CMLS, Cell. Mol. Life Sci. 58 (2001) 1943–1949
1420-682X/01/131943-07 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Research Article

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

M. O'Brien [a], T. McCarthy [b], D. Jenkins [c], P. Paul [a], J. Dausset [d], E. D. Carosella [a] and P. Moreau [a,*]

[a] CEA, Service de Recherches en Hémato-immunologie, DSV/DRM, Hôpital Saint-Louis, Institut d'Hématologie, 1 avenue Claude Vellefaux, 75010 Paris (France), Fax: + 33 1 48 03 19 60, e-mail: moreau@dsvidf.cea.fr
[b] Biochemistry Department, University College Cork (Ireland)
[c] Department of Obstetrics and Gynaecology, University College Cork (Ireland)
[d] Fondation Jean Dausset, 27 rue Juliette-Dodu, 75010 Paris (France)

Received 8 May 2001; received after revision 7 September 2001; accepted 11 September 2001

Abstract. Pre-eclampsia is a disorder of human pregnancy occuring in 5–10% of all births, and represents the leading cause of infant morbidity and mortality and maternal death. In pre-eclampsia, invasion of fetal trophoblasts into maternal arteries during early pregnancy is shallow or absent. Here we examined the hypothesis that *HLA-G*, a non-classical class I *HLA* expressed in cytotrophoblasts, may act as a key gene in pre-eclampsia. We analysed *HLA-G* at the level of transcription and genotyped a silent CAC-CAT polymorphism in exon 3 and a 14-bp insertion/deletion in the 3′ untranslated region. A deficit in levels of the *HLA-G3* transcript was observed in mild pre-eclampsia compared to normal placentas. The distribution of *HLA-G* polymorphisms was different between normal and pre-eclampsia samples. A correlation between the alteration in transcription of the *HLA-G* gene and certain *HLA-G* genotypes was also observed. Thus we provide the first evidence for a possible role of *HLA-G* in genetic susceptibility to, and pathogenesis of pre-eclampsia.

Key words. Pre-eclampsia; *HLA-G*; alternative splicing; polymorphism.

Introduction

Pre-eclampsia (PE) is characterised by elevated blood pressure, oedema and proteinuria associated with end-organ damage in the mother and neonatal prematurity in severe cases [1]. While the disorder only becomes clinically apparent late in pregnancy, pathological changes are evident earlier on. In particular, fetal trophoblast invasion into the myometrium is shallow or even absent in PE, resulting in reduced perfusion of the intervillous space and decreased blood supply to the placenta [2]. This inadequate placentation has been hypothesised to trigger the widespread endothelial cell injury associated with the disease [3]. In normal pregnancies, extravillous cytotrophoblasts which invade deep into maternal spiral arteries express *HLA-G* [4, 5], indeed, HLA-G production is upregulated as cytotrophoblasts differentiate along the invasive pathway in vitro suggesting that HLA-G production is a critical component of the invasion process [6].
The genetic mechanisms underlying susceptibility to PE remain unclear. While early genetic studies focused on a susceptibility locus in the mother [7, 8], several epidemiological studies have since provided evidence for a role of the fetal paternal genotype in the disorder [9]. Several studies have shown association of PE to candidate genes outside the *HLA* complex [10–13]. However, while these

* Corresponding author.

genes may provide some explaination for the hypertension and vascular damage associated with the later phase of the disease, they do not explain the inadequate trophoblast invasion underlying the disorder [2]. In the present study we, therefore, focused on *HLA-G* which seems to be a key component in the invasion process and hence an ideal candidate gene for PE.

HLA-G is an unusual class I *HLA* in that it shows tissue-restricted distribution in cytotrophoblast [5], amniotic cells [14], thymus [15] and endothelial cells of chorionic blood vessels [16]. It also exhibits a limited polymorphism and is subjected to alternative splicing yielding four membrane-bound (HLA-G1 to HLA-G4) and three soluble (HLA-G5, HLA-G6 and HLA-G7) forms [17, 18]. *HLA-G* expression may be induced by interferon-γ [19] and interleukin-10 [20]. The strong expression of *HLA-G* by invasive trophoblasts may, in part, explain maintenance of the fetal semi-allograft during pregnancy, with *HLA-G* inhibiting activation of maternal T and natural killer cells resident in the decidua [21, 22].

A deficit of *HLA-G* protein in PE placentas has been reported [23, 24], and an absence or reduction of *HLA-G* expression by extravillous trophoblasts has also been demonstrated by RNA in situ hybridisation [25].

To evaluate the hypothesis that *HLA-G* may act as a key gene involved in the pathogenesis of PE, we analysed *HLA-G* at the level of transcription and also polymorphism in term placentas from normal and mild PE pregnancies. The results obtained strongly suggest a close association between an observed alteration in *HLA-G* transcription in mild PE and the inheritance of particular *HLA-G* alleles.

Material and methods

Sample collection

Placentas from normal and mild PE pregnancies (Caucasians) were obtained with appropriate informed consent from maternity hospitals in Cork, Ireland. Diagnosis of mild PE required hypertension (an increase in blood pressure of > 15 mm Hg diastolic or > 30 mm Hg systolic from measurement in early pregnancy) and proteinuria in the mother (0.3 g/24 h) as minimum clinical criteria. Pregnancy-induced hypertension (PIH) was defined as an increase in blood pressure as for PE but with no proteinuria. Tissue samples were snap frozen in liquid nitrogen and stored at –80 °C until required for isolation of genomic DNA and total RNA.

Analysis of *HLA-G* Transcripts

Total RNA was extracted from trophoblast tissue using the RNA NOW reagent (Biogentex, Seabrook, Tex.) according to the manufacturer's instructions. The quality of RNA was checked by electrophoresis in a 1.5% agarose-denaturing gel. Complementary DNAs were prepared from 5 µg of total RNA as previously described [20]. G.257F (exon 2 specific) and G.1004R 5′-CCTTTT CAATCTGAGCTCTTCTTT-3′ (exon 5–exon 6 junction) pan-*HLA-G* primers were used to amplify all alternative forms of *HLA-G* transcripts during 35 cycles as previously described [20]. In all PCR reactions, co-amplification with β-actin primers was carried out as a semi-quantitative control of total cDNA (16 cycles to avoid saturation). PCR products were analysed by Southern blot using *HLA-G*-specific probes G.R (exon 2 specific) for pan-*HLA-G* amplification and a β-actin-specific probe [20]. The membranes were exposed to a molecular imager (Biorad, Ivry/Seine, France) and the values obtained for the *HLA-G* signals were normalised to β-actin signals. Because a semi-quantitative methodology was used, levels of *HLA-G* transcription were classified as 'normal', 'reduced' and 'low' rather than associated to numbers. Accordingly, we used ++++ (high), +++, ++ or – (negative) for *HLA-G3* quantification. Analyses were done blinded with regard to status of samples.

HLA-G genotyping

Genomic DNA was prepared from tissue samples using standard techniques. The CAC-CAT polymorphism at codon 93 was genotyped using the glycosylase-mediated polymorphism detection (GMPD) method [26]. The polymorphic site in exon 3 was amplified by an initial PCR using the forward primer G.257F (exon 2) and using as the reverse primer either G.1004R (exon 5–exon 6) for cDNA or 5′-AGGCGCCCCACTGCCCCTGGTAC-3′ (intron 3) for genomic DNA.

In the subsequent nested amplification reaction, the 3′ end of the forward primer was designed so that the first U incorporated downstream of the primer was at the polymorphic site in codon 93. Hence, for typing cDNA, the forward primer was 5′-AACCAGAGCGAGGCCAGTT CT-3′, (exon 2–exon 3), whilst for genotyping genomic DNA, the forward primer used was 5′-GACCGAGGGG GTGGGGCCAGGTTCT-3′ (intron 2 – exon 3). Amplification was carried out using ^{32}P-end-labelled forward primer and the reverse primer 5′-CCTTTGTTCAGCCA-CATTGG-3′ for both genomic and cDNA. Amplified products were subsequently digested and cleavage products were resolved by denaturing gel electrophoresis. For cDNA, the C-93 allele was detected as a 28-nt fragment and the T-93 allele as a 23-nt fragment; for genomic DNA, the C-93 allele was detected as a 32-nt fragment and the T-93 allele as a 27-nt fragment.

For genotyping of the 14-bp insertion/deletion polymorphism, a region encompassing the 3′ untranslated region was amplified using primers 5′-AGAAAGAAGAGCT CAGATTGA-3′ and 5′-GAGAGGTAAGTTATAGCTC AG-3′ for cDNA and primers 5′-TGTGAAACAGCTGC-CCTGTGT-3′ and 5′-AAGGAATGCAGTTCAGCAT

GA-3′ for genomic DNA. For both cDNA and genomic DNA, PCR products were separated by non denaturing polyacrylamide gel electrophoresis.

In cases where placentas were homozygous for one/both of the polymorphisms, *HLA-G* haplotypes could be directly assigned. However, with samples which were heterozygous for both polymorphisms, haplotypes were assigned following allele-specific amplification of cDNA. Specifically, an initial PCR was carried out to amplify either the insertion or deletion alleles: forward primer G.257F and either 5′-CAAAGGGAAGGCATGAACAA ATCTTG-3′ (insertion allele specific) or 5′-GTTCTT GAAGTCACAAAGGGACTTG-3′ (deletion allele specific). In a subsequent nested PCR, the CAC-CAT polymorphism at codon 93 was genotyped as described above.

Statistic analysis

Comparisons of genotype and allele distributions were compared between control and PE samples and performed using Fisher's exact test. Two-tailed probability values (p) were calculated. As PIH is a distinct disease entity from PE, placenta A was not included in statistical analysis.

Results and discussion

Using RT-PCR and Southern blot hybridisations, we first analysed *HLA-G* mRNA alternative splicing in ten normal, one PIH and six mild PE placentas from an Ireland cohort in comparison to the alternative splicing pattern of JEG-3 choriocarcinoma cell line. Amplification experiments with the G.257F (exon 2) and G.1004R (exon 5– exon 6) primer set give rise to three well-defined major bands corresponding to *HLA-G5/HLA-G1*, *HLA-G2/HLA-G4* (same length) and *HLA-G3* mRNA forms (figs 1, 2, table 1). The JEG-3-like pattern was observed in PIH (A sample; fig.1) and all normal placentas but differed in five out of six mild PE placentas, which exhibited a clear deficit in the *HLA-G3* isoform in both primigravida (placentas B, C and D; fig.1) and multigravida (K and O; fig. 2) pregnancies. Although the PE sample size is small, the *HLA-G3* defect appears to be highly specific for PE, because this pattern was never observed in normal placentas including previous unrelated studies performed by our (50 normal samples from France; data not shown) or other [17, 27–29] groups. By increasing the loading of RT-PCR products from PE samples, we could exclude that the observed defect in *HLA-G3* mRNA forms was due to a global deficit in steady-state levels of *HLA-G* trancripts in comparison to normal sample. As expected, molecular imager semi-quantification of *HLA-G*/β-actin RT-PCR products (not shown) revealed small inter-individual differences in the amounts of *HLA-G* transcript in both samples, either due to methodology or individual heterogeneity. Nevertheless, the analysis agreed with visual observations and confirmed published data obtained by others with five PE [24] and ten PE [25] placentas, both providing evidence for a low or very low level of *HLA-G* expression in PE samples in comparison to normal placenta. In situ hybridisation demonstrated that the observed defect in *HLA-G* transcription was not due to reduced numbers of trophoblast cells in PE samples [25].

Hence, mechanisms controlling alternative splicing (*HLA-G3* deficit) but also transcription of the *HLA-G* gene are apparently disturbed in PE and thus might be related to alterations of *HLA-G* expression in invasive cytotrophoblasts.

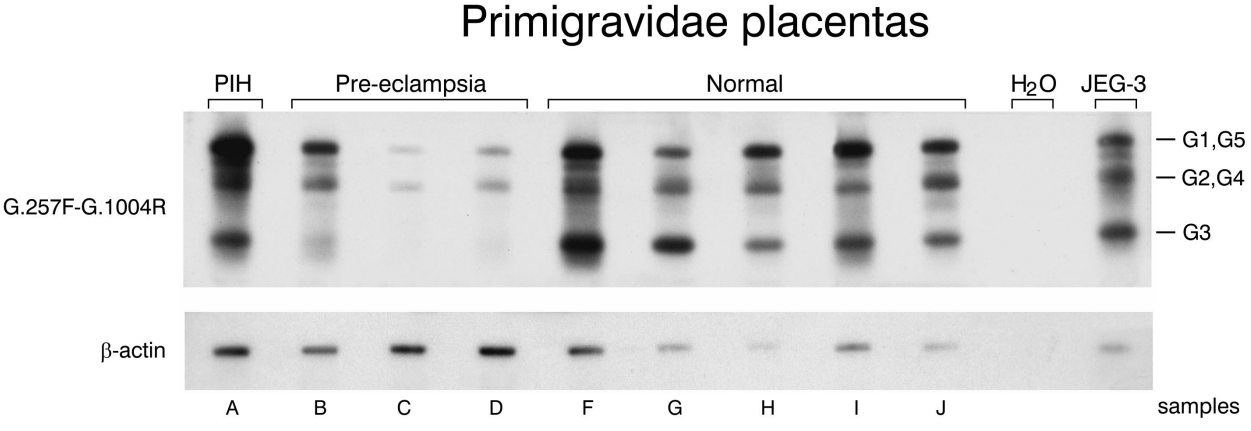

Figure 1. Analysis of *HLA-G* transcription in normal and mild PE placentas from primigravida pregnancies. Southern blots of RT-PCR products obtained using the pan-*HLA-G* primer set G.257F/ G.1004R. G1–G5 indicate alternatively spliced forms of *HLA-G* transcript. β-actin is the internal control. Samples are identified by letters as indicated in table 1. The loading of RT-PCR products from PE samples exibiting low or very low levels of *HLA-G* transcript was increased to confirm that levels of G3 are absent or very reduced in PE samples (even with extensive exposure; data not shown). As previously demonstrated, levels of *HLA-G* transcripts relative to β-actin controls are reduced in PE placentas.

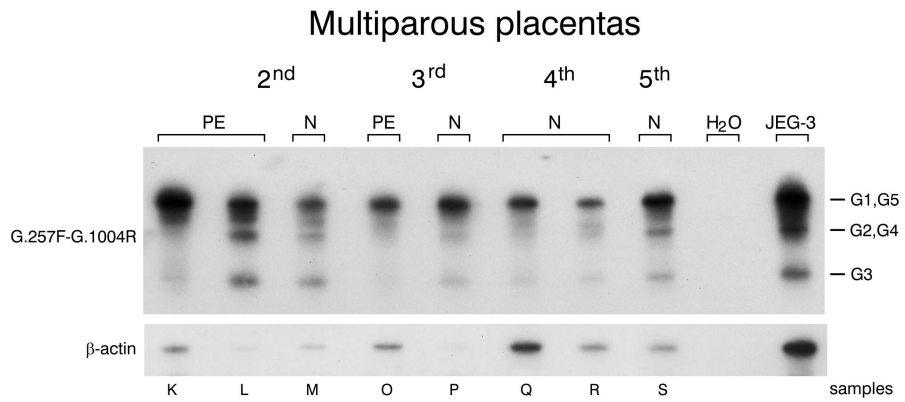

Figure 2. Analysis of HLA-G transcription in normal and mild PE placentas from multigravida pregnancies. Nomenclature is as indicated in figure 1. 2nd, 3rd, 4th and 5th refers to the pregnancy number.

Table 1. Analysis of *HLA-G* transcription and genotype in normal and PE placentas.

Sample	Placenta type	Pregnancy number	*HLA-G* transcription		*HLA-G* genotype		
			overall transcripts	*HLAG3*	C/T-93	I/D-14bp	alleles
A	PIH	1st	normal	++++	CC	DD	C-D, C-D
B	PE	1st	low	–	TT	II	T-I, T-I
C	PE	1st	low	–	CT	II	C-I, T-I
D	PE	1st	low	–	TT	II	T-I, T-I
E	PE	1st	nd	nd	CT	II	C-I, T-I
F	Normal	1st	normal	++++	CC	DD	C-D, C-D
G	Normal	1st	normal	++++	CT	ID	C-D, T-I
H	Normal	1st	normal	++++	CC	DD	C-D, C-D
I	Normal	1st	normal	+++	CC	ID	C-I, C-D
J	Normal	1st	normal	++++	CC	ID	C-I, C-D
K	PE	2nd	low	–	TT	II	T-I, T-I
L	PE	2nd	normal	++++	CC	DD	C-D, C-D
M	Normal	2nd	normal	+++	CC	DD	C-D, C-D
N	Normal	2nd	nd	nd	CT	ID	C-D, T-I
O	PE	3rd	low	–	CT	ID	C-D, T-I
P	Normal	3rd	normal	+++	CC	DD	C-D, C-D
Q	Normal	4th	reduced	++	CT	ID	C-D, T-I
R	Normal	4th	reduced	++	CT	ID	C-D, T-I
S	Normal	5th	normal	+++	CC	DD	C-D, C-D
	JEG		normal	++++	CC	II	C-I, C-I

Normal/reduced/low, levels of *HLA-G* transcription compared to JEG standard; –, very low or no detectable transcript; nd, not determined.

Two reports showed that the short forms of HLA-G, including *HLA-G3*, do not reach the cell surface and thus may have no or limited function [30, 31]. Using a transfected melanoma cell line and untagged molecules, we also investigated the function of truncated HLA-G isoforms and demonstrated both cell surface expression of HLA-G2, -G3 and -G4 and inhibition of immune effector cells [32, 33]. Expression of these isoforms seems to depend on the cell type and/or the presence of chaperoning molecules to which they would be associated. Interestingly, the description of healthy homozygous *HLA-G**0105N (null allele) individuals indicates that HLA-G1 is not necessary for survival [34]. Nevertheless HLA-G2, -G3 and -G6 are produced and may thus play a role during pregnancy [35]. Moreover, the leader peptides of truncated HLA-G isoforms may play a key role in expression of HLA-E [36]. Hence, the reduction in HLA-G3 observed in mild PE may be significant. The HLA-G3 isoform was absent in almost all mild PE samples tested, indicating altered *HLA-G* transcription mechanisms associated with the disease. However, whether altered *HLA-G* transcription is involved in trophoblast invasion (early stages of the disease) or if it is a consequence of placental ischaemia and infarction (late stages of the disease) remains unclear.

Table 2. CAC-CAT polymorphism at codon 93 and 14-pb deletion/insertion (D/I) in the 3′ untranslated region (3′ UTR) related to *HLA-G* alleles (WHO nomenclature).

Allele	Codon 93	3′ UTR
*G*01011*	C	D
*G*01012*	T	I
*G*01013*	C	I
*G*01014*	C	nd
*G*01015*	C	nd
*G*01016*	C	nd
*G*01017*	T	nd
*G*01018*	C	nd
*G*0102*	C	D
*G*0103*	C	I
*G*01041*	C	D
*G*01042*	C	nd
*G*01043*	C	nd
*G*0105N*	T	I
*G*0106*	T	I

nd, not determined.

To assess whether allele-specific transcription was involved, two common polymorphisms were genotyped in both cDNA (n = 6) and genomic DNA (n = 7) from PE samples and normal placentas (table 1). The silent CAC-CAT polymorphism at codon 93 and the 14-bp deletion in the 3′ UTR (table 2) were selected for analysis because both normally occur at a relatively high frequency [37–39].

Genotyping data showed concordance between cDNA and genomic DNA in both normal and PE samples, indicating that *HLA-G* is not subjected to imprinting mechanisms in term placenta. This result agrees with previous studies on normal first-trimester placentas [29, 39] and also indicates that *HLA-G* imprinting is not a factor in PE. In addition, imprinting was not observed at the level of *HLA-G* isoforms using G1/G4- and G5-specific cDNA genotyping (data not shown).

Polymorphism, genotype and allele distributions were compared between normal (n = 11) and PE (n = 7) samples by Fisher's exact test and the results are presented in table 3. Significant differences were observed for the C/T-93 polymorphism when both allele (p = 0.011) and genotype (p = 0.04) distribution were compared, reflecting an excess of the C-93 allele in normal samples. In agreement with this, an excess of the C-93 allele has previously been observed in normal first-trimester placentas [39]. Significant differences were also observed for the I/D-14bp polymorphism due to an excess of the I-14bp allele (p = 0.002) in PE samples. This excess was also evident in the genotype distribution of the I/D-14bp polymorphism, with a significant increase in II-14bp homozygotes (p = 0.02) being observed between control and PE samples. In particular, all primigravidae PE samples were II-14bp homozygotes, while no homozygotes, for the I-14bp allele were observed in controls. The deletion polymorphism was previously genotyped in PE but no significant association was observed [40], probably reflecting the more heterogenous nature of the 13 PE/E samples used compared to the analysis of exclusively mild PE cases in the present study.

Analysis of *HLA-G* allele distribution was carried out following construction of *HLA-G* alleles using the C/T-93 and I/D-14bp polymorphisms, hence giving rise to four possible alleles: C-I, C-D, T-I and T-D. The distribution of these alleles was significantly different between control and PE samples. This reflected an excess of the T-I form (coupled at least with *G*01012*, *G*0105N* or *G*0106* alleles; table 1) in PE samples, contrasting an excess of the C-D haplotype in control samples (p = 0.005).

Thus, differences were observed between PE and normal samples at both the level of *HLA-G* transcription and *HLA-G* genotype. Interestingly, within both sample groups, a correlation was also observed between the level of *HLA-G* transcription and *HLA-G* genotype. Four of five PE placentas which showed the lowest global levels of *HLA-G* transcription, and a low level/absence of the *G3* transcript, were homozygous for the insertion polymorphism. In contrast, normal placentas (with the excep-

Table 3. Statistical analysis of *HLA-G* polymorphisms between normal and PE placentas (Fisher's test).

	Normal	PE	Two-tailed p value
Distribution of polymorphism			
C-93/T-93	18/4 (81.8%)	5/9 (35.7%)	0.011
I-14bp/D-14bp	6/16 (27.3%)	11/3 (78.6%)	0.002
Genotype distribution			
CC/CT, TT	7/4 (63.6%)	1/6 (14.3%)	0.066
TT/CC, CT	0/11 (0%)	3/4 (42.9%)	0.042
II/ID, DD	0/11 (0%)	5/2 (71.4%)	0.002
DD/ID, II	5/6 (45.45%)	1/6 (14.3%)	0.315
Allele distribution			
TI/CD, CI, TD	4/18 (18.2%)	9/5 (64.3%)	0.011
CD/TI, CI, TD	16/6 (72.7%)	3/11 (21.4%)	0.005

/versus. Percentages are given for C-93, I-14bp, CC, TT, II, DD, TI and CD from top to bottom.

tion of sample G) which showed high levels of each *HLA-G* isoform comparable to the JEG control were homozygous for the C-93 allele and had at least one copy of the 14-bp deletion allele. Indeed, the only PE placenta which had the *HLA-G* genotype common in normal placentas, sample L, also showed a high level of *HLA-G* transcription (table 2). The deficit observed in transcription of *HLA-G* and the correlation with *HLA-G* genotype may provide a possible criterion for consideration in the diagnosis and classification of PE, particularly in multigravidae, where diagnosis of PE may be complicated by underlying hypertensive disorders [41].

Recent data provide evidence for association of soluble *HLA-G* plasma levels with *HLA-G* alleles and shows the existence of 'low' or 'high' secretor HLA-G alleles [42]. The possible predisposition for differential functional effects on immune response is thus supported by our finding. Nevertheless, the influence of *HLA-G* polymorphisms on the regulation of *HLA-G* gene transcription, including differential splicing, has yet to be determined. Whether the silent C/T-93 polymorphism of the present study is a genetic marker or plays a direct role in the regulation of differential splicing of *HLA-G* is currently under investigation. Moreover, the presence of the 14-bp insertion was previously shown to generate a 92-bp deletion in the 3′ untranslated region of *HLA-G* mRNA and was considered to represent a cryptic branchpoint sequence for mRNA splicing which may affect mRNA stability [39]. Interestingly, we observed an excess of the I-14bp allele in PE samples associated with the presence of the 92-bp deletion (data not shown). This raises the possibility that this type of mRNA transcript may exhibit different stability. Such a mechanism might, in part, explain the decrease in *HLA-G* mRNA in PE.

In conclusion, the observed quantitative (previous reports) and qualitative (this report) alterations of *HLA-G* transcription seen in PE may render trophoblasts from PE pregnancies susceptible to lysis by maternal natural killer and T cells resident in the decidua. Finally, the observed association between the levels of HLA-G transcription, the specific production of *HLA-G* transcripts and *HLA-G* genotype provides the first argument that *HLA-G* might play a key role in genetic susceptibility to PE.

Acknowledgements. We are grateful to Frances Drummond for technical assistance. We thank the Service Photo de l'Institut d'Hématologie. This work was supported by the Commissariat à l'Energie Atomique and the Société de Secours des Amis des Sciences (M.O'B.).

1 Roberts J. and Redman C. (1993) Pre-eclampsia: more than just pregnancy induced hypertension. Lancet **341:** 1447–1451
2 Lim K. H., Zhou Y., Janatpour M., McMaster M., Bass K., Chun S. H. et al. (1997) Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. Am. J. Pathol. **151:** 1809–1818
3 Wang J. and Trudinger B. (1998) Endothelial cell dysfunction in preeclampsia. J. Nephrol. **11:** 53–56
4 Geraghty D., Koller B. H. and Orr H. (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
5 Kovats S., Main E., Librach C., Stubblebine M., Fisher S. and DeMars R. (1990) A class I antigen, HLA-G, expressed in human trophoblasts. Science **248:** 220–223
6 McMaster M. T., Librach C. L., Zhou Y., Lim K. H., Janatpour M. J., DeMars R. et al. (1995) Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. J. Immunol. **154:** 3771–3378
7 Chesley L. and Cooper D. (1986) Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. Br. J. Obstet. Gynaecol. **93**: 898–908
8 Arngrimsson R., Bjornsson S., Geirsson R., Bjornsson H., Walker J. and Snaedal G. (1990) Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population Br. J. Obstet. Gynaecol. **97:** 762–769
9 Lie R. T., Rasmussen S., Brunborg H., Gjessing H. K., Lie-Nielsen E. and Irgens L. M. (1998) Fetal and maternal contributions to risk of pre-eclampsia: population based study. Br. Med. J. **316:** 1343–1347
10 Harrison G. A., Humphrey K. E., Jones N., Badenhop R., Guo G., Elakis G. et al. (1997) A genomewide linkage study of preeclampsia/eclampsia reveals evidence for a candidate region on 4q. Am. J. Hum. Genet. **60:** 1158–1167
11 Arngrimsson R., Hayward C., Nadaud S., Baldursdottir A., Walker J. J., Liston W. A. et al. (1997) Evidence for a familial pregnancy-induced hypertension locus in the eNOS-gene region. Am. J. Hum. Genet. **61:** 354–362
12 Sohda S., Arinami T., Hamada H., Yamada N., Hamaguchi H. and Kubo T. (1997) Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J. Med. Genet. **34:** 525–526
13 Ward K., Hata A., Jeunemaitre X., Helin C., Nelson L., Namikawa C. et al. (1993) A molecular variant of angiotensinogen associated with preeclampsia. Nat. Genet. **4:** 59–61
14 Houlihan J. M., Biro, P. A., Harper H. M., Jenkinson H. J. and Holmes C. H. (1995) The human amnion is a site of MHC class Ib expression: evidence for the expression of HLA-E and HLA-G. J. Immunol. **154:** 5665–5674
15 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. **21:** 289–298
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 Ishitani A. and Geraghty D. (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
18 Paul P., Adrian Cabestre F., Ibrahim E. C., Lefebvre S., Khalil-Daher I., Vazeux G. et al. (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
19 Yang Y., Chu W., Geraghty D. and Hunt J. (1996) Expression of HLA-G in human mononuclear phagocytes and selective induction by IFN-gamma. J. Immunol. **156:** 4224–4231
20 Moreau P., Adrian-Cabestre F., Menier C., Guiard V., Gourand L., Dausset J. et al. (1999) IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. Int. Immunol. **11:** 803–811
21 Rouas-Freiss N., 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
22 Le Gal F. A., Riteau B., Sedlik C., Khalil-Daher I., Menier C., Dausset J. et al. (1999) HLA-G mediated inhibition of antigen-specific cytotoxic T lymphocytes. Int. Immunol. **11:** 1351–1356
23 Colbern G. T., Chiang M. H. and Main E. K. (1994) Expression of the nonclassic histocompatibility antigen HLA-G by preeclamptic placenta. Am. J. Obstet. Gynecol. **170:** 1244–1250
24 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
25 Goldman-Wohl D. S., Ariel I., Greenfield C., Hochner-Celnikier D., Cross J., Fisher S. et al. (2000) Lack of human leukocyte antigen-G expression in extravillous trophoblasts is associated with pre-eclampsia. Mol. Hum. Reprod. **6:** 88–95
26 Vaughan P. and McCarthy T. (1998) A novel process for mutation detection using uracil DNA-glycosylase. Nucleic Acids Res. **26:** 810–185
27. Moreau P., Carosella E., Gluckman E., Gourand L., Prost S., Dausset J. et al. (1995) Alternative transcripts of the MHC of the non-classical class I HLA-G gene in the trophoblast during the first pregnancy trimester and in the placenta at term. C. R. Acad. Sci. III **318:** 837–842
28 Onno M., Guillaudeux T., Amiot L., Renard I., Drenou B., Hirel B. et al. (1994) The HLA-G gene is expressed at a low mRNA level in different human cells and tissues. Hum. Immunol. **41:** 79–86
29. Hviid T., Moller C., Sorensen 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
30 Mallet V., Proll J., Solier C., Aguerre-Girr M., DeRossi M., Loke Y. W. et al. (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
31 Bainbridge D. R., Ellis S. A. and Sargent I. L. (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
32 Menier C., Riteau B., Dausset J., Carosella E. D. and Rouas-Freiss N. (2000) HLA-G: truncated isoforms can substitute for HLA-G1 in fetal survival. Hum. Immunol. **61:** 1118–1125
33 Riteau B., Rouas-Freiss N., Menier C., Paul P., Dausset J. and Carosella E. D. (2001) HLA-G2, -G3 and -G4 isoforms expressed as non mature cell-surface glycoproteins inhibit NK and antigen-specific CTL cytolysis. J. Immunol. **166:** 5018–5028
34. Ober C., Aldrich C., Rosinsky B., Robertson A., Walker M. A., Willadsen S. et al. (1998) HLA-G1 protein expression is not essential for fetal survival. Placenta **19:** 127–132
35. Castro M. J., Morales P., Rojo-Amigo R., Martinez-Laso J., Allende L., Varela P. et al. (2000) Homozygous HLA-G*0105N healthy individuals indicate that membrane-anchored HLA-G1 molecule is not necessary for survival. Tissue Antigens **56:** 232–239
36 Llano M., Lee N., Navarro F., Garcia P., Albar J. P., Geraghty D. E. et al. (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
37 Harrison G., Humphrey K., Jakobsen I. and Cooper D. (1993) A 14 bp deletion polymorphism in the HLA-G gene. Hum. Mol. Genet. **2:** 2220
38 Kirszenbaum M., Djoulah S., Hors J., Prost S., Dausset J. and Carosella E. D. (1999) Polymorphism of HLA-G gene and protein. J. Reprod. Immunol. **43:** 105–109
39 Hiby S., King A., Sharkey A. and Loke Y. (1999) Molecular studies of trophoblast HLA-G: polymorphism, isoforms, imprinting and expression in preimplantation embryo. Tissue Antigens **53:** 1–13
40 Humphrey K., Harrison G., Cooper D., Wilton A., Brennecke S. and Trudinger B. (1995) HLA-G deletion polymorphism and pre-eclampsia/eclampsia. Br. J. Obstet. Gyn. **102:** 707–710
41 Broughton Pipkin F. (1999) What is the place of genetics in the pathogenesis of pre-eclampsia? Biol. Neonate **76:** 325–330
42 Rebmann V., Van Der Ven K., PaBler 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