A critical review of the role of the major histocompatibility complex in fertilization, preimplantation development and feto-maternal interactions

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From conception to old age, the major histocompatibility complex (MHC) is at the centre of immune responses that aid survival, fitness and adaptation of mammalian species to the environment. Its main function is that of controlling adaptive immunity, particularly T-cell-mediated immunity towards pathogens. In several species, including humans, the MHC is also able to elicit T-cell-mediated immune responses to allogeneic MHC antigens (non-self MHC antigens expressed by another individual from the same species). Although this phenomenon was originally identified in mice by the somewhat unnatural means of tissue transplantation, it was soon realized that it may also play an important role in the natural state, since the mammalian fetus in the maternal uterus is semi-allogeneic, due to the presence of MHC genes inherited from the father. Thus, during normal pregnancy the maternal immune system undergoes changes that lead to tolerance of the fetus. The MHC can play a dual role in the reproduction process: firstly influencing mating choice in some species, affecting the mother–father MHC matching; and secondly influencing the development of the fertilized ovum during the preimplantation period. In this review we examine the role of the MHC at three distinct levels: (i) MHC expression in gametes and its role in fertilization; (ii) MHC expression in placental tissue; and (iii) MHC expression in embryonic tissue. We suggest that the MHC plays a pleiotropic role, both in fitness (survival and reproductive success) and in development, thereby ensuring the survival of the species in future generations.

Key words: cytokines/embryonic development/gametes/MHC/placenta

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Introduction

In mammals the expression of major histocompatibility complex (MHC) genes is developmentally programmed. However, both their precise transcriptional regulation early in ontogeny, and their spatial and temporal location in the developing pre-implantation embryo are not well understood. This subject is important for understanding the regulation and coordinate expression of functional MHC products from the time of fertilization.

The MHC is connected with the survival of the early embryo as a ‘semi-allograft transplant’ during pregnancy (for a historical review see Brent, 1997). Few data derived from studies of early embryogenesis in humans are available, but
there is a significant body of data derived from the mouse. Over the years, considerable evidence has accumulated suggesting that pre- and postimplantation expression of MHC class I in mouse embryos is associated with reproductive performance (Jaffe et al., 1992; Kurpisz and Fernandez, 1992; Sprinks et al., 1993; Fernandez et al., 1995; Jin et al., 1995; Gill, 1996, 1997).

The MHC genes are located on chromosome 6 in humans and chromosome 17 in mice. Three major sub-regions (class I, class II and class III) can be identified according to their differing structure and function. The human complex, called HLA (human leukocyte antigen), consisting of some 20 class I and some 20 class II loci, is the most polymorphic gene system yet identified. Furthermore, the number of known true, so-called ‘classical’, alleles at the class I and class II loci is steadily increasing as additional ethnic populations are investigated.

The ‘classical’ MHC class Ia molecules are encoded by the HLA-A, -B and -C genes and are ubiquitously expressed on the surfaces of adult somatic cells. In addition to these, at least three so-called ‘non-classical’ class Ib genes, HLA-E, -F and -G, have recently been identified, as well as HLA-H, HLA-J, HLA 70 and HLA 92 class I pseudogene sequences. Together, these genes occupy ~1800 kilobases (kb), although the structure of this region is still not fully known (Trowsdale, 1995).

In contrast, the polymorphic MHC class II molecules, encoded by the HLA-DR, DP and DQ loci in humans, show a restricted tissue distribution and are mainly expressed on the surfaces of B lymphocytes, antigen-presenting cells and some cells involved in the control of the immune responses. Numerous other genes have also been identified within the HLA region, some of which encode necessary elements for the effective function of the MHC in antigen presentation, including the proteasome-like molecules LMP-7 and LMP-2 (which cleave foreign peptides into short fragments) and the two transporter molecules associated with antigen processing, known as TAP1 and TAP2. Other genes present encode molecules with unrelated functions, such as tumour necrosis factor (TNFα), steroid 21-hydroxylase and heat shock protein.

With the exception of only a few species (including the South American Tamarin monkey and the Cheetah) the genes of the MHC are polymorphic, are inherited en bloc and are co-dominantly expressed. There is no evidence for either preferential gene sorting or genomic imprinting in the MHC. Thus, in the offspring of an outbred population, such as humans, one set of MHC genes is inherited from the mother and one from the father, generally conferring heterozygosity and continued diversity to the young. In the mouse the majority of non-classical class Ib genes (more than 40) are located in the Q, T and M regions (Elliott et al., 1989; Klein and Horejsi, 1997). They are expressed at lower levels than class Ia, often in a limited range of tissues, and do not exhibit the extensive polymorphism that makes class Ia genes unique within the genome (Klein, 1986). Biochemical and serological studies have demonstrated that at least five distinct class Ib gene products exist in the mouse, designated as Qa-1, Qa-2, Q10, Tla and H-2M (Flaherty, 1976; Wang, et al., 1991; Fisher-Lindahl, 1993). The developmental and tissue-specific expression of the mouse Q5k gene has been reported, with a possible role in early mouse development (Schwemmle et al., 1991; Reyes-Engel et al., 1993). In addition, Qa-2 is thought to correspond to the PED (preimplantation embryonic development) gene product which influences the rate of preimplantation development (Goldbard et al., 1982; Goldbard and Warner, 1982).

In human adult somatic cells, cell-surface expression of a functional MHC class I complex requires co-expression of several molecules, including the MHC class I heavy chain, β2-microglobulin (β2-m), and the peptide transporter molecules TAP1 and TAP2 (Livingstone et al., 1989; Powis et al., 1991, 1992; Germain, 1994). These molecules are essential for peptide loading, enabling class I assembly, stabilization, and transport from the endoplasmic reticulum (ER) to the Golgi apparatus, and thence to the cell membrane, where cell-surface MHC class I loaded with antigenic peptides is presented to circulating T cells (Neefjes and Mombourg, 1993).

Little is understood of the molecular ontogeny of immunological receptors, including the expression of MHC in fetal development. In previous studies we have shown that MHC class I mRNA encoding the heavy chain is present in the mouse zygote from the late 1-cell stage (Sprinks et al., 1993). This expression involves zygotic-derived gene transcription, since mRNA of the paternal origin allele was detected in crosses between mice of differing H-2 haplotypes. The requirement for MHC class I expression in the correct quantitative levels early in development is emphasized by experiments reported by Jaffe et al. (1992), who have shown that mouse embryos over-expressing the class I product H-2Dα fail to develop beyond mid-gestation, whereas control embryos which over-expressed an irrelevant protein developed normally; and by AitAzzouzene et al., 1998, who showed that transgenic mice over-expressing H-2Kb also died at the same stage of embryogenesis. Recently, an impaired breeding phenotype was reported in transgenic mice devoid of β2-m (Fernandez et al., 1996). These observations support the notion that a homeostatic balance of MHC class I transcription is required for optimal embryonic development (Fernandez et al., 1994).

An alternative approach to the study of mice over-expressing MHC is to examine reproductive fitness in MHC-deficient individuals. The MHC is ubiquitous among most mammalian species studied; and among primates, including humans, no individual has been found that lacks the MHC genes (Klein and Horejsi, 1997). In humans the only examples of MHC antigen-negative individuals are patients with bare lymphocyte syndrome, a severe combined immunodeficiency; they do not survive beyond birth (Touraine et al., 1992).
Experiments with animal models (mainly mice) have provided some insight into the role of the MHC in reproduction. It is now possible to apply the technique of targeted gene disruption to selectively confer a negative phenotype to an individual. This technique has been used to study the role of individual MHC genes in the immune system (Grusby and Glimcher, 1995). A large number of transgenic mice, either MHC class I or class II deficient, are now available. Mating of these two types of transgenic mice has yielded animals deficient for both class I and class II. If the MHC were to play a crucial role in reproductive fitness, one would expect the MHC class I and class II deficient mice to be infertile. On the other hand, if the mice were fertile and the MHC plays a role in embryonic development, then one might expect a high degree of fetal resorption or malformation. Although we must await systematic studies on the reproductive capacity of totally MHC-negative mice, partial answers to these question have already been provided by data demonstrating subfertility in mice deficient for MHC class I genes (see below).

In a recent longitudinal study, we have reported that mice lacking the β2-m gene, and thus having a deficit in cell surface MHC class I antigen, display an impaired breeding phenotype with respect to mating and the rearing of pups (Fernandez et al., 1996). Overall these data indicate that, although the β2-m-negative mice appear to reproduce, they are clearly subfertile: a finding consistent with the notion that MHC class I molecules have developmental roles additional to their function in antigen presentation in the adult immune system. It is, however, not clear yet at what specific level the β2-m gene deletion and the MHC class I deficiency could affect the behavioural aspects of reproduction in mice.

Physiology of fertilization and early pregnancy

Gamete precursors in both sexes undergo both meiosis and cellular maturation to complete development. In the male, meiosis (spermatogenesis) and cellular maturation (spermiogenesis) occur in two distinct but continuous stages. Spermatozoa become mature in the male reproductive tract, but they must undergo capacitation in the female reproductive tract to acquire the properties required to fertilize the oocyte.

Unlike sperm maturation, the development of the oocyte is a prolonged and discontinuous process in which oocytes are arrested in prophase of meiosis. Oocyte maturation occurs as individual follicles develop, become dominant and ovulate (Johnson and Everitt, 1995).

Immune regulation during mammalian pregnancy is complex. The earliest contact with foreign antigens in the reproductive process is at sexual intercourse, when spermatozoa, bearing individual-specific MHC antigens and male-specific antigens, are deposited in the female tract. Sensitization rarely occurs, probably because few spermatozoa enter the female tissues to present an immunogenic stimulus. However, this could be a rare cause of infertility. Contact at the vitelline membrane between oocyte and spermatozoa stimulates a complex series of membrane interactions (Almeida et al., 1995; Myles and Primakoff, 1997) primarily involving integrin/disintegrin binding, but other cell surface glycoproteins, including MHC products, may influence sperm-oocyte recognition.

Mammalian reproduction involves a prolonged period of protection to the embryo and fetus, maximized by retaining the fetus within the maternal uterus, often referred to as one of the ‘immunoprivileged sites’ of the body. It is widely accepted that during pregnancy, the mother undergoes profound changes in her immuno-endocrine system, which must be both compatible with maternal well-being and provide the support essential to the developing fetus. Mother and fetus must tolerate one another immunologically and, in addition, both must be able to cope with infection. Clearly this is not an easy balance to achieve since the fetus has MHC antigens, encoded by paternally derived MHC genes, that could be recognized by the maternal immune system. Potentially the mother will see these paternally derived antigens as non-self and mount an immune response to eliminate the fetus. Thus the fetus is effectively an allograft within its mother’s uterus.

Among the changes in maternal physiology that occur during early pregnancy, some of the best understood are hormonal. In humans, implantation of the fertilized blastocyst occurs 6 days after ovulation, and from about this time the luteotrophic hormone human chorionic gonadotrophin (HCG) is produced. HCG binds to LH receptors in the ovaries, stimulating the continued activity of the corpus luteum and elevated progesterone levels, which favour embryo survival. As pregnancy proceeds, the placenta develops and produces high levels of the steroid hormones progesterone and oestrogens, and of the somatomammotrophins (Johnson and Everitt, 1995). Endocrine and immune interactions influencing thymic function and involution during pregnancy have been observed (Clarke and Kendall, 1994). However, the precise molecular connections between maternal hormones and MHC expression are not yet known. It now clear that cytokines, sometimes referred to as ‘the hormones of the immune system’, play a key role in maintaining a maternal environment that is conducive to successful development of the fetus. This is discussed in the next section.

Cytokines and regulation of MHC and immune responses during pregnancy

Unlike the endocrine changes, immunological changes during reproduction are not completely elucidated. It has been postulated that local interactions between cytokines produced by maternal and fetal cells play an important role in the establishment and maintenance of the fetoplacental unit (Wegmann et al., 1993; Hunt and Roby, 1995; Guilbert, 1996; Olah et al., 1996; Vince and Johnson, 1996; Chaouat et al., 1997; DeMo-
raes-Pinto et al., 1997; Simon et al., 1998). These interactions, which affect the normal balance of the maternal immune system and associated cytokines, can stimulate placental growth; but also may regulate the invasion of fetal trophoblast cells within the maternal endometrium. Certain cytokines, in particular interferon gamma (IFN-γ) and TNFα, commonly associated with a Th1 T-cell-mediated immune response, may be classified as pro-inflammatory Th1-type cytokines, although these cytokines are not solely produced by Th1 cells. Others, in particular interleukin (IL)-10, IL-4, IL-5 and IL-13, are classified as anti-inflammatory Th2-type cytokines. In humans this distinction is not as clear as in mice, but there is evidence that during pregnancy there are local changes in the pattern of cytokine expression, inducing a bias away from potentially harmful pro-inflammatory Th1-type cytokines (Wegmann et al., 1993; Guilbert, 1996). Both maternal and fetal cells are involved in cytokine secretion. On the maternal side are numerous macrophages, large granular lymphocytes (LGL), decidual and stromal cells, as well as rare T cells infiltrating the placenta and decidua (Sharkey, 1995; Guilbert, 1996; Robertson et al., 1998). In the fetus there are macrophages, or Hofbauer cells, and trophoblast cells (Sharkey, 1995; Guilbert, 1996; Robertson et al., 1998). Transient, local dominance of the Th2-type pattern would protect the fetal allograft against both adaptive cell-mediated immune attack and the non-specific, inflammatory and phagocytic response.

Pregnancy failure may be explained by several cellular immune-related mechanisms following changes to the normal pregnancy-associated ratio of the two cytokine groupings, especially locally at the maternal-fetal interface. For example, an abnormal cellular immune response involving the Th1-type cytokines, IFNγ and TNFα, has been proposed to account for several reproductive failure (Hill et al., 1992; Ecker et al., 1993; Yamada et al., 1994). It is suggested that the conceptus may be a target of a local cell-mediated immune response, culminating in abortion. In affected women, trophoblast, sperm, microbial or other antigens may activate maternal immune and inflammatory cells (lymphocytes and macrophages), to produce a cellular immune response, mediated by IFN-γ and TNFα. These cytokines have been shown to inhibit in-vitro embryo development and trophoblast growth and function (Hill et al., 1987; Berkowitz et al., 1988; Haimovici et al., 1991). In addition, IFNγ can induce or increase the expression of MHC class I antigens on choriocarcinoma, embryonal carcinoma and trophoblast cells, all of which normally produce little or no MHC class I. This is brought about by influencing MHC gene expression (Anderson and Berkowitz, 1985; Feinman et al., 1987; Bikoff et al., 1991), and by inducing synthesis of TAP proteins (Rodriguez et al., 1997). Such increased MHC antigen expression may well make the trophoblast a target for a maternal cytotoxic immune response. Between 50 and 80% of women with unexplained recurrent spontaneous abortion (RSA) have evidence of an abnormal Th1 cellular immune response to trophoblast antigens, while <3% of women with normal reproductive histories have cellular immunity to these same trophoblast antigens (Mallmann et al., 1991; Yamada et al., 1994). Recently, Piccinni et al. have shown that production of Th2-type cytokines LIF, IL-4 and IL-10 by decidual T cells is reduced in women with unexplained recurrent abortions (Piccinni et al., 1998). Dealtry et al. have found, during the first trimester of pregnancy, production of the Th2-type cytokine IL-13 by placental chorionic villous cytrophoblast and syncytiotrophoblast cells that lie closely associated with IL-13 receptor-bearing maternal macrophages and fetal Hofbauer cells (Dealtry et al., 1998). Taken together these findings suggest that, normally, local Th2-type cytokines, IL-4, IL-10 and IL-13 may act upon cells of the macrophage/monocyte and lymphocyte lineages to limit monocyte-regulated inflammatory responses and Th1-dependent immune responses within the uterus, thus supporting survival of the fetus.

Functional data are given in the mouse by the observations that placentae from the spontaneous resorption-prone mouse mating CBA×DBA/2 produce less IL-4 and IL-10 than the non-resorption-prone CBA×BALB/c mating (Chauvat et al., 1990), and that injection of IL-10 can protect against fetal resorption (Chauvat et al., 1995). However, there are relatively few data relating to Th2-type cytokine expression in humans. Progesterone promotes the expression of Th2-type cytokines by human T cell lines and clones (Piccinni et al., 1995), consistent with the hypothesis that an altered Th1/Th2 balance is associated with pregnancy. Local regulation of the Th2-type expression pattern within the decidua may well involve prostaglandins such as prostaglandin E2 (PGE2), which exerts a powerful influence by switching monocyte cytokine production (Kraan et al., 1995), and moreover, could directly or indirectly induce anergy in uterine T cells (Kelly and Critchley, 1998). Other reports have established that PGE2 not only stimulates IL-10, but is also a potent inhibitor of IL-12 (Kraan et al., 1995), effecting a change in the ratio of these cytokines that is probably the most powerful impetus towards a Th2-type cytokine pattern (Mosmann and Moore, 1991). The first-trimester villous trophoblast is the major source of PGE2 immunomodulator, and maternal macrophages are the major sources of IL-10, therefore both trophoblast and maternal macrophages could contribute to a local cytokine environment in which Th1 T-cell responses responses and inflammatory responses would be severely curtailed in normal successful pregnancy. This would complement the pattern of MHC expression described in the subsequent sections of this review, enabling the fetus to survive in normal pregnancies. In failed pregnancies, however, changes to the cytokine pattern are likely to be significant; stimulating increased MHC expression in the feto-placental unit, and increased local recruitment and activation of inflammatory and cytotoxic immune cells.
Table I. Current state of knowledge on the expression of human leukocyte antigen (HLA) class Ia (classical major histocompatibility complex-A, -B, -C) and HLA class Ib (non-classical HLA-E, -F, -G) on human sperm cell precursors (spermatogenic cells and spermatids), and human mature gametes (spermatozoa and ova)

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<th>Sperm cell precursors</th>
<th>Mature spermatozoa</th>
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<td><strong>HLA-E (+)</strong></td>
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<td><strong>HLA-F (+)</strong></td>
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<td><strong>HLA-G (–)</strong></td>
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Symbols in parentheses indicate presence (+) or absence (–) of HLA class I. NDA: no data available.

MHC gene expression in male gametes

Human MHC expression in both immature and mature sperm cells has been recently reviewed (Hutter and Dohr, 1998). As more refined molecular techniques are developed a detailed molecular pattern is emerging of this controversial subject.

An important question is whether haploid MHC gene expression is possible in human gametes; and if so, would this expression occur at discrete stages of gamete differentiation, or only at the terminal stage of differentiation? The results from early studies were contradictory (Table I) with many investigators reporting the presence of MHC products on human ejaculate sperm (Fellous and Daussel, 1970; Halim et al., 1974; Halim and Festenstein, 1975; Obashi et al., 1990; Chiang et al., 1994; Martin-Villa et al., 1996), and many others reporting its absence (Kamata et al., 1976; Law and Bodmer, 1978; Brodsky et al., 1979; Anderson et al., 1982; Schaller et al., 1993; Patankar et al., 1997).
In contrast, studies utilizing sperm cell precursors or germ cells, rather than mature sperm, have now shown that HLA class Ia products are expressed prior to sperm maturation (Kurpisz et al., 1986; Kurpisz et al., 1987; Janitz et al., 1993; Janitz et al., 1994; Guillaudeux et al., 1996). Moreover, the HLA class Ib products HLA-E and HLA-F have recently been reported to be synthesized on sperm cell precursors (Guillaudeux et al., 1996; Fiszer et al., 1997). Chiang et al. reported expression of HLA-G on mature sperm cells (Chiang et al., 1994) whereas Fiszer et al. reported lack of expression of HLA-G on sperm cell precursors, and lack of HLA-E on mature spermatozoa (Fiszer et al., 1997). These data suggest that, at least for certain class Ib genes, there is a developmentally regulated pattern of expression during spermatogenesis and sperm cell maturation.

The expression on sperm cells of HLA-DR, -DQ and -DP, the major HLA class II subsets, has not been investigated as thoroughly as HLA class I (Table II). The earliest studies reported expression of HLA-DR on mature sperm (Fellous and Dausset, 1970; Barbieri et al., 1990; Obashi et al., 1990; Scofield et al., 1992) whereas Fiszer et al. reported lack of expression of HLA-G on sperm cell precursors, and lack of HLA-E on mature spermatozoa (Fiszer et al., 1997). These data suggest that, at least for certain class Ib genes, there is a developmentally regulated pattern of expression during spermatogenesis and sperm cell maturation.

In conclusion, current data suggest that some degree of developmentally regulated MHC gene expression occurs in male gametes, in particular MHC class I in immature spermatozoa. This argues for a possible role of the MHC in spermatogenesis, perhaps as signal molecules for differentiation.

Hormonal regulation of MHC expression (specifically by sex steroids) has been postulated in females (Clarke and Kendall, 1994), therefore similar regulation may occur in males. In a recent study by Martin-Villa et al. (1996), it was demonstrated that expression of HLA class II molecules on diploid spermatozoa displays a cyclic inverse correlation with inhibin levels in normal human adults. In order to place these findings in a wider context, this work would need to be expanded, ideally in a multicentre World Health Organization study.

In conclusion, current data suggest that some degree of developmentally regulated MHC gene expression occurs in male gametes, in particular MHC class I in immature spermatozoa. This argues for a possible role of the MHC in spermatogenesis, perhaps as signal molecules for differentiation.

### Expression of the MHC in female gametes

There have been few studies addressing the expression of MHC in female gametes and, as with male gametes, the data are rather inconclusive (Tables I and II). Recently, using reverse transcription–polymerase chain reaction (RT–PCR) on human unfertilized oocytes obtained from patients undergoing in-vitro fertilization, Jurisicova et al. detected HLA-G heavy chain mRNA, but not in control cumulus oophorus cells (Jurisicova et al., 1996). Most investigators have not detected classical MHC class I or class II products on the surface of human secondary oocytes (Dohr et al., 1987; Roberts et al., 1992; Fenichel et al., 1995; Patankar et al., 1997).

Previous reports of MHC expression on mouse gametes have also been contradictory (Table III), with some workers...
reporting the presence of MHC class Ia and class II proteins on mature spermatozoa and oocytes (Johnson and Edidin, 1972; Heyner and Hunziker, 1979; Wu et al., 1990), and others reporting their absence (Harding and Wellhausen, 1992). Recently developed RT–PCR-based assays (see next section) have now indicated that class Ib mRNA are present in secondary oocytes, but that class Ia mRNA are absent from both secondary oocytes and post-capacitation spermatozoa (Sprinks et al., 1993; Cooper et al., 1998). So what could be the role of MHC expression on gametes? Current data suggest that some degree of MHC gene expression occurs in the gametes, perhaps as signal molecules. In the light of the most recent data, HLA-E may be a key molecule in the inhibition of natural killer (NK) cell activity, together with peptides of HLA-G origin (Borrego et al., 1998; Braud et al., 1998; Weiss et al., 1998). Furthermore, it can be speculated that after HLA-E translocation to the cell surface, for example in infection, HLA-E may serve as a co-chaperone protein helping to present a peptide to class I-positive cells. This phenomenon may occur in the peri-basal compartment of the human testis. A pre-condition for this mechanism to operate would be a broken blood–testis barrier.

The lack of, or greatly diminished, expression of MHC on mature capacitated spermatozoa suggests that sperm cell ability to fertilize does not depend on either intracellular or cell surface expression of MHC.

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<th>Mature spermatozoa</th>
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<th>MHC class Ib (+)</th>
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<th>MHC class II (+)</th>
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Symbols in parentheses indicate presence (+) or absence (−) of protein products. NDA: no data available.

**MHC expression in placental tissue and the feto-maternal relationship**

The potential role of cytokines in the feto-maternal relationship has already been discussed. However, the survival of the fetal allograft is also aided by other means; such as the lack of significant, immunologically active MHC antigen expression on the surface of cells at the interface with the maternal tissues; masking of T-cell-activating components; and modification of the local immune response (for review see Clark, 1995). Current knowledge of the expression of MHC on placental tissues is summarized in Table IV. MHC masking will be discussed first. The fetus is likely to be protected by the presence of a physical barrier, as originally argued by Medawar (Medawar, 1953). This barrier is negatively charged and rich in sialic acid and mucopolysaccharides. In addition, early in pregnancy the hormones HCG and human placental lactogen (HPL) may act as constituents of the surface layer of glycoproteins, which could protect syncytiotrophoblast cells from immunological attack by the mother. It has also been argued that low levels of paternal antigens may locally tolerate the mother to such antigens by eliciting the formation of maternal blocking antibodies. Local immunosuppression may also occur due to hormones (chorionic gonadotrophins, oestrogen), and most likely involves the removal of antifetal antibody reactivity by trophoblast and placental monocytes. A 10 year prospective study (Ober et al., 1998) of HLA matching and pregnancy outcome in 111 Hutterite couples showed significantly increased fetal loss rates among couples matching for an entire 16-locus haplotype, and for HLA-B and HLA-C. This suggests that insufficient exposure of the mother to paternal ‘foreign’ antigens confers a risk for fetal loss.

The lack of immune activation at the feto-maternal interface could result from several factors. For example, MHC class I and class II antigens are very weakly distributed or absent from normal syncytiotrophoblast (Billington, 1988), except for a truncated form of HLA class Ib on cytotrophoblast cells (Ellis and McMichael, 1990; Kovats et al., 1990); although HLA-C and HLA-C-like mRNA and protein have been demonstrated in first trimester trophoblast (King et al., 1996b). In addition, human trophoblast populations in the chorion laeve and invasive cytotrophoblasts in the placental bed express the unusual class I molecules HLA-E and HLA-G, which could be involved in suppression of maternal responses. Human trophoblasts are resistant to NK cell attack, possibly as a consequence of the presence of HLA-G, which ensures that NK cells detect the trophoblast as normal self. In recent years it has been shown that NK inhibition by HLA-G via the NKTA3 receptor may contribute to the survival of the fetal semi-allograft in the mother during pregnancy (King et al., 1996a; Munz et al., 1997; Rouas Freiss et al., 1997; Le Bouteiller and Lenfant 1997; Verma et al., 1997). HLA-E has also been implicated in interactions with NK cells (Weiss et al., 1998). The expression patterns and possible functions of these non-classical class I MHC molecules are discussed more fully in the section concerning the role of MHC class Ib in reproduction and development.

During implantation of the embryo and at later stages, MHC class Ia molecules disappear from cells which are in immediate contact with the maternal tissue, leaving only low levels of MHC class Ib expression. Current knowledge on the expression of MHC (HLA) class I and class II on blastocysts and preimplantation embryos is summarized in Table V. It is possible, that expression of certain MHC products is advantageous for the survival of the early embryo. It has also been demonstrated that many embryonic cell lines, such as F9,
exhibit defective class I assembly, which can only be rescued by the addition of exogenous antigenic peptide, or by treatment with IFNγ (Bikoff et al., 1991). Further study has revealed that TAP1 mRNA is not normally transcribed in these cells, but can also be induced with IFNγ (Bikoff et al., 1991). Rodriguez et al. (1997) also showed that IFNγ can rescue cell surface expression of HLA class Ia in villous trophoblast by inducing the synthesis of TAP proteins. It has become clear that β2-m deficient mice express low levels of MHC class I molecules (Glas et al., 1994) which might be sufficient for NK protection and successful preimplantation growth. Moreover, Carbone et al. (1993) have demonstrated that expression of free heavy chains at the cell surface correlates with protection against NK killing. The implications of these findings for embryonic development may be considerable and suggest that selective expression of MHC products takes place during development and at the feto-maternal interface. Such expression is potentially subject to modulation, by a fluctuating pattern of Th1-type and Th2-type cytokines, maternal hormones and other cellular interactions.

Unexplained human reproductive failure is quite common and has often been associated with MHC products (Christiansen et al., 1997). However, non-MHC-related mechanisms, such as Th1-type cytokine-mediated immunity, may be involved in such reproductive failure by changing the normal pattern of MHC expression. The Th1-type cytokine, IFN-γ, has been shown to induce MHC antigen expression on placental tissues derived from human trophoblast and human trophoblast cell lines in vitro (Anderson and Berkowitz, 1985; Feinman et al., 1987; Rodriguez et al., 1997). If MHC determinants were to be expressed in vivo, then cytotoxic T-cell attack could occur, resulting in spontaneous abortion. Human teratocarcinoma cell lines frequently express HLA-A, -B and -C antigens, but the degree of expression often varies within the cell population (Andrews and Damjanov, 1985). Quantitative analysis of class I MHC transcripts has discriminated between different levels of class I RNA in choriocarcinoma and embryonal carcinoma cell lines.

### Table IV. Expression of human human leukocyte antigen (HLA) class I and class II on placental tissues

<table>
<thead>
<tr>
<th>Class</th>
<th>Trophoblast cells</th>
<th>Chorion laeve</th>
<th>Amnion cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>King et al. (1997)</td>
<td>Hutter et al. (1997)</td>
<td></td>
</tr>
<tr>
<td>β2-microglobulin (+)</td>
<td>Hutter et al. (1996)</td>
<td>NDA</td>
<td>NDA</td>
</tr>
<tr>
<td>HLA class II (-)</td>
<td>Billington (1988)</td>
<td>NDA</td>
<td>NDA</td>
</tr>
</tbody>
</table>

Symbols in parentheses indicate presence (+) or absence (−) of mRNA and/or protein products. NDA: no data available.
Table V. Expression of human leukocyte antigen (HLA) class I and class II on blastocysts and preimplantation embryos

<table>
<thead>
<tr>
<th>HLA class I (−)</th>
<th>Roberts et al. (1992)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-G (+)</td>
<td>Jurisicova et al. (1996)</td>
</tr>
<tr>
<td>β2-m (+)</td>
<td>Jurisicova et al. (1996)</td>
</tr>
<tr>
<td>HLA class II</td>
<td>NDA</td>
</tr>
</tbody>
</table>

Symbols in parentheses indicate presence (+) or absence (−) of mRNA and/or protein products. NDA: no data available.

In 1982 Goldbard et al. put forward the hypothesis that the time period from fertilization to the first cleavage division and the duration of subsequent cleavage divisions until the blastocyst stage in mouse embryos is under genetic control that may be related to the class Ib gene product, Qa-2 (Goldbard et al., 1982); in which case MHC class I products would have to be functional from the 1-cell stage of development. Unfortunately, limitations of sensitivity in the assay systems available at the time hampered efforts to prove this; and a second major drawback was that the assays were only capable of detecting translated MHC gene products that had assembled and reached the cell surface. In contrast, study of class I gene expression at the mRNA level allows the detection of untranslated and low copy number gene products, potentially providing more information about the regulation of class I gene expression, and its relationship with the control of other associated genes, such as those for β2-m, TAP1 and TAP2, and the low molecular weight proteasome polypeptides, LMP2 and LMP7. However, because a single oocyte or preimplantation mouse embryo contains no more than 50 pg of poly(A)+ mRNA (Clegg and Piko, 1983), and because many mRNA are found in low abundance, the numbers of mouse oocytes and embryos which would be required for the analysis of class I mRNA using conventional techniques such as Northern Blotting are prohibitive.

In 1993 we developed a modified RNA extraction and RT–PCR assay system to determine the time at which class I mRNA are first expressed in preimplantation development, and to study the transcription of paternally derived class I MHC genes in gametes and preimplantation mouse embryos (Sprinks et al., 1993). RT–PCR is, to date, the most powerful assay system for the analysis of gene transcription, involving the use of oligonucleotide primers to specifically and rapidly amplify a target cDNA sequence.

We used oligonucleotide primers specific for two mouse MHC class Ia alleles, H-2Dβ and H-2Dβ, to study the expression of class I transcripts in homozygous or heterozygous one-cell zygotes, 2-cell, 8-cell and blastocyst stage embryos produced by within- and between-strain crosses of congenic H-2b and H-2k mice, differing only at their MHC loci (Sprinks, 1994). Embryo lysates were probed with primer pairs specific either for message from the maternally derived class I H-2D allele, or from its paternally derived counterpart. Although MHC class I alleles are thought to be co-dominantly expressed, the timing of paternal versus maternal class I gene expression in development has not yet been well defined. These experiments showed that mRNA transcribed from maternally and paternally derived class I genes was present, not only in preimplantation embryos at the 2-cell, 8-cell and blastocyst stages of development, but also in the post-syngamy 1-cell embryo, before the first cleavage division. Furthermore, reciprocal crosses, carried out to see whether the sex of parent from which the class I gene was inherited had any effect upon developmental expression, gave no evidence of any imprinting effects or transcriptional regulation due to parental transmission.

In order to test whether paternal mRNA originating from the spermatozoon was introduced at fertilization, or whether mRNA of maternal origin was already present in the egg cytoplasm, pre-and post-capacitation spermatozoa and oocytes were assayed using the same protocol as for the embryos. The results showed conclusively that mRNA encoded by the class I H-2D gene of either allele was not detectable in either spermatozoa or oocytes, leading to the conclusion that de-novo synthesis of embryonic class I message must occur before the first cleavage division in the preimplantation embryo.

Comparisons of the signal intensities from mRNA transcribed from paternally and maternally derived class I genes in the heterozygous embryos indicated approximately equal expression. It was also evident that there were consistent changes in the overall levels of mRNA present. PCR products from 1-cell, 2-cell and blastocyst stage embryos exhibited increasing band intensities with each cleavage division, however, products from uncompact ed 8-cell embryos were surprisingly low, suggesting that regulation of expression may be occurring at this time (Sprinks, 1994).

In order to place these findings in a developmental context, it is important to consider the roles that other MHC-encoded products may play in development. As well as having a putative role in timing cleavage division, the mouse class Ib antigen, Qa-2, appears to be involved in reproductive success, possibly acting as a signalling molecule to influence the cleavage rate of the preimplantation embryo (Warner et al., 1991). The preimplantation embryo development gene (PED) which influences the rate of cleavage divisions in the embryo, is located in the Q subregion of the mouse MHC, and its product is almost certainly the Qa-2 class I antigen (Tian et al., 1992). Since PED expression is dominant in a heterozygous embryo, even when encoded by paternal genes, this provides further evidence that paternally derived MHC gene products are present in the one-cell embryo, otherwise the PED gene could not influence the timing of the first cleavage division (Goldbard et al., 1982; Warner et al., 1988). However, although this appears to be a logical assumption, it has not yet been formally proven. Furthermore, a human counterpart for the murine PED gene has yet to be found, although progress is being made (Warner et al., 1998).
Further evidence for early transcription of other paternally derived gene products in the zygote has come from Ram and Schultz, who demonstrated the expression of a luciferase reporter gene inserted into the male pronucleus of a 1-cell mouse embryo (Ram and Schultz, 1993). Insertion into the female pronucleus did not result in luciferase activity. Taken together these findings suggest that embryonic gene activation initiates in the 1-cell embryo rather than in the 2-cell embryo. In contrast to the luciferase reporter gene experiments, class I gene expression in mouse embryos did not exhibit the paternal/maternal differences mentioned above, raising the possibility that such differences might be artefacts caused by manipulation of the embryo.

However, it is important to note that the transcription of a class I gene is not always followed by the expression of a functional protein product. This is particularly true for the H-2D\(^b\) heavy chain in mice which, unlike the H-2K\(^b\) heavy chain, has been shown to reach the cell surface with or without β2-m expression, where it resides in a partially unfolded conformation (Allen et al., 1986; Williams et al., 1989). More recently, peptide transporter molecules have been implicated in class I surface association of short antigenic peptides to the heavy chain. Whereas cells with deficient expression of peptide transporter genes can transcribe and translate class I genes normally, they are unable to assemble peptide-class I complexes (Monaco et al., 1990).

In summary, these data show that both paternally and maternally derived class I genes are transcribed throughout the whole of preimplantation development, even before the first cleavage division. Therefore, the regulation of class I antigen expression on the embryonic cell surface is almost certainly under post-transcriptional control.

The early onset of expression of MHC class I classical products in preimplantation mouse embryos has been corroborated by others. Arcellana-Panolilio and Shultz showed that in the CD1 Swiss albino mice, H-2 K\(^b\) products were also expressed at all stages of preimplantation development (Arcellana-Panolilio and Shultz, 1994). Recently, comprehensive studies in mice (Cooper et al., 1998) have also corroborated the above findings, and demonstrated that β2-m mRNA transcripts are found in 2-cell embryos and later stages, but not 1-cell embryos. Furthermore, TAP1 transcripts were only detected in blastocysts, whereas TAP2 mRNA was not found at any pre-implantation embryonic stage. At the protein level, MHC class Ia was not seen on the surface of 1-cell embryos, but was detected on the surface of 2-cell embryos and, more strongly, on 8-cell and blastocyst cell membranes. The class Ib antigen, Qa-2, was detected on the surface of 1-cell embryos, whereas β2-m was clearly present on 8-cell embryos and blastocysts. TAPI protein was detected within the blastomeres of embryos at all stages of development.

Taken together these data suggest that, at least for mice, MHC class I complexes composed of heavy chain and β2-m are present at the surface of embryos at very early stages of development. However, they are unlikely to be functional in the normal sense of antigen presentation, due to a lack of functional peptide transporter. Future studies will focus on the introduction of MHC gene anti-sense oligonucleotides which will base pair with the endogenous MHC mRNA to modulate MHC expression during preimplantation development, and provide even more detailed analyses of MHC expression during the earliest stages of life.

### Role of MHC class Ib (non-classical) in reproduction and development

It is of particular interest that in mouse embryos, Qa-2 has been shown to influence dramatically the timing and rate of preimplantation cleavage division, as well as affecting birthweight and litter size, clearly indicating a developmental role both before and after implantation (Warner et al., 1991; Tian et al., 1992). An additional, novel blastocyst-derived MHC sequence linked to H-2D (possibly a mouse analogue of HLA-G) has recently been identified (Sipes et al., 1996; Landel et al., 1997); suggesting that other non-classical class I genes are expressed at this stage of development. Intriguingly, MHC and PED gene alleles, in particular, appear to play a similar role in determining and influencing longevity, since...
mice with faster cleavage rates have shorter life spans than their slower congenic counterparts (Tarin, 1997).

In humans the situation is considerably harder to study. Not only is the arrangement of the MHC genes quite different from that in the mouse (Trowsdale, 1995), but also the physiological and anatomical characteristics of pregnancy are not the same. MHC class Ib genes and their products (HLA-E, -F, -G) have been identified in human embryos, and many investigators believe that they play a role in reproductive success. HLA-E and -F have been reported to be expressed on human sperm cell precursors, but not HLA-G (Fiszer et al., 1997). HLA-E and HLA-G are strongly expressed by extravillous cytotrophoblast (Johnson et al., 1993) and other trophoblast cells (Kovats et al., 1990). Co-dominant expression of various alternatively spliced HLA-G mRNA has been reported in first trimester trophoblast cells (Hviid et al., 1998), although HLA-G protein isoforms produced by cytotrophoblasts appear to be the result of different levels of glycosylation involving N-acetyllactosamine (McMaster et al., 1998). It has been suggested that the extent of trophoblastic invasion at the time of placentation is controlled by decidual large granular lymphocytes (related to NK cells) interacting with this non-classical antigen (King and Loke, 1991; King et al., 1996b, 1997). HLA-G may therefore participate in feto-maternal immunological recognition events throughout pregnancy by interaction with particular groups of maternal effector cells. A novel primate non-classical class I, Mamu-AG, has recently been identified in the rhesus monkey and may play a similar role to primate non-classical class I antigens (Boyson et al., 1997). However, HLA protein expression and function does not appear essential for failure of HLA-G protein expression to have been reported (Cober et al., 1998).

Conclusions

(i) Neither secondary oocytes nor mature spermatozoa appear to require significant levels of class I antigens on their surfaces for fertilization to take place. Although expression during gamete differentiation and maturation may be important.

(ii) In the mouse, mRNAs encoding MHC class Ia, class Ib and β2-m molecules begin to be synthesized soon after conception. MHC class Ia proteins are present on unfertilized oocytes but not on 1-cell embryos, and are again found on 2-cell embryos and at later stages, together with β2-m. These data suggest that maternally synthesized MHC classical class Ia is lost from the embryo soon after conception, but new class Ia molecules are synthesized at the 2-cell stage. However, antigen presentation by these molecules would seem unlikely due to the absence of functional peptide transporter.

(iii) In contrast, MHC non-classical class Ib proteins are present on secondary oocytes and embryos at all stages of preimplantation development and are possibly involved in the regulation of cell differentiation and cleavage division, since in mice the rate of preimplantation development has been correlated with Qa-2 expression.

(iv) Transgenic mice devoid of β2-m display an impaired breeding phenotype and are subfertile. However, since MHC class Ib proteins are present on the surface of 1-cell and 2-cell stage embryos without β2-m it is likely that a complex of class Ib heavy chain and β2-m molecules may be required to influence the rate of early cleavage division.

(v) During and after implantation, the expression of class Ia and class II antigens on placental tissue is switched off, whilst at the same time, class Ib antigen expression is increased, possibly providing a mechanism whereby both T cell- and NK cell-mediated attack can be avoided.

(vi) Also soon after implantation and until parturition, both placentally and maternally derived cytokines establish and maintain an immunological environment in which Th1-type responses are severely curtailed and the fetus can develop unimpaired.

During pregnancy the developing fetus is protected from immunological attack potentially by both a physical barrier, such as negatively charged, sialic acid-rich mucopolysaccharide, and also an immunologically inert layer of placental trophoblast tissue, on which MHC class I antigens are very weakly distributed and from which MHC class II molecules are absent altogether. In addition, certain human trophoblast cell populations in the chorion laeve and invasive cytotrophoblasts in the placental bed express unusual class I molecules that could be involved in suppression of maternal responses such as those mediated by NK cells, for example HLA-G in humans.

It has also been argued that low levels of paternal MHC antigens may induce local alloageneic tolerance by eliciting the formation of maternal ‘cloaking’ antibodies. Maternal antibody transport through the placenta is selective and most likely involves the removal of antifetal antibodies by the trophoblast and placent monocytes, while local immunosuppression may occur due to hormones (chorionic gonadotrophins, oestrogen) and pregnancy factors produced in the placenta.

Thus, there is overwhelming evidence in support of the view that the MHC is intimately involved in numerous aspects of mammalian reproduction. Many of these are still little understood, although it is now becoming clear that the classical role of the MHC in antigen presentation is not the only one. Future studies will almost certainly focus on the expression and function of the non-classical MHC genes during pregnancy as well as the role of MHC antigens and cytokines in recurrent spontaneous abortion. Current observations support the notion that the MHC is critical for the survival and development of the mammalian fetus.

References


Anderson, D.J. and Berkowitz, R.S. (1985) γ-Interferon enhances expression of class I MHC antigens in the weakly HLA\(^A\) human choriocarcinoma cell line BeWo, but does not induce MHC expression in the HLA- choriocarcinoma cell line Jar. J. Immunol., 135, 2498–2501.


DeMoraesPinto, M.I., Vince, G.S., Flanagan, B.F. et al. (1997) Localization of IL-4 and IL-4 receptors in the human term placenta, decidua and amnionchiorionic membranes. Immunology, 90, 87–94.


Fernandez, N., Sprinks, M.T., Cooper, J.C. et al. (1994) Expression and assembly of MHC class I products in preimplantation mouse embryo development. Immunology, 83 (Suppl. 1), 49.


Ober, C., Aldrich, C., Rosinsky, B. et al. (1998b) HLA-G1 protein expression is not essential for fetal survival. Placenta, 19, 127–132.


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