4213.

Kirszenbaum M, Moreau P, Teyssier M, Lafon C, Gluckman E, Dausset J and Carosella E (1995) Evidence for the presence of the alternatively spliced HLA-G mRNA forms in human mononuclear cells from peripheral blood and umbilical cord blood. Hum Immunol 43,237–241.

Kirszenbaum M, Djoulah S, Hors J, Prost S, Dausset J and Carosella ED (1999) Polymorphism of HLA-G gene and protein. J Reprod Immunol 43,105–109.

Kostyu DD, Dawson DV, Elias S and Ober C (1993) Deficit of HLA homozygotes in a Caucasian isolate. Hum Immunol 37,135–142.

Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ and DeMars R (1990) A class I antigen, HLA-G, expressed in human trophoblasts. Science 248,220–223.

Labarrere CA and Althabe OH (1986) Primary chronic abortion, preeclampsia, idiopathic intrauterine growth retardation, hydatidiform mole, and choriocarcinoma: a unifying concept. Am J Reprod Immunol Microbiol 10,156–157.

Langat DK and Hunt JS (2002) Do nonhuman primates comprise appropriate experimental models for studying the function of human leukocyte antigen-G? Biol Reprod 67,1367–1374.

Langat DK, Morales PJ, Fazleabas AT, Mwenda JM and Hunt JS (2002) Baboon placentas express soluble and membrane-bound Paan-AG proteins encoded by alternatively spliced transcripts of the class Ib major histocompatibility complex gene, Paan-AG. Immunogenetics 54,164–173.

Lata JA, Cowchock FS, Jackson LG and Smith JB (1990) Cell surface antigen expression of first trimester chorionic villus samples. Am J Reprod Immunol 22,18–25.

Le Bouteiller P and Blaschitz A (1999) The functionality of HLA-G is emerging. Immunol Rev 167,233–244.

Le Discorde M, Le Danff C, Moreau P, Rouas-Freiss N and Carosella ED (2005) HLA-G*0105N null allele encodes functional HLA-G isoforms. Biol Reprod 73,280–288.

Le Gal F-A, Riteau B, Sedlik C, Khalil-Daher I, Menier C, Dausset J, Guillet J-G, Carosella ED and Rouas-Freiss N (1999) HLA-G-mediated inhibition of antigen-specific cytotoxic T lymphocytes. Int Immunol 11,1351–1356.

LeMaoult J, Le Discorde M, Rouas-Freiss N, Moreau P, Menier C, McCluskey J and Carosella ED (2003) Biology and functions of human leukocyte antigen-G in health and sickness. Tissue Antigens 62,273–284.

LeMaoult J, Krawice-Radanne I, Dausset J and Carosella ED (2004) HLA-G1-expressing antigen-presenting cells induce immunosuppressive CD4+ T cells. Proc Natl Acad Sci USA 101,7064–7069.

Le Rond S, Le Maoult J, Creput C, Menier C, Deschamps M, Le Friec G, Amiot L, Durrbach A, Dausset J, Carosella ED et al. (2004) Alloreactive CD4+ and CD8+ T cells express the immunotolerant HLA-G molecule in mixed lymphocyte reactions: in vivo implications in transplanted patients. Eur J Immunol 34,649–660.

Lee N, Malacko AR, Ishitani A, Chen MC, Bajorath J, Marquardt H and Geraghty DE (1995) The membrane-bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association. Immunity 3,591–600.

Lee N, Llano M, Carretero M, Ishitani A, Navarro F, Lopez-Botet M and Geraghty DE (1998) HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. Proc Natl Acad Sci USA 95,5199–5204.

Leinders-Zufall T, Brennan P, Widmayer P, Chandramani, SP, Maul-Pavicic A, Jager M, Li XH, Breer H, Zufall F and Boehm T (2004) MHC class I peptides as chemosensory signals in the vomeronasal organ. Science 306,1033–1037.

Lenfant F, Pizzato N, Liang S, Davrinche C, Le Bouteiller P and Horuzsko A (2003) Induction of HLA-G-restricted human cytomegalovirus pp65 (UL83)-specific cytotoxic T lymphocytes in HLA-G transgenic mice. J Gen Virol 84,307–317.

Lila N, Rouas-Freiss N, Dausset J, Carpentier A and Carosella ED (2001) Soluble HLA-G protein secreted by allo-specific CD4+ T cells suppresses the allo-proliferative response: a CD4+ T cell regulatory mechanism. Proc Natl Acad Sci USA 98,12150–12155.

Lila N, Amrein C, Guillemain R, Chevalier P, Latremouille C, Fabiani JN, Dausset J, Carosella ED and Carpentier A (2002) Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. Circulation 105,1949–1954.

Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH and Fisher SJ (1997) Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. Am J Pathol 151,1809–1818.

Llano M, Lee N, Navarro F, Garcia P, Albar JP, Geraghty DE and Lopez-Botet M (1998) HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: preferential response to an HLA-G-derived nonamer. Eur J Immunol 28,2854–2863.

Maejima M, Fujii T, Kozuma S, Okai T, Shibata Y and Taketani Y (1997) Presence of HLA-G-expressing cells modulates the ability of peripheral blood mononuclear cells to release cytokines. Am J Reprod Immunol 38,79–82.

Mallet V, Proll J, Solier C, Aguerre-Girr M, DeRossi M, Loke YW, Lenfant F and Le Bouteiller P (2000) The full length HLA-G1 and no other alternative form of HLA-G is expressed at the cell surface of transfected cells. Hum Immunol 61,212–224.

Markow TA and Martin JF (1993) Inbreeding and developmental stability in a small human population. Ann Hum Biol 20,389–394.

Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, Geraghty DE, Hansen JA, Mach B, Mayr WR et al. (2002) Nomenclature for factors of the HLA system, 2002. Tissue Antigens 60,407–464.

Martin-Villa JM, Luque I, Martinez-Quiles N, Corell A, Regueiro JR, Timon M and Arnaiz-Villena A (1996) Diploid expression of human leukocyte antigen class I and class II molecules on spermatozoa and their cyclic inverse correlation with inhibin concentration. Biol Reprod 55,620–629.

Matte C, Lacaille J, Zijenah L, Ward B and Roger M (2000) HLA-G and HLA-E polymorphisms in an indigenous African population. The ZVITAMBO Study Group. Hum Immunol 61,1150–1156.

Matte C, Lacaille J, Zijenah L, Ward B and Roger M (2002) HLA-G exhibits low level of polymorphism in indigenous east Africans. Hum Immunol 63,495–501.

McIntire RH, Morales PJ, Petroff MG, Colonna M and Hunt JS (2004) Recombinant HLA-G5 and -G6 drive U937 myelomonocytic cell production of TGF-beta1. J Leukoc Biol 76,1220–1228.

Menier C, Riteau B, Dausset J, Carosella ED and Rouas-Freiss N (2000) HLA-G truncated isoforms can substitute for HLA-G1 in fetal survival. Hum Immunol 61,1118–1125.

Menier C, Riteau B, Carosella ED and Rouas-Freiss N (2002) MICA triggering signal for NK cell tumor lysis is counteracted by HLA-G1-mediated inhibitory signal. Int J Cancer 100,63–70.

Messer G, Zemmour J, Orr HT, Parham P, Weiss EH and Girdlestone J (1992) HLA-J, a second inactivated class I HLA gene related to HLA-G and HLA-A. Implications for the evolution of the HLA-A-related genes. J Immunol 148,4043–4053.

Monroy A and Rosati F (1979) The evolution of cell-cell recognition system. Nature 27,165–166.

Moore T and Haig D (1991) Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 7,45–49.

Morales P, Corell A, Martinez LJ, Martin VJ, Varela P, Paz AE, Allende LM and Arnaiz VA (1993) Three new HLA-G alleles and their linkage disequilibria with HLA-A. Immunogenetics 38,323–331.

Morales PJ, Pace JL, Platt JS, Phillips TA, Morgan K, Fazleabas AT and Hunt JS (2003) Placental cell expression of HLA-G2 isoforms is limited to the invasive trophoblast phenotype. J Immunol 171,6215–6224.

Moreau P, Paul P, Gourand L, Prost S, Dausset J, Carosella E and Kirszenbaum M (1997) HLA-G gene transcriptional regulation in trophoblasts and blood cells: differential binding of nuclear factors to a regulatory element located 1.1 kb from exon 1. Hum Immunol 52,41–46.

Moreau P, Adrian-Cabestre F, Menier C, Guiard V, Gourand L, Dausset J, Carosella ED and Paul P (1999) IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. Int Immunol 11,803–811.

Nagamatsu T, Fujii T, Yamashita T, Miki A, Kanai T, Kusumi M, Osuga Y, Kozuma S and Taketani Y (2004) Hypoxia does not reduce HLA-G expression on extravillous cytotrophoblasts. J Reprod Immunol 63,85–95.

Navarro F, Llano M, Bellon T, Colonna M, Geraghty DE and Lopez-Botet M (1999) The ILT2 (LIR1) and CD94/NKG2A NK cell receptors respectively recognize HLA-G1 and HLA-E molecules co-expressed on target cells. Eur J Immunol 29,277–283.

Nelson JL, Hughes KA, Smith AG, Nisperos BB, Branchaud AM and Hansen JA (1993) Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis. N Engl J Med 329,466–471.

Nicolae D, Cox NJ, Lester LA, Schneider D, Tan Z, Billstrand C, Kuldanek S, Donfack J, Kogut P, Patel NM et al. (2005) Fine mapping and positional candidate studies identify HLA-G as an asthma susceptibility gene on chromosome 6p21. Am J Hum Genet 76,349–357.

Noci I, Fuzzi B, Rizzo R, Melchiorri L, Criscuoli L, Dabizzi S, Biagiotti R, Pellegrini S, Menicucci A and Baricordi OR (2005) Embryonic soluble HLA-G as a marker of developmental potential in embryos. Hum Reprod 20,138–146.

O'Brien M, McCarthy T, Jenkins D, Paul P, Dausset J, Carosella ED and Moreau P (2001) Altered HLA-G transcription in pre-eclampsia is associated with allele specific inheritance: possible role of the HLA-G gene in susceptibility to the disease. Cell Mol Life Sci 58,1943–1949.

Ober C, Rosinsky B, Grimsley C, van der Ven K, Robertson A and Runge A (1996) Population genetic studies of HLA-G: allele frequencies and linkage disequilibrium with HLA-A1. J Reprod Immunol 32,111–123.

Ober C, Aldrich C, Rosinsky B, Robertson A, Walker MA, Willadsen S, Verp MS, Geraghty DE and Hunt JS (1998) HLA-G1 protein expression is not essential for fetal survival. Placenta 19,127–132.

Ober C, Aldrich CL, Chervoneva I, Billstrand C, Rahimov F, Gray HL and Hyslop T (2003) Variation in the HLA-G promoter region influences miscarriage rates. Am J Hum Genet 72,1425–1435.

Odum N, Ledbetter JA, Martin P, Geraghty D, Tsu T, Hansen JA and Gladstone P (1991) Homotypic aggregation of human cell lines by HLA class II-, class Ia- and HLA-G-specific monoclonal antibodies. Eur J Immunol 21,2121–2131.

Ostensen M and Villiger PM (2002) Immunology of pregnancy – pregnancy as a remission inducing agent in rheumatoid arthritis. Transpl Immunol 9,155–160.

Park GM, Lee S, Park B, Kim E, Shin J, Cho K and Ahn K (2004) Soluble HLA-G generated by proteolytic shedding inhibits NK-mediated cell lysis. Biochem Biophys Res Commun 313,606–611.

Patel RN, Quack KC, Hill JA and Schust DJ (2003) Expression of membrane-bound HLA-G at the maternal-fetal interface is not associated with pregnancy maintenance among patients with idiopathic recurrent pregnancy loss. Mol Hum Reprod 9,551–557.

Paul P, Cabestre FA, Ibrahim EC, Lefebvre S, Khalil-Daher I, Vazeux G, Quiles RM, Bermond F, Dausset J and Carosella ED (2000) Identification of HLA-G7 as a new splice variant of the HLA-G mRNA and expression of soluble HLA-G5-G6, and -G7 transcripts in human transfected cells. Hum Immunol 61,1138–1149.

Penn DJ, Damjanovich K and Potts WK (2002) MHC heterozygosity confers a selective advantage against multiple-strain infections. Proc Natl Acad Sci USA 99,11260–11264.

Penzes M, Rajczy K, Gyodi E, Reti M, Feher E and Petranyi G (1999) HLA-G gene polymorphism in the normal population and in recurrent spontaneous abortion in Hungary. Transplant Proc 31,1832–1833.

Pfeiffer KA, Rebmann V and van der Ven K (2000) Soluble histocompatibility antigen levels in early pregnancy after IVF. Hum Immunol 61,559–564.

Pfeiffer KA, Fimmers R, Engels G, van der Ven H and van der Ven K (2001) The HLA-G genotype is potentially associated with idiopathic recurrent spontaneous abortion. Mol Hum Reprod 7,373–378.

Ponte M, Cantoni C, Biassoni R, Tradori-Cappai A, Bentivoglio G, Vitale C, Bertone S, Moretta A, Moretta L and Mingari MC (1999) Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua-associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor. Proc Natl Acad Sci USA 96,5674–5679.

Potts WK, Manning CJ and Wakeland EK (1991) Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature 352,619–621.

Proll J, Blaschitz A, Hutter H and Dohr G (1999) First trimester human endovascular trophoblast cells express both HLA-C and HLA-G. Am J Reprod Immunol 42,30–36.

Puppo F, Costa M, Contini P, Brenci S, Cevasco E, Ghio M, Norelli R, Bensussan A, Capitanio GL and Indiveri F (1999) Determination of soluble HLA-G and HLA-A-B, and -C molecules in pregnancy. Transplant Proc 31,1841–1843.

Rajagopalan S and Long EO (1999) A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. J Exp Med 189,1093–1100.

Rebmann V, Pfeiffer K, Passler M, Ferrone S, Maier S, Weiss E and Grosse-Wilde H (1999) Detection of soluble HLA-G molecules in plasma and amniotic fluid. Tissue Antigens 53,14–22.

Rebmann V, van der Ven K, Passler M, Pfeiffer K, Krebs D and Grosse-Wilde H (2001) Association of soluble HLA-G plasma levels with HLA-G alleles. Tissue Antigens 57,15–21.

Rebmann V, Busemann A, Lindemann M and Grosse-Wilde H (2003) Detection of HLA-G5 secreting cells. Hum Immunol 64,1017–1024.

Redman CW, McMichael AJ, Stirrat GM, Sunderland CA and Ting A (1984) Class 1 major histocompatibility complex antigens on human extra-villous trophoblast. Immunology 52,457–468.

Redman CW, Sacks GP and Sargent IL (1999) Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 180,499–506.

Rein DT, Breidenbach M, Honscheid B, Friebe-Hoffmann U, Engel H, Gohring UJ, Uekermann L, Kurbacher CM and Schondorf T (2003) Preeclamptic women are deficient of interleukin-10 as assessed by cytokine release of trophoblast cells in vitro. Cytokine 23,119–125.

Rieger L, Hofmeister V, Probe C, Dietl J, Weiss EH, Steck T and Kammerer U (2002) Th1- and Th2-like cytokine production by first trimester decidual large granular lymphocytes is influenced by HLA-G and HLA-E. Mol Hum Reprod 8,255–261.

Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin JN Jr and Bennett WA (1999) Expression of the placental cytokines tumor necrosis factor alpha, interleukin 1beta, and interleukin 10 is increased in preeclampsia. Am J Obstet Gynecol 181,915–920.

Riteau B, Menier C, Khalil-Daher I, Martinozzi S, Pla M, Dausset J, Carosella ED and Rouas-Freiss N (2001a) HLA-G1 co-expression boosts the HLA class I-mediated NK lysis inhibition. Int Immunol 13,193–201.

Riteau B, Rouas-Freiss N, Menier C, Paul P, Dausset J and Carosella ED (2001b) HLA-G2-G3, and -G4 isoforms expressed as nonmature cell surface glycoproteins inhibit NK and antigen-specific CTL cytolysis. J Immunol 166,5018–5026.

Rizzo R, Hviid TV, Stignani M, Balboni A, Grappa MT, Melchiorri L and Baricordi OR (2005a) The HLA-G genotype is associated with IL-10 levels in activated PBMCs. Immunogenetics 57,172–181.

Rizzo R, Mapp CE, Melchiorri L, Maestrelli P, Visentin A, Ferretti S, Bononi I, Miotto D and Baricordi OR (2005b) Defective production of soluble HLA-G molecules by peripheral blood monocytes in patients with asthma. J Allergy Clin Immunol 115,508–513.

Roth I and Fisher SJ (1999) IL-10 is an autocrine inhibitor of human placental cytotrophoblast MMP-9 production and invasion. Dev Biol 205,194–204.

Rouas-Freiss N, Marchal RE, Kirszenbaum M, Dausset J and Carosella ED (1997) The alpha1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors? Proc Natl Acad Sci USA 94,5249–5254.

Rousseau P, Le Discorde M, Mouillot G, Marcou C, Carosella ED and Moreau P (2003) The 14 bp deletion-insertion polymorphism in the 3? UT region of the HLA-G gene influences HLA-G mRNA stability. Hum Immunol 64,1005–1010.

Ryan AF, Grendell RL, Geraghty DE and Golos TG (2002) A soluble isoform of the rhesus monkey nonclassical MHC class I molecule Mamu-AG is expressed in the placenta and the testis. J Immunol 169,673–683.

Sala FG, Del Moral PM, Pizzato N, Legrand-Abravanel F, Le Bouteiller P and Lenfant F (2004) The HLA-G*0105N null allele induces cell surface expression of HLA-E molecule and promotes CD94/NKG2A-mediated recognition in JAR choriocarcinoma cell line. Immunogenetics 56,617–624.

Schmidt CM and Orr HT (1991) A physical linkage map of HLA-A, -G, -7.5p, and -F. Hum Immunol 31,180–185.

Schmidt CM, Ehlenfeldt RG, Athanasiou MC, Duvick LA, Heinrichs H, David CS and Orr HT (1993) Extraembryonic expression of the human MHC class I gene HLA-G in transgenic mice. Evidence for a positive regulatory region located 1 kilobase 5'-to the start site of transcription. J Immunol 151,2633–2645.

Sher G, Keskintepe L, Nouriani M, Roussev R and Batzofin J (2004) Expression of sHLA-G in supernatants of individually cultured 46-h embryos: a potentially valuable indicator of 'embryo competency' and IVF outcome. Reprod Biomed Online 9,74–78.

Sher G, Keskintepe L, Batzofin J, Fisch J, Acacio B, Ahlering P and Ginsburg M (2005) Influence of early ICSI-derived embryo sHLA-G expression on pregnancy and implantation rates: a prospective study. Hum Reprod 20,1359–1363.

Slukvin II, Watkins DI and Golos TG (1999) Tissue distribution of the mRNA for a rhesus monkey major histocompatibility class Ib molecule, Mamu-AG. Tissue Antigens 53,282–291.

Slukvin II, Lunn DP, Watkins DI and Golos TG (2000) Placental expression of the nonclassical MHC class I molecule Mamu-AG at implantation in the rhesus monkey. Proc Natl Acad Sci USA 97,9104–9109.

Solier C, Mallet V, Lenfant F, Bertrand A, Huchenq A and Le Bouteiller P (2001a) HLA-G unique promoter region: functional implications. Immunogenetics 53,617–625.

Solier C, McLaren F, Amadou C, Le Bouteiller P and Joly E (2001b) Detection of transcripts for a soluble form of the RT1-E MHC class Ib molecule in rat placenta. Immunogenetics 53,351–356.

Steinborn A, Rebmann V, Scharf A, Sohn C and Grosse-Wilde H (2003) Placental abruption is associated with decreased maternal plasma levels of soluble HLA-G. J Clin Immunol 23,307–314.

Suarez MB, Morales P, Castro MJ, Fernandez V, Varela P, Alvarez M, Martinez-Laso J and Arnaiz-Villena A (1997) A new HLA-G allele (HLA-G*0105N) and its distribution in the Spanish population. Immunogenetics 45,464–465.

Svejgaard A, Platz P and Ryder LP (1983) HLA and Disease 1982 – a survey. Immunol Rev 70,193–218.

Tamaki J, Arimura Y, Koda T, Fujimoto S, Fujino T, Wakisaka A and Kakinuma M (1993) Heterogeneity of HLA-G genes identified by polymerase chain reaction/single strand conformational polymorphism (PCR/SSCP). Microbiol Immunol 37,633–640.

Tesarik J, Kopecny V, Plachot M, Mandelbaum J, Da Lage C and Flechon JE (1986) Nucleologenesis in the human embryo developing in vitro: ultrastructural and autoradiographic analysis. Dev Biol 115,193–203.

The MHC Sequencing Consortium (1999) Complete sequence and gene map of a human major histocompatibility complex. Nature 401,921–923.

Tregenza T and Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. Mol Ecol 9,1013–1027.

Tripathi P, Abbas A, Naik S and Agrawal S (2004) Role of 14-bp deletion in the HLA-G gene in the maintenance of pregnancy. Tissue Antigens 64,706–710.

Ulbrecht M, Maier S, Hofmeister V, Falk CS, Brooks AG, McMaster MT and Weiss EH (2004) Truncated HLA-G isoforms are retained in the endoplasmic reticulum and insufficiently provide HLA-E ligands. Hum Immunol 65,200–208.

van der Meer A, Lukassen HG, van Lierop MJ, Wijnands F, Mosselman S, Braat DD and Joosten I (2004) Membrane-bound HLA-G activates proliferation and interferon-gamma production by uterine natural killer cells. Mol Hum Reprod 10,189–195.

van der Ven K and Ober C (1994) HLA-G polymorphisms in African Americans. J Immunol 153,5628–5633.

van der Ven K, Skrablin S, Engels G and Krebs D (1998) HLA-G polymorphisms and allele frequencies in Caucasians. Hum Immunol 59,302–312.

van der Ven K, Fimmers R, Engels G, van der Ven H and Krebs D (2000) Evidence for major histocompatibility complex-mediated effects on spermatogenesis in humans. Hum Reprod 15,189–196.

van Lierop MJ, Wijnands F, Loke YW, Emmer PM, Lukassen HG, van der Braat DDMA, Mosselman S and Joosten I (2002) Detection of HLA-G by a specific sandwich ELISA using monoclonal antibodies G233 and 56B. Mol Hum Reprod 8,776–784.

Wedekind C and Penn D (2000) MHC genes, body odours, and odour preferences. Nephrol Dial Transplant 15,1269–1271.

Wedekind C, Seebeck T, Bettens F and Paepke AJ (1995) MHC-dependent mate preferences in humans. Proc R Soc Lond B Biol Sci 260,245–249.

Wekerle T, Kurtz J, Bigenzahn S, Takeuchi Y and Sykes M (2002) Mechanisms of transplant tolerance induction using costimulatory blockade. Curr Opin Immunol 14,592–600.

Wiendl H, Behrens L, Maier S, Johnson MA, Weiss EH and Hohlfeld R (2000) Muscle fibers in inflammatory myopathies and cultured myoblasts express the nonclassical major histocompatibility antigen HLA-G. Ann Neurol 48,679–684.

Wolfberg AJ, Lee-Parritz A, Peller AJ and Lieberman ES (2004) Association of rheumatologic disease with preeclampsia. Obstet Gynecol 103,1190–1193.

Yamashita T, Fujii T, Watanabe Y, Tokunaga K, Tadokoro K, Juji T and Taketani Y (1996) HLA-G gene polymorphism in a Japanese population. Immunogenetics 44,186–191.

Yamashita T, Fujii T, Tokunaga K, Tadokoro K, Hamai Y, Miki A, Kozuma S, Juji T and Taketani Y (1999) Analysis of human leukocyte antigen-G polymorphism including intron 4 in Japanese couples with habitual abortion. Am J Reprod Immunol 41,159–163.

Yan WH and Fan LA (2005) Residues met76 and gln79 in HLA-G alpha1 domain involve in KIR2DL4 recognition. Cell Res 15,176–182.

Yasuda M, Takakuwa K, Higashino M, Ishii S, Kazama Y, Yoshizawa H and Tanaka K (1993) A typical case of reproductive autoimmune failure syndrome in which a patient experienced recurrent abortion, preeclampsia, and intrauterine growth retardation. Am J Reprod Immunol 29,45–47.

Yie SM, Li LH, Li YM and Librach C (2004) HLA-G protein concentrations in maternal serum and placental tissue are decreased in preeclampsia. Am J Obstet Gynecol 191,525–529.

Yie SM, Balakier H, Motamedi G and Librach CL (2005) Secretion of human leukocyte antigen-G by human embryos is associated with a higher in vitro fertilization pregnancy rate. Fertil Steril 83,30–36.

Zeng F and Schultz RM (2005) RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. Dev Biol 283,40–57.

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