IMMUNOGENETICS '98
HLA and Pregnancy: The Paradox of the Fetal Allograft

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The immunologic challenges posed by the evolution of viviparity were described nearly 50 years ago in the classic paper by Medawar (1953), in which he posed the question "how does the pregnant mother contrive to nourish within itself, for many weeks or months, a foetus that is an antigenically foreign body?" (p. 324). At the time this paper was written, the deleterious effects of maternal-fetal Rh incompatibility were well known, but antigens responsible for the rapid and violent rejection of foreign tissues were undiscovered. It was assumed, therefore, that maternal-fetal incompatibility for these as-yet-undefined tissue antigens would be similarly problematic during pregnancy. Today, it is well recognized that allograft rejection is mediated by genes of the major histocompatibility complex (MHC), which include the human leukocyte antigen (HLA) genes (fig. 1), and that maternal-fetal HLA incompatibility is not deleterious during pregnancy. However, the survival of the fetal allograft in mammalian pregnancy remains a paradox.

Despite the fact that the mechanisms that induce tolerance during pregnancy are still not well understood, several features of the immunologic state during pregnancy are clear. For example, the placenta is *not* an immunologically inert barrier, as was suggested by Medawar (1953); rather, maternal and fetal cells are reciprocally transported across the placenta, and a state of mutual tolerance exists between mother and fetus, during normal gestation. Indeed, the transport into the maternal circulation and the long-term survival of fetal cells (Bianchi et al. 1996) and the transport of maternal cells into fetal tissues (Socie et al. 1994; Piotrowski and Croy 1996; Bonney and Matzinger 1997) have been well documented. The fact that immune responses against these cells are not elicited attests to the pregnancy-induced tolerance that is present in both mother and fetus. In addition, fetal tolerance toward maternal cells is long lasting, as evidenced by the lack of responsiveness of adult B cells to noninherited maternal HLA class I antigens (Claas et al. 1988; Bean et al. 1990).

Two additional insights have come to light since Medawar's (1953) classic paper, both of which have influenced the context in which maternal-fetal immunology is considered. First, the classical HLA antigens that are responsible for the rapid rejection of allografts in humans are not present on placental cells at the maternal-fetal interface, and, second, maternal-fetal *in*compatibility, with respect to HLA, actually may be beneficial during pregnancy.

HLA Genes at the Maternal-Fetal Interface

The fetal cells that come into direct contact with maternal tissues in the pregnant uterus are the extravillous cytotrophoblasts, which invade the maternal decidua. These cells do not express any HLA class II genes (i.e., *HLA-DR, HLA-DQ,* or *HLA-DP*), which are strongly immunogenic cell-surface markers in allogeneic transplants. Furthermore, the class I loci *HLA-A* and *HLA-B*, which are expressed in nearly all other nucleated cells, are not expressed in trophoblast-cell populations. Thus, expression of the most polymorphic and antigenic HLA genes is suppressed in cells that are in direct contact with the maternal immune system; instead, the nonclassical, class I gene *HLA-G* is primarily expressed in these tissues (reviewed in Le Bouteiller 1994). In addition, lower and more transient levels of the product of the classical, class I gene *HLA-C* are observed (King et al. 1996). Because *HLA-G* was the first HLA gene to be described in these tissues and because its product is the most abundant HLA protein at the maternal-fetal interface, there has been considerable interest in this gene.

HLA-G is an unusual HLA gene, with respect to several features. First, although its intron-exon structure is identical to that of the other class I genes, a premature stop codon in exon 6 results in a shortened cytoplasmic tail (Geraghty et al. 1987). The functional implications (if any) of this are still unknown. Second, whereas other class I genes are expressed in nearly all nucleated cells, HLA-G protein has been detected only in fetal cells at the maternal-fetal interface (reviewed in Le Bouteiller 1994). Third, HLA-G protein exists in multiple isoforms

Received November 7, 1997; accepted for publication November 12, 1997; electronically published January 28, 1998.

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This article represents the opinion of the author and has not been peer reviewed.

0002-9297/98/6201-0002$02.00

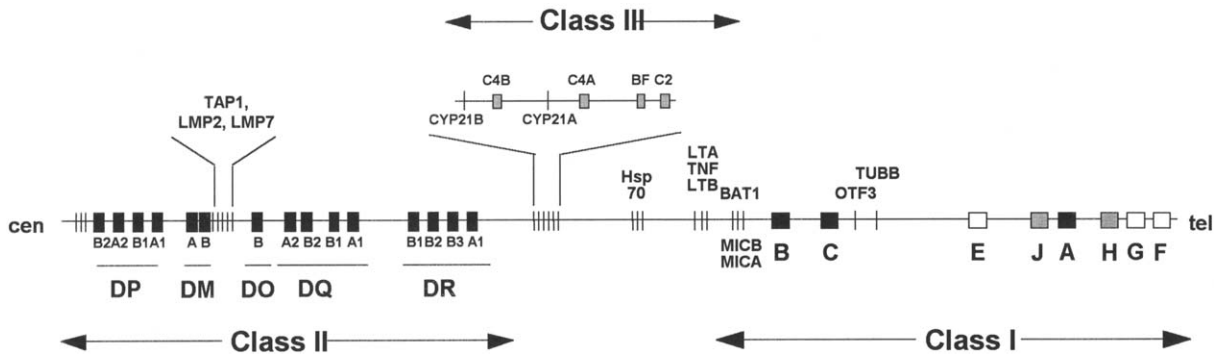

Figure 1 Map of human MHC on chromosome 6p21. Blackened squares represent class Ia (classical) genes, unblackened squares represent class Ib (nonclassical) genes, and gray-shaded squares represent class I pseudogenes (*HLA-H*) or gene segments (*HLA-J*). Class II HLA genes are shown as blackened rectangles. Complement genes are shown as gray-shaded rectangles. All other genes are shown as vertical lines. Modified from the study by Ober and van der Ven (1996).

(fig. 2), as a result of alternative splicing (Ishitani and Geraghty 1992; Fujii et al. 1994). These include transmembrane forms as well as soluble forms that lack the transmembrane and cytoplasmic domains but that include sequences translated from "intronic" mRNA, which are excised from the messages that encode the membrane-spanning HLA-G isoforms. Both transmembrane and soluble forms include full-length isoforms (G1) that retain the α1-domain and the α2-domain, which form the peptide-binding domain (Ishitani and Geraghty 1992; Fujii et al. 1994), similar to the other HLA class I genes. Additional novel isoforms lack either the α2-domain (G2) only or both the α2-domain and the α3-domain (G3) (Ishitani and Geraghty 1992; Fujii et al. 1994). The functions and timing of expression, throughout pregnancy, of the various isoforms are not known, but the α1-domain is capable of inhibiting natural killer (NK) cell activity (Pazmany et al. 1996; Rouas-Freiss et al. 1997). Because NK cells are abundant in the pregnant uterus, one important function of HLA-G may be to protect trophoblast cells from destruction by NK cells. However, the existence of at least five isoforms suggests that HLA-G may serve multiple functions, including antigen presentation (Lee et al. 1995) as well as other as-yet-unknown functions.

Last, *HLA-G* has very little polymorphism at both the nucleotide and amino acid levels (reviewed in Ober and Aldrich 1997). In contrast, the classical HLA genes are among the most polymorphic loci in the human genome. The limited and conservative nature of the amino acid substitutions in *HLA-G* may permit tolerance of the fetal allograft. Furthermore, the distribution of polymorphic residues within the antigen-binding domain is distinct from that seen in the classical HLA genes, suggesting that the evolutionary constraints on *HLA-G* were also different from those that influenced the distribution of polymorphic residues at the classical loci (reviewed in Ober and Aldrich 1997).

An exception to this conservative pattern and number of polymorphic residues in *HLA-G* is found in the African American population, in whom increased diversity has been observed (van der Ven and Ober 1994). Recently, a null mutation in *HLA-G* has been reported in both African American (Ober et al., in press-*a*) and Spanish (Suárez et al. 1997) populations. My colleagues and I have identified homozygotes for this mutation and have demonstrated that the mutation is indeed a null mutation at the protein level, eliminating expression of the predominant HLA-G1 isoforms (i.e., those that include the α2-domain) (Ober et al., in press-*a*). The finding of one multigravida woman (Ober et al., in press-*a*) and one child (C. Ober, C. Aldrich, A. Reed, unpublished data) who are homozygous for this mutation indicates that HLA-G1 protein clearly is not necessary for fetal survival, although reduced levels of HLA-G1 protein in carriers of this mutation may have less obvious negative effects on pregnancy outcome.

This null allele has been named *"HLA-G*105N"* (Suárez et al. 1997) and occurs at frequencies of .074 in African American populations (Ober et al., in press-*a*), .029 in U.S. Hispanic populations (Ober et al., in press-*a*), and .061 in Spanish populations (Suárez et al. 1997); it was absent among 134 U.S. Caucasian chromosomes (Ober et al., in press-*a*). The relatively high frequency of this null allele in two distantly related populations is curious and raises the possibility that reduced expression of HLA-G1 protein in carriers of the *HLA-G*105N* allele may be selected under certain conditions, thereby maintaining the allele at a high frequency in these populations. Because of the high frequency of this allele in populations with a historically high pathogen load, it is tempting to speculate that one function of

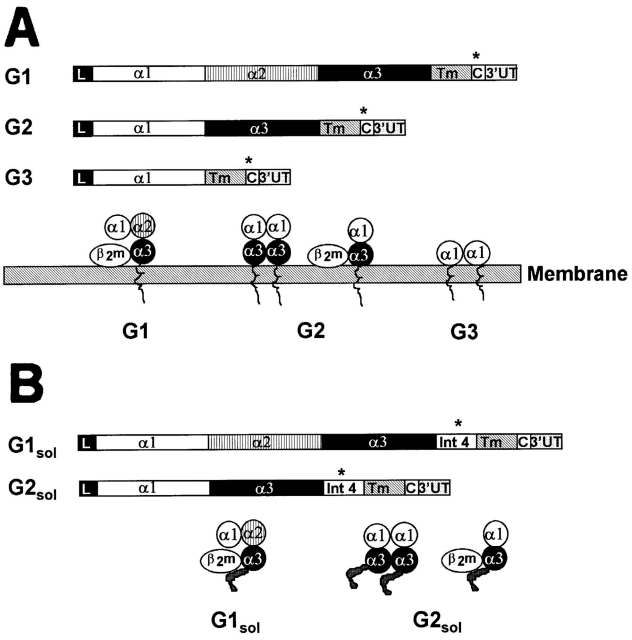

Figure 2 Alternatively spliced isoforms of membrane-bound (*A*) and soluble (*B*) *HLA-G* transcripts and proteins. The full-length transcript (HLA-G1) includes the leader peptide (L) encoded by exon 1, the α1-domain encoded by exon 2, the α2-domain encoded by exon 3, the transmembrane domain (Tm) encoded by exon 4, the cytoplasmic domain (C) encoded by exons 6 and 7, and the 3′ UTR. A premature stop codon in the second codon of exon 6 (indicated by an asterisk [*]) results in a shortened cytoplasmic tail. In the full-length transcript, the α1-domain and the α2-domain form the peptide-binding domain, and the α3-domain binds to β_2-microglobulin (β_2m) at the cell surface, similar to other class I molecules. Exon 3, encoding the α2-domain, is spliced out of the G2 isoforms. Two structures have been proposed for HLA-G2. Two G2 proteins may join at the cell surface and form a class II–like structure (Ishitani and Geraghty 1992; Fujii et al. 1994), or the α3-domain may bind to β_2m at the cell surface (Rouas-Freiss et al. 1997). Both exons 3 and 4 are removed from the G3 isoform, which may be unstable at the cell surface. The two soluble isoforms (*B*) include an additional 21 amino acids, following the α3-domain, which are encoded by nucleotides from intron 4. A stop codon (indicated by an asterisk [*]) terminates translation in intron 4, excluding amino acids encoded by exons 4–6. The additional 21 amino acids are shown as a shaded "tail" following the α3-domain, in the lower part of panel *B*. Modified from the study by Ober and Aldrich (1997).

soluble HLA-G may be to inhibit the proliferation of maternal T cell populations in the pregnant uterus, by induction of cell death, as do soluble HLA-A and HLA-B do in the periphery (Zavazava and Kronke 1996). Maternal T cell populations are remarkably absent from the pregnant uterus (Hunt and Soares 1996), but the mechanisms underlying this observation are unknown. It has been suggested that, in carriers of the *HLA-G*105N* allele, the reduced expression of HLA-G1 soluble protein may be associated with increased numbers of maternal uterine T cells, which could be beneficial in the presence of intrauterine infections (Ober and Aldrich 1997). We currently are exploring this hypothesis in population studies.

Maternal-Fetal HLA Compatibility

Although the classical HLA genes are not expressed in fetal tissues at the maternal-fetal interface, maternal antibodies against paternally derived HLA that are inherited by the fetus are detectable in the circulation of ~20% of primigravidae and ~40% of multigravidae (Payne and Rolfs 1958; van Rood et al. 1958). Presumably, sensitization is elicited by fetal nucleated cells that enter the maternal circulation during pregnancy or at parturition. Regardless of their origin, the presence of anti-HLA antibodies in a significant proportion of healthy pregnancies has demonstrated that sensitization to paternal HLA during pregnancy is not harmful. On the contrary, it has been suggested that these antibodies actually may be beneficial (Beer and Billingham 1976).

The idea that maternal-fetal *in*compatibility, with respect to MHC antigens, is advantageous in mammalian pregnancy was first proposed in the 1960s, on the basis of the observations that placental size was larger among H-2–incompatible murine fetuses, compared with that among compatible fetuses (Billington 1964; James 1965), and that implantation rates were higher for H-2–incompatible murine zygotes, compared with those for compatible zygotes (Kirby 1970). If human fetuses with paternally inherited antigens that are similar to maternal antigens (i.e., histocompatible fetuses) are also at a selective disadvantage during pregnancy, then couples who match for HLA antigens should have poorer reproductive outcomes, compared with couples who do not match for HLA, because only the former can produce compatible fetuses. Indeed, the first two studies to evaluate this hypothesis in humans demonstrated significantly more matching at the HLA-A and HLA-B loci among couples with a history of idiopathic recurrent spontaneous abortion, compared with that among fertile control couples (Komlos et al. 1977; Schacter et al. 1979). However, the >30 subsequent studies of HLA matching in couples with recurrent miscarriage have yielded conflicting results, and no clear relationship between HLA matching and fetal loss can be extrapolated from these retrospective studies (reviewed in Ober and van der Ven 1996). Nonetheless, prospective studies of HLA matching and pregnancy outcome and studies of maternal-fetal HLA compatibility in women with rheumatoid arthritis (RA) have provided support for the hypothesis that HLA-incompatible fetuses are at an advantage in human pregnancy.

HLA Matching and Fetal Loss: Prospective Studies

To elucidate the relationship between HLA matching and pregnancy outcome, my colleagues and I have been conducting prospective, population-based studies of the Hutterites, for >10 years. The Hutterites provide an excellent opportunity to study the relationship between HLA matching and pregnancy outcome, because not only are they among the most inbred human populations (and therefore often match for HLA), but they are also among the most fertile (reviewed in Ober 1995). We have not identified any Hutterite women (among >500) who meet the clinical definition of a recurrent aborter (i.e., at least three spontaneous abortions and no more than one liveborn child), but the overall miscarriage rate is 15.6% (Ober et al., in press-*b*), which is similar to that of outbred couples. Hutterite couples matching for alleles across the entire haplotype or matching for HLA-B antigens had significantly more fetal losses than did couples not matching for HLA (Ober et al., in press-*b*). At the present time, it is not known whether the *HLA-B* locus, per se, or alleles at a locus in linkage disequilibrium with the *HLA-B* alleles account for the observed effects of HLA-B matching on fetal-loss rates among the Hutterites. In this population, matching for alleles at *HLA-B* flanking loci (*HLA-C, TNFa,* and *C4*) also was associated with increased loss rates ($P = .033$, .078, and .043, respectively), which is consistent with an as-yet-unknown locus that is in linkage disequilibrium with *HLA-B* being the primary susceptibility locus. On the other hand, this study considered only serologically defined HLA-B antigens, which are heterogeneous at the molecular level (So 1994). As a result, Hutterite spouses who match for the entire haplotype will match for the same *HLA-B*–locus allele, but spouses matching for serologically defined HLA-B antigens on different haplotypes may not match for the same allele, at the molecular level. This would result in an underestimate of the effects of HLA-B matching on fetal loss. Regardless, these studies demonstrate that HLA matching is a significant risk factor for sporadic fetal loss among the Hutterites.

Maternal-Fetal HLA Compatibility and Autoimmune Disease

Despite the fact that class II antigens are not expressed on fetal cells at the maternal-fetal interface, the production of maternal HLA antibodies directed against paternally derived class II genes (*HLA-DR* and *HLA-DQ*) indicates that fetal class II antigens are recognized by the maternal immune system. Furthermore, although it is likely that sensitization to fetal HLA occurs most commonly at parturition, when fetal blood spills into the maternal circulation, there is evidence to suggest that fetal cells expressing HLA class II antigens are recognized by the mother's system during pregnancy.

The latter hypothesis is supported by a study of 34 pregnant subjects with RA (Nelson et al. 1993). There were significantly more maternal-fetal disparities, with respect to alleles at the *HLA-DRB1, HLA-DQB1,* and *HLA-DQA1* loci, in pregnancies that were characterized by improvement or remission of the RA, compared with pregnancies characterized by active RA (Nelson et al. 1993). This study suggests that, during pregnancy, not only does the maternal immune system recognize fetal class II genes but that the maternal immune response to these antigens differs depending on whether the fetus is compatible or incompatible, with respect to these genes. For example, the recognition of self antigens on fetal cells actually may augment an autoimmune response, whereas recognition of disparate class II antigens on fetal cells may divert the maternal immune response away from an autoimmune response.

The Paradox of the Fetal Allograft

The evolution of viviparity in mammalian pregnancies presented unique challenges to the maternal immune system during pregnancy. Immunological tolerance of an allogeneic fetus had to be accomplished without sacrifice to the antigenic diversity that is critical for the survival of the species. The expression of the minimally polymorphic HLA-G molecule at the maternal-fetal interface may facilitate the induction of local tolerance and may function to inhibit NK-cell activity, perform antigen presentation, and perhaps immunomodulate the maternal T cell populations in the uterus. On the other hand, recognition of the classical, polymorphic HLA in the maternal periphery may elicit additional protective responses during pregnancy and may contribute to the maintenance of genetic diversity at HLA loci.

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