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Role of HLA-G, HLA-E and KIR2DL4 in Pregnancy

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KEYWORDS HLA-E; HLA-G; KIR2DL4; NK cells; Normal pregnancy; recurrent spontaneous abortion

ABSTRACT Mammalian pregnancy has always been considered as immunological enigma. During pregnancy various immunological components at fetomaternal interface contribute to the immunosuppression which may allow semiallogenic fetus to survive. Along with Th2 shift, some other cytokines also play an important role in directing the normal pregnancy. Further modulation of immunological effector cells like T cells, macrophages and NK cells are also involved. Further, the lack of HLA class I expression at fetomaternal interface assists in escaping the fetus from maternal immune response, moreover, simultaneous expression of non classical HLA-G and HLA-E at fetomateral interface helps in downregulation of NK cells. NK cells interact with HLA-G via their KIR2DL4 receptors. We have reviewed the role of these molecules and how the loss of function in any of these components can lead to immunodysregulation hence leading to rejection of the fetus.

INTRODUCTION

During mammalian pregnancy fetus is protected from rejection by maternal immune response, although mother has to maintain adequate immune efficiency to protect developing fetus from infections. An immunosuppressive state develops during pregnancy contributing fetal survival and growth. Though fetus was initially thought to be present in an immune privileged site, it is now known that it is continuously exposed to maternal immune response. Cells of fetal origin have been found in maternal circulation and they persist even after delivery (Bonney and Matzinger 1997) which may evoke maternal immune response. The hypothesis about uterus as an immune privileged site has been rejected, as in pregnancy uterus is able to reject non paternal tissue grafts as well as bacteria and viruses. Lack of fetal rejection, even after exposure of fetal cells to maternal immune response, suggests tolerance or immunological suppression of maternal immune responses specific to fetus. The key molecules and exact mechanism that induces immunological tolerance have not been well defined.

With the exception of HLA-C, no molecules of the HLA Ia (classical) loci are expressed on the fetometrnal interface (King et al. 2000). At fetomaternal interface, however, HLA Ib (non classical) molecules i.e. HLA-G and HLA-E which are implicated in down regulation of NK cell activity are expressed (Kovats et al. 1990; King et al. 2000; Ishitani et al. 2003). Because of confined expression of HLA-G at fetometrnal ineterface and it's potential to downregulate maternal immune response, it is speculated that it may play a critical role in the maintenance of normal pregnancy. As HLA-E can downregulate NK cell cytotoxicity, along with HLA-G it may also be involved in immmunosuppression at maternofetal interface. HLA-G functions by interacting with KIR2DL4, an NK cell receptor, to regulate NK cell activity. This review is focused on the role of all the three components, and how abnormality in this immunoregulatory circuit may affect immunoregulation.

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HLA Independent Immune Mechanisms in Normal Pregnancy

The lack of maternal rejection of the allogenic fetus, suggests that adaptation of the immune system occurs during normal pregnancy. Some components of the HLA dependent and independent mediators of immune system favor the fetal acceptance (Fig. 1).

Th cells and their cytokines are the most extensively studied components of immunology in human pregnancy. Th2 shift of cytokines during pregnancy (Wegmann et al. 1993) is

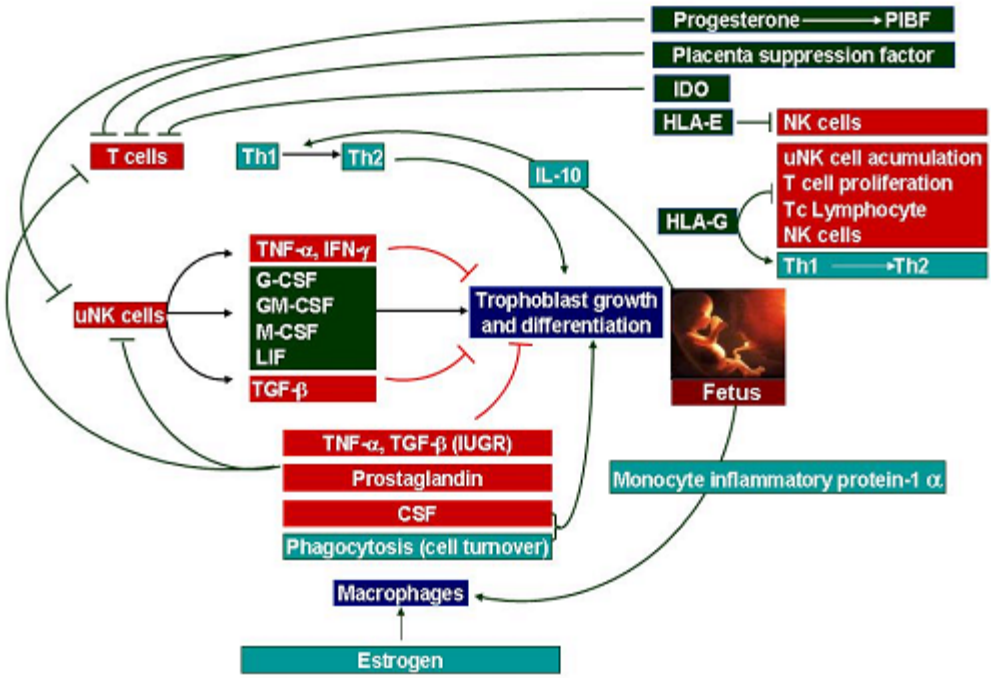

Fig.1. Immune mechanism in normal pregnancy- Fetus and maternal immune system both contribute to maintain normal pregnancy, which is manifested with Th2 shift of cytokines, which may be brought upon with estrogen and progesterone hormones and IL-10 & HLA-G antigens of the fetus. Fetus contributes IDO that blocks T lymphocyte proliferation and placenta suppression factor that inhibits CTL proliferation. NK cells secret CSFs and LIF, which is essential for trophoblast growth and differentiation. NK cells also secrets TNF-α, INF-γ, TGFβ that may inhibit trophoblast growth, thus growth is carried out in balanced and regulated way. Macrophages play an important role in cellular turnover while implantation and they also release prostaglandin that inhibits NK cells and CTL response. In preeclampsia and IGUR they can also release Th1 type cytokines.

supported by the evidence that women with rheumatoid arthritis get a temporary remission during gestation (Da Silva and Spector 1992), where as systemic lupus erythematosis, flare up during pregnancy (Varner 1991). Th2 shift of cytokines may be caused by hormones (Piccinni et al. 1998; 1995). Progesterone produces PIBF (progesterone induced blocking factors) in lymphocytes (Szekeres-Bartho et al. 1997) that suppress mitogen-induced lymphocyte proliferation, activation of NK cells and TNF production by NK cells. Placenta contributes placenta suppressor factor, which inhibits human mixed lymphocyte reaction (MLR), proliferation of CTLL-2 cells and cytotoxic T lymphocyte (CTL) activity.

About 50-90% of leukocytes in the decidua are NK cells. uNK cells may be involved in the regulation of extent of trophoblastic growth, differentiation and invasion. One of the functions of uNK cells is cytokine secretion (Vince and Johnson 2000), most of which influence trophoblast growth and placentation. The secreted CSF (Colony stimulating factors) is responsible for trophoblast cell proliferation and differentiation (Garcia-Lloret et al. 1994). Further Leukaemia inhibitory factor (LIF) secreted by these uNK cells promotes implantation (Nachtigall et al. 1996). Probably to have control over the growth of trophoblast cells they also secrete transforming growth factor b (TGFb) that inhibits trophoblast growth and differentiation (Karmakar and Das 2002). uNK cells cytokine profile also includes TNF-a & IFN-g which are Th1 cytokines and may be detrimental for trophoblast cells. These molecules of innate immunity may be involved against infection and pathogens. However, their ability to affect HLA-

Ia deficient trophoblast cells may be countered by HLA-E and –G, which are ligand for killer cell inhibitory receptors of NK cells.

During pregnancy continuously changing physical interaction at fetomaternal interface and appropriate growth and function of placenta requires a constant turn over of cells that are carried out by apoptosis **(**Uckan et al. 1997). Macrophages are involved in the clearance of these apoptotic cells to prevent any inflammatory response and thus fetal rejection. Macrophages are also implicated in cytokine secretion and in preeclamptic women due to apoptotic overload and insufficient clearance leads to the secretion of proinflammatory TNF-a (Pijnenborg et al. 1998). There are various physiological and immunological alterations taking place during pregnancy and thus outcome may be a result of their combined activity which is shown in Figure-1.6.

Another important molecule implicated is human leukocyte antigens (HLA). Their role is also interesting as normal gestation is accompanied by expression of non classical class I antigens i.e. HLA-G and HLA-E on fetomaternal interface.

HLA-G: Immunomodulator of Pregnancy

Ellis et al in 1986 identified expression of HLA-Ib on human non-villous cytotrophoblast cells (Ellis et al. 1986), and later on it was named as HLA-G. Because of its confined expression in certain organs in the body including fetomaternal interface, it generates great interest as regard to its possible function and organ distribution. HLA-G has also been documented on placental extra villous cytotrophoblasts (Carosella et al. 2000), certain tumors (Urosevic et al. 2001; 2002), thymus, eye and kidney (Carosella et al. 1996; Onno et al. 1994) but not on the villous cytotrophoblasts or syncytiotrophoblasts (Chumbley et al. 1993). This suggested that it has a role in the maintenance of tolerance during pregnancy. Later on its role in tumor escape mechanism in various cancers and in organ transplantation has also been explored (Lila et al. 2002). HLA-G expression is also well documented in skin inflammations (Aractingi et al. 2001; Khosrotehrani et al. 2001), muscle inflammation (Wiendl et al. 2000), multiple sclerosis (Fainardi et al. 2003), nonrejected allografts (Lila et al. 2000; Creput et al. 2003), tumors (Tripathi and Agrawal 2006) and in the course of HIV infection (Lozano et al. 2002). Various studies have substantiated the role of HLA-G in immunoregulation. HLA-G expressed on HLA class I deficient trophoblast cells, could counter the immunesurveillance by NK cells. HLA-G is also able to inhibit the transendothelial migration of NK cells. HLA-G interaction with ILT-2 and/or some unknown inhibitory NK receptor may regulate adhesion, possibly by affecting the expression of adhesion molecules on NK cells (Forte et al. 2001). This information suggest that HLA-G at fetomaternal interface may be preventing transplacental migration of uNK cells, as well as neutralizing the accumulated NK cells through interacting with their killer inhibitory receptors. Other possible functions of HLA-G may be regulation of cytotoxic response. HLA-G can suppress proliferation of T lymphocytes. It has been shown that HLA-G induces suppression of $CD4^+$ lymphocyte proliferation (Bainbridge et al. 2000) and its interaction with killer inhibitory receptors of T-cells which further inhibits antigen specific HLA-restricted CTL response (Fournel et al. 2000). HLA-G1 transfected APCs can inhibit proliferation of $CD4^+$ T cells and induce $CD4^+$T cell anergy. A unique feature of HLA-G is alternative splicing which leads to the formation of seven isoforms, four of which are membrane bound (G1-G4) and three are soluble isoforms (G5-G7). Further HLA-G5, which is a soluble isoform is shown to induce apoptosis of activated $CD8^+$ cells through activation of Fas/Fas-L pathway (Fournel et al. 2000). These soluble HLA antigens could be used as a marker for the success of IVF. Secretion of soluble HLA-G by in vitro fertilized human embryos is associated with a higher pregnancy rate (Fuzzi et al. 2002; Yie et al. 2005a) and that reduced levels of soluble HLA-G during early pregnancy correlate with a higher incidence of preeclampsia (Yie et al. 2005b). HLA-G may also influence cytotoxic T lymphocytes and uNK cells to change their cytokine profile and thus shift immune response towards type 2 in favor of normal pregnancy (Kanai et al. 2001). HLA-G locus is oligomorphic, as only fifteen alleles are reported till date, compared to other highly polymorphic HLA class I antigens. Recent studies have focused on mutation analysis of the HLA-G gene as it has been shown that different alleles of HLA-G can have different impact on functionality and stability of the protein. All fifteen alleles of HLA-G gene defined till date can be differentiated by four nonsynonymous substitutions which define five

major alleles, where as nonsynonymous substitutions further subdivide each allele into its subtype (Agrawal and Pandey 2003). Association studies in past few years have debated the role of HLA-G in pregnancy, as there is poor reproducibility of HLA-G allelic association with RSA (Table 1).

Different alleles have been shown to affect isoform concentration and hence are expected to influence the functionality of protein in a unique way (Rebmann et al. 2001, Hviid et al. 2003). The 14 bp deletion/insertion polymorphism of HLA-G exon-8 is responsible for the alternative splicing of the HLA-G transcript. It provides a cryptic branch point that induces alternative splicing resulting into the removal of surrounding 92 bases from the mature transcript. Pentameric initial AUUUG of 14 b sequence is probably involved in deadenylation and subsequent decay of mRNA. The absence of such a motif in the 92 base deleted transcripts may provide more stable mRNA. However, the opposite occurs with 14 base deletion (Rousseau et al. 2003) and this indel polymorphism has been associated with success of pregnancy (Matte et al. 2000, Tripathi et al. 2004). Another contribution of HLA-G in manipulation of immunological response is related to the expression of HLA-E. HLA-G provides its leader peptide that enables surface expression of HLA-E antigens. As HLA-E antigens are also reported to have NK cell regulating properties. HLA-G may regulate the expression of HLA-E antigens and hence can indirectly affect the NK cell response at fetomaternal interface during pregnancy.

HLA-G provides its immunoregulatory functions by interacting with various inhibitory receptors: ILT2, ILT4, and KIR2DL4 (Colonna et al. 1997; Rajagopalan and Long 1999). ILT2 and ILT4 has broader expression profile i.e. on lymphoid & myeloid cells and myeloid cells respectively, where as expression of KIR2DL4 is NK cell specific. Similarly ILT2 and ILT4 has broader ligand specificity i.e. various classical HLA class I molecules, though have a higher affinity for HLA-G (Shiroishi et al. 2003), but specific ligand of KIR2DL4 is HLA-G.

HLA-E: Another Player in Pregnancy

Another important component of the immunological network at fetomaternal interface is HLA-E. Compared to HLA-G, HLA-E expression is not only confined to fetomaternal interface but has wider tissue distribution including T cells, B cells, activated T lymphocytes and various other cells (Houlihan et al. 1995). However, their immunoregulating properties and dependence on HLA-G peptide for expression is suggestive of immune regulating properties affecting pregnancy outcome.

Immunoregulatory functions of HLA-E

This non-classical class I antigen was originally mapped to chromosome 6p21.3 but later on was officially named as HLA-E (Koller et al. 1998). HLA-E antigens are identified as ligand of a subset of immunoglobulin superfamily of NK cell receptors and their interaction with KIR of NK cells may be responsible for inhibition of killer activities of NK cells (Vales-Gomez et al. 2000). HLA-E specifically interacts with CD94/NKG2A that leads to the recruitment of the phosphatase SHP-1 to phosphorylated tyrosine of NKG2A and results in the inhibition of NK cells (Carretero et

Table 1: HLA-G association studies for recurrent spontaneous abortion (RSA)

S. No.	*Reference*	*Study*	*Patients*	*Controls*	*Association*	*Allele associated*
1	Karhukorpi et al 1997	Case control	38 RSA couple	26 fertile couple	No	-
2	Penzes et al 1999	Case control	21 RSA couple	72 fertile couple	No	-
3	Yamashita et al 1999	Case control	20 RSA couple	54 fertile couple	No	-
4	Pfeiffer et al 2001	Case control	78 RSA couple	52 fertile women	Yes	G*01013 & G*0105N
5	Aldrich et al 2001	Cohort	113 RSA couple	-	Yes	G*0104 & G*0105N
6	Hviid et al 2002	Case control	61 RSA couple	47 fertile couple	Yes	G*0106
7	Ober et al 2003	Cohort	42 Hutterite women	-	Yes	Promoter region -725C/G
8	Hviid et al 2004	Case control	29 IVF, 61 RSA women	93 fertile women	Yes	14 bp indel in exon-8
9	Abbas et al 2004	Case control	120 RSA women	120 fertile women	No	-
10	Tripathi et al 2004	Case control	120 RSA women	120 fertile women	Yes	14 bp indel in exon-8
11	Yan et al 2006	Case control	69 RSA women	146 fertile women	No	-

al. 1998). This immunoregulatory property has been confirmed in 721.221 cells that lacks HLA-A, -B, -C and –G but expresses HLA-E and prevents the lysis by NK cells expressing CD94/NKG2 inhibitory receptors (Borrego et al. 1998). This immunomodulating activity of HLA-E may be helpful in the success of pregnancy, as >90% of CD 56+ lymphocytes of decidua are constituted by CD94/NKG2 + NK cells (Gudelj et al. 1996). This immunoregulatory activity suggests a possible role of HLA-E molecules in protection of fetus from maternal immune response in normal pregnancy.

Interaction of HLA-E with Peptides and Their Role in Surface Expression

As already known HLA class Ia antigens play a central role in immune recognition. They make a complex with a nonpolymorphic peptide and a β-2 microglobulin and are transported to the surface of the cell to present this peptide to CD8+ T cells. These T cells specifically recognize various peptides and thus discriminate between self and non-self. Contrary to HLA Ia antigens HLA-E is less polymorphic but its immunoregulatory property indicate that it plays a role in immuno-surveillance by presenting a limited set of peptide particularly of HLA-I origin (Borrego et al. 1998). The surface expression of HLA-E is required hence is regulated by the binding of the peptides derived from MHC class I leader sequence. The leader sequence from HLA-A, -B, -C and –G, but not from –F, suffice the prerequisite for the organization and surface expression of HLA-E (Lee *et al.* 1998). Both HLA-E and –F posses shorter leader peptides and hence are not able to bind and stabilize HLA-E. These peptides are highly conserved, with only minor variations at position 2, 7 and 8 (O'Callaghan and Bell 1998) (Table 2). The variation at position 2 which binds to the pocket 'B' of HLA-E has significant impact, as replacement of methionine or leucine here by threonine present in certain HLA-B molecule can inhibit their interaction to HLA-E molecule (Braud et al. 1998). Along with position 2 another substantial anchor residues are identified at position 7 (leucine / valine) and at position 9 (leucine).

There are evidences that viruses provide peptide that either mimic HLA-I leader peptide or manages to interact with HLA-E leading their expression which in turn counter the NK cell attack. Human cytomegalovirus (HMCV) codes for UL40 protein that has a leader peptide identical to the leader segment of human HLA-Cw that binds with the HLA-E (Tomasec et al. 2000). HCV peptide of core 35-44 amino acid binds and stabilizes HLA-E surface expression, and results in the protection from NK cell cytotoxicity. Blocking experiments with monoclonal antibodies confirmed that the resistance of target cells was by CD94/NKG2A receptors of NK cells (Nattermann et al. 2005a). HIV derived peptide HIV p 24 $_{14\text{-}22}$ has been identified as a candidate peptide that can bind with HLA-E, resulting enhanced expression of HLA-E and impaired NK cell function (Nattermann et al. 2005b). Peptides capable of interacting with HLA-E do not always inhibit NK cell lysis, but some time they help in identification of altered cells and favor their elimination. HLA-E can also present peptide derived from the leader sequence of hsp60, which inhibits recognition by NK cell inhibitory receptor, CD94/NKG2A, and thus helps recognition as well as elimination of stressed cells in a peptide dependent manner (Michaelsson et al. 2002).

Ability of HLA-E to down regulates the NK cell activity depends on affinity of its interaction with ligand or peptide as well as with NK cell receptors. In a recent study, affinity of peptides of various origins with HLA-E was investigated and, it was found that the HLA-G leader sequence showed the strongest affinity (Kaiser et al. 2005, Llano et al. 1998). As HLA-E is also expressed on fetomaternal interface that normally expresses only HLA-G, it can be expected to perform unique immunomodulating activities in the placenta.

HLA-E Polymorphism

A very high level of polymorphism is maintained in HLA class I classical antigens under selective pressure of presenting diverse pathogenic antigens. Contrary to this HLA-E exhibits very low degree of allelic polymorphism, as it is involved in presenting a limited set of peptides suggesting their role in well regulated and predicted immunosurveillance and immuno regulation. Two nonsynonymous alleles of HLA-E are present in human population **(**Geraghty et al. 1992; Matte et al. 2000), maintained through strong balancing selection (Grimsley and Ober 1997), distinguished by a single sequence dimorphism at position 107, arginine (E*0101; HLA-E^R) or glycine (E*0103; HLA-E^G). Though

this dimorphism exists in equal frequencies but some studies have shown the association of this locus with susceptibility to nasopharyngeal carcinoma (Grimsley et al. 2002), type I diabetes mellitus (Hirankarn et al. 2004) and susceptibility to HIV infection (Hodgkinson et al. 2000). It has been demonstrated that association of HLA-E*0101 allele with RSA (Lajoie et al. 2006). These observations are in line with the evidences about the biophysical differences between both alleles (Tripathi et al. 2006). It was shown that HLA-E^R always has a lower peptide affinity and thus decreased surface expression than HLA-E^G (Strong et al. 2003). Also the thermal stability of HLA-E^G – peptide complexes were higher as compared to HLA-E^R –peptide complex (Strong et al. 2003). As accuracy of peptide loading is very essential for stability and surface expression, it may affect immuno modulating properties of HLA-E indirectly.

KIR (Killer Immunoglobulin Type Receptors): KIR2DL4

Another important player involved in the interactions at fetomaternal interface is the NK cell receptors. Role of NK cell killer inhibitory receptors are pivotal, as they are implicated in HLA-G and HLA-E mediated NK cell inhibitory activities. Two human NK cell receptor families have been described (Fig. 2). One NK cell receptor family is designated as C type lectin superfamily which is type II membrane-bound glycoprotein, CD94 with an external lectin like domain, that heterodimerizes with one member of NKG2 (A, B, C, E, H) family of proteins (Lazetic et al. 1996). Out of two human NK cell receptor families, HLA-E specifically interacts with subtypes of CD94/NKG2 receptors (Braud et al. 1998). Another family belongs to the immunoglobulin superfamily. They are known either as killer inhibitory receptors or killer activator receptors, according to the type of transmitting signals their ligation with class I molecule induces. KIRs with long cytoplasmic domains are designated as inhibitory receptors that contain cytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIM) i.e. KIR2DL and KIR3DL whereas those with short cytoplasmic domains are deisgnated as activating receptors that associate with the adapter DAP12 via a lysine residue in their transmembrane region i.e. KIR2DS and KIR3DS. The KIR (Killer Immunoglobulin type Receptors) gene family currently comprises of 15 genes and two pseudo genes encompassing a 100-200 Kb region of the Leukocyte Receptor Complex (LRC) located on chromosome 19 (19q13.4) (Trowsdale 2001). KIR genes constitute haplotypes in the LCR region, which exhibits extensive variation in the number and type of KIR genes present. All KIR haplotypes are flanked by KIR3DL3 (centromeric end) and by KIR3DL2 (telomeric end), together with the centric KIR3DP1 and KIR2DL4. These flanking KIRs which limit two regions of variable KIR gene content constitute the framework genes (Wilson et al. 2000; Vilches and Parham 2002).

Expressions of KIR genes are not universal in all NK cells, but each NK cell consists of specific profile of KIR genes. Exceptionally, KIR2DL4 being evolutionarily conserved, framework member of the KIR gene family, is expressed by all KIR haplotypes and in all NK cells. Various receptors interacting with HLA-G has been reported i.e. ILT2, ILT4, KIR2DL4, BY55 and CD94/NKG2A (Navarro et al. 1999; Colonna et al. 1998; Rajagopalan et al. 1999; Anumanthan et al. 1998; Borrego et al. 1998). All these receptors have broader expression profile and broader ligand specificity, but KIR2DL4 receptor is only expressed on NK cells and that is specific to HLA-G but not to other HLA-I antigens including HLA-E. Using polyclonal antisera, KIR2DL4 protein has been reported on the surface of all peripheral NK cells and uNK cells (Rajagopalan and Long 1999). It is expressed in a codominant manner contrasting with at least some other KIR that may be monoallelically expressed (Chan et al. 2003).

The structure of KIR2DL4 is also divergent and transmits a complex signal to NK cells to inhibit their cytotoxicity (Rajagopalan et al. 2001). Functionally, KIR2DL4 has been reported to be an inhibitory receptor for peripheral NK cells and uterine NK cells, but ligation of KIR2DL4 with mAb results in the activation of IFN-ã secretion but not cytotoxicity in peripheral NK cells. These different functional activities of KIR2DL4 may be due to ITIM in its cytoplasmic domain and a charged residue in the transmembrane domain. Both these domains in KIR2DL4 have been shown to be functional using chimeric receptors (Yusa et al. 2002; Faure and Long 2002). This suggests KIR2DL4 may mediate different functions under different circumstances.

Further it is known that KIR2DL4 activates cytokine production, but not cytotoxicity, in resting NK cells from peripheral blood (Rajagopalan et al.

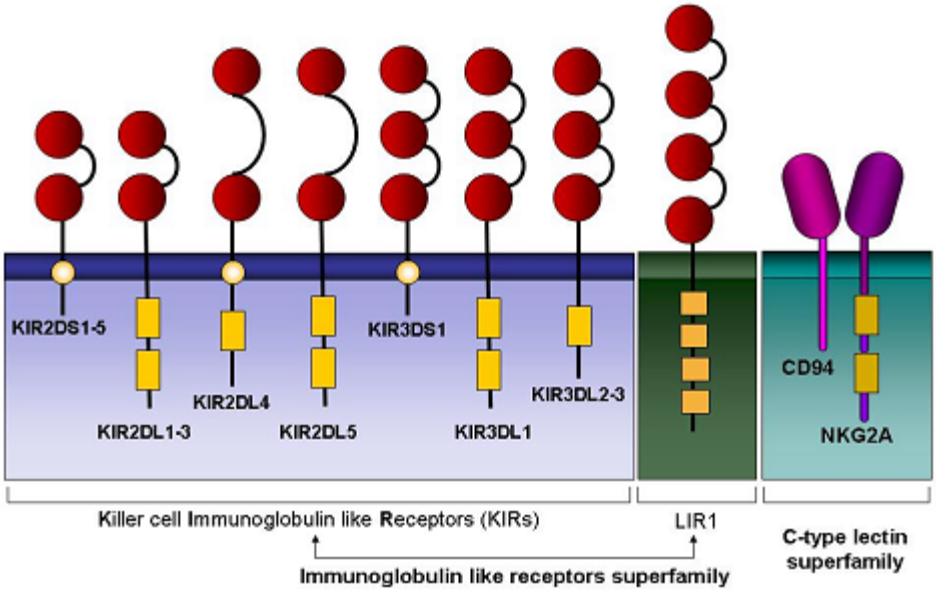

Fig. 2. Types of NK cell receptors- Killer cell immunoglobulin like receptors are further classified as KIR2D or 3D depending upon presence of 2 or 3 extracellular immunoglobulin like domain. Further their nomenclature as KIRDS or KIRDL depends on the presence of short or long cytoplasmic tail. KIRs with long cytoplasmic tail consist of Immunoreceptor tyrosine based inhibitory motif (ITIM), which conveys inhibitory signal to NK cell. Where as KIRs with short cytoplasmic tail do not consist of ITIM but posses a charged amino acid in their transmembrane domain that may be involved in interaction with DAP-12, and can convey activating signals to NK cells. Another group of receptors immunoglobulin like transcript (ILT) or leukocyte immunoglobulin like receptors (LIR) is characterized by the presence of 2 or 4 Ig like extracelluler domains. ILTs along with KIRs constitute immunoglobulin like receptors superfamily. Another group of receptors are C-type lectin superfamily which consists of disulphide linked heterodimer of CD94 glycoprotein and one member of NKG2.

2001). Recently it is demonstrated that the interaction of KIR2DL4 with soluble HLA-G activates a proinflammatory / proangiogenic response (Rajagopalan et al. 2006). Cytokine production by NK cells are very essential step in implantation as it plays an important role in remodeling of the maternal vasculature (Parham 2004), which is required to establish adequate blood supply to the fetus, absence of which may lead to preeclampsia.

KIR2DL4 possesses synonymous and non synonymous substitutions as well as indel polymorphism, thus constituting 15 different alleles (Table 3). Of these alleles there are only seven distinct amino acid sequences, defined by non synonymous substitutions. These alleles can be further subdivided by synonymous substitutions and single nucleotide deletion at the end of exon-6, which encodes the transmembrane domain (Witt et al. 2002). This deletion of adenine at the end of exon-6 results in inconsistent recognition of the intron-6 splice signal, leading to exon 6 skipping during mRNA production. Due to alternate splicing, a fraction of the mRNA produced by these alleles include the transmembrane region but is missing the cytoplasmic region. This indel polymorphism results in two isoforms, either with full length 'F' or with deleted transmembrane region 'D TM'.

Full length allele i.e. 10A produces only a single mRNA that appears to code for a classical membrane bound receptor that includes an inhibitory ITIM motif in its cytoplasmic tail. The alternate allele i.e. 9A has a deletion of 1 adenine in a series of 10 adenines at the end of the transmembrane exon-6. The 9A allele produces a small amount of mRNA that includes the transmembrane exon 9ATr. However, the frame shift mutation caused by the deletion results into the stop codon, at the 4th codon of exon 7, the

Table 3: Polymorphic sites showing different alleles of KIR2DL4

Allele	*Exon-3*					*Exon-5*							*Exon-6*	*Exon-7*			*Exon-9*					
Codon	*30*	*64*	*66*	*72*	*78*	*109*	*115*	*137*	*146*	*161*	*182*	*186*	*206*	*231*	*234*	*248*	*316*	*317*	*318*	*321*	*347*	*348*
Nucleotide	*192*	*293*	*301*	*317*	*336*	*429*	*446*	*514*	*540*	*584*	*649*	*659*	*721*	*796*	*805*	*845*	*1051*	*1052*	*1057*	*1066*	*1144*	*1145*
2DL4*00101	A	G	A	T	A	C	A	A	C	C	A	G	T	T	T	A	A	G	G	C	T	A
2DL4*00102	-	-	-	-	-	-	-	G	-	-	G	-	-	C	-	-	-	-	A	-	-	-
2DL4*0010301	-	-	-	-	-	-	-	G	-	-	G	-	-	C	-	-	-	-	-	-	-	-
2DL4*0010302	-	-	-	-	-	-	-	G	-	-	G	-	-	C	-	-	-	-	-	-	-	-
2DL4*00104	-	-	-	-	-	-	-	-	-	-	G	-	-	C	-	-	-	-	-	-	-	-
2DL4*00201	-	-	-	-	-	-	G	-	-	-	G	C	-	-	-	-	-	-	-	-	-	-
2DL4*00202	-	-	-	-	-	-	G	G	-	-	G	C	-	-	-	-	-	-	-	-	-	-
2DL4*003	-	C	T	-	-	-	-	G	-	-	G	-	-	C	C	G	-	-	A	G	C	C
2DL4*004	-	C	-	-	-	-	G	G	-	-	G	C	-	C	-	-	-	-	-	G	C	C
2DL4*005	G	-	-	-	-	-	G	G	-	-	G	C	-	C	-	-	-	-	-	-	-	-
2DL4*00601	-	-	-	-	-	T	G	G	-	-	G	C	A	C	-	-	-	-	-	-	-	-
2DL4*007	-	-	-	A	G	-	G	G	-	-	G	C	-	-	-	*	T	-	-	-	-	-
2DL4*0080101	-	-	-	-	-	-	G	G	-	-	G	C	-	-	-	*	-	-	-	-	-	-
2DL4*0080102	-	-	-	-	-	-	G	G	-	-	G	C	-	-	-	*	-	C	-	-	-	-
2DL4*0080103	-	-	-	-	-	-	G	G	-	-	G	C	-	-	-	*	-	-	-	-	-	-
2DL4*0080201	-	-	-	-	-	-	G	-	-	-	G	C	-	-	-	*	-	-	-	-	-	-
2DL4*0080202	-	-	-	-	-	-	G	-	-	-	G	C	-	-	-	*	-	-	-	-	-	-
2DL4*009	-	-	-	-	-	-	G	G	-	G	G	G	-		-	*	-	-	-	-	-	-
2DL4*0010	-	-	-	-	-	T	G	G	A	-	G	C	-	C	-	-	-	-	-	-	-	-
2DL4*0011	G	-	-	-	-	-	G	G	-	-	G	C	-	-	-	*	-	-	-	-	-	-

Alleles and various polymorphic sites of KIR2DL4 receptors: There are ~ 22 polymorphic sites in the exonic region that assign the presence of various alleles. Exon and codon numbering is according to Immuno polymorphic database (IPD)-http://www.ebi.ac.uk/ipd/index.html. IPD database consists of ~22 alleles of KIR2DL4 but out of which only 11 codes for different proteins, which are differentiated by nonsynonymous substitutions or frameshift generated by deletion mutation. In the database Allele 0010301 and 0010302, 0080101 and 0080103, & 0080201 and 0080202 are similar in coding sequences. In database sequencing of KIR2DL4*00105 and KIR2DL4*00602 are in pending.

first cytoplasmic exon, suggesting a protein with a truncated cytoplasmic tail (Fig. 3).

The protein product from this mRNA could conceivably be expressed on the membrane but would lack the inhibitory ITIM motif. The other mRNA transcript produced by the 9A allele (9A ÄTM) is more abundant but omits the transmembrane exon, making it unlikely that any translated protein could be expressed on the membrane. As via KIR2DL4, HLA-G confers down regulation of NK cells during normal pregnancy, the reduced expression of membrane bound receptor or absence of ITIM motif, may exert some pathophysiological impact on the success of pregnancy.

Junction of Triad at Fetomaternal Interface

During gestation numerous endocrinological changes occur, mostly to change the micro-environment at the fetomaternal interface in the favor of pregnancy. The interplay of various cytokines, pregnancy specific hormones and enzymes like IDO can provide maternal tolerance towards semiallogenic fetus in an HLA indepen-dent manner.

The role of HLA, being a determinant of histocompatibility, could be anticipated in acceptance of semiallogenic fetus by mother. Initial evidences about classical HLA I suggested increased sharing among couples could lead to rejection of fetus (Thomas et al. 1985). The reason could be lack of adequate initial maternal immune response to fetal antigens that produces protective antibodies against fetus (Beer et al. 1981) and homozygosity of recessive lethal alleles that are in linkage disequilibrium with specific HLA haplotypes (Thomas et al. 1985). However, lack of expression of classical HLA class I and II antigens, except for a possible weak expression of HLA-C, provides no obvious reasons for these observations (King et al. 2000). The expression of nonclassical HLA-G at fetomaternal interface and their immunoregulating properties generated lots of speculation about their role in pregnancy. Their expression in various pathologies also suggest their implication in immunoregulation.

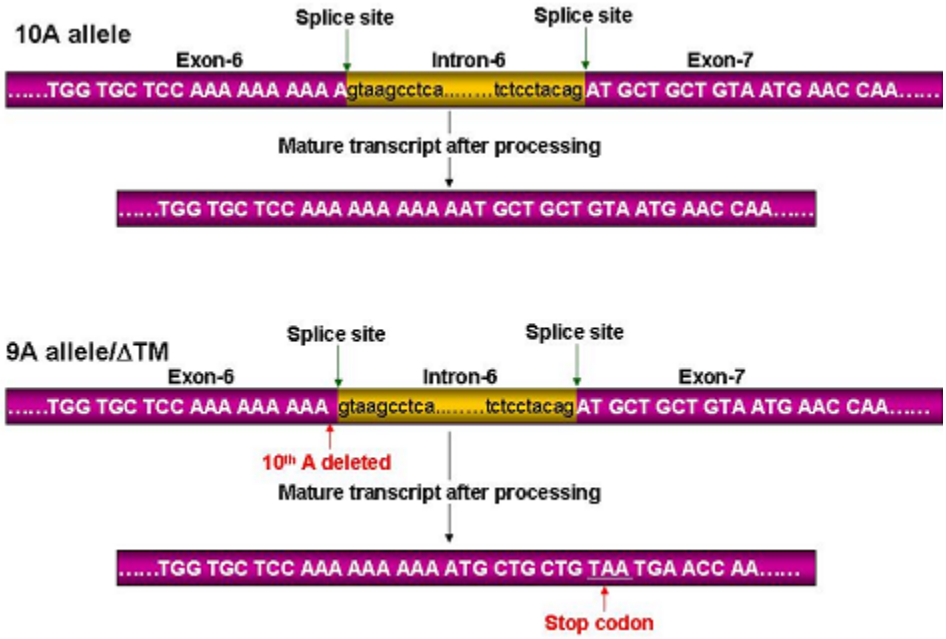

Fig. 3. Deletion of Adenine in 9A allele results DTM domain: Poly (A) tract is present just before splice signal for intron-6. In 10A allele after splicing of primary transcript a mature transcript is produced that code for functional protein. In 9A allele 10th adenine of poly (A) tract is deleted that results in frame shift of mature transcript and generation of stop codon at fourth codon of exon-7. Generation of stop codon results a protein lacking cytoplasmic domain.

The debate over the immunoregulatory role of HLA-G antigen further heated up with the finding of HLA-G*0105N (the null) allele and the existence of homozygotic individuals with this mutation (Ober et al. 1998; Casro et al. 2000). These individuals could not encode functional HLA-G1 or -G5 protein isoforms, but they manage to survive. This finding suggested towards the presence of other alternative pathway of immunoregulation during pregnancy. However, several studies have shown that the other HLA-G isoforms lacking exon 3 (with the frameshift mutation in 0105N) might have some of the same functions as the full-length isoforms (Sala et al. 2004; Le Discorde et al. 2005). Though HLA-G*0105N allele can not produce functional HLA-G1 isoform, it may generate other HLA-G isoforms, such as membrane-bound HLA-G2 and -G3 and the soluble HLA-G6 and -G7 proteins. These may substitute for HLA-G1 and -G5 (Le Discorde et al. 2005), thus taking over the immune tolerogeneic function of HLA-G.

Another HLA counterpart with NK cell regulating properties present at fetomaternal interface is HLA-E. Expression depends on the loading of correct peptide on HLA-E, protection of target cells by NK cells further depends on the interaction between HLA-E/peptide complex and CD94/NKG2 receptor of NK cells. Peptides can affect the affinity between NKG2/CD94 receptor and HLA-E/peptide complex since comparison of peptides of various origins has revealed that HLA-G derived peptides can confer strongest interaction between receptor and peptide complex (Kaiser et al. 2005). This property emphasizes role of HLA-E expression at the fetomaternal interface, as HLA-G is confined to placental trophoblast cells. HLA-E is co expressed in all cells that express HLA-G and HLA-E binds with the HLA-G derived nonamer peptides in these cells (Ishitani et al. 2003). Even the HLA-G 0105N null allele, which does not encode functional HLA-G1 or -G5 protein isoforms (Ober et al. 1998), could provide peptide for HLA-E expression. Thus raises the possibility that HLA-E could function even in the absence of functional HLA-G (Sala et al. 2004). In this study it was seen that HLA-G*0105N-transfected cells express surface HLA-E to a similar extent as the unmutated HLA-G gene, whereas HLA-G1 cell surface expression was undetectable. This finding suggests that HLA-E functions not only in the presence but also in the absence of fully functional HLA-G. In absence of fully functional HLA-G, HLA-E at least in part may compensate their activity. Thus it could be anticipated that simultaneous loss of both nonclassical HLA antigens could affect NK cell regulation at fetomaternal interface during pregnancy.

The lack of reproducibility is often seen in HLA and RSA association studies. This suggests that additional mechanisms that synergize with the outcome of these HLA antigens may have not have been taken into consideration in these experiments. As HLA-G conveys NK cell regulation via inhibitory receptors of NK cells so their role may be speculative in the functioning of HLA antigens during pregnancy. One such NK cell killer cell inhibitory receptor is KIR2DL4 which is specific to HLA-G and is present in all NK cells (Valiante et al. 1997). Though various receptors have interaction with HLA antigens but most have broader expression profile and broader ligand specificity, only KIR2DL4 receptor is expressed on NK cells and is specific to HLA-G but not to other HLA-I antigens including HLA-E (Rajagopalan and Long 1999).

Interaction of HLA-G and KIR2DL4 is decisive for NK cell regulation; hence any mutation in either molecule would enhance the NK cell mediated cytolysis. In classical HLA class I molecules residues from 77~83 of á1 domain has been proposed to be crucial for KIR recognition. Further comparison of different HLA and KIR interaction revealed that they may share some common feature of binding to their ligands recognizing residues from 77~83 (Natarajan et al. 2002). As in this region Met ^{76} and Gln^{79} are unique to HLA-G among all the alleles, it was anticipated and later was confirmed as crucial sites for KIR2DL4 recognition (Yan and Fan 2005). On the other hand among substitutions of KIR2DL4 one indel mutation at the end of exon-6 is expected to affect the functionality of this molecule. Hence it can be anticipated that this allele may disrupt the down regulation of NK cells, and thus may affect the outcome of pregnancy.

Analysis of KIRs (Witt et al. 2004) and HLA-G gene (Table-1) have resulted either in the lack of any association or contradictory results respectively. It would be interesting to study HLA-G and KIR2DL4 genes in combinations to ascertain the role of these molecules in pregnancy as they function collectively during NK cell regulation. Any alteration in the functioning of either of the two components would affect NK

cell regulation and in turn reproductive success. It is also demonstrated that combination of specific repertoire of inhibitory receptors of the KIR family (inhKIR), and fetal HLA-Cw antigens could provide a spectrum of risk for pre-eclampsia (Hiby et al. 2004). Mothers homozygous for group A KIR haplotypes with fetus of HLA- C2 genotype (either HLA-C2 homozygous or heterozygous) are at the highest risk of pre-eclampsia (Hiby et al. 2004). A recent study has suggested that a limited maternal repertoire of inhibiting KIRs (inhKIRs) and/or lack of maternal inhKIR-fetal HLA-C epitope matching may predispose to miscarriage (Varla-Leftherioti 2005). Similarly studies about the HLA-G and -E specific inhibitory receptor of NK cells i.e. KIRs, ILT2 and NKG2/CD94 family of receptors and study of their combinations with HLA counterparts could reveal some interesting results, further demonstrating immunoregulating properties of these HLA antigens in pregnancy.

In conclusion, there are various factors affecting pregnancy but data about their specific contribution in the pregnancy outcome is often controversial. Non classical HLA antigens could play immunoregulatory role at fetomaternal interface via inhibitory receptors of NK cells. Simultaneous study of HLA antigens and their KIR counterparts could reveal their functioning in NK cell regulation. HLA-G and HLA-E has similar properties and may compensate their activity hence their study in combinations may represent cumulative contribution by non classical HLA antigens at fetomaternal interface.

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