Studies in humans suggest that reproductive failure may be influenced by immunological factors or by genes encoding immunological factors and regulatory mechanisms controlling immunological expression. Using molecular methods, immunological factors can be clearly studied in an immunogenetic context. One example, the major histocompatibility complex (MHC), known as the human leukocyte antigens (HLA) in humans and MHC in other mammals, affects many different stages of reproduction. Studies in some outbred, and in closely related, human populations indicate that HLA, or HLA-linked, genes and HLA regulatory factors affect gamete development, embryo cleavage, blastocyst and trophoblast formation, implantation, fetal development and survival. Studies in non-human mammals indicate that MHC, or MHC-linked, genes such as the grc complex, Ped/Qa-2, t haplotypes and MHC regulatory factors, have similar reproductive effects. Human reproductive failure may also be a consequence of disruption of interacting factors, including interactions between HLA antigens, cytokines and natural killer (NK) cells. In this review, we highlight the importance of immunogenetic and interacting factors in human reproductive failure. We argue that studies in closely related human populations and animal models may contribute to a better understanding of the ways in which immunogenetic and interacting factors are involved in human reproduction.

Key words: early pregnancy loss/HLA/pregnancy/recurrent spontaneous abortion/reproductive failure

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Introduction
As discussed in Part I (Roy Chaudhury and Knapp, 2001), many factors involved in humoral and cellular immune responses may influence human reproductive failure. Traditionally, most of these factors have been studied from an immunological perspective and are identified by immunological assaying for antibodies and/or antigens. Unfortunately, most immunological assays for antibodies and/or antigens are unstandardized and difficult to replicate. For this reason, controversy surrounds the relative importance of many immunological factors in human reproductive failure. In contrast, molecular methods may provide greater reproducibility, reliability, accuracy and may be used to standardize immunological assays. Molecular methods may also enable researchers to investigate how genes encoding immunological factors or regulatory mechanisms controlling gene expression affects reproduction. Until recently, few studies have investigated the influence of immunogenetic factors during reproductive failure and their importance in reproduction remains unclear and often controversial. Animal models have also improved our understanding of immunogenetic factors. Increasingly, it is becoming clear that human reproduction is a dynamic interplay between immunological and immunogenetic factors (interacting factors) during pregnancy. The disruption of
these immunological and immunogenetic networks may often lead to reproductive failure. Although, this also remains a relatively unexplored area of research, our review demonstrates that interactions between immunological and immunogenetic factors must also be studied.

Due to the complicated nomenclature for human reproduction (see Part I: Roy Chaudhury and Knapp, 2001), different categories of reproductive failure will be defined as follows. Infertility is the failure to conceive after frequent unprotected intercourse (Reiss, 1998). Unexplained infertility occurs when no cause of infertility can be identified after full clinical investigation of both partners (Reiss, 1998). Miscarriage will be defined as the spontaneous loss of a pregnancy with a gestational age of ≤24 weeks (Regan, 1997; Reiss, 1998). Recurrent miscarriage is defined as the loss of three or more consecutive pregnancies prior to week 24 of gestation (Reiss, 1998). Depending on the study, recurrent spontaneous abortion (RSA) is defined as the loss of two or more (or even three or more), clinically detectable pregnancies without reference to the week of gestation. Reproductive failure is defined as the inability to conceive or to carry a pregnancy to term. Reproductive success is defined as the ability to conceive and carry a pregnancy to term and reproductive outcome refers to both reproductive failure and success.

**Immunogenetic factors**

While an extensive spectrum of immunological factors can affect reproductive outcome, numerous genetic associations with human reproductive failure suggest that immunogenetic factors may also influence reproduction (Figure 1). These factors include proteins encoded by genes and the transcriptional and translational mechanisms, which regulate gene expression during different stages of reproduction. Some of these factors have been traditionally studied from an immunological perspective. However, it is important to determine how the genes and regulatory mechanisms of these factors influence pregnancy. Genetic methods such as cloning, sequencing and expression analysis are now utilized to identify and study immunogenetic factors. However, to definitely demonstrate the direct influence of genes in pregnancy, the production of animals with inactivated (null or knockout) genes are invaluable models when human studies are not possible. Knockout mice have now provided convincing evidence of the importance of factors, e.g. cytokines, leukaemia inhibitory factor (LIF), mucin-1 (MUC1) and natural killer (NK) cells during pregnancy, supporting the need for further immunogenetic research.

Historically, many immunogenetic studies have focused upon the influence of human leukocyte antigen (HLA) genes during reproduction. Major histocompatibility complex (MHC) antigens have been well-studied in humans and many other mammals, and it has been suggested that the MHC may play a dual role in reproduction. First, it may influence mate choice in some species (for review, see Grob et al., 1998) and second, the MHC may affect many different stages of reproduction (Fernandez et al., 1999). Thus, studies of the immunogenetic effects of HLA, or HLA-linked, genes in human reproduction provide an example, or framework, to study the function of other immunogenetic factors during pregnancy.

**HLA expression and function**

The human MHC, or HLA, may play critical roles throughout pregnancy by influencing gamete development, embryo cleavage, blastocyst and trophoblast formation, implantation, fetal development and survival (Figure 1). The HLA complex contains over 150 loci spanning ~4 Mb of DNA on chromosome 6p21 (Browning and McMichael, 1996). These genes encode proteins controlling cell–cell interactions and regulating immune responses (Ober and van der Ven, 1997) by presenting peptides to T-cells to form immunogenic complexes capable of T-cell stimulation (Browning and McMichael, 1996). The MHC is divided into three regions, class I, II and III (Ober and van der Ven, 1997). In class I and II loci allelic polymorphism is the highest amongst all functional mammalian genes, with some loci exceeding 100 alleles (Apple and Erlich, 1996). This diversity is significant for MHC regulation of cellular interactions and organ transplantation (Browning and McMichael, 1996). The class I region includes the classical, or class Ia genes (HLA-A, HLA-B and HLA-C), the non-classical, or class Ib genes (HLA-E, HLA-F and HLA-G) and the pseudogenes, HLA-H, HLA-J, HLA-K and HLA-L (Geraghty et al., 1992a,b; Messer et al., 1992). Class I molecules are heterodimers of HLA-encoded α chains with the non-MHC-encoded β2m. Generally, the function of class I molecules is to present peptides derived from endogenously synthesized, cytosolic proteins to CD8 cytotoxic cells. Similarly, the class II HLA-DR, -DP and -DQ molecules consist of HLA-encoded α and β chain heterodimers (Apple and Erlich, 1996) whose function is to present peptides derived from exogenous proteins or from endogenously synthesized cell-surface glycoproteins, to CD4 helper cells (for review, see Browning and McMichael, 1996). Non-HLA class II genes encode proteins involved in antigen-processing and transport, e.g. TAP1, TAP2, LMP7 and LMP2. The class III region also contains genes involved in immune response, although these molecules do not directly contribute to antigen processing or T-cell presentation (Browning and McMichael, 1996; for review, see Aguado et al., 1996). MHC genes are inherited as haplotypes and are co-dominantly expressed. Historically, HLA typing was performed by microcytotoxicity (Terasaki et al., 1964; Amos et al., 1969). However, recent advances in molecular genetics have enabled more precise and extensive HLA typing.

HLA expression varies throughout gametic and somatic tissues. HLA expression on male gametes remains controversial. Early studies detected HLA antigens on ejaculated spermatozoa, but some groups reported their absence (for review, see Fernandez et al., 1999). Sperm cell precursors, or germ cells, have consistently shown class Ia expression prior to sperm maturation (Kurpisz et al., 1986, 1987; Janitz et al., 1993, 1994; Guillaudeux et al., 1996). Class Ib, HLA-E and HLA-F, expression has been detected on sperm cell precursors (Guillaudeux et al., 1996; Fiszer et al., 1997). Chiang et al. (1994) reported HLA-G expression on mature sperm cells. However, a recent study (Hiby et al., 1999) did not verify this. Class II male gametic expression has been less thoroughly investigated than class I expression and sample contamination in early studies may have confounded detection, since class II gametic expression has not been recently reported. Generally, there is some HLA expression on male gametes, particularly class I expression on immature spermatozoa.
zoa. There have been fewer, even less conclusive studies on female gametic HLA expression (Fernandez et al., 1999). Another group (Jurisicova et al., 1996) reported HLA-G mRNA in unfertilized oocytes using reverse transcription–polymerase chain reaction (RT–PCR). Most other researchers have not detected oocyte class I or class II products (Dohr et al., 1987; Roberts et al., 1992; Fenichel et al., 1995; Patankar et al., 1997). Reports of class I and II expression in rodent gametes is also contradictory (for review, see Kurpisz et al., 1995; Fernandez et al., 1999). The lack of, or low, gametic HLA expression suggests that fertilization does not depend on HLA expression. However, HLA gametic antigens may function as signal molecules, or they may be involved in gamete differentiation and maturation (for review, see Hutter and Dohr, 1998; Fernandez et al., 1999). A schematic diagram of HLA expression during early development is presented in Figure 2.

In somatic tissues, HLA distribution varies with each antigen. Class II expression tends to be restricted to B lymphocytes, macrophages endothelial cells and activated T-cells. Class Ia genes, HLA-A, -B and -C, are ubiquitously distributed and expressed on nearly all nucleated cells. Recently, studies have reported more extensive tissue distribution, and greater potential functions, for class Ib HLA-E, -F and -G than previously considered (Hammer et al., 1997a; Le Bouteiller and Blaschitz, 1999). For example, HLA-E is expressed in most tissues, including adult and fetal thymus and liver, lymph nodes, spleen, resting T and B cells, activated T-cells, skin, mucosa colon and eosinophils. HLA-F transcripts are found in resting T and B cells, activated T-cells, peripheral lymphocytes, fetal thymus, liver, skin, adult tonsil and lymphoblastoid cell lines (Ober and van der Ven, 1997; Wainwright et al., 2000). Low HLA-G mRNA expression levels have been detected in adult peripheral B and T lymphocytes (Kirszenbaum et al., 1994), mononuclear phagocytes (Yang et al., 1996) and progenitor haematopoietic stem cells (CD34+) from umbilical cord blood (Kirszenbaum et al., 1995). In mature tissues, HLA-G mRNA has been found in keratinocytes (Le Bouteiller and Lenfant, 1996), lymphoid and melanoma malignant cells (Amiot et al., 1996; Paul et al., 1998), thymus (Crisa et al., 1997), the anterior portion of the eye, skin, lung, kidney, ovary, colon and intestine (Carosella et al., 1996). Importantly, HLA-G expression is unlike other class I genes, since it can be transcribed as different isoforms and is predominantly expressed at the maternal–fetal interface.

**HLA expression and function at the maternal–fetal interface**

Fetal and maternal cells are in close contact with one another at the maternal–fetal interface, especially at the implantation site, in
the maternal arteries and in the intervillous space (Benirschke and Kaufmann, 1995). Generally, first trimester trophoblast cells transcribe more class I mRNA and protein than term trophoblast cells (Kovats et al., 1990; Wei and Orr, 1990). During various stages of gestation, distinct trophoblast subpopulations contain different levels of class I mRNA and translated proteins (Hammer et al., 1997b). Generally, HLA-A and -B membrane-bound molecules have not been detected in any trophoblast subpopulations during pregnancy (Galbraith et al., 1981; Sunderland et al., 1981a; Faulk et al., 1982; Johnson and Stern 1986; Kovats et al., 1990; Shorter et al., 1990; Hunt, 1992; Le Bouteiller, 1994). However, HLA-A and -B transcripts and translated intracellular products have been detected in several trophoblast cell types, including villous cytotrophoblast cells (Guillaudeux et al., 1995). In contrast, both HLA-C mRNA and protein expression has been identified in invasive extravillous cytotrophoblast cells in the cell islands, placental septa, and the basal plate, and interstitial, intramural and intraluminal cytotrophoblast cells, as well as trophoblastic giant cells and endovascular cells (Chumbley et al., 1994; King et al., 1996a; Hutter et al., 1996; for review, see Le Bouteiller and Lenfant, 1996; Hammer et al., 1997a; Proll et al., 1999; King et al., 2000a). Finally, Hammer et al. (1997b) reported that all class Ia molecules are heterogeneously expressed in the amnion epithelium, an embryoblast-derived cell layer covering the amnion cavity which is not in contact with maternal tissues.

The class Ib molecules have different distributions at the maternal–fetal interface. HLA-E transcripts have been detected in several trophoblast cell subpopulations isolated from first trimester or term human placenta, in term chorionic membrane and in amnion epithelial cells (Wei and Orr, 1990; Guillaudeux, et al., 1995; Houlihan et al., 1995). In vivo, HLA-E membrane-bound proteins have been detected in amnion epithelial cells and trophoblast cells (Houlihan et al., 1995; King et al., 2000b). Recently, HLA-E protein has been found to associate with the class III molecules, TAP and calreticulin, and dissociates upon binding of class I leader sequences. Without these leader sequences, HLA-E cannot be expressed upon cell surfaces (Braud et al., 1998a,b; Lee et al., 1998). HLA-F also binds TAP and calreticulin, although it is not expressed as a membrane-bound protein (Wainwright et al., 2000).

Various HLA-G expression patterns at the maternal–fetal interface have been described using different methodologies (Le Bouteiller and Blaschitz, 1999). The most precise distributions have been determined by specific anti-HLA-G monoclonal antibodies (Bensussan et al., 1995; Lee et al., 1995; McMaster et al., 1995, 1998; Loke et al., 1997; Chu et al., 1998). In vivo, HLA-G protein is expressed in first trimester placentae, in populations of extravillous cytotrophoblast cells including invading extravillous cytotrophoblast, endovascular and interstitial trophoblast, placental bed giant cells and cytotrophoblast of the chorion laeve (Bulmer et al., 1991; Chumbley et al., 1994; Hutter et al., 1996; King et al., 1996b; Le Bouteiller and Blaschitz, 1999). HLA-G mRNA expression in extravillous cytotrophoblasts has been reported in second trimester placenta and term membranes (Sunderland et al., 1981b; Ellis et al., 1990; Kovats et al., 1990; Wei and Orr, 1990; Yelavarthi et al., 1991; Chumbley et al., 1994) and HLA-G protein has been detected in term extravillous cytotrophoblast (Blaschitz et al., 1997). Only one group has reported in-vivo expression of HLA-G in syncytiotrophoblast (Chu et al., 1998). HLA-G expression has also been reported in the endothelial cells of fetal vessels present in the mesenchymal core of chorionic villi (Blaschitz et al., 1997) and in amniocchorion and amniotic fluid (McMaster et al., 1998). In fetal tissues, HLA-G has been reported in the eye, thymus (Shukla et al., 1990), first trimester liver (Houlihan et al., 1995), lung, heart and kidney (Carosella et al., 1996).

Although placental HLA-G expression occurs throughout gestation (Le Bouteiller et al., 1999), the onset of HLA-G expression during early development remains uncertain (Hiby et al., 1999). HLA-G mRNA and protein was detected in 40% of human pre-implantation embryos at each cleavage stage up to the blastocyst and high expression was correlated with a high embryonic cleavage rate. Blastocysts with detectable HLA-G mRNA had significantly greater mean numbers of blastomeres per embryo at 24 and 48h after fertilization than HLA-G-negative blastocysts, suggesting that HLA-G expression reflects increased embryo cleavage rates and is correlated with more successful implantation (Jurisicova et al., 1996). In contrast, other authors (Hiby et al., 1999) did not detect HLA-G mRNA in human 2–8-cell embryos, nor in blastocysts. Moreover, some research groups reported that HLA-G expression decreases as gestation proceeds (Kovats et al., 1990; Wei and Orr, 1990); others found high levels of HLA-G mRNA in term placenta (Hiby et al., 1999). Thus, there is currently no consensus regarding the onset and progression of HLA-G expression during human development.

Using RT–PCR and different sets of HLA-specific primers, six different HLA-G transcriptional isoforms (HLA-G1 to -G6) have been detected in unpurified trophoblast cells from human first trimester and term placentae (Ishitani and Geraghty, 1992; Fujii et al., 1994; Kirszenbaum et al., 1994; Onno et al., 1994; Moreau et al., 1995), from villous cytotrophoblast cells of purified term placenta and in-vitro differentiated syncytiotrophoblast (Guillaudeux et al., 1995). Recently, Hiby et al. (1999) detected all six isoforms in first trimester and term placentae, highly purified villous and extravillous trophoblast cells and JEG-3 and 221-G cell lines. Unfortunately, there remain technical difficulties in isoform detection due to variations in methodological preparation (for review, see Le Bouteiller et al., 1999).

The importance of HLA-G expression during pregnancy remains unknown. Functionally, HLA-G differs from other class Ib genes (for review, see Ober et al., 1996; Ober and Aldrich, 1997) since it preferentially binds nonamer peptides (Lee et al., 1995; Diehl et al., 1996) and may present viral antigens to T-cells, thereby playing a role in immunosurveillance during pregnancy. Few viruses infect fetal cells, so limited HLA-G diversity may suffice for T-cell viral presentation. Although fetal extravillous trophoblast cells express HLA-G, pathogen infection through maternal blood occurs primarily in the intervillous space, where exposed fetal villous syncytiotrophoblast generally lack HLA-G expression (Loke et al., 1999). Low polymorphism may eliminate strong maternal immune responses or induce maternal tolerance. Maejima et al. (1997) suggested that HLA-G expression may modulate maternal tolerance of pregnancy by shifting Th1/Th2 cytokine profiles in human decidua. Hiby et al. (1999) suggested that HLA-G may be relatively unimportant in gametogenesis or early embryogenesis and may, instead, play a role in later developmental processes such as implantation. This proposal is supported by a study by McMaster et al. (1995), in which HLA-G
Production by early gestation cytotrophoblast stem cells was critical for in-vivo and in-vitro cytotrophoblast differentiation during invasion of maternal tissues. Moreover, mouse studies indicate that non-classical class I expression increases during and after implantation (Fernandez et al., 1999). HLA-G may also sustain trophoblast growth and prevent reproductive loss. Research by Hamai et al. (1999), who studied interleukin (IL)-2 supplementation on the growth of HLA-G positive (BeWo, JEG-3) and negative (JAR) trophoblastic cell lines for IL-2 receptors, found HLA-G positive cell lines were not influenced by IL-2 addition. However, negative lines exhibited significantly decreased proliferation when cultured with IL-2. HLA-G transfection of negative lines eliminated the IL-2 growth-inhibitory effect. The authors suggested that since IL-2 stimulates T-cell proliferation and induces lymphocyte-activated-killer (LAK) cells, HLA-G expression may enable trophoblast cells to evade cell damage. Inadequate HLA-G expression could lead to IL-2 mediated growth suppression and IL-2 expression may be up-regulated, or HLA-G expression may be down-regulated, in women experiencing reproductive failure (Hamai et al., 1997; Lim et al., 1998). Interestingly, HLA-G expression was either absent, or reduced, in extravillous trophoblast cells in a sample of 10 pre-eclamptic placentae, suggesting that low fetal HLA-G expression may result in maternal rejection (Goldman-Wohl et al., 2000).

Recently, Le Bouteiller and Blaschitz (1999) suggested that HLA-G could regulate placental angiogenesis and soluble HLA-G isofoms may act as immunosuppressors during pregnancy. Regulated and quantitative shifts of HLA-G isofoms during development have been observed. Thus, isofoms may perform diverse functions in the placental environment (Ishitani and Geraghty, 1992). Unfortunately, the specific roles of HLA-G isofoms during pregnancy remain unknown. Limited in-vitro and in-vivo studies present conflicting results on the importance of the HLA-G1 isofom. Mallet et al. (2000) reported that HLA-G1 was expressed as a membrane-bound protein at the cell surface of murine and human (JAR) trophoblast cell lines, but HLA-G2, -G3 and -G4 transcripts were retained in the endoplasmic reticulum. This suggests that HLA-G1 expression may be important during pregnancy. However, Ober et al. (1998a) identified one adult and one first term placenta, which were homozygous for a single base pair deletion in exon 3 of HLA-G1. These findings suggest that HLA-G1 expression is not essential for survival. While there is no consensus for humans, the observation that other primates possess HLA-G homologues with isoforms (Boyson et al., 1997) supports the argument that HLA-G isoforms could be important during pregnancy.

HLA-G polymorphism may also be a target for maternal anti-fetal immune responses (Klein, 1990). 'Abnormal' HLA-G alleles may fail to be recognized by the maternal immune system during pregnancy, resulting in reproductive loss (Yamashita et al., 1999). While initial studies reported that HLA-G was non-polymorphic (Geraghty et al., 1987; Kovats et al., 1990), the majority of recent studies have reported limited HLA-G polymorphism at both the nucleotide and amino acid levels (Alizadeh et al., 1993; Morales et al., 1993; Ober et al., 1996; Yamashita et al., 1996; Hviid et al., 1997; Hiby et al., 1999). One study of 45 African Americans detected extensive non-synonymous HLA-G variation (van der Ven and Ober, 1994). This may reflect the general finding that many loci in African populations are more polymorphic than the same loci in other populations (Tishkoff et al., 1996). Notably, most studies focus on HLA-G polymorphisms in exons 2 and 3 (encoding the α1 and α2 domains) because of class Ia homology. However, if HLA-G performs novel functions, polymorphisms in other regions of the molecule may also be important (for review, see Ober and Aldrich, 1997).

To summarize, HLA may play critical roles during many different stages of pregnancy. Although the importance of HLA antigens in human gamete development and fertilization remains controversial, these antigens could influence early embryonic cleavage, blastocyst/trophoblast development and implantation (Figure 2). In particular, the unique characteristics and distribution of the HLA-G antigen at the maternal–fetal interface suggests that the HLA-G gene may have a critical role during early stages of reproduction.

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**Figure 2.** HLA expression varies throughout pregnancy and controversy surrounds the distribution and relative importance of HLA antigens during early development. This figure summarizes published reports on HLA expression from gametogenesis to implantation. HLA-la = HLA-A, -B and -C loci. " = expression of HLA antigen and " = lack of expression. See text for further details.
HLA sharing in outbred individuals

The earliest studies of outbred individuals suggested that HLA antigen expression influenced fetal development, and subsequent reproductive outcome (Figure 1). During the 1960–70s, studies suggested that fetuses inheriting paternal MHC antigens that differed from maternal antigens (histoincompatible pregnancies) may have a selective survival advantage compared with conceptuses inheriting paternal MHC antigens that did not differ from maternal antigens (histocompatible pregnancies) (Billington, 1964; Kirby, 1970; Beer and Billingham, 1976). A histocompatible pregnancy may be unrecognized by the maternal immune system, or elicit an inappropriate maternal response resulting in reproductive failure. Thus, couples with RSA may be aborting histocompatible fetuses due to parental HLA similarity (Christiansen, 1996). Early retrospective studies reported increased HLA sharing amongst couples with RSA compared with fertile controls (Komlos et al., 1977; Schacter et al., 1979). However, studies during the last two decades have demonstrated conflicting results. Ober and van der Ven (1997) reviewed over 30 studies, from the late 1970s to the mid-1990s, and found that ~50% of these studies reported greater HLA sharing in couples with RSA than controls. There was little agreement on which HLA locus or loci were important in RSA. Some studies reported RSA associations with class I loci, class II loci or increased sharing over all loci. Discrepancies have arisen since many studies utilized small sample sizes or different study designs (Ober, 1999), particularly with respect to the selection and stratification of fertile and RSA couples. These studies also suffered from limitations inherent in retrospective studies, often failing to address the effects of HLA sharing in couples not selected on the basis of reproductive histories (Creus et al., 1998). Studies also differed in tissue typing methodology and the number of antigens studied. Older studies utilized serological HLA typing, which is influenced by both typing reagent quality and the cell isolation techniques employed. Indeed, a large, multicentre study reported that >25% of serologically typed HLA-DR antigens were incorrect, when compared with DNA–restriction fragment length polymorphism (RFLP) methods (Lorentzen et al., 1997). Additionally, it is difficult to serologically resolve broad antigens into potentially important subtypes (Doherty and Donaldson, 1991). For example, while four HLA-DQ serological specificities can be identified, DNA-based methods can detect 22 DQB and DQA alleles (Bodmer et al., 1995). Thus, differences in methodology and reliability can confound attempts to compare data concerning HLA sharing between published studies (Wagenknecht et al., 1997). The majority of recent studies, using DNA-based techniques, have not detected increased sharing of HLA-DR and/or DQA alleles in couples with RSA (Christiansen et al., 1989; Ito et al., 1992; Takakuwa et al., 1992; Laitinen et al., 1993; Wagenknecht et al., 1997). Increased HLA-G (Karhukorpi et al., 1997; Yamashita et al., 1999) or HLA-C sharing among couples with recurrent miscarriage has not been observed either (Christiansen, 1999). Only Ober et al. (1993) reported a significant increase in HLA-DQ sharing amongst couples with RSA compared with controls using PCR single-specific oligonucleotide probe hybridization (PCR–SSOP). Despite the controversial relationship between HLA sharing and reproductive failure, there is a general consensus that the degree of HLA sharing in RSA couples has no impact on the prognosis of subsequent pregnancies (Smith and Cowchock, 1988; Christiansen et al., 1994; Recurrent Miscarriage Immunotherapy Trialist Group, 1994).

Other studies have investigated HLA sharing in couples experiencing unexplained infertility (for review, see Ober and van der Ven, 1997), since a subgroup of couples with unexplained infertility may experience peri-implantational losses (Collins et al., 1983; Wilcox et al., 1988) due to increased HLA similarity (Creus et al., 1998). Some studies found no significant differences in HLA sharing between couples with unexplained infertility and fertile couples (Nordlander et al., 1983; Persitz et al., 1985). Others reported significantly higher HLA sharing in couples with unexplained infertility (Coulam et al., 1987). An alternative method to investigate peri-implantational loss, involves the study of parental HLA sharing in infertile couples undergoing assisted reproductive technology (ART). Although few studies have utilized this method (Weckstein et al., 1991; Balasch et al., 1993; Ho et al., 1994; Creus et al., 1998), two larger projects are notable. Ho et al. (1994) studied HLA-A, -B, -C, -DR and -DQ loci in 76 couples with unexplained infertility and found a significant excess of HLA sharing (two or more antigens; $P = 0.015$). In all, 36 couples who did not achieve pregnancy with IVF had increased HLA-DQ sharing compared with couples with successful pregnancies (Jin et al., 1995). Recently, Creus et al. (1998) compared 50 infertile couples who did not achieve a pregnancy after three or more IVF cycles with at least two transferred embryos, to 50 fertile couples who achieved a pregnancy after one IVF treatment. They found that three or more implantation IVF failures were significantly associated with two or more antigens shared between partners ($P < 0.05$).

Other studies have examined HLA antigen frequency among women with RSA since many maternal autoimmune diseases involving fetal loss are associated with HLA antigens or haplotypes (for review, see Ober, 1990). A few studies have reported statistically significant associations between recurrent miscarriage and class II HLA-DR antigens (especially for the serological types DR1 and DR3). Other studies have found contrary results. Christiansen et al. (1999) conducted a meta-analysis of 18 case-control studies of the frequency of the DR1 and DR3 antigens among women with recurrent miscarriage. The authors found that the HLA-DR1 antigen was associated with increased susceptibility to recurrent miscarriage. Other studies have detected no significant class I antigen associations with RSA. For example, no significant differences in HLA-E or -G allele frequencies, were detected between RSA women and controls using DNA-based methods (Steffensen et al., 1998; Penzes et al., 1999). A few studies, focusing on RSA occurrence amongst the relatives of women with RSA, reported an increased RSA prevalence amongst first-degree biological relatives and suggest the existence of a familial predisposition to RSA (Johnson et al., 1988; Alexander et al., 1988; Christiansen et al., 1990a; Ho et al., 1991; Christiansen, 1996). Christiansen et al. (1990b) reported that sisters who shared both parental HLA haplotypes with probands, experienced significantly higher miscarriage rates than sisters who shared only one, or no, parental HLA haplotypes with the proband.

Some prospective studies have followed up subsequent reproductive outcome in women and couples experiencing RSA.
For example, Sbracia et al. (1996) investigated the expression of HLA-A, -B and -DR alleles using serological typing in 57 and 30 RSA and fertile couples respectively. The researchers documented reproductive outcome for three years. A significant increase in HLA-DR sharing was observed in couples that aborted during the follow-up period, compared with couples and controls who achieved a livebirth (P < 0.03 and 0.02 respectively). The HLA-B44/DR5 haplotype frequency was also significantly increased in the same study (P < 0.03). It is important to note, however, that couples received different forms of psychological and immunotherapy during the follow-up period and reproductive outcome may be confounded by these factors. Christiansen et al. (1996) studied 234 women with RSA and 360 controls for HLA-DR and -DQ antigens using PCR sequence specific primers (PCR-SSP) and RFLP and reported subsequent reproductive outcome. Significantly greater numbers of women with HLA-DR1 and/or -DR3 miscarried (62%), compared with those negative for both allogenotypes (29%; P = 0.025).

Although it has been suggested that parental histocompatibility, or that HLA homozygosity, may result in preferential abortion of fetuses, few studies have investigated whether histocompatible or homozygous fetuses have been preferentially aborted. Wenk and Boughman (1989) typed HLA-A and -B antigens in 2569 healthy parents and children and found that parental antigen sharing did not result in a significant deficit of histocompatible nor homozygous children. Ober et al. (1993) identified HLA-DQA1 and -DQB1 alleles in 40 abortuses and 31 liveborn children of 68 couples with RSA. Significantly more couples with RSA shared two HLA-DQA1 alleles compared with fertile controls (P = 0.031). Although haplotype frequencies of HLA-DQA1/DQB1 in parents with RSA and aborted fetuses were not significantly different from controls (Steck et al., 1995), fewer than expected HLA-DQA1 compatible fetuses were observed. Assuming the numbers of compatible and incompatible pregnancies are equal at conception, the researchers suggested that histocompatible HLA-DQA1 fetuses may be selectively aborted during early pregnancy and HLA-DQA1 incompatible fetuses may have better survival rates (Steck et al., 1995). Thus, HLA molecules may significantly contribute to reproductive outcome. Modern techniques of HLA typing, and further molecular studies, should provide more detailed understanding of the influence of the HLA during pregnancy.


detailed understanding of the influence of the HLA during pregnancy.

Part II: Immunogenetic and interacting factors

These studies suggest that HLA genes are associated with reproductive outcome in outbred individuals. Importantly, the genes influencing reproductive outcome may not be HLA genes per se, but closely linked susceptibility genes, or genetic defects, which play critical roles in pregnancy maintenance, fetal development and survival (Creus et al., 1998; Kostyu, 1994). HLA genes could be markers for these susceptibility genes if they are retained in preferential allelic combinations over long periods of time, i.e. linkage disequilibrium. The HLA complex displays particularly high levels of linkage disequilibrium. Class III genes BF, C2, C4A, C4 have been found in linkage disequilibrium (Kostyu, 1994). Class I linkage disequilibrium has been found for HLA-A and -B, HLA-B and -C or HLA-A extending to HLA-G (Morales et al., 1993). Interestingly, HLA-G demonstrates striking levels of linkage disequilibrium with most (but not all) HLA-A alleles, even among distantly related populations (Le Bouteiller and Blaschitz, 1999). Furthermore, linkage disequilibrium can extend from class I (at the telomeric end) to class II (close to the centromere), with some ancestral haplotypes extending from HLA-A to -DR (2000 kb) or to -DP (4000 kb) (Dauss et al., 1978; Awdeh et al., 1983; Raum et al., 1984; Kay et al., 1988; van Endert et al., 1992). Linkage disequilibrium increases the probability that individuals with identical HLA antigens at one locus will also have identical antigens at a second HLA locus (Ober, 1990). Therefore, closely related individuals are also more likely to share haplotypes by virtue of these haplotypes being identical by descent. Studies on parental HLA sharing between closely related individuals, rather than outbred couples, may thus detect the effects of greater HLA homozygosity and MHC-linked genes in linkage disequilibrium.

HLA sharing in closely-related individuals

Population-based and prospective studies have been conducted in Hutterite communities (Ober et al., 1983, 1985, 1988, 1992, 1998b; Ober, 1995). The small number of founding Hutterite HLA haplotypes and reproductive isolation increases the probability that couples will share HLA antigens and other linked loci. The Hutterites are amongst the most fertile human population ever studied (Sheps, 1965; Ober, 1995). Contraception is prohibited and large families are desired (Ober and van der Ven, 1997). From their research with Hutterites during the last 16 years, Ober et al. suggested that HLA-DR, or HLA-DR linked genes, may affect fertilization, pre- or peri-implantational embryonic survival, and HLA-B, or HLA-B, linked genes may contribute towards recognized fetal loss. Initial studies demonstrated longer birth intervals among couples sharing HLA-A or -B antigens (Ober et al., 1983). Median time intervals to produce 10 children was 13.73 years in couples sharing no antigens, 14.52 years among couples sharing one antigen, and 19 years for those sharing two or more antigens. Longer median intervals from marriage to each birth were also observed for Hutterite couples sharing HLA-A, -B or -DR alleles, compared with Hutterite couples not sharing alleles (Ober et al., 1985, 1988). When the effects of individual loci were analysed, significantly longer birth intervals and smaller completed family sizes (P = 0.041) were detected with HLA-DR parental sharing (Ober et al., 1985, 1988). In a subsequent 5 year prospective study of 104 couples and 154 pregnancies, a significant increase in time from menstruation after birth to the first pregnancy was associated with parental HLA-DR compatibility (P = 0.015; 5.1 months to pregnancy in couples sharing one or more DR alleles versus 2 months in couples sharing no alleles). In the same study, fetal loss was significantly associated with HLA-B sharing (P = 0.041, with adjustment for age, gravidity, kinship). Another 10 year prospective study of 111 Hutterite couples with 251 pregnancies, revealed significantly increased fetal losses in couples sharing the entire 16-locus class I, II, III, haplotype (P = 0.002). Amongst individual loci, significant fetal loss rates were detected for sharing of HLA-B (P = 0.019), HLA-C (P = 0.033) and C4 (P = 0.043), although fetal losses from HLA-C and C4 sharing may have been due to their close proximity to HLA-B (Ober et al., 1998b). Laitinen et al. (1991) also found that RSA was correlated with HLA-A and -B
sharing in Finnish populations. Like the Hutterites, the Finns have been reproductively isolated with reduced genetic diversity. Laitinen (1993) studied 14 HLA genes in 56 Finnish couples by serological and DNA-based methods, and found significantly greater frequencies of 12 HLA haplotypes (most extended from HLA-A to -DP) in couples with RSA compared with controls (P < 0.001). Interestingly, four of these ‘risk’ haplotypes were also identified in Ober’s (1995) Hutterite study.

Studies among the Hutterites and Finns suggest that particular HLA haplotypes may have deleterious effects upon fetal viability. A low frequency of 12 HLA ‘risk’ haplotypes, most extending from HLA-A to -DP, were reported amongst 29 newborns born to Finnish couples during a 2 year follow-up period (Laitinen, 1993). Ober et al. found a 64.4% deficit of Hutterite homozygous individuals for a five locus haplotype (HLA-A, -B, -C, -DR and -DQ), which may reflect the additive or interactive effects of selection against homozygous HLA-B and DR fetuses (Ober, 1995). Needless to say, more studies are required to determine what HLA haplotypes or loci are commonly associated with reproductive failure.

Although it has been suggested that parental histocompatibility, resulting in offspring HLA homozygosity, may lead to preferential abortion of fetuses, few studies have investigated whether histocompatible or homozygous fetuses are preferentially aborted. Studies in outbred populations remain inconclusive. Whereas in Hutterites, no significant deficit of homozygous, or histocompatible, children were detected with high resolution typing of over 1000 parents and children for HLA-B, or a 16 loci HLA haplotype (Ober et al., 1998b). Alternatively, Ober et al. (1998b) has suggested that fetal/maternal histocompatibility may influence reproductive outcome. That is, a fetus recognizing maternal tissues as non-self (a heterozygous compatible pregnancy) may have survival advantages over a homozygous compatible fetus, which would not recognize maternal tissues as non-self. This suggestion may be supported by the observation that Hutterites experience greater losses of HLA-B homozygous fetal/maternal compatible pregnancies, than heterozygous compatible pregnancies (Ober et al., 1998b).

HLA regulation

Regulatory mechanisms controlling the expression of HLA, or HLA-linked genes, may also play a role in reproductive outcome. Mutations, duplications, translocations or aberrant regulation could alter HLA expression at the maternal–fetal interface and lead to reproductive failure. Class I and II expression at the maternal–fetal interface is regulated by various transcriptional and post-translational mechanisms, extracellular viruses and cytokines (Browning and McMichael, 1996). Placental class Ia transcriptional regulation is modulated by cis- and/or trans-acting regulatory mechanisms. cis-Regulatory regions comprise CpG-rich nucleotide sequences (CpG islands) located in the 5′ region of many class I coding sequences (Boucraut et al., 1993a; Le Bouteiller, 1994). Methylation of CpG islands can reduce transcription. In the HLA class I null trophoblast-derived JAR cell line, significantly reduced class Ia expression was reproducibly caused by CpG island methylation. However, only HLA-E expression appeared unmethylated and transcriptionally active. Studies on trophoblast cells from human term placenta differed.
Part II: Immunogenetic and interacting factors

Elsen et al., 1998). An interferon (IFN)-γ inducibility control sequence has also been reported in the HLA-G promoter (Hunt et al., 1998). Recently, Lefebvre et al. (1999) reported that IFN-responsive regulatory regions outside the HLA-G promoter are involved in transcriptional and post-transcriptional mechanisms of IFN-γ activation of HLA-G. Moreover, a distal 250 bp regulatory element, located 1.1 kb upstream from the first HLA-G exon, directs specific HLA-G transcription in mouse placenta (Schmidt et al., 1995).

Class II regulation is also governed by transcriptional and post-translational processes. Although modulation primarily occurs on a transcriptional level involving cis- and trans-acting elements. Several cis-acting 5′ elements and proximal promoter regulatory sequences, containing the X, Y and W/Z box elements, are important for class II expression (Wassmuth, 1996). Interestingly, a mutation involving the lack of transcription factor binding to class II promoters has been linked to some congenital defects leading to severe combined immunodeficiencies (Wassmuth, 1996).

To escape immune detection by class I cytotoxic lymphocytes, some viruses have developed mechanisms which regulate HLA expression in infected cells or transformed cell lines (Israel and Kourilsky, 1996). HLA-DR expression can be down-regulated by the varicella-zoster virus which causes varicella (chickenpox) (Abendroth et al., 2000). Class I expression can be modulated by N-myc, c-myc and v-ras oncogenes (Bernads et al., 1986) adenoviruses, the HIV-1 Tat protein, the herpes simplex virus (HSV) and the cytomegalovirus (HCMV) (Israel and Kourilsky, 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996). HSV and HCMV infections have also been implicated in miscarriage and RSA (Kriel et al., 1996).

The interactions of HLA or HLA-linked genes with genes on different chromosomes may also influence the development of late-onset diseases. For example, interactions between susceptibility loci for multiple sclerosis have been described for HLA alleles on chromosome 6 and genes on chromosome 17 (Karpjuk et al., 1997). Additionally, Pandey et al. (1999) demonstrated that particular homozygous genotypes for tumour necrosis factor (TNF)-α and immunoglobulin allotypes (GM and KM) interacts with class II HLA-DQα1, contributing to an increased relative risk to non-insulin-dependent diabetes mellitus (NIDDM). Furthermore, HLA alleles are associated with developmental and malignant diseases and other auto-immune conditions (for review, see Jin et al., 1995).

It has also been argued that individuals with fewer different MHC alleles have less resistance to pathogenic infection (Doherty and Zinkernagel, 1975; Hughes and Nei, 1988). Homozygosity for HLA class II alleles may increase susceptibility for common variable immunodeficiency (CVID) (De La Concha et al., 1999). Considering that HLA alleles are associated with numerous diseases, mutations within HLA, or HLA-linked, genes could have pleiotrophic effects, manifesting HLA associations between two or more diseases. Rheumatoid arthritis has been associated with decreased fecundity (Nelson et al., 1993) and also RSA (Shelton et al., 1994). Finally, homozygosity for an as yet unidentified gene in linkage disequilibrium with the HLA reduces the susceptibility for both type I diabetes and coeliac disease (Lie et al., 1999), and mutations in this gene may lead to increased susceptibility for these diseases.

MHC expression and sharing in animal models

Although the relative importance of HLA expression at the maternal–fetal interface in humans remains unresolved, MHC
expression can be studied in many other animals, including rodents and non-human primates. The mouse MHC region (H-2) has been studied the most extensively. The H-2 complex spans 4000 kb on chromosome 17 (Klein et al., 1981) and contains more than 30 genes which are grouped as class I, II, III and class II region-encoded modifiers. Of particular interest are the class I genes, consisting of class Ia (classical) K, D, and L loci and the class Ib (non-classical) Q, T, and M loci. As in humans, class Ia products have greater expression, tissue distribution and polymorphism than class Ib antigens (Fernandez et al., 1995). Recent mouse data demonstrated that class Ia, Ib and β2m were synthesized soon after conception. Class Ia proteins were detected on unfertilized oocytes, were absent on 1-cell embryos, present on 2-cell embryos and later stages with β2m. Class Ib expression was detected on secondary oocytes and embryos at all stages of preimplantation development. During and after implantation, class Ia and II antigens were switched off in placental tissue but class Ib antigen expression increased (Fernandez et al., 1999).

MHC distribution at the maternal–fetal interface in other mammalian species, including sheep, pigs, cows and horses has also contributed to our general understanding of MHC expression during pregnancy (for reviews, see MacCluer et al., 1988; Gautschi and Gaillard, 1990; Amills et al., 1998).

Several MHC homologues have been isolated in non-human primates, including chimpanzees, the rhesus monkey and the cotton-top tamarin (Boyson et al., 1995). The rhesus monkey MHC complex has been extensively studied, since both humans and the rhesus monkey have similar discoid placentae with villous morphology, similar fetal membrane organization (Luckett, 1974; Benirschke and Miller, 1982) and homologous MHC class I loci (Boyson et al., 1996a). HLA-A, -B, -E, -F, and -G orthologues (Mamu-A, -B, -E, -F and -G) have been identified in the rhesus monkey (Miller et al., 1991; Otting and Bontrop, 1993; Boyson et al., 1995, 1996a,b). Although Mamu-G is an HLA-G orthologue, it is a pseudogene (Boyson et al., 1996b). Slukvin et al. (1998) identified another class I gene, Mamu-AG, which shares characteristics with HLA-G; including a truncated cytoplasmic domain, alternative splicing, limited polymorphism, multiple isolectric isoforms and conserved residues important for NK-receptor recognition (Boyson et al., 1997). Unlike HLA-G, it is primarily expressed in first trimester villous syncytiotrophoblasts (Slukvin et al., 1998). Importantly, both HLA-G and Mamu-AG are primarily expressed at the maternal–fetal interface with restricted and low-level expression in other tissues (Slukvin et al., 1999).

Research among non-human species living under more controlled conditions, suggests that specific MHC genes, or MHC-linked genetic regions, affect fertility, reproduction and development. These genes affect fertility, number of offspring, offspring birth and weaning weights, and offspring viability. For example, studies on the rhesus monkey, pigs and horses revealed significant effects of MHC sharing on reproductive outcome. In macaques, significantly more ‘unsuccessful’ couples shared MnLA-A antigens than did ‘successful’ couples (Knapp et al., 1996). In pigs, parental sharing, or particular maternal antigens, resulted in decreased litters (Renard et al., 1985; Gautschi and Gaillard, 1990). Particular haplotypes influenced maternal ovulation rates (Rothschild et al., 1984), piglet birth and weaning weights (Rothschild et al., 1986; Gautschi and Gaillard, 1990) and piglet mortality (Kristensen et al., 1980). In horses, specific dam MHC alleles, specific sire alleles and sire–dam incompatibility were associated with foaling rate (MacCluer et al., 1988). Swine and cheetah studies have also indicated MHC-homozygote offspring deficiency (Gautschi and Gaillard, 1990; Yuuki and O’Brien, 1990). Additionally, MHC effects have been correlated with chicken egg number (Gavora et al., 1986), rabbit ovulation rates and embryonic loss (Gill, 1996). Thus, it is possible that MHC or MHC-linked genes could dramatically affect fertility and reproduction.

The rat gre region, closely linked to the MHC, is ~50 kb and incorporates several class I genes and pseudogenes. While wildtype rats are fertile, homozygotes for the deleted region are small, have increased prenatal mortality, are sterile (males) or subfertile (females) and are highly susceptible to chemical carcinogens (Cortese Hassett et al., 1989). An uncharacterized homologous mouse gre region may map distal to H-2D (Vincek et al., 1990). Although no human homologue has been identified, Gill (1994) suggests that MHC genes associated with reproductive disorders in the rat and human map to the same approximate chromosomal positions. Like HLA gene epistasis in humans, the gre region interacts epistatically with the tail anomaly lethal gene (Tal) in rats (Schaid et al., 1982; Gill et al., 1984; Gill, 1987).

Multiple murine genes involved in spermatogenesis and embryogenesis, known as t haplotypes, map to chromosome 17, which is homologous to human chromosome 6 (Dobrovolskai-Zavadkskaia, 1927; Chesley, 1932; Chesley and Dunn, 1936; Bennett, 1975; Silver, 1985). Phenotypic effects of t haplotype genes include, taillness, embryonic homozygote death, heterozygote male sterility and developmental abnormalities caused by embryonic complex lethal genes (tcl). The tcl genes, organized into different complementation groups, disrupt particular embryonic stages of development (Bennett, 1975; Artzt et al., 1982; Silver, 1985; Klein, 1986). Heterozygous mice for two different complementation groups are viable but sterile and homozygous embryos of the same complementation group fail to survive. For example, homozygosity for the t12 complementation group results in failure of blastocyst formation and implantation (Shin et al., 1984). Homozygosity for t9 leads to blastocyst implantation without ectodermal organization (Ark et al., 1991) and homozygosity for t9 results in primitive streak outgrowth and duplication of neural tissue during days 8–10 (Bennett and Dunn, 1960; Ben-Shaul et al., 1983). Interestingly, some t haplotypes of these complementation groups map to the mouse MHC H-2 complex (i.e. near H-2Q and H-2K). For example, the tcl-w5 haplotype, which causes ectoderm degeneration before embryo differentiation, maps 200 kb of H-2K and is inseparable by recombination (Shin et al., 1984; Artzt et al., 1988). Kostyuk (1994) suggests that mutations, deletions, duplications, or abnormal expression of possible HLA-linked t homologues, may be responsible for some of the HLA associations with various reproductive or developmental disorders. However, only a few human t homologous genes have been mapped. TCP11 maps 2 Mb centromeric to the HLA (Ragoussis et al., 1992) while TCP1 and TCP10 are unlinked to the HLA and map to the long arm (q) of chromosome 6 (Blanche et al., 1992; Masuno et al., 1996). Thus, human t homologous haplotypes may not exist, since mapped t homologue genes are separated over several chromosomal regions (Bibbins et al., 1989). Although few studies have

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investigated the existence of human t haplotypes, many researchers have mapped genes within or near the H-2 region and HLA, which are involved in gametogenesis, embryogenesis and fetal development and survival (Table I). These genes encode proteins, which either have direct effects upon fertility and fetal development and survival, or encode transcription factors, which could regulate expression of MHC and MHC-linked genes. The functions of these genes have primarily been determined in rodents, although some human homologues have been found to play similar roles in human fertility, fetal development and survival. Considering that the HLA complex displays particularly high levels of linkage disequilibrium, it is likely that these homologues are linked to the HLA.

Studies of the Ped gene illustrate the utility of studying the function of rodent MHC or MHC-linked genes for comparison with humans. The mouse Ped (pre-implantation embryonic development) gene of the H-2 region, directly affects embryonic viability and fetal development. This gene encodes the class Ib, Qa-2 antigen. The Ped phenotype manifests itself during the first cleavage division (Goldbard et al., 1982) and is present at the 2-cell stage (Warner et al., 1987; Xu et al., 1994). The Ped (fast) allele is dominant and associated with faster in-vitro and in-vivo cleavage rates. The Ped slow allele shows a 5 h delay in the first cleavage division (Xu et al., 1993). Notably, the Ped fast allele is associated with significantly greater cell numbers in the inner cell mass and the trophectoderm, larger litter sizes and greater birth weights and weaning weights (Warner et al., 1991; McElhinny et al., 1998). Moreover, Ped may influence longevity since Ped fast strains seem to have shorter life spans than Ped slow strains (Tarín, 1997). Ped expression may also be controlled by genes that regulate apoptosis, since mRNA and protein of the Bcl-2 and caspase gene families that control apoptosis have been detected in pre-implantation embryos. A homeostatic mechanism, by genes that regulate cell survival (e.g. Ped), and those that regulate cell death (e.g. Bcl-2), may determine the overall viability of pre-implantation embryos (Warner et al., 1998). A human Ped homologue may exist since fertilized IVF oocytes have different cleavage rates and fast-developing human pre-implantation embryos have better survival rates than slow-developing embryos (Bolton et al., 1989; Ziebe et al., 1997; Sakkas et al., 1998). Since Qa-2 is encoded by class Ib genes, the putative human homologue could be either HLA-E, -F or -G. Subsequently, 108 spare day 3 human preimplantation embryos were examined for class Ib mRNA by RT–PCR. HLA-E mRNA was detected in 84% of 86 embryos, HLA-G mRNA was detected in 44% of 88 embryos and HLA-F was detected in 0% of 17 embryos (Cao et al., 1999). These authors do not believe that HLA-E is the Ped gene since it differs in tissue distribution from Qa-2. The HLA-E mouse homologue has also recently been suggested to be Qa-1 (for review, see Long, 1998). It is also unlikely that HLA-F is the Ped gene, since it was not detected in day 3 human embryos. HLA-G has previously been suggested to be the Ped homologue (Juriscova et al., 1996) since Qa-2 and HLA-G both have similar peptide ligand repertoires, form truncated membrane-bound and soluble forms and have been detected in pre-implantation embryos and trophoblast (Stroynowski and Tabaczewski, 1996). However, HLA-G and Qa-2 differ in several respects. HLA-G lacks the interferon response sequence (IRS) in the 5′ region of Qa-2. They also differ in tissue distribution (Wei and Orr, 1990; Schmidt and Orr, 1993) and bind different types of peptides (O’Callaghan and Bell, 1998). Unlike Qa-2, HLA-G is not linked to the cell surface by a glycosylphosphatidylinositol (GPI) linkage (Cao et al., 1999). Moreover, the mouse gene, Blastocyst MHC, may be the HLA-G homologue since the protein has a similar peptide-binding region to HLA-G, a truncated cytoplasmic tail and is transcribed in blastocyst and placenta (Sipes et al., 1996). Cao et al. (1999) also suggested that the putative human Ped gene is linked to HLA-G and may be HLA-C. Similar to Qa-2, HLA-C is also linked by a GPI linkage to the cell surface and may be involved in reproductive events since it is expressed on extravillous trophoblast (King et al., 1997b). Although Ped is a class Ib gene and HLA-C is a class Ia gene, these classifications differentiating between class I and class II genes may be too specific (Hughes et al., 1999). Finally, Qa-2 is encoded by two class Ib genes, Q7 and Q9, suggesting that several HLA class I genes (Cao et al., 1999) or perhaps other HLA-linked genes could contribute to a human Ped phenotype. These studies clearly demonstrate the value of investigating MHC, or MHC-related, genetic effects in animal models to determine how human homologues could have similar functions in human fertility, reproduction and development.

Interactions: cytokines and HLA expression

Several factors have been associated with one another among women with recurrent miscarriage. Christiansen et al. (1989) reported 13% of non-pregnant women with recurrent miscarriage were positive for two or more serological markers associated with autoimmune disorders compared with 2% of controls (P = 0.01). Kwak et al. (1992) found that 16% of women with recurrent miscarriage were positive for at least two autoantibodies compared with 8% of controls (P = 0.06). This indicates that multiple immunological factors may influence pregnancy outcome, and when combined with immunogenetic factors, they could form intricate networks and complex feedback loops. In this context, HLA expression may play a pivotal role by regulating interactions between factors, e.g. cytokines and natural killer (NK) cells (Figure 3).

Figure 3. Immunological and immunogenetic factors form networks and feedback loops which influence human reproduction. See text for details. LIF = leukaemia inhibitory factor; MUC-1 = mucin-1.
<table>
<thead>
<tr>
<th>Human gene</th>
<th>Full name</th>
<th>Human location</th>
<th>Murine gene</th>
<th>Murine location</th>
<th>In-vitro or in-vivo protein function</th>
<th>Expression of mRNA or protein</th>
<th>References</th>
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<tr>
<td>BATs (BAT1-9)</td>
<td>HLA-B associated transcripts</td>
<td>Telomeric end of class III</td>
<td>Bats</td>
<td>H-2 region</td>
<td>Member of DEAD-box family of ATP-dependent RNA helicases&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Various tissues and cell lines&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Spies et al. (1989)</td>
</tr>
<tr>
<td>HKE4 and HKE6</td>
<td>KE4, KE6, mouse, homologue</td>
<td>Centromeric end of HLA</td>
<td>Ke4, Ke6</td>
<td>H2Ke1-5 = 17p18.4–18.49</td>
<td>Ke6 is involved with kidney function&lt;sup&gt;b&lt;/sup&gt;</td>
<td>HKE4 = placenta, lung, kidney, pancreas&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Abe et al. (1988)</td>
</tr>
<tr>
<td>DDR1 (NEP, EDDR1, NTRK4, TRKE, member 1 DDR, CAK)</td>
<td>Discoidin domain receptor family,</td>
<td>6p21.3</td>
<td>Nep</td>
<td>17C</td>
<td>Protein tyrosine kinase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Fetal and adult tissues&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Di Marco et al. (1993)</td>
</tr>
<tr>
<td>NFYA</td>
<td>Nuclear transcription factor Y, α</td>
<td>6p21</td>
<td>Nfya</td>
<td>17p26</td>
<td>Transcription factor?</td>
<td>Many cell types&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Becker et al. (1991)</td>
</tr>
<tr>
<td>NOTCH4</td>
<td>Notch</td>
<td>6p21.3</td>
<td>In3</td>
<td>17p18.7</td>
<td>Endothelial development&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Endothelial cells in embryonic and adult tissues&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Li et al. (1998)</td>
</tr>
<tr>
<td>POU5FL (OCT3, OCT4)</td>
<td>Pou domain, class 5, transcription factor 1</td>
<td>100 kb telomeric to HLA-C</td>
<td>Pou5f</td>
<td>17p19.23</td>
<td>Transcription factor</td>
<td>Oocytes before and after fertilization&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Okamoto et al. (1990)</td>
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<tr>
<td>RXRβ</td>
<td>Retinoid X receptor, β</td>
<td>6p21.3</td>
<td>Rxrβ</td>
<td>H-2 region</td>
<td>Hinds 5' enhancers&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Striatum&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hamada et al. (1989)</td>
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</table>

<sup>a</sup> Asp-Glu-Ala-Asp, 1 of 8 highly conserved sequence motifs.<br><sup>b</sup> Member of DEAD-box family of ATP-dependent RNA helicases.<br><sup>c</sup> Member of gene family that controls neuron development, differentiation and maintenance.<br><sup>d</sup> Essential for class II MHC expression. Recognition of CCAATT elements.<br><sup>e</sup> Involved in stem cell signalling to trophectoderm differentiation. Down-regulated during endoderm and mesoderm differentiation.<br><sup>f</sup> Controls dopaminergic mesolimbic pathway.
<table>
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<tr>
<th>Human gene</th>
<th>Full name</th>
<th>Human location</th>
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<th>Expression of mRNA or protein</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>MAPK(p38-δ)</strong> Mitogen-activated protein kinase</td>
<td>6p21.3</td>
<td>p38-δ</td>
<td>17A3-B</td>
<td>Member of kinase family that transduces extracellular stimuli into intracellular signals, controlling gene expression</td>
<td>Adult lung, testis, kidney and gut epithelium&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hu et al. (1999)</td>
<td></td>
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<tr>
<td><strong>TCP11</strong></td>
<td>T-complex homologue 11</td>
<td>6p21.3-p21.2</td>
<td>Tcp11</td>
<td>Distal inversion of the t-complex</td>
<td>Closely linked to a Zn finger gene, ZNF76, member of GLI Kruppel family&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Testis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Ragoussis et al. (1992)</td>
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<tr>
<td><strong>TCTE1</strong></td>
<td>T-complex-associated-testis-expressed 1</td>
<td>Centromeric to HLA</td>
<td>Tcte-1</td>
<td>17p23.50</td>
<td>Transcriptional regulator&lt;sup&gt;?&lt;/sup&gt;</td>
<td>Testis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sarvetnick et al. (1989) Sarvetnick et al. (1990) Kwiatkowski et al. (1991)</td>
</tr>
<tr>
<td><strong>PIM1</strong> Oncogene PIM-1</td>
<td>Centromeric to HLA-DP</td>
<td>Pim1</td>
<td>In distal inversion</td>
<td>Proto-oncogene&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Differentiating embryonic cells, adult spermatids and leukaemias&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Nagarajan et al. (1986) Sorrentino et al. (1988) Amson et al. (1989) Domen et al. (1987) Saris et al. (1991)</td>
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<td><strong>CDKNIA</strong> (CIP, WAF1, p21)</td>
<td>Cyclin-dependent kinase inhibitor 1A</td>
<td>6p21.2</td>
<td>Waf1</td>
<td>Proximal to H-2 region</td>
<td>Inhibits cyclin kinase activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>In most tissues&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Huppi et al. (1994) Demetrick et al. (1995)</td>
</tr>
<tr>
<td><strong>FGD2</strong> Facioscapulohumeral dystrophy 2</td>
<td>6p21.2</td>
<td>Fgd2</td>
<td>17 orthologue</td>
<td>Guanine nucleotide exchange factor&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Diverse tissues&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pasteris and Gorski (1999)</td>
<td></td>
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<tr>
<td>Human gene</td>
<td>Full name</td>
<td>Human location</td>
<td>Murine gene</td>
<td>Murine location</td>
<td>In-vitro or in-vivo protein function</td>
<td>Expression of mRNA or protein</td>
<td>References</td>
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<td>AEGLI</td>
<td>Acidic Epididymal</td>
<td>6p21.1-p21.2</td>
<td>Aeg1</td>
<td>H-2M region</td>
<td>Sperm surface protein involved in gamete fusion&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Epididymal cells&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hayashi et al. (1996)</td>
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<td></td>
<td>Glycoprotein-like1</td>
<td>(orthologue)</td>
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<td></td>
<td>Coats sperm entering epididyma&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Epididymis, ductus deferens, seminal plasma, spermatozoa&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yoshino et al. (1998)</td>
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<td>Causes sperm negative charge&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Regulated by androgen&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Aeg antibodies block fertilization&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Purified Aeg binds to oocyte and inhibits fusion&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>RUNX2</td>
<td>Runt-related</td>
<td>6p21</td>
<td>Cbfa1</td>
<td>17p28.07</td>
<td>Osteoblast-specific transcription factor&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Osteoblast cells&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>(CBFA1, AML3, PEBP2A, OSF2)</td>
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<td></td>
<td></td>
<td>Controls osteoblast differentiation during embryonic development&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>Ducy et al. (1997)</td>
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<td>Controls bone matrix deposition of differentiated osteoblasts postnatally&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>CCND3</td>
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<td>Controls cell cycle&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Inaba et al. (1992)</td>
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<td></td>
<td>Controls cell division and differentiation in the germline&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Motokura et al. (1992)</td>
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<td>Important for decidualization&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Xiong et al. (1992)</td>
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<sup>a</sup>Human protein.  
<sup>b</sup>Rodent protein.
Cytokines are small, secreted, membrane-bound proteins that act through cell-surface receptors and generally induce changes in gene expression within their target cells (Parham, 2000). They also play critical roles in pathogen response and regulation of cellular growth and differentiation. Currently, cytokines are known to influence several stages of reproduction (Figure 1). Cytokines e.g. ILs, IFNs and TNFs, can modulate HLA expression throughout pregnancy (Figure 1). IL-4 can induce class II expression and IL-10 can increase class I and II expression (Parham, 2000). Specifically, IL-10 enhances HLA-G transcription in human first trimester cultured trophoblast cells. IL-10 also up-regulates HLA-G surface-expression in peripheral blood monocytes (PBMC)s, while other classical class I and class II genes are simultaneously down-regulated (Moreau et al., 1999). IFNs can induce class I mRNA and protein expression from various types of class I negative cells (Wan et al., 1987). In vitro, IFNs can induce class Ia expression, but not all of the class Ib antigens (for review, see Singer and Maguire, 1990). IFNs can induce class I expression from day 8.5 embryonic cells, which lack class I expression (Ozato et al., 1985; Miyazaki et al., 1986). Generally, IFN-γ is the most potent cytokine since it is an inducer of Th1 responses and a negative regulator of B cell differentiation and proliferation (O’Neil et al., 1999). Specifically, IFN-γ induces the formation of a nuclear binding protein, which binds to class I flanking regulatory sequences, increases class I expression and decreases class II expression (Brown et al., 1993). IFN-γ may also be an important cytokine involved in maternal–fetal interactions since IFN-γ can induce some cell lines to express HLA-G (Anderson and Berkowitz, 1985) and it enhances class Ia mRNA expression in amnion epithelial cells (Kovats et al., 1990; Wei and Orr, 1990). Interestingly, King et al. (2000a) reported that IFN-γ significantly up-regulated in-vivo surface expression of HLA-C compared with HLA-G expression in extravesicular trophoblast cells. Furthermore, cytokines are known to influence each other. For example, IFN-γ is regulated by the cytokine IL-1β, which can suppress IFN-γ induced class II expression (Rohn et al., 1999).

TNFs may also play critical roles in pregnancy maintenance. For example, TNF enhances T-cell proliferation, modulates T-cell receptor expression, enhances NK activity and regulates B cell functions (Aguado et al., 1996). TNF treatment of primary and established cell lines increased class I cell-surface expression (Collins et al., 1986). TNF treatment of human endothelial and dermal cells increased class I protein and mRNA expression 8–10-fold and 100-fold respectively. Overall, cytokines can have different, yet simultaneous, effects on HLA expression on cells involved in reproductive events. These effects may be directly related to the proliferation and immunotolerance of trophoblast, or fetal, cells. Unfortunately, precise cytokine/HLA interactions and timing of interactions during pregnancy remains unresolved.

**Interactions: NK cells and HLA expression**

NK cells may also contribute to human reproduction during many stages of pregnancy (Figure 1). Peripheral NK cells are found throughout the body’s circulatory system and a subpopulation of NK cells, uterine NK cells can be found at the maternal–fetal interface. Functionally, NK cells are capable of cytotoxic killing of target cells (Parham, 2000) and studies continue to investigate whether reproductive outcome is influenced by changes in NK cell number, function or target-cell recognition (for review, see Roy Choudhury and Knapp, 2001). NK cell recognition of HLA antigens may be important during reproduction since NK binding to HLA antigens can activate, or inhibit, NK target cell killing (Yokoyama and Seaman, 1993), or alter NK cytokine secretion (Loke and King, 2000). Three families of peripheral NK inhibitory and activatory receptors, which recognize HLA antigens, have been identified. The Killer cell immunoglobulin-like receptors (KIR), bind HLA-B and -C (Biassoni et al., 1995; Gumperz et al., 1995; Mandelboim et al., 1996; Gumperz et al., 1997). The CD94/NKG2 type II membrane glycoprotein of the C-type lectin superfamily, binds HLA-E, rather than a broad range of HLA class I antigens as previously suggested (Borrego et al., 1998; Braud et al., 1998a; Lee et al., 1998; Brooks et al., 1999) and the Immunoglobulin-like transcript (ILT) family (Samaridis and Colonna, 1997; Cosman et al., 1997), binds HLA-A, -B, -C and -G (Borges et al., 1997; Colonna et al., 1997; Cosman et al., 1997; Fanger et al., 1998; Vitale et al., 1999).

Uterine NK cells also express KIR, CD94/NKG2 and ILT receptors (Hiby et al., 1997), but assays of peripheral NK and uterine NK receptors in the same individual, reveal different proportions in these cells. Moreover, the expression patterns of these NK receptors vary between women (Verma et al., 1997). Thus, for these reasons understanding the interactions of uterine NK cells with HLA expressing cells at the maternal–fetal interface requires clear identification of uterine NK receptors (see Roy Choudhury and Knapp, 2001).

Uterine NK cells are in close association with invading extravillous trophoblast cells in the uterus (King et al., 1998), which express HLA-C, -E and -G antigens (King et al., 1996b; King et al., 2000a,b,c). Therefore, HLA expression may regulate NK activity at the maternal–fetal interface. Peripheral and uterine NK cell recognition of HLA-C has been demonstrated by several groups (Colonna et al., 1993; Moretta et al., 1993; Vitale et al., 1995). Interestingly, uterine NK cells have higher proportions of KIR receptors for HLA-C and CD94/NKG2 receptors for HLA–E than peripheral NK cells (King et al., 1997a; King et al., 2000c). HLA-E binding to CD94/NKG2 uterine NK receptors inhibits cytotoxicity to cell lines. However, uterine NK cells were unable to kill trophoblast cells when monoclonal antibodies blocked CD94/NKG2 receptors or HLA class I antigens, or when class I antigens were removed by acid. This suggests that in-vivo trophoblast HLA-E binding with CD94/NKG2 receptors on uterine NK cells may regulate functions other than inhibiting cytotoxicity during reproduction (King et al., 2000b).

Many studies have focused upon NK receptor recognition of the HLA-G antigen due to the unique distribution of HLA-G at the maternal–fetal interface. Uterine NK cell recognition of HLA-G expressing extravillous trophoblast may inhibit NK trophoblast lysis. Chumbley et al. (1994) first reported that HLA-G transfection in a B lymphoblastoid cell line protected first trimester trophoblasts from decidual NK cell lysis. This has been confirmed by many groups (Deniz et al., 1994; Pazmany et al., 1996; Mandelboim et al., 1997; Rouas-Freiss et al., 1997a,b; Soderstrom et al., 1997; Colonna et al. 1998; Navarro et al., 1999; Ponte et al., 1999). HLA-G1 and -G2 isoforms also can inhibit cell lysis by peripheral NK cells (Perez-Villar et al., 1997; Rouas-Freiss et al., 1997a,b). In addition, HLA-G1 inhibited NK cell

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movement across porcine endothelial cells (Durling et al., 2000), suggests that different HLA-G isoforms may have specialized functions (Ishitani and Geraghty, 1992).

The type of uterine NK receptors, which bind HLA-G remains controversial. However, in-vitro studies using 221-HLA-G transfectants as target cells and peripheral blood NK cells as effector cells may not parallel in-vivo interactions at the maternal–fetal interface (Le Bouteiller and Blaschitz, 1999). Earlier studies demonstrated that CD94/NKG2 recognized HLA-G. Yet later studies demonstrated that NK cells recognized HLA-E bound to HLA-G leader peptides, allowing HLA-E surface expression and recognition by the CD94/NKG2A receptor (Pende et al., 1997; Perez-Villar et al., 1997; Soderström et al., 1997; Llano et al., 1998; Navarro et al., 1999). ILT2 and ILT4, members of the ILT family, directly bind HLA-G and inhibit target cell lysis (Colonna et al., 1997; Colonna et al., 1998; Allan et al., 1999; Navarro et al., 1999; Vitale et al., 1999). However, the broad specificity of ILT2 and ILT4 for other HLA class I antigens suggests that HLA-G binding to these NK receptors does not provide a unique 'fetal' signal at the maternal interface (King et al., 2000c). Several KIR receptors that bind HLA-G and inhibit NK cell lysis have been identified, including p49 (Cantoni et al., 1998) and KIR2DL4 (Ponte et al., 1999, Rajagopolan and Long, 1999). Interestingly, HLA-G1 recognition by NK KIR receptors inhibited target killing of K562 cell lines. These cell lines lacked HLA-E expression, demonstrating that HLA-G1 was capable of inhibiting NK cytotoxicity without NK CD94/NKG2 receptor recognition of HLA-E (Khalil-Daher et al., 1999). One KIR receptor, KIR2DL4 may display both inhibitory and activatory functions (King et al., 2000c), suggesting a unique role for the interaction of HLA-G and KIR2DL4 at the maternal–fetal interface. Yet the distribution of this NK receptor remains controversial since Rajagopolan and Long (1999) reported that all peripheral cells express KIR2DL4, while Ponte et al. (1999) reported that only some first trimester uterine NK cells and all term placental NK cells expressed KIR2DL4.

In summary, studies indicate that NK cells are capable of recognizing HLA expressing trophoblast during pregnancy and may inhibit cytotoxic trophoblast killing. King et al. (2000c) suggest that placentation, or cytokine secretion, may be regulated by a variety of CD94/NKG2, KIR and ILT uterine NK cell receptors, which recognize HLA expressing trophoblast cells. Thus, reproductive failure may occur if trophoblast cells lack or have aberrant HLA expression or if NK cells lack appropriate inhibitory receptors (Lopez-Botet et al., 1996).

Interactions: cytokines and NK cells

Both cytokines and NK cells play fundamental roles in pregnancy maintenance. Not surprisingly, NK cells and cytokines act together to orchestrate regulatory pathways of pregnancy. For example, IFN-γ, IFN-α, IFN-β, IL-2, IL-7, IL-6, IL-12 and IL-15 exert direct and potent stimulatory effects on NK activity (King and Loke, 1990; Naume and Espevik, 1994; King et al., 1999). These activities can include NK proliferation, NK or LAK non-destructive killing or NK cytokine secretion (Naume and Espevik, 1999). Interestingly, HLA-G1 recognition by NK KIR receptors restricted killing or NK cytokine secretion (Naume and Espevik, 1999). Importantly, there may be critical differences in NK and cytokine pathways between mice and humans. In mouse decidua, γδ T-cells play a dominant role in Th1/Th2 cytokine profile production at the maternal–fetal interface; whereas in human decidua, CD56+ CD16− NK cells and macrophages seem to predominate (Clark, 1991; Maruyama et al., 1992; Chernyshov et al., 1993). Moreover, human decidua have no obvious counterpart for the mouse’s large, NK-lineage metrial gland-type cells, which secrete nitric oxide and cytokines (Croy, 1990; Parr et al., 1995; Hunt et al., 1997). However, the smaller CD56+ human cells can
also produce cytokines and possibly nitric oxide (Saito et al., 1993). Thus, different cytokines affect peripheral and uterine NK cell activity and NK cells are capable of their own cytokine secretions (Jokhi et al., 1993, 1994c; King et al; 1995). Future studies should elucidate the type and temporal sequence of cytokine/NK interactions at the maternal–fetal interface and determine whether the inappropriate regulation of these pathways contribute to reproductive failure.

Other interacting factors

In addition to the interactions between NK cells, the HLA cytokines, there are even more potential interactions between factors during reproduction. Cytokines, e.g. TNF-α, IL-1α, IL-6, IL-4 and transforming growth factor (TGF)-β interact with LIF (Lorenzo et al., 1994; Wetzler et al., 1994; Arici et al., 1995; Delage et al., 1995; van Eijk et al., 1996), which may influence human reproduction from gamete development through implantation (for review, see Roy Chaudhury and Knapp, 2001). LIF transcription is induced by IL-1α, IL-1β, TGF-β, and TNF-α (Allan et al., 1990; Arici et al., 1995). Cytokines are also secreted by many other immune cells, including γδ T-cells. Some authors suggest that these endometrial/decidual cells may be important for maternal–fetal interactions (Janeway et al., 1988; Mincheva-Nilsson et al., 1992). In non-pregnant mice, γδ T-cells mediate both Th1 and Th2 responses (Bluestone et al., 1991; Chien et al., 1996; Fu et al., 1994; Weintraub et al., 1994; Hsieh et al., 1996; Tanaka et al., 1996); and they can secrete cytotoxic, inflammatory Th1 cytokines, e.g. IL-2, when cultured in vitro with mouse or human trophoblast cells (Clark et al., 1999). They also can secrete Th2 cytokines, promoting antibody production, such as IL-10 and TGF-β2, in the decidua of mice experiencing high rates of fetal resorptions (Pu et al., 1994; Hsieh et al., 1996; Arck et al., 1997). Although associating a particular cytokine profile with γδ T-cells remains controversial (for review, see Szekeres-Bartho et al., 1999). In contrast, cytokine expression is correlated with production of LIF. Piccinni et al. (1998) demonstrated that LIF-producing T-cells were down-regulated by Th1 cytokines, IL-12, IFN-γ and IFN-α, and up-regulated by Th2 cytokines, IL-4 and progesterone. Decreased LIF and Th2 (IL-4, IL-10) cytokine production by decidual T-cells was also detected in women with recurrent miscarriage compared with fertile controls. Other factors expressed in the endometrium, e.g. MUC1, may also be important for NK interactions. Mucins may act as anti-adhesion molecules on the uterine epithelium and MUC1 down-regulation may be critical for successful implantation (for review, see Chaudhury and Knapp, 2001). Zhang et al. (1997) demonstrated that MUC1 expression can inhibit NK target cell lysis in a dose-dependent way. Since activated NK (LAK) cells are capable of killing trophoblast cells (Drake and Head, 1989), MUC1 may inhibit NK lysis at the maternal–fetal interface. These studies demonstrate just a limited number of the potential interactions that may occur between factors during pregnancy. The disturbance of any of these pathways, or the aberrant, or lack of expression, of factors could thereby, contribute to reproductive failure. Thus, while the independent effects of each factor is important, there remains a critical need to investigate the interactions between factors during pregnancy.

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Summary and conclusion

Although the role of HLA during gamete development and fertilization remains controversial, HLA tissue-specific distribution may directly influence embryo cleavage through post-parturition (Figure 1). For this reason, HLA distribution at the maternal–fetal interface is an important field of investigation. The expression of HLA-G and -C on extravillous trophoblast cells (King et al., 1998, 2000a,b) suggests that these molecules may interact with maternal immune cells to inhibit fetal rejection. HLA-C may be the human homologue of the Ped phenotype, which directly affects embryonic and fetal development (Cao et al., 1999). HLA-G exhibits unique characteristics suggesting that it may be involved in critical maternal–fetal interactions. HLA-G expression is present throughout gestation. It demonstrates lower polymorphism than other class I loci and possesses unique structural properties and transcriptional isoforms (Le Bourteiller et al., 1999). It has been suggested that HLA-G may prevent reproductive failure since it could provide fetal protection from pathogens. HLA-G may also prevent maternal rejection of the fetus, modulate decidual cytokine shifts which regulate reproductive events and regulate cytotoxic trophoblast differentiation and trophoblast growth (for review, see Le Bourteiller and Blaschitz, 1999). While reproductive failure could also result from parental sharing of HLA loci or haplotypes, 30 years of research in outbred couples has produced little agreement regarding the most important HLA locus or loci responsible for reproductive failure (Ober and van der Ven, 1997). Discrepancies in studies of outbred couples may be due to differences in study sample sizes, design and methodology. Studies often differ in the selection and stratification of fertile and RSA couples. Interestingly, recent studies based upon DNA techniques have not detected increased class II nor class I parental sharing in RSA couples (Christiansen et al., 1989; Ito et al., 1992; Takakuwa et al., 1992; Laitinen et al., 1993; Karhukorpi et al., 1997; Wagenknecht et al., 1997; Yamashita et al., 1999). Intermittent associations between parental HLA sharing and reproductive failure may reflect linkage disequilibrium in some outbred populations. However, many confounding variables may complicate studies of outbred populations. Additionally, HLA antigen frequencies in women with RSA and subsequent reproductive success remains controversial. Importantly, it has not been demonstrated that parental histocompatible, or offspring homozygous for particular HLA loci, results in preferential abortion in outbred families.

In contrast, studies in closely related populations suggest that some HLA loci, or haplotypes, are associated with fetal loss and increased birth intervals (Ober et al., 1993; Laitinen, 1993). Studies in genetically isolated groups also demonstrated a deficiency of histocompatible offspring in particular HLA haplotype pairs (Laitinen 1993; Ober 1995). Thus, immunogenetic effects such as linkage disequilibrium, on reproductive outcome may be more easily detected among isolated, closely related populations than among outbred groups. The majority of studies of outbred couples suffer from the limitations of retrospective studies and most couples were selected on the basis of reproductive histories (Creus et al., 1998). Reproductive outcome in outbred groups is confounded by use of contraception, small family sizes and high genetic diversity. Considering the fact that MHC genes are the most polymorphic loci known among
vertebrates (Klein and Figueroa, 1986), closely related individuals that exhibit less genetic diversity and similar HLA haplotypes are more likely to share other homoyzous regions in the genome. Therefore, the HLA may serve as a marker for overall genome homoyzosity. In outbred couples, high amounts of HLA polymorphism may confound detection of genetic effects upon reproductive failure since individuals rarely share many HLA alleles. By studying closely related individuals it may be possible to detect HLA, or HLA-linked genes and regions, which affect fertility, reproduction and development. Human homologues of murine genes that directly affect gametogenesis and embryogenesis have also been identified (Table I). Human homologues of the Ped gene, the g~ complex and X haplotypes influencing fertility, reproduction and development, may also exist. These HLA genes, or genetic regions, could influence a multitude of networks between immunological factors involved in reproductive events. For example, the BYSL gene, which maps to 6p21.1 near the HLA, encodes a protein that forms a complex with trophinin and testing in trophoderm cells during implantation, mediating adhesion between trophoblast and endometrial cells (Suzuki et al., 1999).

Thus, interactions between factors may be crucial to achieve fertility and to maintain a pregnancy. As described, cytokine activity may influence HLA expression at the maternal–fetal interface since IFNs and TNFs cytokines can induce HLA class I surface expression in class I negative cells (Wan et al., 1987; Collins et al., 1986) and IFN-~ can control MHC expression via transcriptional regulatory pathways (Brown et al., 1993). Cytokines may also affect NK functions at the maternal–fetal interface since they have direct and potent stimulatory effects upon NK activity (King and Loke, 1990). Since activated NK (LAK) cells can kill trophoblast cells (Drake and Head, 1989), and are capable of secreting cytokines which have appropriate trophoblast receptors (King et al., 1999), both cytokines and NK cells could provide important regulatory pathways for pregnancy maintenance. Moreover, NK cells possess receptors which recognize HLA antigens, e.g. HLA-G, -C and -E, expressed on extravillous cytotrophoblast cells (King et al., 1998, 2000a,b,c). Antigen binding to receptors can alter NK target cell killing and many groups have demonstrated that HLA-G expression can inhibit uterine NK target killing of trophoblast cell lines (for review, see Lanier, 1999). This demonstrates just a few of the potential factor networks that may function throughout human reproduction.

Thus, successful reproduction is a dynamic interplay between immunological and immunogenetic factors, as illustrated in Figure 1. Detection and further study of these factors requires highly specific assaying during particular stages of pregnancy. While only a few interactions have been reported during, and after, blastocyst formation, fewer studies have been undertaken during early gamete development, fertilization and embryo cleavage. Thus, animal models can serve as reasonable alternatives to human studies, due to the difficulties of investigating these processes during human reproduction. This review demonstrates the importance of immunogenetic factors and interacting factors and suggests that the disruption of interacting networks may also contribute to human reproductive failure.

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