Major histocompatibility antigen expression on the bovine placenta: its relationship to abnormal pregnancies and retained placenta

C.J. Davies a,*, J.R. Hill b, J.L. Edwards c, F.N. Schrick c, P.J. Fisher d, J.A. Eldridge a, D.H. Schlafer d

a Department of Veterinary Microbiology and Pathology & Center for Reproductive Biology, College of Veterinary Medicine, Washington State University, P.O. Box 647040, Pullman, WA 99164-7040, USA
b CSIRO Livestock Industries, Armidale, NSW 2350, Australia
c Department of Animal Science, Institute of Agriculture, Tennessee Agricultural Experiment Station, The University of Tennessee, Knoxville, TN 37996, USA
d Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

Abstract

In viviparous animals, regulation of expression of major histocompatibility complex (MHC) class I antigens by the trophoblast cells, which constitute the outermost layer of the placenta, seems to be critical for maternal immunological acceptance of an allogeneic fetus. Cattle are unusual in this regard, since the bovine trophoblast cells, in specific regions of the uterine/placental interface, normally express MHC class I antigens during the third trimester of gestation. This expression appears to be biologically relevant as MHC class I compatibility between a cow and her fetus has been associated with an increased incidence of placental retention. We have found significant differences in lymphocyte populations, cytokine production, and trophoblast cell apoptosis in the placentomes of MHC-compatible and -incompatible pregnancies at parturition. This suggests that maternal immunological recognition of fetal MHC class I proteins triggers an immune/inflammatory response that contributes to placental separation at parturition in cattle. Early in pregnancy, a complete shutdown of MHC class I expression by trophoblast cells appears to be critical for normal placental development and fetal survival. In bovine somatic cell nuclear transfer (SCNT) pregnancies, there is an extremely high rate of fetal loss between days 30 and 90 of pregnancy. We have shown that in bovine SCNT pregnancies, between days 34 and 63 of gestation, there is both abnormal expression of MHC class I antigens by trophoblast cells and an abnormal accumulation of lymphocytes within the uterine stroma. Consequently, it is likely that activation of the maternal mucosal immune system, within the uterus at the same time when placentomes are being established, interferes with

* Corresponding author. Tel.: +1 509 335 7106; fax: +1 509 335 8529.
E-mail address: cdavies@vetmed.wsu.edu (C.J. Davies).
the process of placentome development and leads to immune-mediated abortion. Our data suggest that bovine MHC-compatible pregnancies provide a unique model for studying regulation of the uterine immune system, as well as immune-mediated placental rejection.

© 2004 Elsevier B.V. All rights reserved.

**Keywords:** Bovine placenta; Major histocompatibility complex; Reproductive immunology; Retained placenta; Immune-mediated abortion

---

### 1. Introduction

In viviparous animals, an allogeneic fetus must develop within its mother’s uterus. Consequently, maternal immunological acceptance of the fetus is vital for reproductive success. In most mammals, tolerance of the fetal allograft appears to involve both a lack of expression of polymorphic major histocompatibility complex (MHC) antigens on the trophoblast cells that cover the exterior of the placenta as well as the maintenance of an immunologically quiescent or immunosuppressive state of the uterus.

The MHC region got its name because alloantigens encoded by this genetic region are extremely potent at stimulating rejection of transplanted tissues or organs. It is now known that the MHC encodes two types of extremely polymorphic cell surface glycoproteins, the class I and class II proteins, that present peptide antigens to T lymphocytes. The ‘classical’, MHC class I molecules are expressed on most somatic cells and present peptides derived from an animal’s own proteins, or from proteins of intracellular pathogens, to cytotoxic/suppressor T lymphocytes (CTL). In most species that have been studied, trophoblast cells do not express ‘classical’, class I molecules (Hunt et al., 1987; Gogolin-Ewens et al., 1989; Loke, 1989; Donaldson et al., 1990; Low et al., 1990; Kydd et al., 1991). Consequently, lack of MHC class I expression is believed to protect the placenta from attack by the maternal immune system. This hypothesis is substantiated by experiments demonstrating that allografts expressing classical, class I antigens do not survive in the uterus (Beer and Billingham, 1974; Reimers and Dziuk, 1974; Hansen et al., 1986). Pregnancy or pseudopregnancy in rats, or progesterone treatment in sheep, prolonged allograft survival but did not protect allografts from eventual rejection (Beer and Billingham, 1974; Hansen et al., 1986). Additional class I genes encode ‘non-classical’, class I molecules. The ‘non-classical’, class I genes are much less polymorphic and have restricted cellular expression. Furthermore, the products of these genes appear to have distinct functions. An example of a ‘non-classical’, class I gene is HLA-G, encoded in the human leukocyte antigen (HLA) complex. In humans, HLA-G is expressed specifically on invasive, cytotrophoblast cells and may protect these cells from attack by CD4+ T lymphocytes and/or natural killer (NK) cells (Ellis et al., 1986, 1990; Kovats et al., 1990; Le Bouteiller, 2000; Bainbridge et al., 2000, 2001a; Park et al., 2004a). MHC class II molecules are usually expressed only on professional antigen-presenting cells (APC): dendritic cells, macrophages, and B lymphocytes. This pattern of expression is consistent with their function, which is to present peptides from extracellular pathogens or proteins to helper T lymphocytes. Under normal circumstances, MHC class II antigens are not expressed on trophoblast cells.
2. Trophoblast cell MHC class I expression in normal bovine pregnancies

Numerous studies have failed to detect the expression of the highly polymorphic, ‘classical’ MHC class I antigens by trophoblast cells of various species (Hunt et al., 1987; Gogolin-Ewens et al., 1989; Loke, 1989; Donaldson et al., 1990; Kydd et al., 1991). However, in some species, it was found that subpopulations of trophoblast cells expressed monomorphic, ‘non-classical’ MHC class I proteins. An example of this is the expression of HLA-G by human, invasive cytotrophoblast cells (Ellis et al., 1986, 1990; Kovats et al., 1990). Low et al. (1990) reported finding areas of MHC class I expression in the interplacentomal region of three of six bovine placentas, examined by immunohistochemistry. In a subsequent study, we used immunohistochemistry to quantitate MHC class I expression in three regions of the uterine/placental interface: the interplacentomal region, the arcade region where the placenta covers the luminal surface of the placentomes, and the villous/crypt region within the placentomes (Davies et al., 2000). During the fourth month of pregnancy, on an average, only 2% of interplacentomal trophoblast cells expressed class I antigens. However, during the sixth and eighth months of pregnancy, and at parturition, 26–100% of interplacentomal trophoblast cells expressed class I antigens. As pregnancy progressed, class I expression was also upregulated in the arcade region of the placentomes, reaching maximum expression just prior to parturition, when on average, 62% of arcade trophoblast cells expressed class I antigens. We never detected the presence of class I antigens on trophoblast cells of the cotyledonary villi by immunohistochemistry. However, another group has reported detecting both MHC class I mRNA and protein in cotyledonary, binucleate trophoblast cells isolated from placentomes collected on day 275 of pregnancy, which is 4–15 days prior to normal parturition (Ellis et al., 1998; Bainbridge et al., 2001b). The difference between our results and those of other investigators (Ellis et al., 1998; Bainbridge et al., 2001b) probably reflects the difference in the time relative to parturition when examination was carried out, or the fact that these investigators did not distinguish between class I antigen expression by villous and arcade binucleate trophoblast cells.

Placentomes are the areas of interface, providing tight attachments between maternal and fetal membranes, for nutrient exchange. They are formed by branching fetal cotyledonary villi, which grow down into the maternal caruncular crypts in a finger-in-glove arrangement (Schlafer et al., 2000). A novel finding, in our study, on uterine and placental class I antigen expression in cattle was that the endometrial epithelial cells of the maternal crypts lacked detectible class I expression, throughout pregnancy (Davies et al., 2000). We know of no other species in which class I antigen expression by endometrial epithelial cells is shut down.

Bovine binucleate trophoblast cells produce a number of important steroids and proteins including progesterone, placental lactogen, and the pregnancy-associated glycoproteins (Reimers et al., 1985; Duello et al., 1986; Myers and Reimers, 1988; Roberts et al., 1995). The binucleate cells are unique in that they migrate across the interface and fuse with endometrial epithelial cells forming hybrid trinucleate cells (Wooding and Wathes, 1980; Wooding, 1982, 1992). If these trinucleate cells were to express maternal MHC class I antigens, they would almost certainly present fetal peptides. Presentation of fetal peptides by maternal class I glycoproteins could provoke attack by maternal cytotoxic T lymphocytes. Consequently, lack of MHC class I expression by cryptal, endometrial epithelial cells may be an important mechanism for protecting the fetus from immune-mediated rejection.
Bainbridge et al. (2001b) reported that class I mRNA isolated from bovine binucleate cells corresponded to both, classical and non-classical class I proteins. Recently, we profiled the mRNA expression at term in intercotyledonary trophoblast cells, and in fetal and maternal lymphocytes. We found that the trophoblast cells expressed both, classical and non-classical, class I genes and that the percentage of transcripts corresponding to non-classical genes was much higher in trophoblast cells than in lymphocytes (Davies, unpublished). In addition, different non-classical, class I mRNA were isolated from trophoblast cells with distinct MHC genotypes. It is, therefore, likely that the classical class I proteins, and possibly some of the non-classical proteins, expressed by bovine trophoblast cells are capable of stimulating a maternal, anti-placental immune response.

3. MHC class I compatibility and placental retention

Tight attachment of the placenta to the maternal endometrium must be maintained throughout pregnancy. On the other hand, placental attachments must be rapidly broken down at parturition. The importance of placental separation at term in cattle is underscored by the clinical problem of placental retention. The overall rate of placental retention in cattle is approximately 6.6% (Joosten et al., 1987). Established risk factors for placental retention include abortion, stillbirth, twin births, dystocia, induction of parturition, metabolic disorders, or short gestation length (Larson et al., 1985; Joosten et al., 1987). Nevertheless, only about a third of retained placentas are associated with these risk factors. Even in normal pregnancy and delivery, there is a 4.1% incidence of placental retention (Joosten et al., 1987, 1991b). Recent data suggest that in these cases placental retention may be due to failure of immune-mediated rejection of the placenta at parturition.

Gunnink (1984a–d) was the first to suggest that an inflammatory response was involved in normal placental separation. In a series of elegant experiments, this investigator demonstrated that bovine placentomes from cows with normal placental separation contained a chemotactic factor for leukocytes and that this factor was lacking in placentomes from cows with retained placentas. In addition, he demonstrated that blood leukocytes from cows with retained placentas responded poorly to chemotactic stimuli, presumably because they were not activated. A recent study also found that blood neutrophils from cows with retained placenta were in a less activated state than those from cows with normal placental separation (Kimura et al., 2002). Furthermore, this study provided evidence indicating that one of the chemotactic factors in placentomes at parturition is IL-8. Another recent study found that placentomal macrophages from cows that released their placentas normally contained immunoreactive acid phosphatase, but that cows with retained placentas had much lower levels of this lysosomal enzyme (Miyoshi et al., 2002). In a particularly intriguing study, Joosten and coworkers (Joosten et al., 1991a; Joosten and Hensen, 1992) found that placental retention following normal parturition was associated with MHC class I compatibility, or identity, between a dam and her calf. One explanation for this finding is that the presence of classical, class I antigens on trophoblast cells stimulates a beneficial trophic, and/or anti-inflammatory, immune response that is required for normal placental maturation (Wegmann, 1987; Joosten and Hensen, 1992). Alternatively, immunological recognition of trophoblast, class I proteins at parturition could initiate a destructive, but necessary, im-
immune response that results in the breakdown of placentomal attachments and placental release.

Another interesting observation, although not necessarily linked to immune function, is that in pregnancies with normal placental separation, virtually all placentomal binucleate cells degranulate prior to parturition; however, in cattle with retained placentas binucleate cell degranulation is incomplete (Williams et al., 1987; Gross et al., 1991). We have used immunohistochemistry with the SBU-3 monoclonal antibody (mAb) to study the process of binucleate cell degranulation (Lee et al., 1985, 1986a, b, 1990; Morgan et al., 1989; Schlafer et al., 2000). This antibody reacts with one of the pregnancy-associated glycoproteins (Atkinson et al., 1993; Xie et al., 1997). In prepartum samples, collected following the drop in progesterone that occurs less than 24 h before parturition, we found that virtually all SBU-3-positive binucleate cells had fused with endometrial epithelial cells and degranulated (Schlafer et al., 2000).

Based on these findings, we proposed the hypothesis that an immune/inflammatory process triggered by periparturient expression of foreign class I molecules on placentomal trophoblast cells is required for normal placental separation. To test this hypothesis, we conducted a breeding study with the objective of comparing cellular and molecular events at parturition in MHC-compatible and -incompatible pregnancies. Prepartum samples, consisting of intact placentomes, and full-thickness, apposed, interplacentomal uterine and placental tissues, were collected from two groups of five heifers, one carrying MHC-incompatible and the other MHC-compatible pregnancies. The samples were collected during cesarean sections, performed under local anesthesia, within 24 h of normal parturition as determined by a drop in progesterone to <2 ng/ml (Matsas et al., 1992). In addition, postpartum samples, consisting of intact placentomes with caruncular and cotyledonary tissues, were collected per vagina, within half an hour of parturition, from five heifers carrying MHC-incompatible and four heifers carrying MHC-compatible pregnancies. Immunohistochemistry was used to assess the level of trophoblast and endometrial epithelial cell class I expression, the number of binucleate trophoblast cells, the leukocyte populations in the uterus and placenta, and the amount of immunoreactive IL-2 and TNF-α. Apoptosis was assessed both by end-labeling of fragmented DNA with Klenow (FragEL DNA Fragmentation Detection Kit; Calbiochem) and by quantitation of cells with apoptotic bodies in Hoechst 33342-stained sections.

The extent of class I expression by interplacentomal and placentomal arcade trophoblast cells were not significantly different in compatible and incompatible pregnancies. However, compatible pregnancies had significantly greater numbers of SBU-3-positive binucleate cells at term than incompatible pregnancies, both in placentomal villi (Wilcoxon rank sum test $P = 0.037$) and in arcades ($P = 0.005$). In our previous studies, we had detected a greater than 10-fold increase in the number of CD68+ (mAb EMB11; DAKO Corp., Carpinteria, CA) fetal macrophages in the cotyledonary villi between the sixth and the eighth month of pregnancy (Bielefeldt-Ohmann et al., 1988; Ackermann et al., 1994; Schlafer et al., 2000). Fetal macrophage counts in the MHC-compatible and -incompatible pregnancies were not significantly different. However, with the exception of one case, in the maternal, caruncular crypts of cesarean section samples from the incompatible pregnancy group ($n = 4$), there were significantly more macrophages than in the caruncular crypts of the compatible pregnancies ($n = 5$; Wilcoxon rank sum test, $P = 0.028$ for manual counts and $P = 0.014$ for counts done by digital image processing).
We assessed both maternal and fetal lymphocyte populations in the interplacentomal, placentomal arcade, and placentomal villous/crypt regions of the interface. In addition to the MHC-compatible and -incompatible term pregnancies described above, nine pregnancies at earlier stages of gestation, including three each in the fourth, sixth, and eighth month, were also examined. Cryostat sections that included both fetal and maternal tissues were stained using mAb specific for CD2 (CC42, BioSource International, Camarillo, CA), CD3 (MM1A, VMRD, Pullman, WA), CD4 (CC30, BioSource), CD8 (CC63, BioSource), and γδ-TCR (GB21A, VMRD). Although lymphocyte populations in all regions of the uterus changed over the course of pregnancy, significant differences between MHC-compatible and -incompatible pregnancies were restricted to the maternal arcade region. In MHC-incompatible pregnancies, there were significantly fewer arcade cytotoxic/suppressor T lymphocytes at parturition than during gestation (Wilcoxon rank sum test, \( P = 0.001 \) for CD2 and \( P = 0.023 \) for CD8). This decrease in the number of arcade CTL did not occur in MHC-compatible pregnancies. Consequently, the numbers of CTL were significantly different in compatible and incompatible pregnancies (Wilcoxon rank sum test, \( P = 0.008 \) for CD2 and \( P = 0.014 \) for CD8). Presumably, the decline in the arcade CTL population in MHC-incompatible pregnancies was a consequence of recognition of foreign, paternally encoded class I antigens. However, the mechanism underlying this decline is not known. In both mice and horses, pregnancy-associated induction of transient CTL tolerance to paternal MHC antigens has been described (Tafuri et al., 1995; Jiang and Vacchio, 1998; Zhou and Mellor, 1998; Baker et al., 1999). Furthermore, there is evidence for a Fas-mediated clonal deletion of trophoblast-specific CTL in mice (Jiang and Vacchio, 1998). It is therefore possible that uterine inflammation at parturition is initiated by indirect, helper T-lymphocyte recognition of processed MHC class I peptides, presented by MHC class II molecules on maternal antigen-presenting cells in the caruncular crypts, rather than direct recognition of MHC class I antigens by reactivated CTL in the placentomal arcades.

The most exciting findings involved cytokine production and apoptosis. In comparison to incompatible pregnancies, compatible pregnancies had dramatically reduced amounts of immunoreactive IL-2 in endometrial epithelial and trophoblast cells at term (mAb IL-2 14.1, VMRD). In addition, maternal and fetal macrophages in MHC-compatible pregnancies at term stained more intensely with antibodies against TNF-α than macrophages in incompatible pregnancies (mAb 2C4-1D3 and rabbit anti-rBoTNF-α, both gifts from Dr. Ted Elsasser). This may reflect release of immunoreactive TNF-α in MHC-incompatible pregnancies. We also found fragmented DNA, indicative of apoptosis, in virtually all endometrial epithelial and trophoblast cells in prepartum, cesarean section samples from MHC-incompatible but not compatible pregnancies (Klenow FragEL DNA Fragmentation Detection Kit; Calbiochem, Cambridge, MA). To confirm that these cells were undergoing apoptosis, we counted apoptotic bodies in sections stained with the DNA stain Hoechst 33342 (Molecular Probes, Eugene, OR). Apoptotic bodies are generally detected during the later stages of apoptosis. Consequently, the proportion of villous trophoblast cells with apoptotic bodies was significantly greater in samples collected postpartum than those collected at cesarean section or during gestation (Kruskal–Wallis test, \( P < 0.001 \)). However, MHC-compatible and -incompatible pregnancies were not significantly different in the parameters compared.
It is clear that there are significant differences in the events that occur at parturition in MHC class I-compatible and -incompatible pregnancies. Presumably, these differences are due to immunological recognition of class I proteins. However, recognition may involve indirect recognition by helper T lymphocytes rather than direct recognition by CTL. Activation of T helper type 1 (Th1) cells and macrophages, with release of IFN-γ, TNF-α, and other inflammatory mediators, may induce apoptosis of trophoblast cells and endometrial epithelial cells resulting in placental release. There are probably also redundant mechanisms that contribute to placental release. Some of the differences observed between MHC-compatible and -incompatible pregnancies may be due to a delay in molecular and cellular events in compatible pregnancies rather than their complete absence. Nevertheless, MHC compatibility has significant molecular consequences and can result in an increased incidence of retained placentas (Joosten et al., 1991a; Joosten and Hensen, 1992).

4. MHC class I expression and immune-mediated abortion

Bovine pregnancies established by somatic cell nuclear transfer (SCNT), often referred to as cloning, fail at a much higher frequency than those established by in vivo or in vitro fertilization (IVF) (reviewed by Edwards et al., 2003). Much of the pregnancy failure in SCNT pregnancies is associated with abnormal placental development (Stice et al., 1996; Hill et al., 2000). Recently, Hill et al. (2002) reported that trophoblast cells from 34- to 63-day-old SCNT pregnancies expressed MHC class I antigens. In contrast, trophoblast cells from age-matched control pregnancies were completely negative for class I expression. Another striking feature of days 34–63 SCNT pregnancies was the presence of large lymphoid aggregates, and an increased number of scattered CD3+ lymphocytes, in the uterine stroma of the SCNT embryo transfer (ET) recipients (Hill et al., 2002).

Immunohistochemical characterization of uterine lymphocytes in SCNT pregnancies revealed that the majority of lymphocytes in lymphoid aggregates were CD4+ helper T lymphocytes (Fig. 1). The aggregates also contained CD8+ T lymphocytes, B lymphocytes, and a small number of γδ-TCR-positive T cells (Figs. 1 and 2). The large number of CD4+ T lymphocytes in lymphoid aggregates suggests that in SCNT pregnancies the primary mode of immunological response to trophoblast class I antigens may involve indirect recognition. Indirect recognition is a well-established mechanism of graft rejection (Benichou et al., 1998; Game and Lechler, 2002). In cattle, binucleate trophoblast cells migrate across the interface and fuse with endometrial epithelial cells leading to the formation of hybrid giant or trinucleate cells (Wathes and Wooding, 1980; Wooding and Wathes, 1980). The hybrid cells are short lived, and dead cells with pyknotic nuclei have been observed within the endometrium and inside the mononuclear trophoblast cells (Wathes and Wooding, 1980; Wooding and Wathes, 1980). It is likely that uterine macrophages, and/or dendritic cells, also phagocytize debris from dead giant and trinucleate cells and that these cells process and present trophoblast antigens. We do not currently have any data regarding the specificity of the endometrial lymphocytes present in SCNT pregnancies. Demonstration that these cells are specific for fetal MHC class I antigens is an important objective that we would like to pursue in the near future. However, demonstration of indirect recognition of fetal MHC class I proteins by CD4+ helper T lymphocytes would require that the appropriate class I
Fig. 1. Photomicrographs of uterine lymphoid nodules from SCNT pregnancies stained by immunohistochemistry with mAb for lymphocyte subset markers: (A) CD2, mAb CC42; (B) CD4, mAb CC30; (C) CD8, mAb CC63; and (D) γδ TCR, mAb GB21A (CC42, CC30, and CC63 – BioSource International, Camarillo, CA; GB21A – VMRD, Pullman, WA). Antigen-positive cells were visualized using an avidin–biotin amplification kit and the red chromogen 3-amino 9-ethyl carbazole (AEC; Zymed Laboratories, San Francisco, CA); sections were counterstained with hematoxylin. The majority of lymphocytes in the lymphoid nodules were CD4+ helper T lymphocytes. In addition, the nodules contained a moderate number of CD8+ cytotoxic/suppressor T lymphocytes and a few γδ-T cells. Bars represent 100 μm.

proteins be cloned and expressed in vitro, so that purified class I proteins could be used in lymphocyte proliferation or activation assays.

Bovine placentome development begins around day 30 of pregnancy (Schlafer et al., 2000). We have shown that by day 34 of pregnancy SCNT trophoblast cells express class I antigens (Hill et al., 2002). Furthermore, in a recent study that employed microarrays to compare gene expression in individual day 7 blastocysts produced by SCNT and in vitro fertilization, Pfister-Genskow et al. (submitted for publication) found that MHC class I genes were upregulated in SCNT blastocysts, derived from a somatic tissue cell line created from a 58-day-old bovine fetus. The authors used immunofluorescence to confirm that SCNT blastocysts expressed MHC class I antigens, while IVF blastocysts did not. Expression of class I antigens by SCNT blastocysts suggests that class I expression on the placenta results from incomplete reprogramming of somatic cell nuclei by egg cytoplasm. Abnormal class I expression early in pregnancy may result in presentation of fetal class I peptides by uterine antigen-presenting cells just when mature placentation should be developing. Presentation of foreign, fetal class I peptides by MHC class II molecules of maternal, uterine APC would likely induce a massive CD4+ T-lymphocyte response similar to the response seen in SCNT pregnancies (Fig. 1). The response in SCNT pregnancies is probably an inflammatory
T-cell response with the production of mediators such as IFN-γ. To determine if this is the case, we are using real-time RT-PCR to evaluate uterine and placental cytokine mRNA transcription in SCNT and control pregnancies. In theory, inflammatory mediators could upset the normal hormone/cytokine balance, modulate cell surface protein expression, and interfere with placentome development.

If SCNT pregnancies fail as a result of trophoblast cell class I expression, which induces a maternal anti-placental immune response that interferes with placentome development, MHC class I compatible SCNT pregnancies, in which the fetus carries only class I antigens also carried by the ET recipient, should have much better fetal survival rates than MHC-incompatible SCNT pregnancies. It is likely that the greatest improvement would be between days 30 and 90 when placentation is occurring. Although we have not had the opportunity to do a controlled experiment to test the hypothesis that MHC class I compatible SCNT pregnancies have significantly improved fetal survival rates, we have examined the level of MHC class I compatibility in successful and unsuccessful SCNT pregnancies. In SCNT pregnancies produced at the University of Tennessee in 2001 and 2002, we observed a substantial difference in the number of successful pregnancies with different SCNT donor cell lines. Consequently, we used a microarray-based MHC typing system, and genomic MHC class I sequencing, to characterize the MHC haplotypes carried by the donor cell lines and most of the ET recipients (Park et al., 2004b). The objective was to see if there was a difference in MHC compatibility in successful and unsuccessful pregnancies. Because the SCNT donor cell lines were derived from Jersey cows and the ET recipients were either Angus or Angus crosses, none of the pregnancies were completely MHC-compatible. Nevertheless, the data strongly support the hypothesis that MHC compatibility results in improved fetal survival. The two SCNT donor cell lines with substantial numbers of successful pregnancies were both derived from MHC homozygous cows. In contrast, the SCNT
Table 1
Comparison of abortion rates with MHC homozygous and heterozygous SCNT cell lines

<table>
<thead>
<tr>
<th>SCNT cell line</th>
<th>Year</th>
<th>MHC type</th>
<th>Number of embryos transferred</th>
<th>Number of pregnant ET recipients</th>
<th>Day 28&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Day 90&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Day 200&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Term&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC class I homozygous SCNT cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UT3888</td>
<td>2001</td>
<td>AH68/AH68</td>
<td>48</td>
<td></td>
<td>29</td>
<td>21</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>UT3888</td>
<td>2002</td>
<td>AH68/AH68</td>
<td>21</td>
<td></td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>UT4585</td>
<td>2002</td>
<td>AH67/AH67</td>
<td>39</td>
<td></td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>–</td>
<td>–</td>
<td>108</td>
<td></td>
<td>45</td>
<td>34</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>MHC class I heterozygous SCNT cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UT4381</td>
<td>2001</td>
<td>AH12/AH68</td>
<td>48</td>
<td></td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UT4472</td>
<td>2002</td>
<td>AH12/AH68</td>
<td>5</td>
<td></td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>–</td>
<td>–</td>
<td>53</td>
<td></td>
<td>21</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> MHC class I haplotypes (AH) carried by the SCNT donor cell lines were determined by MHC class I microarray typing and genomic MHC class I sequencing.

<sup>b</sup> Day 28 pregnancy rates following transfer of a single SCNT embryo to each ET recipient were not significantly different for the two groups (P = 0.80).

<sup>c</sup> Abortion rates for class I homozygous and heterozygous SCNT cell lines were compared for three time periods: from days 28 to 90, days 28 to 200, and day 28 to term. For all three time periods, abortion rates were significantly different at P < 0.001 (chi-square test).

Donor cell lines with poor fetal survival were from MHC heterozygous cows (Table 1). Furthermore, many of the ET recipients carried MHC haplotypes that were closely related to the haplotypes of the two MHC homozygous cell lines. Day 28 pregnancy rates following transfer of a single SCNT blastocyst to each ET recipient were similar, with 42% for MHC homozygous donor cell lines and 40% for MHC heterozygous donor cell lines (P = 0.80). However, the abortion rates from days 28 to 90, days 28 to 200, and day 28 to term were significantly different with the chi-square test giving a probability of P < 0.001 for the null hypothesis (Table 1). Furthermore, analysis of the microarray class I typing data revealed that the homozygous SCNT cell lines expressed only a few class I peptides that were not expressed by the ET recipients that carried SCNT fetuses beyond day 90 of pregnancy. Consequently, it seems unlikely that the strong association between MHC homozygous SCNT fetuses and improved fetal survival is simply a coincidence.

Results from two lines of investigation in this area may seem to contradict our hypothesis that a substantial portion of the fetal mortality in bovine SCNT pregnancies is due to inappropriate expression of MHC class I antigens by placental trophoblast cells and immune-mediated abortion. Studies conducted with MHC class I transgenic mice have demonstrated that in allogeneic pregnancies, trophoblast class I expression during the second half of pregnancy does not result in increased fetal mortality (Tafuri et al., 1995; Rogers et al., 1998; Shomer et al., 1998; Zhou and Mellor, 1998; Ait-Azzouzene et al., 2001). In mice, instead of increased fetal mortality, trophoblast class I expression during the second half of pregnancy induced both cytotoxic T-cell and B-cell, class I allotype-specific tolerance (Tafuri et al., 1995; Zhou and Mellor, 1998; Ait-Azzouzene et al., 2001). Cattle also routinely tolerate MHC class I expression on trophoblast cells during the third trimester of
pregnancy (see Section 2 above; Davies et al., 2000). The transgenic mice discussed thus far expressed class I proteins on trophoblast cells during the second half of pregnancy. Transgenic mice, with a class I transgene under the control of a housekeeping gene promoter, expressed MHC class I proteins early in pregnancy and were unable to survive beyond midgestation (Ait-Azzouzene et al., 1998). In these transgenic mice, fetal death was not due to immunological rejection, since it occurred in syngeneic as well as allogeneic pregnancies. Although this study demonstrates that in mice MHC class I expression early in embryogenesis can have profound negative effects, it does not provide direct support for our hypothesis. Nonetheless, in both mice and cattle abnormal class I expression early in pregnancy seems to be detrimental, while class I expression later in pregnancy is tolerated. Other studies that need to be considered are those showing that in sheep and rodents allogeneic skin grafts transplanted into the uterus are rejected; however, progesterone administration delays the rejection (Beer andBillingham, 1974; Reimers and Dziuk, 1974; Hansen et al., 1986; Hansen, 1998). In sheep, progesterone has both direct and indirect immunosuppressive effects (Hansen, 1998). During early pregnancy, progesterone stimulates the production of uterine milk protein, which inhibits lymphocyte function. However, the concentration of progesterone within the ovine uterus is probably not high enough to have a direct immunosuppressive effect until after day 50 of pregnancy, when placental progesterone production is sufficient to maintain pregnancy (Hansen, 1998). In cattle, the placenta never produces enough progesterone to maintain pregnancy. Therefore, it is doubtful that the concentration of progesterone in the bovine uterus is ever sufficient to directly inhibit lymphocyte activation. We do not believe that the immunosuppressive effects of uterine milk protein would be sufficient to suppress immunological rejection of SCNT fetuses during the first trimester of bovine pregnancy.

5. Conclusions

Histocompatibility complex class I expression in bovine trophoblast cells is tightly regulated and biologically relevant. Our findings suggest that in cattle, maternal immunological recognition of fetal MHC class I proteins expressed by trophoblast cells triggers an immune/inflammatory response that contributes to placental separation at parturition. This is an intriguing example of adaptation of the immune system for a function distinct from protection against pathogens. It also appears that aberrant MHC class I expression by trophoblast cells of SCNT fetuses early in pregnancy induces immune-mediated abortion. In conclusion, we believe that bovine MHC-compatible pregnancies provide a unique model for studying regulation of the immune system at the uterine/placental interface, as well as both normal and abnormal, immune-mediated placental rejection.

Acknowledgements

Funds for this work were provided by: USDA NRICGP grant 96-35203-3356; a grant from Cyagra Inc., Elizabethtown, PA; USDA CSREES Animal Health Formula Funds provided by the Animal Health Research Center, Washington State University; and funds
from the Robert Fast Food Animal Research Endowment of the College of Veterinary Medicine, Washington State University. Funds for the data generated at The University of Tennessee using somatic cell nuclear transfer were provided by USDA Hatch funds and the State of Tennessee through the Tennessee Agricultural Experiment Station Department of Animal Science. The University of Tennessee Food Safety Center of Excellence, and USDA NRICGP grant 99-35208-8402.

References


