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ARTICLE

Cell bio-imaging reveals co-expression of HLA-G and HLA-E in human preimplantation embryos

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Valerie Shaikly graduated with a BSc Honors degree in life sciences from the University York in 1990. She then began training as a clinical embryologist at the Hammersmith Hospital, London. Whilst working as a senior embryologist she completed a Master's degree in health promotion in infertility at the University of East London. Valerie began her research in reproductive immunology whilst working as a senior embryologist at the Assisted Reproduction and Gynaecology Centre, London. In 2009, she was awarded a PhD from the University of Essex. Her research has focused on the investigation of factors associated with human preimplantation embryo development and foetal maternal tolerance during the course of in-vitro fertilisation.

Abstract The non-classical major histocompatibility complex (MHC) class Ib antigens, termed HLA-G and HLA-E, have been associated with fetal maternal tolerance. The role of HLA-G in the preimplantation embryo remains unclear although immunoprotection, adhesion and cell signalling mechanisms have been suggested. Unlike HLA-G, HLA-E protein expression has not been previously studied in preimplantation embryos. Embryos and model trophoblast cell lines JEG-3 and BeWo were labelled with the HLA-G- and HLA-E-specific monoclonal antibodies MEMG9 and MEME07. Flow cytometry, confocal microscopy and single particle fluorescence imaging techniques were employed to investigate the spatial and temporal expression of these receptors. Lipid raft analysis and adhesion assays were performed to investigate the role of these receptors in cell membrane domains and in promoting adhesion by cell-to-cell contact. HLA-E and HLA-G were co-localized in the trophectoderm of day 6 blastocysts. Analysis on trophoblast cell lines revealed that 37% of HLA-G and 41% of HLA-E receptors were co-localized as tetramers or higher order homodimer clusters. HLA-G receptors did not appear to play a role in either cell adhesion or immunoreceptor signalling via lipid raft platforms on the cell membrane. A possible role of HLA-G and HLA-E in implantation via immunoregulation or modulation of uterine maternal leukocytes is discussed.

KEYWORDS: embryo, human, MHC, reproductive immunology

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Introduction

The complex mechanisms involved in the immunological acceptance of a fetus and placenta, which are semi-allogenic to the mother, have not yet been fully elucidated. In humans, it has been proposed that the immune privilege of the fetus is linked with expression of the human leukocyte antigens (HLA) class I subsets (Blaschitz et al., 2001; Guleria and Sayegh, 2007).

The receptors (also termed antigens) HLA-G and HLA-E are encoded on chromosome 6 of the human major histocompatibility complex (MHC) and have been designated as non-classical HLA class Ib molecules because they exhibit less polymorphism than their classical HLA class Ia (HLA-A, -B, -C) counterparts. HLA-E is ubiquitously expressed whereas HLA-G has a restricted tissue distribution (Lee et al., 1998; LeMaoult et al., 2003; Rodgers and Cook, 2005). Both HLA-E and HLA-G exhibit low polymorphism compared with HLA class I classical genes; they are highly conserved and present a limited repertoire of peptides to T-cells (Rodgers and Cook, 2005). Cell-surface expression of HLA-E heavy chain requires the formation of a non-covalent complex with β_2-microglobulin and a leader peptide antigen, derived from the N-terminal sequence of other HLA class I molecules such as HLA-G or HLA-C (Borrego et al., 1998).

HLA-G was the first non-classical MHC class Ib antigen identified on extravillous trophoblast (Kovats et al., 1990). Later HLA-E was also detected on extravillous trophoblast (King et al., 2000). Evidence suggesting that HLA-G and HLA-E can separately modulate the activity of local target cells that express specific receptors has been produced. It was found that HLA-G suppresses the allo-proliferative response of uterine mononuclear cells (Riteau et al., 1999). HLA-G also serves to modulate production of cytokines and angiogenic factors by natural killer (NK) cells to allow trophoblast invasion and differentiation (van der Meer et al., 2004). Soluble HLA-G has also been shown to regulate angiogenesis through soluble HLA-G1/CD160-mediated anti-angiogenic activity, which may play a role in the vascular remodelling of maternal spiral arteries during pregnancy (Fons et al., 2006). HLA-E is known to bind with CD94/NKG2A inhibitory receptors on uterine natural killer cells, thus providing protection for trophoblast cells from NK cytotoxicity (Trundley and Moffett, 2004). Cell-surface HLA-E expression is dependent on the co-expression of HLA-G, thus their concomitant cell-surface expression may provide immunoprotection of the fetus from the maternal immune system. Bhalla et al. investigated the expression of HLA-G and HLA-E in the placentas of women with normal pregnancy and recurrent miscarriage. They found no difference in expression between these two groups and reported expression of HLA-G on invading extravillous trophoblast and HLA-E in-several decidual cell types including the extravillous trophoblast (Bhalla et al., 2006). Ishitani et al. (2003) reported co-expression of HLA-E and HLA-G in placental tissues and model cell lines; HLA-G was also expressed independently of HLA-E. In addition, it was noted that the expression of HLA-E was not homogenous among cells of a given type with some cells not showing expression.

Previous studies have shown that HLA-G protein is synthesized early in human embryogenesis (Jurisicova et al., 1996; Shaikly et al., 2008; Yao et al., 2005). The function of the early synthesis of HLA-G remains unclear; however, Fuzzi et al. (2002) reported that secretion of the soluble form of HLA-G by preimplantation embryos is associated with implantation potential. Similar findings have since been reported by other workers (Vercammen et al., 2008).

Although the expression of HLA-G has been reported in human embryos, no detailed studies have been conducted regarding HLA-E protein expression. In one study, Cao et al. (1999) reported HLA-E mRNA in 84% of preimplantation embryos analysed.

It has been suggested that HLA-G may play a role in cell adhesion (Odum et al., 1991; Sanders et al., 1991) and may therefore promote blastocyst attachment and cellular apposition (Le Bouteiller, 2002). A further putative role for HLA-G is as a cell-signalling molecule. It has been observed that the murine orthologue of HLA-G, known as H-2 Qa-2, is expressed in mouse embryos that exhibit fast cleavage and high implantation potential (Warner et al., 1987). These authors report that the Qa-2 molecule is localized in lipid rafts and therefore implicated in cell signalling via lipid raft associated proteins (Comiskey et al., 2003); these workers also noted HLA-G association with lipid rafts and postulate that HLA-G may also serve as a signalling molecule.

This study used experiments designed to investigate whether HLA-E and HLA-G display physical association on the trophectoderm of the blastocyst. Trophectoderm cells are the first cells that come into contact with the maternal decidua and thus mechanisms of signalling and adhesion might be in place to promote correct cellular apposition and uterine implantation. Confocal microscopy and single particle fluorescence imaging (SPFI) were employed to investigate the spatial and oligomeric distribution of HLA-E and HLA-G at a single cell level on the cell surface of blastocysts and model trophoblast cell lines JEG-3 and BeWo. Quantification of cell-surface receptors of HLA-G and HLA-E was determined on cell culture populations by flow cytometry. Lipid raft formation and adhesion assays were also carried out to test whether HLA-G promotes the formation of membrane raft domains and cellular attachment.

Materials and methods

Source of human embryos

Ethical approval for this study was obtained from the University of Essex ethical committee and the Human Fertilisation and Embryology Authority (HFEA; project licence R0165). Embryos were donated with informed consent by patients treated at the Assisted Reproduction and Gynaecology Centre, London. Ovarian stimulation, oocyte retrieval and insemination were carried out as previously described (Taranissi et al., 2005). Embryos were allocated to this study on day 6 after embryo transfer and freezing of blastocysts suitable for cryopreservation. Cryopreserved embryos donated to this research were thawed using the Vitrolife Thaw Kit (Vitrolife, Englewood, USA) and allowed to equilibrate in culture overnight. For cell imaging studies, thawed and fresh blastocysts that were defined to be of morphological good quality with clear trophectoderm and inner cell mass were selected.