CMLS, Cell. Mol. Life Sci. 56 (1999) 356–362
1420-682X/99/040356-07 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Research Article

Detection of HLA-E and -G DNA alleles for population and disease studies

E. Gómez-Casado†, J. Martínez-Laso†, M. J. Castro, P. Morales, J. Trápaga, M. Berciano, E. Lowy and A. Arnaiz-Villena*

Departamento de Inmunologia y Biología Molecular, Hospital 12 de Octubre, Universidad Complutense, E-28041 Madrid (Spain), Fax + 34 91 3908399, e-mail: antonio.arnaiz@inm.h12o.es

Received 14 July 1999; received after revision 25 August 1999; accepted 25 August 1999

Abstract. HLA-E and -G genes show a restricted polymorphism encoding for molecules whose variability is limited at the peptide binding site. Fourteen alleles that give rise to only three productive proteins for HLA-G (*0101, *0103 and *0104) and five alleles with three different proteins for HLA-E (*0101, *0102 and *0103) have been described. Expression of these molecules is low and found in many tissues for HLA-E; HLA-G protein is expressed in extravillous trophoblast cells and thymic epithelium. Molecular studies have shown how HLA-G and HLA-E bind to natural killer (NK) cells immunoglobulin and lectin-type inhibitory receptors. HLA-E may act as a sentinel of the cell; if classical class I and HLA-G are being expressed, HLA-E molecules may reach the cell surface and inhibit the lysis by NK cells. Most findings are consistent with the hypothesis that HLA-E and -G proteins may be tolerogenic molecules at either the T-cell receptor (TcR) (inflammation, graft rejection) or NK level, switching off cells which usually attack foreign (including foetus) or self (autoimmune) antigens. A low HLA-E and -G polymorphism is observed in humans, and their allele frequencies are mostly homogeneous in the populations tested so far. Many studies to detect these alleles are now being performed in isolated populations and also in pregnancy-associated pathologies. In the present paper, standard and detailed techniques to detect HLA-E and -G DNA polymorphism are reported and discussed.

Key words. HLA-E; HLA-G; placenta; trophoblast; SSO; RFLP; SBT; CD94/NKG2; NKIR.

Nonclassical human major histocompatibility complex (MHC, also named HLA) class I or MHC class Ib genes have been distinguished from classical MHC class I molecules by their limited polymorphism [1–10] and lower level of expression on the cell surface. The HLA-E gene [1] is located between HLA-C and -A loci. The HLA-G [11] is located telomeric to the HLA-A locus, showing a high degree of sequence similarity with HLA-A2, both at the nucleotide and amino acid levels [12]. HLA-E and -G are currently being widely studied in order to establish a relationship between their low polymorphism and their possible function.

HLA-E is expressed ubiquitously but at the transcriptional level; HLA-G is certainly expressed in extravillous cytotrophoblast and also in some thymic epithelial cell subpopulations [13, 14], and may be that in some macrophages [15], fetal endothelial cells [16] and, although conflicting results were published, may be in syncytiotrophoblast [17]. This restricted expression suggests that HLA-G products may play an important role in the mother's immune tolerance to the semiallogenic foetus [18]. It has been suggested that HLA-G expres-

† The contribution by E. Gómez-Casado and J. Martínez-Laso is equal, and the order of authorship is arbitrary.
* Corresponding author.

sion on target cells protects them from natural killer (NK) cell-mediated lysis through NK-inhibitory receptors. Although conflicting results have been obtained on the identification of NK-cell receptors recognising HLA-G [19], it is now admitted that HLA-G interacts with the following killer inhibitory receptors belonging to the immunoglobulin (Ig) superfamily: leukocyte Ig-like receptor (LIR)-1/Ig-like transcript (ITL)-2 [20, 21], ITL-4 [22] and p49 [23]. Another member of the KIR family named KIR2DL4 has recently been described. It is structurally different in the configuration of its two extracellular Ig domains, transmembrane and cytoplasmatic domains. This inhibitory receptor binds to cells expressing HLA-G but not to cells expressing other HLA class I included HLA- E [24].

HLA-E expression on the cell surface is dependent on the nonamer peptides which are derived from the peptide leader from classical class I antigens and preferentially from HLA-G. On the cell surface, HLA-E is able to bind inhibitory CD94/NKG2A or activating CD94/NKG2C lytic receptors [25].

There are six major alternatively spliced forms of HLA-G messenger RNA (mRNA) [26]: HLA-G1, -G2, -G3 and -G4, which encode membrane-bound proteins containing 3, 2, 1 and 2 external domains, respectively and two HLA-G soluble forms (-G5 and -G6; also designated as HLA-G1s and HLA-G2s, [27]). The latter are encoded by mRNA(s) *-G1* and *-G2* that retain intron 4, which is partially translated. On the other hand, no alternatively spliced forms have been found for HLA-E.

During the 12th International Histocompatibility Workshop and Conference, our group set up the first standardised protocol for HLA-E and -G DNA typing by polymerase chain reaction-specific sequence oligoprobes (PCR-SSO) and restriction fragment length polymorphism (RFLP); this allowed us to study more than 400 individuals belonging to different populations [28, 29]. However, although these techniques were used by many laboratories, they have never been published. In this paper, this methodology is detailed, for the first time, with improvements that include the detection of all the new -E and -G alleles described so far.

Materials and methods

HLA-E PCR-SSO typing

PCR amplification. Genomic DNA from peripheral blood mononuclear cells was amplified by using the PCR. In order to obtain the complete exon 2, intron 2 and exon 3 HLA-E sequences, a specific 5′ primer (E25′:TGTGAATTCTCTACCGGGAGTAGAGAGG) and a specific 3′ primer (E33′:AGCCCTGTGGACCCTCTT) were used [3]. PCR reactions included 10 × PCR buffer (10 mM Tris-HCl pH 8.4 at 24 °C, 50 mM KCl, 0.1 mg/ml gelatin, 0.02% NP40, 1.5 mM $MgCl_2$, final concentrations), 125 μM 2′ deoxyribonucleoside-5′ triphosphate (dNTP) mix, 1 μM of each primer and 0.025 U/μl Taq Polymerase (Perkin Elmer) in a final volume of 100 μl. The PCR conditions consisted of 30 cycles of 94 °C, 30 s; 65 °C, 1 min; and 72 °C, 1 min in a programmable heat block (9600 Perkin Elmer). The PCR products obtained were electrophoresed in a 2% agarose gel and detected by staining with ethidium bromide.

Oligonucleotide labelling. Specific oligonucleotides for HLA-E (table 1) were labelled with dig-ddUTP (Boehringer Mannheim, Germany) following the manufacturer's recommendations.

Dot/slot blotting. PCR products were blotted onto a nylon membrane (Amersham, UK) as follows: i) for denaturation, 80 μl of the amplification reaction was taken, and 320 μl of NaOH 0.5 M was added to it. This was mixed well and maintained at room temperature for 10 min. 400 μl of ammonium acetate 2 M was added, and this was mixed well and put in ice; ii) 50 μl of this final mixture was transferred to a spot of the nylon membrane previously soaked in ammonium acetate 1 M. Once the sample had passed through the membrane, 500 μl of ammonium acetate 1 M was added. The membrane was dried at room temperature and then dried to completion at 80 °C for 2 h (Baking), or illuminated with a 254-nm ultraviolet (UV) lamp for 5 min. Alternatively, PCR products can be blotted directly onto the membrane (1 μl) and the membranes dried at room temperature. With constant gentle agitation, the membranes were wet with 0.4 M NaOH for 10 min and then equilibrated with ammonium acetate 2 M. The membranes were dried at room temperature and then dried to completion as described above.

Prehybridisation. Each membrane was placed in a 50-ml tube with hybridisation buffer (6 × SSPE, 5 × Magic Denhardt, 0.1% Lauryl sarcosine, 0.02% SDS, 0.2 ml/cm^2) for 30 min at 42 °C.

Hybridisation. The hybridisation solution used in prehybridisation was removed, and the hybridisation solution (0.2 ml/cm^2) containing the dig-ddUTP-labelled oligonucleotide probe was added. This was incubated for 3 h at 42 °C.

Table 1. Specific oligonucleotide probes used for HLA-E typing.

Exon 2		
Name	*DNA sequence (5′ → 3′)*	*Specificities*
E2011	GGCTCCCACTCCTTG	0101,0102,01031,01032
E2021	CGAGTGAACCTGCGG	0101,0102,01031
E2022	CGAGTGAATCTGCGG	01032
E2031	CTGCGCGGCTACTAC	0101,01031,01032
E2032	CTGCGGCGCTACTAC	0102
Exon 3		
Name	*DNA sequence (5′ → 3′)*	*Specificities*
E3011	CCCGACAGGCGCTTC	0101,0102
E3012	CCCGACGGGCGCTTC	01031,01032,0104
E3021	CACCAGAGAGCCTAC	0101,0102,01031,01032
E3022	CACCAGGGAGCCTAC	0104

Washing. This process was done as follows: With constant gentle agitation, the membrane was rinsed in 100 ml per membrane of 2 × SSPE, 0.1% SDS at room temperature for 5 min, twice. The membrane was washed in 100 ml of tetramethylammonium chloride (TMAC) solution (50 mM Tris-HCl (pH 8.0), 3 M TMAC, 0.5 M EDTA, 0.1% SDS) at room temperature for 10 min. The membrane was washed twice in 100 ml of preheated TMAC solution at 50 °C for 15 min and then washed in 100 ml of 2 × SSPE.

Chemiluminescent detection. The following washes were done at room temperature, with constant shaking. The wash volume was approximately 100 ml per 100 cm^2 of membrane. This was washed immediately in buffer 1 (100 mM Tris-HCl, pH 7.2, 150 mM NaCl) for 5 min. This was washed in buffer 2 (buffer 1 containing 1% blocking reagent, Boehringer) for 30 min and incubated in buffer 1 containing AP-antibody anti-dig (75 mU/ml, dilution 1/10,000) for 30 min. Next, this was washed in buffer 1 for 15 min, twice, and then in buffer 3 (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$) for 5 min. The membrane was incubated in buffer 3 for 5 min. The membranes were wet with the CDP-star substrate solution (Phototope-star Detection kit for nucleic acids, New England Biolabs, UK) and then dried briefly (but not completely) and exposed with an X-ray film for a variable time (5 min or more).

Colour development for alkaline phosphatase. This was the same as the chemiluminescent protocol but using buffer 3 containing nitroblue tetrazolium choloride (NBT) solution (30 µl of NBT stock per 10 ml of buffer 3) and X-Phosphate (30 µl of X-Phosphate stock per 10 ml of buffer 3) (Boehringer Manhein, Germany) instead of CPD-star substrate. NBT stock: 100 mg/ml nitroblue tetrazolium salt in 70% dimethyl formamide. X-Phosphate stock: 50 mg/ml 5-Bromo-4-chloro-3-indodyl phosphate in dimethyl formamide. Incubation was done in complete darkness. The colour was developed in a few minutes and was completed in one day (do not shake). The membrane was washed in 50 ml of TE 10/1, pH 8.0, to stop the colour development.

Table 2. Specific oligonucleotide probes used for HLA-G typing.

Exon 2		
Name	*DNA sequence (5′ → 3′)*	*Specificities**
G 2.1	GTGGACGA-CACGCAGTTC	01011, 01012, 01013, 01014, 01015, 01016, 01017, 01018, 0102, 0104, 0105N
G 2.2	GTGGACGAC-TCGCAGTTC	0103
G 2.3	GAGGGGCCA-GAGTATTGG	01012, 01013, 01017, 01018, 01041, 0105N
G 2.4	GAGGGGC-CGGAG-TATTGG	01011, 01014, 01015, 01016, 0102, 0103, 01043
G 2.5	AG-GAGGGGCCC-GAGTATT	01042
G 2.6	AACAC-CAAGGCT-CACGCA	01014
G 2.7	GAGCGGGAG GGGCCG-GAG	0102
Exon 3		
Name	*DNA sequence (5′ → 3′)*	*Specificities**
G 3.1	TCTCATACC-CTCCAGTGG	01012, 01017, 0105N
G 3.2	TCCGACG-GTCGC-CTCCT	01013, 01015, 01017
G 3.3	TC-CGACGGACG-CCTCCTC	01011, 01012, 01014, 01016, 01018, 0102, 0103
G 3.4	CGCCTCATC-CGCGGGTAT	01041, 01042, 01043
G 3.5	CGCCTCCTC-CGCGGGTA	01011, 01012, 01013, 01014, 01015, 01016, 01017, 01018, 0102, 0103, 0105N
G 3.6	GAAC-GAGGACT-GCGCTCC	0105N
G 3.7	AACGAGGAC-CTGCGCTCC	all but 0105N

*G*01014, -01015, -01016, -01017 and -01018 have been recently described. HLA-G*01011 and -G*01016 cannot be distinguished each other in exons 2 and 3.

HLA-G PCR-SSO typing

PCR amplification. Genomic DNA was amplified using PCR. In order to obtain the complete exon 2, intron 2 and exon 3 HLA-G sequences, a specific 5′ primer (G-25′: TCCATGAGGTATTTCAGCGC) and a specific 3′ primer (G-33′: GGTACCCGCGCGCTGC-AGCA) were used [5]. PCR reactions included 10 × PCR buffer, 125 µM dNTP mix, 1 µM of each primer and 0.025 U/µl Taq Polymerase (Perkin Elmer) in a final volume of 100 µl. The PCR conditions consisted of 30 cycles of 96 °C, 15 s; 55 °C, 15 s; and 72 °C, 1 min in a programmable heat block (9600 Perkin Elmer). The PCR products obtained were electrophoresed in a 2% agarose and detected by staining with ethidium bromide. Specific oligonucleotides for HLA-G (table 2) were labelled with dig-ddUTP as described above. Also, Dot/slot-blot was the same as that used for HLA-E typing.

Hybridisation. Each membrane was placed into a 50-ml tube with TMAC solution (10 ml) as hybridisation buffer containing the dig-ddUTP-labelled oligonucleotide probe. It was incubated with rotation for 3 h at 54 °C.

Washing. With constant gentle agitation, the membrane was rinsed in 100 ml per membrane of 2 × SSPE, 0.1%

SDS at room temperature for 5 min, twice. The membrane was washed in 100 ml of TMAC solution [50 mM Tris-HCl (pH 8.0), 3 M TMAC, 0.5 M EDTA, 0.1% SDS] at room temperature for 10 min. The membrane was washed in 100 ml of preheated TMAC solution at 60 °C for 15 min, twice. Membranes hybridised with oligoprobes G2.2, G3.2 and G3.6 had to be washed with preheated TMAC solution at 63 °C for 15 min. The membrane was then washed in 100 ml of 2 × SSPE. The detection steps were the same as described above

HLA-G PCR-RFLP-DNA typing

PCR amplification. Genomic DNA was amplified using PCR. To obtain exon 2 and exon 3 HLA-G sequences separately, two specific primer sets, G-25′: TCCATGAGGTATTTCAGCGC/G-23′: CTGGGCCGGAGTTACTCACT and G-35′: CACACCCTCCAGTGGATGAT/G-33′: GGTACCCGCGCGCTGCAGCA, were respectively used [5]. PCR reactions included 10 × PCR buffer, 125 μM dNTP mix, 1 μM of each primer and 0.025 U/μl Taq Polymerase (Perkin Elmer) in a final volume of 100 μl. The PCR conditions consisted of 30 cycles of 96 °C, 15 s; 55 °C, 15 s; and 72 °C, 1 min in a programmable heat block (9600 Perkin Elmer).

Digestion. Following the HLA-G exon 2 or exon 3 amplification, aliquots (10 μl) of the reaction mixture were digested with the restriction endonucleases as follows: i) exon 2 amplifications were digested using MspI, HinfI, AsuI and ApaI, and ii) exon 3 with AcyI, PpuMI and BseRI following the manufacturers' recommendations. The digestion products were size-resolved in a 6% acrylamide gel or 2% Nusieve agarose and detected by staining with ethidium bromide. Interpretations of the possible results are shown in table 3.

HLA-E and -G sequence-based typing (SBT)

HLA-E and -G PCR products from both chromosomes comprising exon 2, intron 2 and exon 3 sequences were identified by differential migration in 2% agarose gel electrophoresis and purified with a Qiaquick gel extraction kit (Qiagen, Hilden, Germany). Double-stranded DNA templates were sequenced using the Sanger's dideoxy chain terminator method, with dye-labelled dideoxy terminators. DNA samples were analysed in a Perkin Elmer (Foster City, CA) 373A automated DNA sequencer as previously [30]. HLA-E and -G amplification primers were also used as sequencing primers. Due to relatively low genetic polymorphism, SBT can be applied for HLA-E and -G typing because ambiguous positions were assigned correctly.

Results and discussion

HLA-E polymorphism. HLA-E alleles detected by PCR-SSO typing do not show significantly different frequencies among 479 unrelated healthy individuals (HLA-A, -B, -C phenotyped) belonging to four different ethnic groups: Caucasoids (Spanish, French, Hungarians, Danish and Italians), Orientals (Thai-Chinese, Chinese and Japanese), and Asian Indians and Negroids (South Africans) [28]. Initially, the E*0101, 0102, 0103 and 0104 alleles and some unusual typing patterns were found ([28], fig. 1). A sequence-based typing strategy of these patterns revealed the existence of a new allele, E*01031, and the absence of E*0104 in the Spanish population [8]. No linkage disequilibrium was found between HLA-E and any of the classical class I (HLA-A, -B, -C) or class Ib (HLA-G) loci [28]. Many populations remain to be studied, and new HLA-E variants may be found. However, this locus seems to

Table 3. PCR-RFLP HLA-G allelism determination.

	Enzymes	Bands (bp)	Specificities
Exon 2			
	MspI	113+125	01011, 01014, 01015, 01016, 0102, 0103, 01043
		238	01012, 01013, 01017, 01018, 01041, 01042
	HinfI	79+125	0103
		106+175	all but 0103
	AsuI	118+125	01014
		80+125	all but 01014
	ApaI	120+161	01042
		281	all but 01042
Exon 3			
	AcyI	45+231	all but 01013, 01015, 01017
		276	01013, 01015, 01017
	PpuMI	108+168	all but 0105N
		276	0105N
	BseRI	40+236	all but 01041, 01042, 01043
		276	01041, 01042, 01043

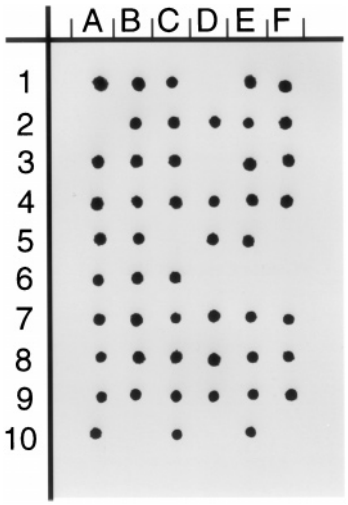

Figure 1. Chemiluminescent signals (points) correspond to positive hybridisation patterns of the E2021 oligoprobe (exon 2) which detects the E*0101, 0102 and 01031 alleles. The localisation of each individual sample in the membrane is achieved by a double code based on one number (column) and one letter (i.e. 1A = individual 1).

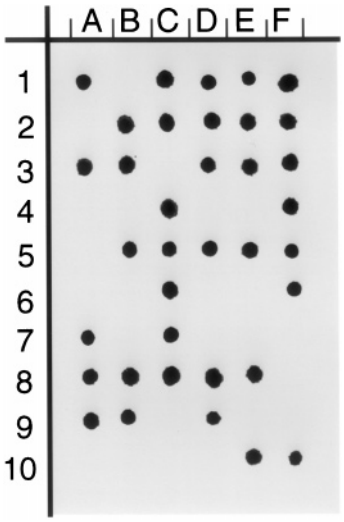

Figure 2. The membrane shown contains the G3.2 oligoprobe (exon 3), which detects G*01013, 01015 and 01017 alleles. The localisation of each individual sample in the membrane is achieved by a double code based on one number (column) and one letter (i.e. 1A = individual 1).

have low polymorphism at the DNA level, which gives rise only to three different proteins with nonsynonymous changes at codons 83, 107 and 157. This restricted polymorphism may be tightly related to modulation of NK cell function, particularly at the fetal-maternal interface, by presenting nonamers derived from the leader peptide of -G and other class I proteins [31–33]. *Pongidae* also show little polymorphism [34–36].

HLA-G polymorphism. The HLA-G alleles detected did not show significantly different frequencies among 514 unrelated healthy individuals who were also HLA-A, -B and -C phenotyped and belonged to four different ethnic groups: Caucasoids (Spanish, French, Hungarians, Danish and Italians), Orientals (Thai-Chinese, Chinese and Japanese), and Asian Indians and Negroids (South Africans) [29]. The HLA-G*01014, 01015, 01016, 01017, 01018, 01042 and 01043 alleles have recently been described and admitted by the WHO Nomenclature Committee [7, 9, 35, 37]. Hence, these alleles were not tested for the populations then studied. New probes have now been designed to detect these alleles, and specificities for existing probes have been modified in order to type these new alleles (see table 2). All HLA-G alleles can be identified by PCR-SSO, except G*01011/01016, because they only differ at the beginning of exon 4 by a single base substitution (see http://www.anthonynolan.com/HIG/seq/pep/text/g.pt.txt). Figure 2 shows an example of the PCR-SSO typing with the oligoprobe G3.2 that detects the G*01013, 01015 and 01017 alleles. On the other hand, restriction enzymes and PCR-RFLP techniques are able to recognise all polymorphic positions except those at the beginning of exon 3 for G*01012, 01017 and 0105N (the equivalent one to G3.1 probe) and those located in exon 4 for G*01016 and G*01042. An example of the G*0105N detection by PpuMI restriction enzyme is shown in figure 3.

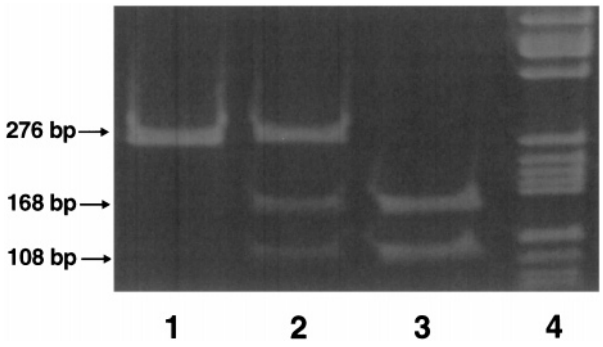

Figure 3. Restriction pattern with the enzyme PpuMI. Lane 1: no cut of the PCR fragment for an individual G*01011/01011 is observed. Lane 2: a G*01011/0105N heterozygous cell. Lane 3: a G*0105N/0105N homozygous cell. Lane 4: Molecular weight markers.

Linkage disequilibria had already been found between HLA-G and -A in Spaniards [5]. Also, a significant A2-G*01011 linkage disequilibrium is found in all other populations studied [7, 29, 38, 39] and may be the most ancient A/G linkage disequilibrium already present in ancestral hominoid populations [29]. The finding of strong and selective A/G linkage disequilibria with several HLA-A alleles suggests the existence of true physical distance variations in the A/G segment in the different HLA haplotypes [40]. If there were a hot-spot recombination site between the HLA-A and -G it, would probably hinder A/G allelic associations.

It has been postulated that HLA-G molecules may send negative signals from foetal cytotrophoblast to maternal NK cells in order to avoid foetal rejection [26, 31, 32], particularly the soluble isoforms [41]. Variability among HLA-G alleles is located at codons 31, 54, 57, 69, 93, 107 and 110. Only aminoacid residues at positions 31 and 110 show nonsynonymous changes [29], but they do not maintain specific contacts either with antigen binding site or T-cell receptor. On the other hand, the HLA-G molecules do not show the three hypervariable regions at the peptide binding site like other HLA antigen-presenting molecules. Studies in primates also show that polymorphism is restricted, indicating the presence of selective forces to maintain invariance during at least the past 50 million years [35, 42]. However, an antigen-presenting role for MHC-G molecules is not discarded [43, 44].

Acknowledgements. This work was supported by the Ministerio de Educación (PM-57-95 and PM-96-21) and Comunidad de Madrid (06-70-97 and 8.3/14/98) grants.

1 Mizuno S., Trapani J. A., Koller B. H., Dupont B. and Yang S. Y. (1988) Isolation and nucleotide sequence of a cDNA clone encoding a novel HLA class I gene. J. Immunol. **140:** 4024–4030
2 Koller B. H., Geraghty D. E., Shimizu Y., DeMars R. and Orr H. T. (1988) HLA-E. A novel HLA class I gene expressed in resting T lymphocytes. J. Immunol. **141:** 897–904
3 Ohya K., Kondo K. and Mizuno S. (1990) Polymorphism in the human class I MHC locus HLA-E in Japanese. Immunogenetics **32:** 205–209
4 Pook M. A., Woodcock V., Tassabehji M., Campbell R. D., Summers C. W., Taylor M. et al. (1991) Characterization of an expressible nonclassical class I HLA gene. Hum. Immunol. **32:** 102–109
5 Morales P., Corell A., Martinez-Laso J., Martin-Villa J. M., Varela P., Paz-Artal E. et al. (1993) Three new HLA-G alleles and their linkage disequilibria with HLA-A. Immunogenetics **38:** 323–331
6 Yamashita T., Fujii T., Watanabe Y., Tokunaga K., Tadokoro K., Juji T. et al. (1996) HLA-G gene polymorphism in a Japanese population. Immunogenetics **44:** 186–191
7 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
8 Gomez-Casado E., Martinez-Laso J., Vargas-Alarcón G., Varela P., Díaz-Campos N., Alvarez M. et al. (1997) Description of a new HLA-E (E*01031) allele and its frequency in the Spanish population. Hum. Immunol. **54:** 69–73
9 Kirszenbaum M., Djoulah S., Hors J., Le Gall I., de Oliveira E. B., Prost S. et al. (1997) HLA-G gene polymorphism segregation within CEPH reference families. Hum. Immunol. **53:** 140–147
10 Suarez M. B., Morales P., Castro M. J., Fernandez-Soria V., Varela P., Alvarez M. et al. (1997) A new HLA-G allele (HLA-G*0105 N) and its distribution in the Spanish population. Immunogenetics **45:** 464–465
11 Geraghty D. E., Koller B. H. and Orr H. T. (1987) A human major histocompatibility complex class I gene that encodes a protein with a shortened cytoplasmic segment. Proc. Natl. Acad. Sci. USA **84:** 9145–9149
12 Kirisits M. J., Kunz H. W. and Gill T. J. (1991) Analysis of the sequences similarities of classical and non-classical class I genes. In: HLA 1991, vol. 1, pp. 1021–1030, Tsuji K., Aizawa M. and Sasuzuki T (eds), Oxford University Press, Oxford
13 Crisa L., McMaster M. T., Ishii J. K., Fisher S. J. and Salomon D. R. (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
14 Mallet V., Blaschitz A., Crisa L., Schmitt C., Fournel S., King A. et al. (1999) HLA-G in the human thymus: a subpopulation of medullary epithelial but not CD83(+) dendritic cells expresses HLA-G as a membrane-bound and soluble protein. Int. Immunol. **11:** 889–898
15 Yang Y., Chu W., Geraghty D. E. and Hunt J. S. (1996) Expression of HLA-G in human mononuclear phagocytes and selective induction by IFN-gamma. J. Immunol. **156:** 4224–4231
16 Blaschitz A., Lenfant F., Mallet V., Hartmann M., Bensussan A., Geraghty D. E. et al. (1997) Endothelial cells in chorionic fetal vessels of first trimester placenta express HLA-G. Eur. J. Immunol. **27:** 3380–3388
17 Chu W., Fant M. E., Geraghty D. E. and Hunt J. S. (1998) Soluble HLA-G in human placentas: synthesis in trophoblasts and interferon-gamma-activated macrophages but not placental fibroblasts. Hum. Immunol. **59:** 435–442
18 King A. and Loke Y. W. (1991) On the nature and function of human uterine granular lymphocytes. Immunol. Today **12:** 432–435
19 Mandelboim O., Pazmany L., Davis D. M., Valés-Gómez M., Reyburn H. T., Rybalov B. et al. (1997) Multiple receptors for HLA-G on human natural killer cells. Proc. Natl. Acad. Sci. USA **94:** 14666–14670
20 Colonna M., Navarro F., Bellon T., Llano M., Garcia P., Samaridis J. et al. (1997) A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. J. Exp. Med. **186:** 1809–1818
21 Navarro F., Llano M., Bellon T., Colonna M., Geraghty D. E. 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
22 Colonna M., Samaridis J., Cella M., Angman L., Allen R. L., O'Callaghan C. A. et al. (1998) Human myelomonocytic cells express an inhibitory receptor for classical and nonclassical MHC class I molecules. J. Immunol **160:** 3096–3100
23 Cantoni C., Verdiani S., Falco M., Pessino A., Cilli M., Conte R. et al. (1998) p49, a putative HLA class I-specific inhibitory NK receptor belonging to the immunoglobulin superfamily. Eur. J. Immunol. **28:** 1980–1990
24 Rajagopalan S. and Long E. O. (1999) A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. J. Exp. Med. **189:** 1093–1100
25 Braud V. M., Allan D. S. J., O'Callaghan C. A., Soderstrom K., D'Andrea A., Ogg G. S. et al. (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature **391:** 795–799

26 Carosella E. D., Dausset J. and Kirszenbaum M. (1996) HLA-G revisited. Immunology Today **17:** 407–409
27 Fujii T., Ishitani A. and Geraghty D. E. (1994) A soluble form of the HLA-G antigen is encoded by a messenger ribonucleic acid containing intron 4. J. Immunol. **153:** 5516–5524
28 Arnaiz-Villena A., Chandanayingyong D., Chiewsilp P., Diaz-Campos N., Fan L., Fauchet R., et al. (1997) HLA-E polymorphism: 12th International Histocompatibility Workshop study. In: Genetic Diversity of HLA. Functional and Medical implications, vol. 1, pp. 151–154, Charron D. (ed.), EDK, Sèvres
29 Arnaiz-Villena A., Chandanayingyong D., Chiewsilp P., Fan L., Fauchet R., Fumiaki N. et al. (1997) HLA-G polymorphism: 12th International Histocompatibility Workshop study. Genetic Diversity of HLA. Functional and Medical Implications, vol. 1, pp. 155–159, Charron D. (ed.), EDK, Sèvres
30 Arnaiz-Villena A., Timon M., Corell A., Perez-Aciego P., Martin-Villa J. M. and Regueiro J. R. (1992) Primary immunodeficiency caused by mutations in the gene encoding the CD3-γ subunit of the T-lymphocyte receptor. N. Engl. J. Med. **327:** 529–533
31 Rouas-Freiss N., Marchal-Bras Goncalves R., Menier C., Dausset J. and Carosella E. D. (1997) Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. Proc. Natl. Acad. Sci. USA **94:** 11520–11525
32 Rouas-Freiss N., Marchal R. E., Kirszenbaum M., Dausset J. and Carosella E. D. (1997) The α1 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
33 Braud V. M., Allan D. S. J. and McMichael A. J. (1999) Functions of nonclassical MHC and non-MHC-encoded class I molecules. Curr. Opin. Immunol. **11:** 100–108
34 Suarez B., Morales P., Castro M. J., Fernandez-Soria V., Recio M. J., Perez-Blas M. et al. (1997) Mhc-E polymorphism in Pongidae primates: the same allele is found in two different species. Tissue Antigens **50:** 695–698
35 Arnaiz-Villena A., Martinez-Laso J., Alvarez M., Castro M. J., Varela P., Gomez-Casado E. et al. (1997) Primate Mhc -E and -G alleles. Immunogenetics **46:** 251–266
36 Arnaiz-Villena A., Alvarez-Tejado M., Morales P., Gomez-Casado E., Castro M. J., Recio M. J. et al. (1999) Evolution of MHC-G in primates: a different kind of molecule for each group of species. J. Reprod. Immunol. **43:** 111–125
37 Marsh S. G. E. (1998) Nomenclature for factors of the HLA system, update February 1998. WHO Nomenclature Committee for Factors of the HLA System. Tissue Antigens **51:** 584–585
38 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
39 Ober C. and Aldrich C. L. (1997) HLA-G polymorphisms: neutral evolution or novel function? J. Reprod. Immunol. **36:** 1–21
40 Geraghty D. E., Pei J., Lipsky B., Hansen J. A., Taillon-Miller P., Bronson S. K. et al. (1992) Cloning and physical mapping of the HLA class I region spanning the HLA-E-to-HLA-F interval by using yeast artificial chromosomes. Proc. Natl. Acad. Sci. USA **89:** 2669–2673
41 Castro M. J., Morales P., Catálfamo M., Fernández-Soria V., Suárez B., Varela P. et al. (1998) Lack of HLA-G soluble isoforms in Graves-Basedow thyrocytes and complete cDNA sequence of the HLA-G*01012 allele. Eur. J. Immunogenet. **25:** 311–315
42 Castro M. J., Morales P., Fernández-Soria V., Suárez B., Recio M. J., Alvarez M. et al. (1996) Allelic diversity at the primate Mhc-G locus: exon 3 bears stop codons in all Cercopithecinae sequences. Immunogenetics **43:** 327–336
43 Le Bouteiller P. and Blaschitz A. (1999) The functionality of HLA-G is emerging. Immunol. Rev. **167:** 233–244
44 Morales P., Martinez-Laso J., Castro M. J., Gomez-Casado E., Alvarez M., Rojo R. et al. (1999) An evolutive overview of the MHC-G polymorphism: clues about the unknown function(s). In: The Major Histocompatibility Complex: Evolution, Structure and Function, Kasahara M. and Takahata N. (eds), Springer, Tokyo