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Lack of human leukocyte antigen-G expression in extravillous trophoblasts is associated with pre-eclampsia

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Pre-eclampsia, a common complication of first pregnancies, is thought to result from a poorly perfused placenta and may reflect an abnormal maternal immune reaction to the hemiallogenic fetus. Human leukocyte antigen (HLA)-G, a major histocompatibility tissue-specific antigen expressed in extravillous trophoblast cells (fetal-derived), may protect trophoblasts from maternal–fetal immune intolerance and allow these cells to invade the uterus. Through RNA in-situ hybridization analysis, we studied the expression pattern of HLA-G in normal placentae and placentae from pregnancies complicated by severe pre-eclampsia. In normal placenta we found HLA-G expression in the anchoring extravillous trophoblasts with an increasing gradient of expression in the more invasive cells. However, in nine out of 10 pre-eclamptic placentae HLA-G expression was absent or reduced. We conclude that HLA-G is normally expressed in invasive trophoblasts and HLA-G expression is defective in most pre-eclamptic placentae. We propose that trophoblasts lacking HLA-G are vulnerable to attack by the maternal immune system. These defective trophoblasts will be unable to invade the maternal spiral arteries effectively, thereby developing vessels which cannot adequately nourish the developing placenta. This poorly perfused placenta may initiate the systemic cascade of events associated with pre-eclampsia.

Key words: HLA-G/placenta/pre-eclampsia/spiral arteries/trophoblast

Introduction

Pre-eclampsia, a hypertensive disease of human pregnancy, is a major cause of infant and maternal mortality worldwide and is resolved by childbirth. Pre-eclampsia is primarily found as a complication of first pregnancies. It is characterized by proteinuria, pregnancy-induced hypertension and oedema and can induce a cascade of potentially life-threatening systemic problems (Lefant *et al*., 1990). It has been postulated that pre-eclampsia in primigravidae stems from a poorly perfused placenta which develops as a result of maternal immune reaction to the hemiallogenic fetus (reviewed in Loke and King, 1997; Taylor, 1997).

Evidence which implicates the placenta as the source of pre-eclampsia centres on the abnormal trophoblast–uterine interaction manifested as defects in the morphology of the placental bed consistently observed in this syndrome (Zuspan, 1988; Redman, 1991a,b). In normal pregnancy the extravillous trophoblasts anchor the placenta to the uterus and invade the maternal tissue and vascular system (Pijnenborg, 1990). These invading trophoblasts remodel the maternal spiral arteries by displacing the endothelial lining and destroying most of the musculoelastic tissue of these arteries (Brosens *et al*., 1967; Boyd and Hamilton, 1970; Zhou *et al*., 1997a). These arteries are thus converted to vessels with low resistance and high capacitance necessary for increased blood flow (reviewed in Pijnenborg, 1990; Loke and King, 1997). In pre-eclampsia, invasive trophoblasts are fewer in number and invasion is shallow (Redman, 1991a). As a direct result the maternal vessels do not undergo the complete spectrum of physiological changes and the mean external diameter of the myometrial vessels is less than half that of vessels found in normal pregnancies (Brosens *et al*., 1972; Gerretsen *et al*., 1981; Khong *et al*., 1986; Moodley *et al*., 1989; Zhou *et al*., 1997b). These defects in pre-eclampsia lead to a placenta which is relatively hypoxic and fails to meet the fetal demands for increased blood flow. In its more severe forms the defective placenta may cause intrauterine growth retardation (IUGR).

Pre-eclampsia is thought to stem either from an underlying maternal medical problem that may only be evident during the added physiological demands of pregnancy or as a primary problem of maternal fetal immune recognition (Ness and Roberts, 1996). Classic pre-eclampsia, which can complicate 1–10% of first pregnancies, may be immunological in origin because it is most likely to occur in primigravidae and in multiparous women with a new sexual partner (Need, 1975; Roberts *et al*., 1990; Sibai *et al*., 1995). In a recent study, an increased incidence of pre-eclampsia was found in pregnancies resulting from donor sperm insemination as compared to partner insemination (Smith *et al*., 1997). These lines of investigation suggest that pre-eclampsia develops as a consequence of maternal immune response to the hemiallogenic fetus and that prior exposure to paternal antigen is protective (Redman, 1991b). Pre-eclampsia has also been used as an

example of the 'genetic conflict' theory of evolution (Moore and Collins, 1997; Schuiling *et al*., 1997).

The question of how the maternal immune system tolerates the hemiallogenic fetus was answered in part when it was found that trophoblasts do not express the classic highly polymorphic HLA class l antigens, thus protecting trophoblasts from attack by maternal T cell-mediated alloreactivity. However, trophoblasts would still be vulnerable since natural killer (NK) cells lyse cells devoid of class l molecules. Expression and function of the major histocompatibility genes throughout fertilization and embryogenesis was recently reviewed (Fernandez *et al*., 1999). The discovery that HLA-G, a class lb molecule of low polymorphism and tissue specificity, is expressed on extravillous trophoblasts, helped explain how maternal NK cells may tolerate the invasive villi (Ellis *et al*., 1986; Geraghty *et al*., 1987; Kovats *et al*., 1990; reviewed in Bouteiller *et al*., 1999). The demonstration, in a functional assay, that expression of HLA-G protects cells from lysis by NK cells highlights a role for HLA-G in maternal tolerance of trophoblast cells (Pazamany *et al*., 1996; Rous-Freiss *et al*., 1997; reviewed in Yokayama, 1997). Recent work using transfectants of HLA-G and NK clones suggests that protection afforded by HLA-G may be due to its regulation of HLA-E (when present) through its leader sequence (Navarro *et al*., 1999). The significance of these results to the situation in the placenta is yet unknown. Protection of trophoblasts from attack by NK cells is of critical importance, since in a normal pregnancy trophoblasts closely associate with NK cells at the implantation site, where they represent the major lymphocyte population (reviewed in Loke and King, 1997; King *et al*., 1997, 1998). However, NK cells do not act independently to control placentation (Johnson *et al*., 1997). Among the factors that may play a role in placentation and invasion of trophoblasts are: Fas and its binding to Fas ligand, cytokines produced at the implantation site, integrins, adhesion molecules, and proteolytic systems involved in the degradation of the extracellular matrix (reviewed in Cross *et al*., 1994; Uckan *et al*., 1997).

We began our study with a characterization of HLA-G expression in the three trimesters of normal pregnancies. We next compared HLA-G expression in placentae of primigravidae complicated by severe pre-eclampsia to normal term placenta controls. Based on our results we propose that aberrant HLA-G expression prevents trophoblasts from appropriately invading the maternal tissues and vascular system. This failure of invasion may subsequently result in the placentation defects leading to the reduced utero-placental blood flow observed in pre-eclampsia and initiate the systemic problems associated with this disorder. Our results are in agreement with and expand those of Hara *et al*., who demonstrated an altered expression of HLA-G in pre-eclamptic placentae using immunohistochemistry on frozen sections (Hara *et al*., 1996).

Materials and methods

Clinical criteria

Placentae from normal pregnancies were obtained from women with uncomplicated pregnancies and with no prepregnancy medical problems.

The pre-eclamptic patients were diagnosed with severe pre-eclampsia according to established criteria (ACOG, 1996): proteinuria ⩾5 g protein in 24 h (3+ urine dipstick), hypertension ⩾160 mmHg systolic or ⩾110 mmHg diastolic, and IUGR of the fetus <10th percentile for gestational age.

First and second trimester placentae were obtained from elective pregnancy terminations. Normal third trimester placentae and placentae of pregnancies complicated by pre-eclampsia were taken from normal deliveries or Caesarean sections in accordance with the protocol of the Human Subjects Committee approved by our institution.

In-situ hybridization

Tissues were formalin-fixed and paraffin-embedded. Sections (5 μm) were floated onto Superfrost/Plus slides (Menzel-Glaser, Braunschweig, Germany). Radioactive RNA in-situ hybridization was performed according to published methods (Millen and Hui, 1996), with some modifications. We investigated chromogen methods and found that, given the high levels of alkaline phosphatase in the placenta, the radioactive method that we employed gave more reliable results. Prior to in-situ hybridization the slides were baked for 40 min at 55°C and deparaffinized in xylene and rinsed in graded alcohols followed by 0.5×SSC (1×SSC is 0.15 mol/l sodium chloride and 0.015 mol/l sodium citrate). Slides were treated with proteinase K, 20 μg/ml (Sigma Chemical Co., St Louis, MO, USA) for 7.5 min at 37°C. Acetylation of the slides was for 10 min at room temperature in 0.1 mol/l triethanolamine/acetic anhydride. Slides were rinsed in PBS and 0.5×SSC and dehydrated through graded alcohols and air dried at room temperature. Hybridization with [^{35}S]UTP-labelled antisense riboprobe (5×10^5 c.p.m./slide) was performed in 50 μl of 50% formamide hybridization buffer (32) at 55°C in a humidified chamber. After an overnight incubation the slides were rinsed in 2×SSC/0.1% 2-mercaptoethanol and treated with 20 μg/ml RNase A (Sigma) for 30 min at 37°C. The slides were washed for 2 h in 0.1×SSC/0.1% 2-mercaptoethanol at 55°C, rinsed in 0.5×SSC and air-dried. Slides were dipped in Kodak NTB-2 emulsion (Eastman Kodak Co., Rochester, NY, USA) and exposed for 1–2 weeks at 4°C and developed according to the manufacturer's instructions at 15°C. The slides were counterstained with haematoxylin and eosin and photographed under bright and darkfield microscopy. The normal and pre-eclampsia in-situ slides were read blind to their origin although the placental pathology of pre-eclampsia was readily identifiable in some of the sections. All experiments were performed on duplicate slides.

In-situ ribobrobes

The HLA-G probe (Geraghty *et al*., 1987) was a 451 bp *Pvu*II fragment from exon 8 (3′ untranslated region of HLA-G in order to avoid cross hybridization to other class 1-related genes) cloned into bluescript (Stratagene, CA, USA). Sense and antisense riboprobes were prepared (Millen and Hui, 1996) with T3 and T7 RNA polymerase respectively (Boehringer Mannheim, Germany), from linearized plasmids and digested with RNase-free DNase (Boehringer Mannheim). The antisense human cytokeratin probe (Oshima *et al*., 1986) was linearized with *Apa*I (New England Biolabs, Beverly, MA, USA) and transcribed with T3 RNA polymerase. [^{35}S]UTP (New England Nuclear, Boston, MA, USA) was incorporated into the riboprobe labelling reactions.

Immunohistochemistry

Anti-cytokeratin immunohistochemistry, with a broad spectrum keratin monoclonal primary antibody, was performed on serial sections according to the Histostain Plus kit (Zymed Lab-SA System, South

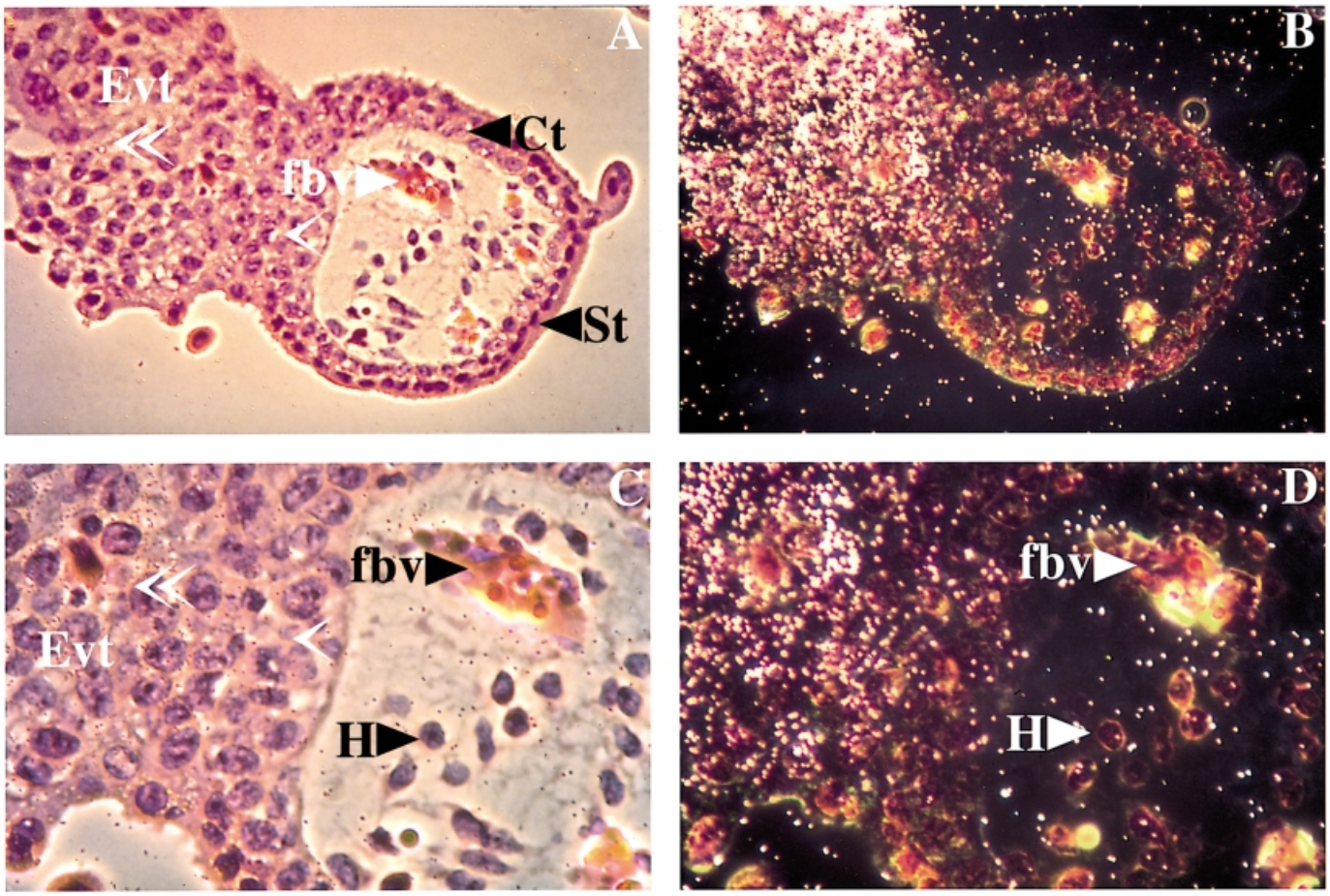

Figure 1. Antisense HLA-G RNA in-situ hybridization of first trimester placenta. A paraffin-embedded first trimester placental section hybridized with an antisense HLA-G riboprobe, counterstained with haematoxylin and eosin and photographed under brightfield (**A**, **C**) and darkfield (**B**, **D**) microscopy [original magnification ×200 (**A**, **B**) and ×800 (**C**,**D**)]. Positive expression of HLA-G is evident in extravillous trophoblasts (Evt) of the cell columns with proximal cells (one open arrowhead) showing less HLA-G expression than more distal invasive cells (two open arrowheads). Syncytiotrophoblast (St), Hofbauer cells (H) fetal blood vessels (fbv) and cytotrophoblasts (Ct) are negative for HLA-G expression.

San Francisco, CA, USA), and developed with their AEC chromogen mixture.

Results

Expression of HLA-G RNA in normal first trimester placentae

We initially characterized HLA-G expression in normal placentae. RNA in-situ hybridization of normal first trimester paraffin-embedded placental sections revealed HLA-G expression only in trophoblast cell columns of the anchoring villi (Figure 1A–D). We did not detect HLA-G expression in the mesenchymal stroma, Hofbauer cells (macrophages), villous cytotrophoblasts, fetal blood vessels, or in the syncytiotrophoblast. The red blood cells appear bright in darkfield microscopy (Figure 1B,D) but exposed silver grains are not present above background levels. Furthermore, we found an increasing gradient of expression of HLA-G in the more invasive (distal) end of cell columns. Using a sense HLA-G probe, we detected no specific hybridization above background, confirming the specificity of the probe (Figure 2A,B). In HLA-G in-situ hybridization of second trimester placentae, we observed expression in invasive trophoblasts at the implantation site of the placental bed (data not shown).

Expression of HLA-G RNA in normal third trimester and pre-eclampsia placentae

Table I summarizes the clinical profiles of the patients we chose to study. Twelve normal and ten placentae complicated by severe pre-eclampsia (ACOG, 1996) were included. There were no known prepregnancy underlying medical conditions in either the normal or pre-eclampsia patients. Mode of delivery, 5′ APGAR score, weight of the infant, gestational age and age of the mother as well as reason for Caesarean section are indicated.

We hybridized tissue sections from the 12 normal placentae and 10 pre-eclampsia placentae with the HLA-G antisense riboprobe. We observed HLA-G expression in extravillous trophoblasts in normal placenta at the site of implantation and proceeding through Nitabuch's layer into the decidua (Figure 3A,B) and in trophoblast cell islands. In 9/10 pre-eclampsia placentae HLA-G expression was either absent or reduced in the invasive extravillous trophoblasts at the implantation site (Figure 3D,E,G,H). Non-specific probe hybridization to fibrin

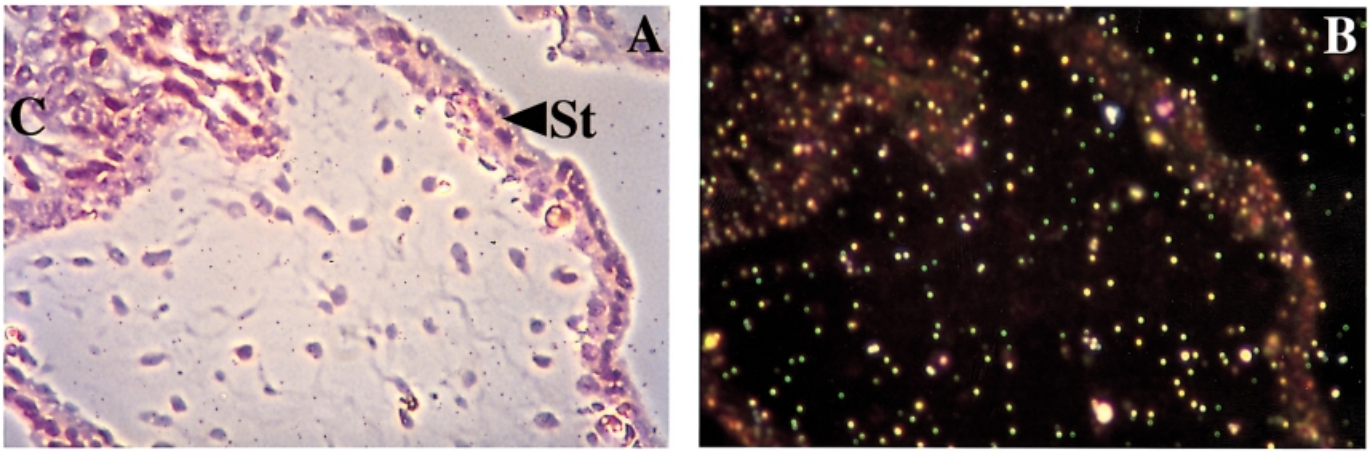

Figure 2. Sense HLA-G RNA in-situ hybridization of first trimester placenta. A paraffin-embedded first trimester placental section hybridized with a sense HLA-G riboprobe, counterstained with haematoxylin and eosin and photographed under brightfield (**A**) and darkfield (**B**) microscopy (original magnification ×400). No hybridization above background levels is observed. Syncytiotrophoblast (St) and a cell column (C) are indicated.

Table I. Clinical profiles of the patients in the study

Patient	Age (years)	Parity	PIH	Proteinuria	Mode of delivery	Ges. age (years)	Weight	5′ APGAR	Comments
Normal									
1	27	3	ϕ	ϕ	C/S	38	2735	10	Breech presentation, twins
2	37	1	ϕ	ϕ	C/S	40	2960	10	Fetal distress
3	33	3	ϕ	±	C/S	38	3125	10	2 prior C/S
4	29	2	ϕ	ϕ	C/S	39	4220	9	1 prior C/S
5	30	1	ϕ	+	C/S	40	2910	10	Breech presentation
6	36	4	ϕ	ϕ	C/S	38	2805	10	2 prior C/S
7	29	1	ϕ	ϕ	C/S	40	2985	10	Fetal distress
8	35	3	ϕ	±	C/S	37	2885	9	Fetal distress
9	31	3	ϕ	ϕ	Normal	40	3095	10	
10	24	2	ϕ	ϕ	Normal	39	3325	10	
11	28	2	ϕ	ϕ	Normal	40	3580	10	
12	27	1	ϕ	±	Normal	41	3580	10	Fetal distress
PE									
1	25	1	Severe	3+	C/S	29	640	8	Fetal distress
2	22	1	Severe	3+	C/S	29	830	9	Breech presentation
3	29	1	Severe	3+	C/S	38	2700	10	
4	32	1	Severe	3+	Normal	31	680	0	Intrapartum fetal death
5	26	1	Severe	3+	C/S	35	1638	9	Fetal distress
6	23	1	Severe	3+	C/S	40	2055	10	Fetal distress
7	28	1	Severe	3+	C/S	35	1340	10	HELLP syndrome
8	29	1	Severe	3+	Vacuum	37	2035	9	
9	30	1	Severe	3+	C/S	30	800	9	Fetal distress
10	27	1	Severe	3+	C/S	33	1340	10	Breech presentation

PIH = pregnancy-induced hypertension; Ges. = gestational; C/S = Caesarean section; PE = pre-eclampsia; HELLP = hemolysis, elevated liver enzymes and low platelets; ϕ = none; ± = trace 0.1 g/l; 1+ = 0.3 g/l; 3+ = 3.0 g/l.

deposition was evident in most specimens. In the pre-eclampsia placentae characterized as reduced we could occasionally detect focal clusters of HLA-G positive trophoblasts that had invaded into the maternal decidua. Positive cytokeratin immunohistochemistry of the next section in the series (Figure 3C,F,I) identified extravillous trophoblasts in the decidua. We also detected hybridization to trophoblasts in sections from all of the placentae included in this study through RNA in-situ hybridization analysis with an antisense cytokeratin riboprobe (data not shown). This confirmed that RNA was intact in all of the placentae. Table II summarizes the placental pathology and in-situ results. The placental pathology of all 12 normal placentae was unremarkable but anatomical anomalies typical of pre-eclampsia (i.e. infarcts and decidual vascular lesions) were observed in the majority of pre-eclamptic placentae.

Discussion

We examined 12 normal term placentae and 10 placentae of pre-eclampsia for HLA-G expression using RNA in-situ hybridization analysis. In all normal term placentae we detected HLA-G in the extravillous trophoblasts of the implantation site. In sharp contrast to these results we detected no or reduced HLA-G expression in 9/10 placentae complicated by pre-eclampsia. Thus, we have found a strong correlation between lack of expression of the HLA-G found in the

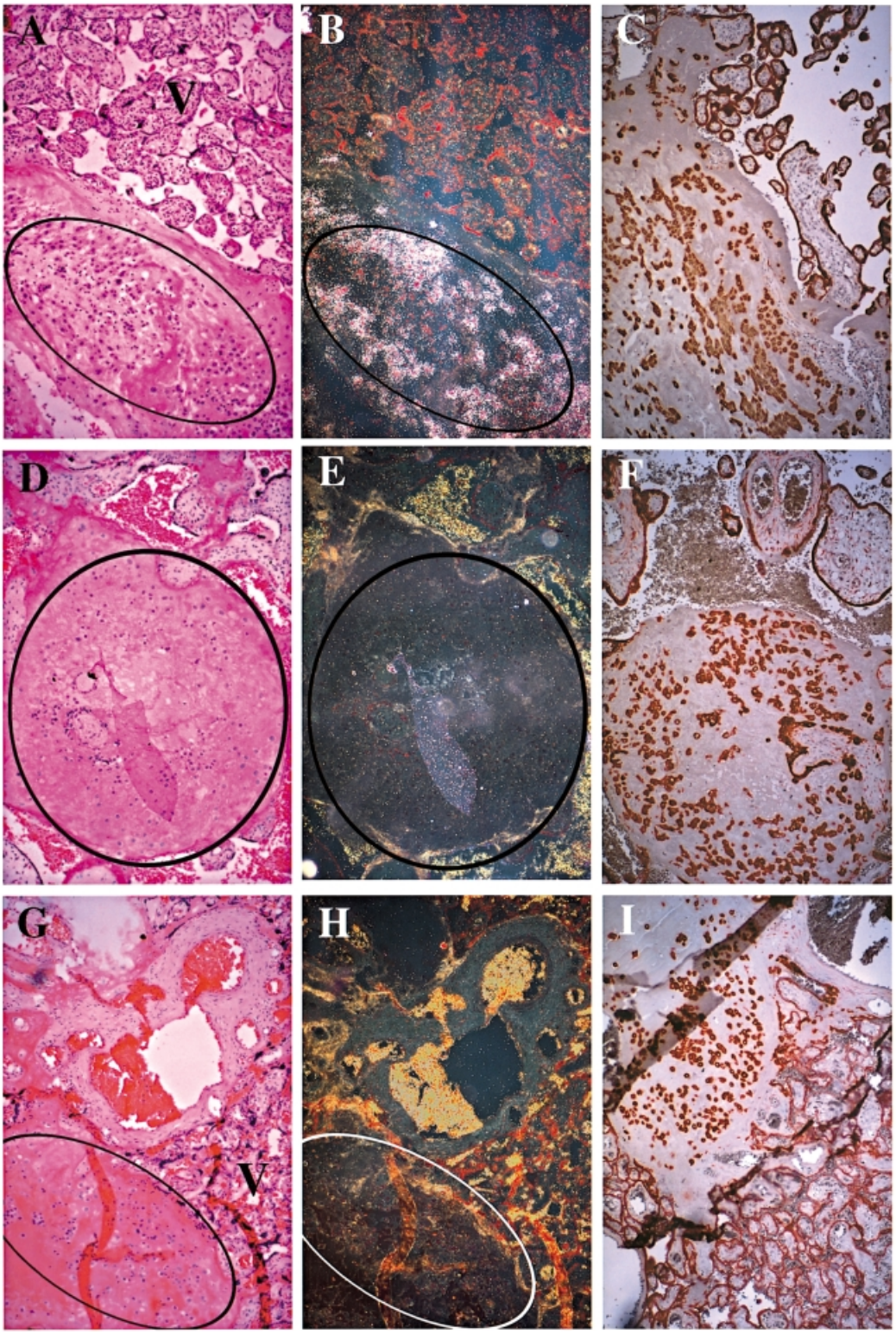

Figure 3. Antisense HLA-G RNA in-situ hybridization of normal third trimester placenta and pre-eclampsia placenta. Normal third trimester placental sections (**A**, **B**, **C**) and pre-eclampsia sections from two patients (**D** ,**E**, **F**) and (**G**, **H**, **I**) were hybridized with an antisense HLA-G riboprobe (**A**, **B**, **D**, **E**, **G**, **H**) and counterstained with haematoxylin and eosin. The same slide was photographed under brightfield (**A**, **D**, **G**) and darkfield (**B**, **E**, **H**) microscopy (original magnification ×200). Extravillous trophoblast cells that have infiltrated the decidua are circled in the in-situ sections. Blood appears yellowish in the darkfield photographs. Positive hybridization to the HLA-G riboprobe is detected in the extravillous trophoblasts of the normal placenta (**A**, **B**) but not in pre-eclamptic placentae (**D**, **E** and **G**, **H**). Positive cytokeratin immunohistochemistry of the next section in each block (**C**, **F**, **I**) confirms the presence of extravillous trophoblasts in all sections. Chorionic villi are indicated (V).

Table II. Summary of placental pathology and HLA-G in-situ hybridization

Patients	Pathology report	HLA-G *in situ*
Normal		
1	Normal term placenta	Positive
2	Normal term placenta	Positive
3	Normal term placenta	Positive
4	Normal term placenta	Positive
5	Normal term placenta	Positive
6	Normal term placenta	Positive
7	Normal term placenta	Positive
8	Normal term placenta	Positive
9	Normal term placenta	Positive
10	Normal term placenta	Positive
11	Normal term placenta	Positive
12	Normal term placenta	Positive
Pre-eclampsia		
1	Small placenta with mature histological features, villous oedema, infarcts and normoblast in villous vessels, recent thrombosis of stem villous vessels	Positive
2	Placenta with no unusual features	Negative
3	Term placenta with infarct, mild fibrinoid change of decidual vessels	Negative
4	Small second trimester placenta with infarcts and signs of chronic abruption, fibrinoid necrosis, and acute atherosis of decidual vessels	Reduced
5	Small preterm placenta with infarcts and intervillous fibrin deposition	Negative
6	Small term placenta, focal oedema, intervillous fibrin deposition	Negative
7	Small placenta with infarcts and placental haematomas	Negative
8	Small term placenta Negative/reduced	
9	Small preterm placenta, placental infarcts	Negative/reduced
10	Mature placenta with retroplacental haematoma, fibrinoid necrosis of decidual vessels	Negative

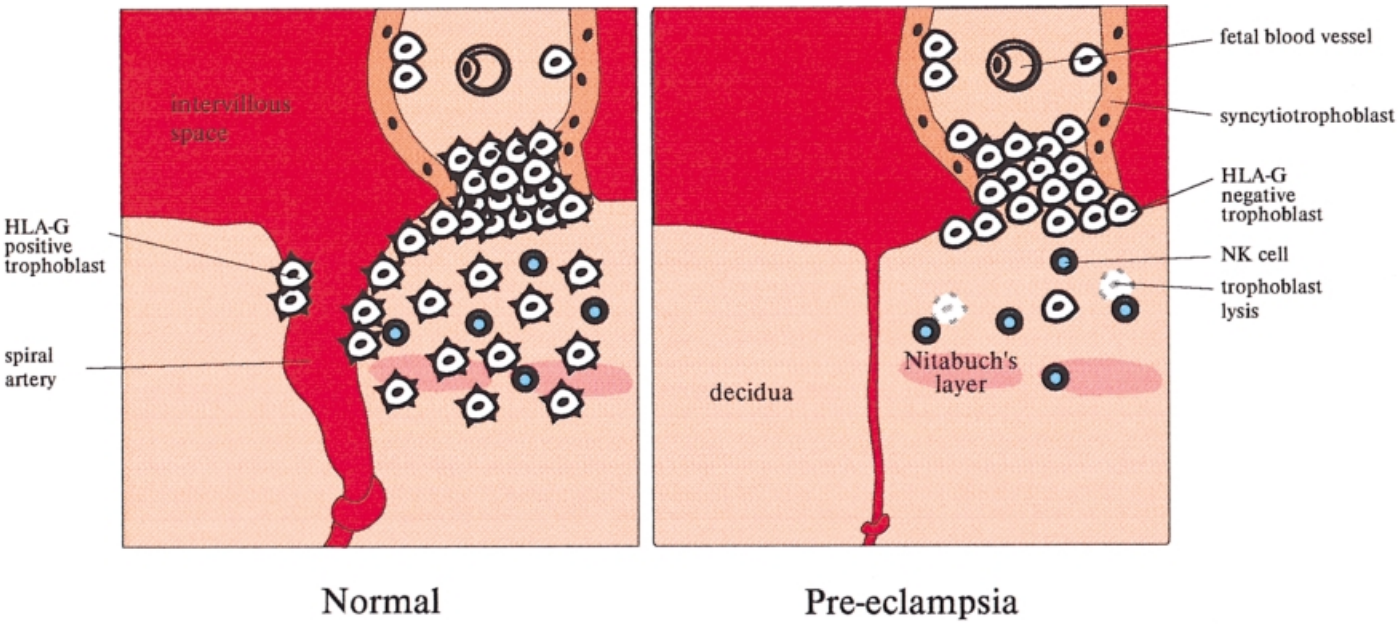

Figure 4. Model of HLA-G expression in the placenta. HLA-G is normally expressed only in anchoring extravillous trophoblasts, not in syncyciotrophoblast nor in cytrotrophoblasts. The extravillous trophoblasts normally invade the uterus and the maternal spiral arteries, and remodel the arteries for increased blood flow. HLA-G protects these invasive cells from lysis by natural killer (NK) cells and is the laissez-passer necessary for successful trophoblast endovascular and interstitial invasion. When HLA-G expression is lacking, as it is in pre-eclampsia, trophoblasts invading the decidua are lysed by NK cells. Spiral arteries are not remodelled and remain narrow, resulting in a poorly perfused placenta. HLA-G (dark knobs on cell).

extravillous trophoblasts at the fetal–maternal interface and severe pre-eclampsia complicated by IUGR. These data give molecular support to the hypothesis that pre-eclampsia is caused by localized aberrant immune recognition between the mother and fetus at the utero-placental interface. In pre-eclampsia placentae characterized as negative/reduced we could occasionally detect focal areas with clusters of HLA-G expressing trophoblasts. These clusters of HLA-G positive trophoblasts were found to have invaded the decidua and crossed Nitabuch's layer. We conclude that if trophoblasts

express HLA-G they can invade the uterus even if the pregnancy is complicated by pre-eclampsia, suggesting that HLA-G expression is a necessary precondition for invasion.

Our criterion for choosing patients in this study was those patients whose pre-eclampsia was most probably of immune origin. We therefore confined our pre-eclampsia patients to primigravidae with no medical problems prior to pregnancy. We included only women considered to have severe pre-eclampsia [pregnancy-induced hypertension (PIH) and proteinuria] and obvious IUGR as a further parameter of severity. Because the severe pre-eclampsia cases were often delivered by Caesarean section, we included 8/12 normal patients delivered by Caesarean section, none of whom had chronic placental insufficiency. We chose to study HLA-G at the level of RNA expression in an in-situ hybridization analysis. We used an antisense riboprobe which hybridizes to the 3′ untranslated region of the gene to minimize the possibility of cross-reactivity to other HLA molecules. The use of RNA in-situ hybridization was an advantage in this study since we wanted to analyse paraffin-embedded placental sections in order to obtain maximum resolution of tissue morphology and because many of our clinical samples were available only as archival paraffin-embedded preparations. Immunohistochemical data were not obtained in this survey since despite our best efforts we found that all available HLA-G-specific antibodies bound successfully only in frozen, not in paraffin, sections. In agreement with our results, Colbern *et al*., using an RNA protection assay, demonstrated reduced HLA-G expression in the placentae of pre-eclampic patients (Colbern *et al*., 1994). However, when their results were normalized to cytokeratin, which is expressed by all trophoblasts, HLA-G expression was similar to normal controls. Their finding may be related to the reduced number of extravillous trophoblasts found in pre-eclampsia. By using RNA in-situ analysis we were able to avoid this bias.

In normal first trimester placenta we observed HLA-G expression only in extravillous trophoblasts of the anchoring columns. In second and third trimester placentae we detected HLA-G in the extravillous trophoblasts at the implantation site and trophoblast cell islands. In our normal first trimester placentae we were able to detect an increasing gradient of HLA-G expression in the more distal portion of the trophoblast cell columns. This suggests that expression of higher levels of HLA-G is correlated with increased invasiveness. Our data support the immunohistochemistry data of McMaster *et al*. where expression was found only in the extravillous trophoblasts (McMaster *et al*., 1995). In normal placentae our RNA in-situ results parallel those found for immunohistochemistry and suggests that RNA and protein regulation are coincident in trophoblasts. This supports the reliability of using RNA in-situ analysis for our study. Our data contrast in part with others (Yelavarthi *et al*., 1991; Chumbley *et al*., 1993) who detected HLA-G expression in first trimester cytotrophoblasts (the stem cells) and in placental villous mesenchymal cells. Our results describe a more straightforward pattern of regulation where HLA-G is repressed in the stem cell cytotrophoblast and expressed in one of the differentiating cell types, the anchoring trophoblast lineage. One possible explanation for the difference in our results is the use of different methods of detection of in-situ RNA, each method with its inherent background problems. Recently, HLA-G expression in endothelial cells has been detected through immunohistochemistry on frozen sections with an antibody specific for a soluble HLA-G (Blaschitz *et al*., 1997). We used a probe which hybridized to the 3′ untranslated region of HLA-G. Differential splicing of the HLA-G RNA or our use of paraffin sections may explain differences in our results.

The correlation between the development of pre-eclampsia and primigravidae is suggestive of a defect in fetal–maternal immune recognition. Our work provides molecular evidence that this immune problem may be mediated by defective HLA-G expression. Yet definitive proof for an essential role for HLA-G remains elusive as progress in the field is hampered by lack of a counterpart for HLA-G in an animal model system. There is also the possibility that NK cells in conjunction with HLA-G may be involved in protection of the placenta from viral infection (Johnson *et al*., 1997; Shust *et al*., 1998) Our data are in agreement with the recent observations of Hara *et al*. who used HLA-G immunohistochemistry and placentae of pre-eclampsia (Hara *et al*., 1996). We propose a model (Figure 4) where appropriate HLA-G expression in the extravillous trophoblasts is the ‘laissez-passer’ necessary to allow these cells to invade the maternal decidua and vascular system. HLA-G is first detected in an increasing gradient of expression in cell columns of anchoring villi. In turn these invasive cells mediate the changes which provide the enhanced uterine perfusion needed for maintaining an adequate blood supply during pregnancy. When HLA-G is not expressed appropriately, as we found in pre-eclampsia placentae, the trophoblasts lack the necessary ‘entry permit’ and are prevented from invading the uterus, as the mother’s NK cells will lyse these trophoblasts lacking HLA-G. Without appropriate trophoblast invasion the maternal arteries are not remodelled. This results in reduced utero-placental blood flow and initiates the cascade of systemic events manifested as pre-eclampsia.

Acknowledgements

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