Why is the fetal allograft not rejected?1

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ABSTRACT: In viviparous species, the conceptus must be protected from a potentially hostile maternal immune system. The major histocompatibility complex (MHC) is a genetic region that encodes MHC class I and class II proteins, which present peptide antigens to T lymphocytes and induce graft rejection. The MHC, class II proteins are only expressed on professional, antigen-presenting cells. However, classical, MHC class I proteins are expressed on all nucleated somatic cells. Protection of the conceptus from immune-mediated rejection involves downregulation of classical MHC class I antigen expression on trophoblast cells, which form the external epithelial layer of the placenta, and maintenance of an immunologically favorable immunosuppressive environment in the uterus. Normally, bovine trophoblast cells do not express MHC class I antigens before d 120 of pregnancy. However, during the last third of gestation, trophoblast cells in the interplacentomal and arcade regions of the placenta express classical, MHC class I proteins, which could potentially induce fetal rejection, as well as nonclassical, MHC class I proteins. A human, nonclassical, MHC class I antigen, human leukocyte antigen G, is an important immunoregulatory factor required for the maintenance of pregnancy. In cattle, MHC class I expression during the last third of pregnancy has no adverse effects and probably contributes to placental separation at parturition. However, somatic cell nuclear transfer (SCNT) conceptuses, the majority of which are aborted between d 30 and 90 of pregnancy, had trophoblast cell expression of MHC class I antigens before d 34 of pregnancy. In conjunction with increased trophoblast MHC class I expression, SCNT pregnancies exhibited a marked increase in the number of stromal lymphocytes in the uteri of surrogate dams. A retrospective study found that SCNT pregnancies established using MHC class I-homozygous cell lines, in which the immunological barrier is greatly reduced, had significantly improved fetal survival from d 28 to term (51% survival for MHC-homozygous and 5% for MHC-heterozygous SCNT fetuses). Consequently, it appears that the high rate of fetal mortality in SCNT pregnancies is due, at least in part, to inappropriate expression of trophoblast, MHC class I antigens resulting in immune-mediated placental rejection. This suggests that appropriate regulation of MHC class I genes is critical for immunological acceptance of an allogeneic conceptus.

Key words: abortion, bovine, immunology, major histocompatibility complex, reproduction

INTRODUCTION

In 1953, Peter Medawar presented a seminal lecture at a meeting of the Society for Experimental Biology. During his lecture, 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?” (Medawar, 1953; Billington, 2003). Medawar recognized that, from the perspective of the maternal immune system, a conceptus is a semiallogeneic tissue that should be rejected. Consequently, in viviparous species the conceptus must be protected from the potentially hostile maternal immune system. Many details of how maternal tolerance to the conceptus is established and maintained are still an enigma. Nevertheless, it appears that 3 factors contribute to protection of the conceptus from immune-mediated rejection: (1) anatomical separation of the mother and fetus by isolation of the fetus within the placenta; (2) downregulation of polymorphic major histocompatibility complex (MHC) antigen expression on the tropho-

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blast cells that form the external epithelial layer of the placenta; and (3) maintenance of an immunologically favorable, immunosuppressive environment in the uterus (Raghupathy, 2001; Hunt et al., 2005).

The MHC is a genetic region that encodes 2 types of extremely polymorphic cell-surface proteins, the MHC class I and class II proteins. Before delineation of the function of the MHC proteins, the MHC was identified because alloantigens encoded in this region stimulated acute rejection of transplanted tissues. Major histocompatibility complex class I and class II proteins are assembled from different subunits; however, their overall structure is similar, and they both present peptide antigens to antigen receptors on T lymphocytes.

There are 2 types of MHC class I genes, classical and nonclassical (Kelley et al., 2005). Classical, class I genes are highly polymorphic and encode proteins that are expressed on most somatic cells. The classical, MHC class I proteins present peptides from an animal’s own proteins or from intracellular pathogens to T-cell receptors on CD8+, cytotoxic-suppressor T lymphocytes. In addition, classical, class I proteins serve as ligands for inhibitory receptors on NK cells and other leukocytes.

The nonclassical class I genes are monomorphic or oligomorphic and have restricted cellular expression. The products of the nonclassical, class I genes have diverse functions, but one important function is to act as ligands for inhibitory leukocyte receptors, including receptors encoded in the natural killer complex and the leukocyte receptor complex (Shiroishi et al., 2003; LeMaoult et al., 2004; Parham, 2005). An example of a nonclassical, MHC class I protein that is important in reproduction is human leukocyte antigen G (HLA-G). The HLA-G protein is expressed specifically by invasive cytotrophoblast and modulates immune responses in the human uterus (Hunt et al., 2005).

Major histocompatibility complex, class II proteins are usually only expressed on professional, antigen-presenting cells (i.e., dendritic cells, macrophages, and B lymphocytes) and present peptides derived from extracellular pathogens or proteins to CD4+, helper T lymphocytes. Major histocompatibility complex, class II proteins are never expressed on trophoblast cells. Expression of nonclassical, MHC class I proteins by trophoblast cells is believed to inhibit activation of uterine leukocytes and to protect the conceptus from immune-mediated rejection (Ishitani et al., 2003; Hunt et al., 2005). In contrast, trophoblast expression of the highly polymorphic, classical, class I proteins has the potential to trigger rejection of the fetal-placental allograft, probably through indirect allorecognition, in which fetal MHC class I antigens are internalized by maternal, antigen-presenting cells and presented as bound, MHC class II peptides to CD4+, helper T lymphocytes (Benichou et al., 1998; Game and Lechler, 2002).

The objectives for this article are (1) to review what is known about expression of classical and nonclassical MHC class I genes in the bovine placenta, and (2) to discuss the role of aberrant MHC class I expression in immune-mediated abortion of somatic cell nuclear transfer (SCNT) fetuses.

**TROPHOBLAST EXPRESSION OF MHC CLASS I GENES IN NORMAL BOVINE PREGNANCIES**

In most species, mature trophoblast cells do not express immunoreactive, classical, MHC class I proteins (Hunt et al., 1987; Gogolin-Ewens et al., 1989; Donaldson et al., 1990). However, it is normal for bovine trophoblast cells in the interplacentomal and arcade regions of the placenta to express immunoreactive, membrane-bound, MHC class I proteins during the last third of pregnancy (Davies et al., 2000). In contrast, trophoblast cells of the cotyledonal villi, which are in intimate contact with the caruncular crypt cells, never express immunoreactive, membrane-bound, MHC class I proteins.

Although, in theory, expression of the classical, MHC class I proteins could induce fetal rejection, expression of classical, MHC class I proteins by the bovine trophoblast during the last third of pregnancy does not have an adverse effect on pregnancy outcome and is probably beneficial. Compatibility, or identity, of the MHC between a dam and her conceptus has been associated with an increased incidence of placental retention at parturition (Joosten et al., 1991). Furthermore, comparison of MHC class I-compatible and -incompatible pregnancies revealed reduced leukocyte activation, as well as endometrial epithelial and trophoblast cell apoptosis, in MHC class I-compatible pregnancies (Davies et al., 2004).

Molecular analysis of the MHC class I genes expressed in bovine interplacentomal trophoblast cells revealed expression of classical and nonclassical, MHC class I genes (Davies et al., 2006). There was substantial variation in the ratio of classical to nonclassical gene expression among pregnancies, with nonclassical sequences accounting for 34 to 79% of the transcripts. Four bovine leukocyte antigen, nonclassical (BoLA-NC) loci were identified: BoLA-NC1, BoLA-NC2, BoLA-NC3, and BoLA-NC4. The bovine nonclassical transcripts exhibited several characteristic features of nonclassical, MHC class I genes, including limited polymorphism; premature stop codons; alternative splicing in the transmembrane domain; and putative, nonclassical, transmembrane domain, amino acid motifs (IPI and VPI). Transcripts for 1 allele, BoLA-NC1*50501, had a complete deletion of exon 5, which encodes the transmembrane domain.

A unique characteristic of the human HLA-G and murine preimplantation embryo development (Ped) loci is that they encode membrane-bound and soluble, MHC class I isoforms (HLA-G and murine Qa-2 antigens, respectively), which interact with leukocyte inhibitory receptors such as immunoglobulin-like transcript-2 (ILT2) and immunoglobulin-like transcript-4 (ILT4).
and induce immunosuppression (Comiskey et al., 2003; Shiroiishi et al., 2003; Hunt et al., 2005).

Transfection experiments were conducted with 2 classical and 4 nonclassical bovine MHC class I alleles encoded by 1 MHC haplotype (AH11; C. J. Davies, unpublished results). In these experiments, 3 of the 4 nonclassical, MHC class I genes (BoLA-NC1, BoLA-NC2, and BoLA-NC4) encoded proteins that could not be detected on the membrane of transfected murine P815 cells by flow cytometry. Therefore, these genes probably encode soluble rather than membrane-bound, MHC class I isoforms. The products of these loci may be delivered to the maternal side of the maternal-fetal interface, where they could act as soluble immunoregulatory proteins, by the fusion of bovine binuculate trophoblast cells with maternal, endometrial epithelial cells (Wooding and Wathes, 1980; Wooding, 1992, 1982).

In summary, bovine trophoblast cells in the interplacentomal and arcade regions of the maternal-fetal interface express classical, as well as nonclassical, MHC class I proteins during the third-trimester of pregnancy. Expression of classical, MHC class I proteins is important because these proteins have the potential to induce immune-mediated abortion. Identification of the nonclassical, MHC class I genes expressed in bovine trophoblast cells is significant because secreted and cell-surface, nonclassical, MHC class I proteins appear to be vital regulators of uterine immune function.

**IMMUNE-MEDIATED ABORTION IN SOMATIC CELL NUCLEAR TRANSFER PREGNANCIES**

Immune-mediated abortion has been difficult to study because of a lack of suitable animal models that exhibit abnormal patterns of placental MHC class I expression. The majority of bovine SCNT conceptuses, often referred to as clones, are aborted between d 30 and 90 of pregnancy (Edwards et al., 2003). Expression of MHC class I antigens was demonstrated on trophoblast cells of 34- to 36-d-old SCNT conceptuses, whereas age-matched controls had no MHC class I expression (Hill et al., 2002; Davies et al., 2004). Abnormal MHC class I expression has been demonstrated in SCNT blastocysts, indicating that MHC class I expression starts early in embryonic development (Pfister-Genskow et al., 2005).

In conjunction with increased trophoblast, MHC class I expression, the uteri of SCNT surrogate dams had a marked increase in the number of individual and aggregated stromal lymphocytes (Hill et al., 2002; Davies et al., 2004). Immunohistochemical characterization of the uterine lymphocytes revealed that the lymphoid aggregates were composed predominantly of CD4+ helper T lymphocytes (Davies et al., 2004). The large number of CD4+ T lymphocytes suggests that the primary mode of immunological recognition is indirect recognition, with peptides from processed, fetal, MHC class I proteins being presented by maternal antigen-presenting cells. Real-time, reverse transcription, PCR analysis of endometrial cytokine gene expression in 1 SCNT pregnancy and 1 control pregnancy revealed an inflammatory profile, including a 42-fold increase in interleukin-12 gene expression in the SCNT pregnancy (C. J. Davies, unpublished results).

In a retrospective study, it was found that 2 particularly successful SCNT cell lines were derived from MHC class I-homozygous cattle (Davies et al., 2004). Somatic cell nuclear transfer pregnancies established using MHC class I-homozygous cell lines, in which the immunological barrier is greatly reduced, had significantly improved fetal survival from d 28 to term compared with pregnancies established using MHC-heterozygous SCNT embryos (51% survival for MHC-homozygous and 5% for MHC-heterozygous SCNT fetuses). Consequently, it appears that the high rate of fetal mortality in SCNT pregnancies is due, at least in part, to inappropriate expression of trophoblast, MHC class I antigens and immune-mediated placental rejection.

**CONCLUSIONS**

Cattle are unusual in that they express MHC class I antigens on trophoblast cells in the interplacentomal and arcade regions of the placenta during the third trimester of pregnancy. Trophoblast expression of classical, MHC class I proteins probably triggers a uterine inflammatory response at parturition, which contributes to placental separation. In addition to expressing highly polymorphic, classical, MHC class I antigens, bovine trophoblast cells express nonclassical, MHC class I proteins, which are encoded by 4 class I genes. Most bovine nonclassical isoforms appear to be secreted proteins, which may act as immunosuppressive factors.

Early in pregnancy, there is aberrant expression of MHC class I antigens on trophoblast cells of SCNT conceptuses. In addition, SCNT pregnancies have far more lymphocytes and lymphoid aggregates in the maternal endometrium than control pregnancies. Remarkably, in a retrospective study, SCNT fetuses derived from MHC class I-homozygous cell lines had a significantly greater fetal survival rate than SCNT fetuses from MHC-heterozygous cell lines. These findings suggest that a substantial proportion of the pregnancy loss in SCNT pregnancies is the result of immune-mediated rejection of the placenta.

Recent findings in cattle and other species suggest that appropriate regulation of classical and nonclassical, MHC class I genes in the trophoblast cells that form the outermost layer of the placenta is critical for maternal immunological acceptance of the fetoplacental allograft. In addition to tight regulation of MHC class I genes, a complex network of hormones, cytokines, and other regulatory factors contribute to maternal immunological tolerance of a semi- or fully allogeneic conceptus.
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LITERATURE CITED


