Invasive equine trophoblast expresses conventional class I Major Histocompatibility Complex antigens

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Summary

Monoclonal antibodies and alloantisera were used in an indirect immunohistochemical assay to determine the expression of class I and class II Major Histocompatibility Complex (MHC) antigens by equine placental cells and the endometrial tissues at the fetal-maternal interface. MHC class I antigens were expressed at high density on the surface of the trophoblast cells of the chorionic girdle at days 32–36, just prior to their invasion of the endometrium. The mature gonadotrophin-secreting cells of the endometrial cups, which are derived from the chorionic girdle cells, had greatly reduced levels of MHC class I antigen expression while no MHC class I antigens were detectable on the non-invasive trophoblast cells of the allantochorion, except in small isolated patches. MHC class I antigens immunoprecipitated from chorionic girdle cells with either monoclonal antibodies or alloantisera had a relative molecular mass of 44,000, which was identical to that of MHC class I antigens precipitated from lymphocytes with the same reagents. MHC class II antigens were not detected on any trophoblast cells, although they were expressed at high levels by the endometrial glandular and luminal epithelium immediately bordering the endometrial cups. MHC class I antigens were also expressed at high levels by endometrial tissues in the area of the cups. The high level of MHC class I antigen expression by endometrial glands within and bordering the cups was in sharp contrast to the greatly reduced class I antigen expression by the mature endometrial cup cells themselves. Polymorphic, paternal, MHC class I antigens that are expressed by the invasive trophoblast cells of the chorionic girdle probably provide the antigenic signal for maternal anti-fetal alloantibody production in early horse pregnancy. Reduced expression of these antigens by terminally differentiated endometrial cup cells may represent a strategy for the protection of the conceptus from maternal immunological attack and destruction that is distinct from prevention of primary maternal sensitization.

Key words: horse, MHC, trophoblast, Major Histocompatibility Complex, antigens.

Introduction

The rarity of harmful maternal anti-fetal immune responses in normal human pregnancy can be explained in large measure by the failure of tissues at the fetal–maternal interface to express immunogenic, paternally inherited MHC antigens. The expression of class I MHC antigens by human cytotrophoblast cells appears to be limited to the truncated, non-polymorphic products of the HLA-G (HLA 6.0) locus. Immunohistochemical and immunoprecipitation studies using the antibody W6/32 indicate that expression of these non-HLA-A,B,C gene products is closely regulated, with detectable antigen expression by villous and extravillous cytotrophoblast cells but no expression by syncytiotrophoblast cells that form the primary exchange surface of the human placenta (Redman et al. 1984; Ellis et al. 1986, 1989, 1990; Kovats et al. 1990). Similar molecules have been described in the baboon on villous syncytiotrophoblast (Stern et al. 1987).

The lack of conventional MHC antigens on human trophoblast may account for the low incidence of maternal sensitization to paternally inherited alloantigens (Redman et al. 1987). In a prospective study of spontaneous abortion, Regan (1987) found an incidence of cytotoxic antibody to paternal lymphocyte antigens of less than 40% in primi-and multi-parous women four weeks postpartum. Furthermore, of women who did produce antibody, most did so only after 28 weeks of gestation. Similar figures have been reported by others (Doughty and Gelsthorpe, 1976; Barnstable and Bodmer, 1978). Circulating cytotoxic effector cells specific for paternal lymphocytes are rarely detected; in one study they were found in only 2 of 19 pregnant
women (Redman et al. 1987; reviewed in Sargent et al. 1987). In women who are sensitized, perhaps as a result of transfusion of fetal lymphocytes into the maternal circulation, the novel class I antigens found on human trophoblast cells would not be expected to act as targets for the cell- or antibody-mediated cytotoxic effector mechanisms that are induced (Redman et al. 1987; Sargent et al. 1987).

In horses, the apparent absence of harmful effects resulting from maternal sensitization to fetal MHC antigens is more difficult to explain (Antczak and Allen, 1984). Mares regularly produce high titered cytotoxic alloantibodies to paternal MHC antigens in early pregnancy after mating with stallions carrying foreign MHC alleles (Bright et al. 1978; Allen, 1979; Antczak et al. 1982, 1984). The predictability and vigor of this response suggests that the inciting cells may form an integral part of the fetal–maternal interface, yet despite the regular occurrence of potentially harmful cytotoxic antibodies fetal development is apparently unaffected (MacCluer et al. 1988; Park et al. 1989). The appearance of antibody by day 60 of pregnancy, shortly after the development of the endometrial cups, lead to the hypothesis that cup cells are the source of paternal MHC antigen which stimulates maternal antibody production (Antczak et al. 1984; Allen et al. 1984). This hypothesis has proved difficult to test, but indirect evidence has suggested that little or no MHC antigen is expressed by endometrial cup cells (Allen et al. 1984; Crump et al. 1987).

Endometrial cups are derived from the invasive trophoblast cells of the chorionic girdle (Allen et al. 1973). The chorionic girdle is a well-circumscribed annular band of tissue found almost at the equator of the day 34 equine conceptus (Fig. 1A and B). The conceptus at this stage is a spherical structure approximately 8 cm in diameter which is immobilized by the muscle tone of the uterus and not yet firmly implanted in the uterus. Trophoblast cells of the non-invasive allantochorion on the surface of the conceptus are held in close contact with the luminal epithelium of the endometrium. The epitheliochorial placental unit begins developing around day 40 as the allantochorion forms villi, which interdigitate with corresponding crypts in the endometrium, providing for nutrient exchange throughout pregnancy (reviewed by Allen, 1982).

The trophoblast cells of the chorionic girdle invade the uterine epithelium between days 36 and 38 and lose contact with the conceptus. After approximately 24 h in the epithelium they migrate into the sub-epithelial stroma, where they greatly enlarge, cease dividing, become binucleate and begin secreting equine chorionic gonadotrophin (eCG). By day 43 the densely packed invasive trophoblast cells have formed the endometrial cups, which comprise a variable number of nodules, each 1 to 2 cm in diameter, arranged in a circle in the endometrium (Fig. 1C) (Allen et al. 1973). Leukocytes, primarily lymphocytes, accumulate around the developing endometrial cups and gradually increase in number as gestation progresses (Allen, 1979). The only known function of the endometrial cups is production of eCG (Allen and Moor, 1972; Allen et al. 1973) which is detectable in maternal blood between days 40 and 120, by which time the cups have degenerated and sloughed into the uterine lumen (Allen, 1979).

We demonstrated previously that alloantisera to parental MHC class I antigens bound to cells of the chorionic girdle but not to the non-invasive trophoblast cells of the allantochorion (Crump et al. 1987). To further investigate the relationship between MHC antigen expression by trophoblast cells and maternal cytotoxic antibody production, we have characterized the expression of MHC class I and class II antigens on the equine trophoblast and endometrium using monoclonal antibodies and we have determined the relative molecular mass of the MHC class I antigens expressed by the chorionic girdle.

**Materials and methods**

**Tissue collection**

Endometrial tissues from non-pregnant mares and endometrial, placental and fetal tissues from pregnant mares at various stages of gestation were obtained from horses in the herd of the Equine Genetics Center at Cornell University. Conceptuses from mares between days 34 and 36 of pregnancy were collected by non-surgical uterine lavage and tissues from other mares up to day 60 of pregnancy were collected by surgical hysteroscopy as described (Antczak et al. 1987a). Endometrial biopsy specimens were obtained from a non-pregnant mare at intervals throughout an estrus cycle and from a pregnant mare following removal of a day 34 conceptus.

**MHC typing of horses**

Equine Lymphocyte Antigen (ELA, the MHC of the horse) types were assigned to horses after tissue typing using a panel of characterized alloantisera (Bernoco et al. 1987).

**Antibody reagents**

The monoclonal antibodies and alloantisera used in this study were described previously (Donaldson et al. 1988). Seven mouse monoclonal antibodies (A131 (Spear et al. 1985), H58A (Davis et al. 1987), PA2.2 (Brodsky and Parham, 1982), 115.1, 116.1, 117.1, and 117.2 (Donaldson et al. 1988)) were used to detect MHC class I proteins. The antibodies are of the IgG1 isotype except for H58A which is of the IgG2a isotype and 117.1 which is of the IgG2b isotype. Antibody H58A was a gift from Dr W. Davis of Washington State University; A131 was a gift from Dr J. Kornbluth of the University of Pennsylvania; and PA2.2 was a gift from Dr P. Parham of Stanford University. The rat monoclonal antibody F71.8, which is specific for a surface molecule of trophoblast cells, was used to identify endometrial cup cells (Antczak et al. 1987).

Alloantisera s2474 and s2488 identify polymorphic equine MHC antigens encoded by two or more class I loci (Donaldson et al. 1988). Antisera 2474 reacts with products of the ELA-B, but not the ELA-A, locus of the MHC haplotype carried by stallion 0834. Antiserum 2488 was raised across a whole MHC haplotype barrier and reacts with products of both the ELA-A and ELA-B loci of the same stallion. Stallion 0834 carries the ELA-A2 antigen and is homozygous for all detectable MHC class I and class II antigens. The alloantisera...
were used to precipitate MHC molecules from radio-labelled chorionic girdle cells from pregnancies stilled by stallion 0834. F39.2 is a rat monoclonal antibody of the IgG2a isotype directed against equine MHC class II antigens (Crepaldi et al. 1986). Mouse and rat monoclonal antibodies to canine parvovirus (Parrish et al. 1982) and normal horse serum (NHS) were used as negative controls.

The monoclonal anti-MHC antibodies appear to bind to non-variant (monomorphic) determinants of equine class I MHC molecules; the alloantisera, by definition, bind to polymorphic determinants of the ELA-A and ELA-B MHC class I antigens (Donaldson et al. 1988).

**Immunofluorescence**

The chorionic girdle was dissected from the extra-embryonic membranes of day 34 concepuses and the girdle cells were gently scraped from the basement membrane using a scalpel blade. The resulting sheets and clumps of cells were then passed through a 22 g hypodermic needle to reduce the tissue to small clumps and single cells mechanically. The cells were labelled using an indirect immunofluorescence assay (Crepaldi et al. 1986) with MHC class I specific monoclonal antibodies and FITC-conjugated goat anti-mouse immunoglobulin. After labelling, the cells were viewed using a Leitz microscope under UV illumination and photographed using Kodak Tri-X Pan film.

**Radiolabelling and cell lysis**

For radiolabelling, small sheets of allantochorion were gently scraped and processed mechanically as described above for chorionic girdle cells. Equine peripheral blood lymphocytes (PBL) were isolated from heparinized samples of venous jugular blood using methods described previously (Antczak et al. 1982).

Chorionic girdle cells, allantochorion cells and PBL were radiolabelled using 125Iodine (Amersham, Arlington Hts., IL) by a lactoperoxidase method described previously (Donaldson et al. 1988). Aliquots of PBL containing 1×10^6 cells and aliquots of trophoblast cells equivalent in packed volume to a pellet containing 2×10^9 PBL were labelled. After labelling and washing cells were lysed in a total of 1 ml of 0.5/0.5 buffer (10 mM Tris–HCL, 140 mM NaCl, 1 mM phenylmethylsulphonyl fluoride (PMSF), 5 mM ethylene diamine tetraacetate (EDTA), pH 7.6), containing a final concentration of 0.5 % (v/v) Triton-X 100.

**Immunoprecipitation**

Precipitation of antigen–antibody complexes was performed as described previously (Donaldson et al. 1988). Briefly, 1.0 ml of fresh lysate was precleared by incubation with 50 μl of normal mouse and 50 μl of normal rat sera or 100 μl of normal horse serum at 4°C for 4 h followed by two precipitations each with 100 μl (packed volume) of protein A agarose beads (Bethesda Research Laboratories, Gaithersburg, MD). For specific immunoprecipitations 100–175 μl of precleared lysate containing 1×10^9 TCA-precipitable cts min^-1 was incubated with 20 μl of allantiserum, 50 μl of tissue culture supernatants containing monoclonal antibody, or 1 μl of ascites containing high titered monoclonal antibody. The antibodies were incubated with lysate at 4°C overnight. Immune complexes were recovered by incubating the lysate/antibody mixture with 40 μl of 50 % v/v protein A agarose beads for 1 h at 4°C. Reacted beads were washed 4 times in 0.5/0.5 buffer before final resuspension in sample buffer (10 % glycerol, 5 % 2-mercaptoethanol, 2.3 % SDS, 0.0625m Tris–HCl pH 6.8, 0.01 % bromophenol blue).

**SDS-PAGE**

SDS-PAGE of immunoprecipitation samples was performed using the discontinuous method of Laemmli (1970), using 12 % acrylamide separating and 5 % acrylamide stacking gels. Relative molecular mass markers from 14 300 to 205 000 were used (Sigma, St. Louis, MO). Dried gels were autoradiographed at −70°C using XAR-5 film (Eastman Kodak, Rochester, NY).

**Immunohistochemistry**

Small pieces of fetus, chorionic girdle and allantochorion, endometrial cup with or without allantochorion, and endometrium were snap frozen in OCT embedding medium (Miles Scientific, Naperville, IL) and stored at −70°C until use. Tissue sections were cut 4–6 μm thick using a cryostat at −25°C and were fixed for 10 min in acetone at 4°C. The sections were labelled using an indirect immunoperoxidase assay as previously described (Antczak et al. 1987b; Crump et al. 1987), with the monoclonal antibody reagents listed above in the first stage and peroxidase conjugated goat anti-mouse or goat anti-rat antibodies (Organon Teknika-Cappel, West Chester, PA) as the second stage reagents.

**Analysis of immunohistochemical labelling**

The intensity of immunohistochemical labelling by the six anti-MHC class I specific monoclonal antibodies employed in this study varied from tissue to tissue, reflecting the density of cellular MHC class I antigen expression. Labelling of cell types in tissue sections by each antibody was assessed subjectively as NEGATIVE, WEAK, or STRONG and assigned a numeric score of 0, 1 or 2, respectively. However, each antibody did not behave identically and they could be divided into two groups on the basis of labelling intensity. Strong labelling of tissues was observed more frequently with one group of antibodies (H58A, A131, 117.1 and 117.2) than with another group (115.1, 116.1 and PA2.2). Within each group there was little variation in labelling intensity. Five patterns of antigen expression were defined. Tissues that were labelled STRONG (with antibodies from the strong labelling group) and STRONG (with antibodies from the weakly labelling group) were assigned a labelling score of 4; tissues that labelled STRONG and WEAK, respectively, were assigned a score of 3; tissues that labelled STRONG and NEGATIVE, respectively, were assigned a score of 2; tissues that labelled WEAK and NEGATIVE, respectively, were assigned a score of 1; and tissues labelled NEGATIVE and NEGATIVE, respectively, were assigned a score of 0. Thus, the intensity of labelling with members of the second, less reactive, group of antibodies was used to distinguish differences in MHC class I antigen expression by tissues which were strongly labelled by members of the first group of antibodies. Similarly, labelling by members of the first, more reactive, group of antibodies was used to distinguish between tissues not labelled at all by members of the second group. Using this score the levels of MHC class I antigen expression by several fetal, placental and endometrial tissues were objectively compared (see Table 1).

**Results**

(1) Chorionic girdle cells express MHC class I but not MHC class II antigens at a time when fetal cells express both MHC class I and class II antigens

Between days 33 and 35, prior to invasion of the endometrium, trophoblast cells of the chorionic girdle were labelled intensely by all of the anti-MHC class I
Table 1. Summary of anti-class I MHC labelling scores for equine fetal, placental and endometrial tissues

<table>
<thead>
<tr>
<th>Tissue*</th>
<th>Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal uterine lymphocytes</td>
<td>4</td>
</tr>
<tr>
<td>Trophoblast of the chorionic girdle</td>
<td>4</td>
</tr>
<tr>
<td>(invasive trophoblast, pre-invasion)</td>
<td></td>
</tr>
<tr>
<td>Endometrial luminal and glandular epithelium and connective tissue near cup</td>
<td>3-4</td>
</tr>
<tr>
<td>Fetal liver and kidney</td>
<td>2</td>
</tr>
<tr>
<td>Endometrial cup cells</td>
<td>1</td>
</tr>
<tr>
<td>(invasive trophoblast, post-invasion)</td>
<td></td>
</tr>
<tr>
<td>Trophoblast of the allantochorion</td>
<td>0‡</td>
</tr>
<tr>
<td>(non-invasive trophoblast)</td>
<td></td>
</tr>
</tbody>
</table>

* Frozen sections of equine fetal, placental and endometrial tissues were produced as described in the Materials and methods section.
† Tissues were labelled using a panel of MHC class-I-specific monoclonal antibodies and scored from 0 (negative) to 4 (strong labelling), as defined in the Materials and methods section.
‡ Except in small, isolated patches, where labelling of varying intensity was observed (see text and Fig. 8).

antibodies used. This level of MHC class I expression (labelling score 4, Table 1) was indistinguishable from that of lymphocytes in endometrial sections (see below). In striking contrast, a monoclonal antibody to equine MHC class II antigens failed to label the chorionic girdle cells (Fig. 2A and B).

The non-invasive trophoblast cells of the allantochorion membranes adjacent to the chorionic girdle were not labelled with any antibodies to MHC class I or class II antigens (labelling score 0, Table 1; Fig. 2C and D). The trophoblast cells of the allantochorion were strongly labelled, however, by a panel of five monoclonal antibodies directed against trophoblast restricted molecules (data not shown, see Antczak et al. 1987b; Oriol et al. 1989).

Fetal organs at day 35 contained cells that were labelled by antibodies to both MHC class I and class II antigens (Fig. 2E and F). Hematopoietic cells of fetal liver and glomeruli and intertubular connective tissue of fetal kidney were strongly labelled by anti-MHC class II antibody F39.2 and by anti-MHC class I antibodies of the more reactive group (labelling score 2, Table 1). Muscle, skin and nervous tissue from day 35 fetuses were not labelled by either anti-MHC class I or class II antibodies.

(2) Chorionic girdle cells express paternally inherited, polymorphic, MHC class I antigens

The MHC class I specific monoclonal antibodies 117.1 and H58A and alloantisera s2488 and s2474 precipitated proteins with an apparent relative molecular mass of 44 000, similar to conventional equine MHC class I antigens, from lysates of surface iodinated chorionic girdle cells. No bands were observed with a relative molecular mass of 12 000–14 000, which is consistent with previous observations that equine β-2 microglobulin is not labelled by 125Iodine using the lactoperoxidase method (A. Lew, personal communication, 1987). No differences in the estimated relative molecu-

lar mass were detected when several different anti-MHC class I antibodies were tested (Fig. 3).

Monoclonal antibodies 117.1 and H58A were used in a subsequent experiment to precipitate antigens from chorionic girdle cell and lymphocyte lysates carrying the same MHC antigens. No differences in the relative molecular mass were observed in the autoradiograph bands produced in adjacent lanes of the same SDS-PAGE gel (Fig. 4). The anti-class I antibodies did not precipitate molecules from radiolabelled allantochorion cell lysates (data not shown). The anti-class II MHC antibody F39.2 precipitated molecules from lymphocyte lysates with the characteristic weight of equine class II MHC antigens (Crepaldi et al. 1986), but no bands were observed when the antibodies were used with chorionic girdle cell lysates (data not shown).

The immunoprecipitation results described above...
Fig. 6. Immunohistochemical labelling of frozen sections of mature (D50–60) endometrial cups. Photomicrographs of entire endometrial cups labelled with monoclonal antibodies as described below. Scale bar=1800 μm. (Legend: allantochorion, ac; endometrial cup, ec; endometrium, em). (A) Antibody 116.1, specific for MHC class I antigens. There is no detectable labelling of endometrial cup cells or of the overlying allantochorion. There is strong labelling of the epithelium of endometrial glands within the endometrial cup and in adjacent endometrium. (B) Antibody H58A, specific for MHC class I antigens. There is weak sparse, patchy labelling of the epithelium of endometrial glands within the endometrial cup cells. There is no detectable labelling of endometrial cup cells. There is no detectable labelling of endometrial cup cells. There is strong labelling of endometrial lumenal epithelium, and subjacent endometrial stromal cells. Scale bar=50 μm. (C) Antibody H58A, specific for MHC class I antigens. There is a focus of strong labelling of trophoblast cells of the allantochorion (arrow). There is no detectable labelling of adjacent allantochorion trophoblast cells or of other cells of the allantochorion. There is no detectable labelling of endometrial cup cells. Scale bar=130 μm. (D) Antibody H58A, specific for MHC class I antigens. There is no detectable labelling of trophoblast or other cells of the allantochorion. There is strong labelling of the endometrial lumenal epithelium, endometrial glandular epithelium and endometrial vascular endothelium. Scale bar=130 μm. (C) Antibody H58A. There is a focus of strong labelling of trophoblast cells of the allantochorion (arrow). There is no detectable labelling of other trophoblast cells or other cells of the allantochorion. There is strong labelling of the endometrial lumenal epithelium, and subjacent endometrial stromal cells. Scale bar=50 μm.

were obtained after iodination of intact, live, chorionic girdle cells, suggesting that the equine MHC class I antigens were expressed on the surface of the invasive trophoblast cells. To confirm this observation, freshly isolated chorionic girdle cells were labelled with monoclonal antibodies to class I MHC antigens using an indirect immunofluorescence assay. The antibodies labelled the surface of the chorionic girdle cells intensely and uniformly (Fig. 5). When acetone-fixed cells were used the sharp definition of cell surfaces resulting from localized fluorescence of membrane antigens was absent and the cells were diffusely labelled (data not shown).

Fig. 3. Immunoprecipitation of MHC class I molecules from chorionic girdle cells using alloantisera and monoclonal antibodies. SDS–PAGE of MHC class I molecules precipitated from 125I-labelled chorionic girdle cells from a conceptus from mare 2157 (ELA-A9/-A19) sired by stallion 0834 (ELA-A2/-A2) using monoclonal antibodies 117.1 and H58A and alloantisera s2488 and s2474. The monoclonal antibodies and the alloantisera are described in the Materials and methods. H58A was in ascites fluid, 117.1 was in ascites (asc) and tissue culture supernatant (sn). Mouse anti-Canine Parvovirus and Normal Horse Serum (NHS) are negative controls. The mobility of relative molecular mass standards is indicated (×10^-5).
Fig. 4. Immunoprecipitation of MHC class I molecules from chorionic girdle cells and lymphocytes using monoclonal antibodies. SDS-PAGE of MHC class I molecules precipitated from $^{125}$I-labelled chorionic girdle cells (G) from a conceptus from mare 2155 (ELA-A2/-A9) sired by stallion 2505 (ELA-A3/-A3) or peripheral blood lymphocytes (L) from stallion 2505 using monoclonal antibodies 117.1 and H58A. Mouse anti-Canine Parvovirus was used as a negative control. The mobility of relative molecular mass standards is indicated ($\times 10^{-3}$).

(3) Mature endometrial cup cells express undetectable or very low levels of MHC class I antigens and undetectable levels of MHC class II antigens

In distinct contrast to the intense labelling of the invasive trophoblast cells of the chorionic girdle by the panel of anti-MHC class I antibodies (Fig. 2), mature endometrial cup cells, which are derived from the chorionic girdle cells, failed to be labelled, or were labelled only weakly by this same panel of antibodies (labelling score 1, Table 1). Fig. 6A is a low power photomicrograph of a frozen section of an entire endometrial cup labelled with anti-MHC class I monoclonal antibody 116.1. The red staining is restricted to endometrial glands within the cup and other maternal tissues surrounding the cup. Fig. 6B, which shows a similar endometrial cup section labelled with a monoclonal antibody restricted to trophoblast cells, highlights the extent of invasion of the endometrial cup cells.

Frozen sections of day 50–60 endometrial cups from 12 horse pregnancies were examined. The cup cells were not labelled by members of the less reactive group of antibodies (Fig. 7A), but they were labelled weakly by one or more antibodies of the more reactive group (Fig. 7C). There was no consistent hierarchy of labelling intensity among members of the more reactive group of monoclonal antibodies, and none of the antibodies labelled cup cells from all 12 pregnancies. The weak labelling of the cup cells by any single anti-MHC class I reagent was always uniform within an individual endometrial cup. The weak intensity of labelling of the endometrial cup cells by the monoclonal antibodies to MHC class I antigens is emphasized by the strong intensity of labelling of adjacent endometrial glands with the same antibodies (Fig. 7C), or by the strong labelling of the cup cells themselves with monoclonal antibodies to trophoblast molecules (Fig. 7D). There was no labelling of endometrial cup cells from any pregnancy with the anti-MHC class II monoclonal antibody (Fig. 7B).

The non-invasive trophoblast cells of the allantochorion, which were not labelled by the panel of anti-MHC class I antibodies between days 33 and 35, remained class I negative between days 50 and 60 (labelling score 0, Table 1). By this stage of pregnancy, the characteristic interdigitations between allantochorion and maternal endometrium of the mature equine placenta had formed (Fig. 8B). Two exceptions to this pattern were occasionally observed: first, in discrete patches of allantochorion overlying some endometrial cups (Fig. 8A); and second, in small patches comprising a few cells in allantochorion away from an endometrial cup (Fig. 8C). In the latter cases, labelling of tropho-
blast cells with anti-class I MHC antibodies was often associated with poor attachment of the allantochorion to the underlying endometrium. Labelling of trophoblast cells of the allantochorion was of variable intensity, with strong labelling most often observed over endometrial cups. Labelling of trophoblast cells of the allantochorion with the anti-MHC class II antibody F39.2 was never detected (not shown).

(4) Endometrial tissues express high levels of MHC class I and class II antigens

The undetectable or weak labelling of endometrial cup cells with anti-MHC class I antibodies was distinct from the strong labelling of maternal endometrial tissues, especially of the lymphocytes which were aggregated around most cups. The epithelium of endometrial glands within or near the endometrial cups (Figs 6A, and 7A and C) and the lumenal epithelium overlying the cups (Fig. 8A) were always intensely labelled by all the MHC class I specific antibodies (labelling score 4, Table 1). The labelling intensity of these epithelial cells was similar to that of the lymphocytes. The labelling of endometrial epithelial cells away from the endometrial cups with MHC class I antibodies was highly variable and often diminished a short distance away from the cup.

The anti-class II MHC antibody strongly labelled aggregates of lymphocytes and endometrial luminal and glandular epithelium at the margin of endometrial cups. In addition, non-endocrine cells within the cups such as vascular endothelium, small clusters of lymphocytes, and poorly defined filamentous cells were labelled. The labelling of epithelial cells was patchy or absent a short distance from the margin of the endometrial cups, with the distance from an individual cup being roughly proportional to the number of accumulated lymphocytes. Many cases even the epithelial cells of glands near the center of endometrial cups were unlabelled (Fig. 7B). Labelling of glandular epithelium with the anti-MHC class II antibody was rarely detected in the pregnant endometrium away from endometrial cups.

Discussion

Although many mechanisms may work together to achieve the relative immunological inactivity of the conceptus in normal pregnancy, the rarity of anti-MHC antibody responses in most species can be explained largely by the low levels of expression of antigenic MHC molecules by the trophoblast cells of the placenta (Beer and Sio, 1982; Chaouat et al. 1983; Lala et al. 1983; Antczak and Allen, 1984, 1989; Redman et al. 1987; and Antczak, 1989). The situation in horses is exceptional. Pregnant mares almost invariably mount strong anti-paternal cytotoxic alloantibody responses during first pregnancy, apparently without harmful effect on the developing conceptus (Allen et al. 1984; Antczak et al. 1984). The antibody responses are directed virtually exclusively against paternally inherited class I MHC antigens (Donaldson et al. 1988).

Prior to invasion of the endometrium on days 36–38, the trophoblast cells of the chorionic girdle expressed very high levels of MHC class I antigens (Fig. 2A and B). By day 60, in contrast, the mature endometrial cup cells, which are derived from the chorionic girdle cells, expressed extremely low levels of MHC class I antigens that were undetectable or only barely detectable by monoclonal antibodies used in an indirect immunohistochemical assay (Figs 6 and 7). Class I antigens were not detected on non-invasive trophoblast cells of the allantochorion at any stage of pregnancy, except in small isolated patches usually found over endometrial cups (Fig. 8). Because only mononuclear antibodies, and not alloantisera, were used to detect class I antigens on non-invasive trophoblast, it was not possible to determine if the rare expression of class I antigens by those cells represents paternal, polymorphic (and hence antigenic) MHC antigen, or non-polymorphic MHC antigens similar to those expressed by human cytotrophoblast cells (Ellis et al. 1990; Kovats et al. 1990).

By the same reasoning, it is not clear if the class I antigens expressed in low amount on mature endometrial cup cells (Fig. 7C) are maternal, polymorphic antigens. However, earlier experiments using absorption of alloantisera suggested that the cup cells might express low levels of paternal MHC antigens (Allen et al. 1984).

Class I MHC antigens were not detected on the conceptus prior to day 25, and between days 25 and 30 class I antigens were only detected on those conceptuses in which the chorionic girdle was identifiable morphologically (data not shown). Experiments to identify precisely the gestational age at which class I MHC antigens first appear are difficult because of the extreme fragility of the conceptus and the indistinct morphology of the developing chorionic girdle before day 30. The hypothesis that chorionic girdle cells are the only trophoblast cell population that expresses MHC class I antigens is consistent with the observation that secondary antibody responses in pregnant mares previously sensitized to paternal MHC antigens are not observed prior to day 40 (Antczak et al. 1984).

The pattern of expression of MHC antigens in equine trophoblast populations described here may clarify earlier observations linking equine pregnancy and histocompatibility. First, it is likely that chorionic girdle cells are the source of antigen for the maternal anti-paternal MHC alloantibody responses detected regularly in early horse pregnancy. Previous experiments, which focussed on the mature eCG-secreting endometrial cup cells, failed to establish that isolated endometrial cup tissue could induce primary or secondary anti-MHC antibody responses when used to immunize horses (Crump et al. 1987). The appearance of anti-MHC antibodies 2–3 weeks after migration of class-I positive chorionic girdle cells into the endometrium is consistent with the time required for a primary immune response (Antczak et al. 1984).

Second, the reduced expression of MHC class I antigens by endometrial cup cells may render the mature cup cells resistant to lysis by alloantibody or
cytotoxic lymphocytes. The intense lymphocytic infiltration in and around the endometrial cups appears to result in the destruction of the cups, which are sloughed into the lumen of the uterus by day 120 (Allen, 1979). The nature of the cellular infiltrate and its immunological significance remain unresolved, however. The 2- to 3-month lifespan of the cups in vivo suggests that maternal immune responses are not very effective in destroying the cup cells. These results may explain the absence of major effects on the outcome of equine pregnancy that can be ascribed to MHC compatibility or incompatibility between sire and dam (MacCluer et al. 1988; Park et al. 1989).

Emergent data from several species indicate that MHC class I antigen expression in trophoblast cells is more complex than previously supposed. In mice and rats, polymorphic MHC class I antigens have been detected on trophoblast cells. The levels of expression are very low, however, and while some investigators have reported expression by all trophoblast cell populations (Colavincenzo and Lala, 1984, 1985; Hunt and Soares, 1988) other workers have reported that expression is limited to specific trophoblast subpopulations, primarily to spongiotrophoblast cells (Jenkins and Owen, 1980; Singh et al. 1983; Billington and Burrows, 1986). Differential expression of MHC class I antigens by trophoblast cells has been reported in the rat placenta (Kanbour et al. 1987; Fowler et al. 1990). Labyrinthine and spongiotrophoblast cells were shown to express a non-classical class I MHC antigen in both a membrane-bound and a soluble form, while only spongiotrophoblast cells expressed polymorphic class I MHC antigens (Fowler et al. 1990).

In humans, no polymorphic class I antigens have been detected on any trophoblast populations. The class I HLA-G molecule has been detected in a cell-associated and a soluble form in experiments using villous cytotrophoblast cells (Kovats et al. 1990) and a similar molecule (HLA 6.0) is expressed by extravillous cytotrophoblast cells (Ellis et al. 1990). The villous syncytiotrophoblast cells do not appear to express these unusual class I molecules (Sunderland et al. 1981; Redman et al. 1984; and Ellis et al. 1986).

In horses, the expression of high levels of MHC class I antigens appears to be a developmentally regulated, stage-specific phenomenon, restricted to the first phase of invasive trophoblast cell development. Reduced MHC class I expression by the mature endometrial cup cells, in the second phase of invasive trophoblast cell development, may represent a defence against alloimmune rejection mechanisms which are distinct from the prevention of sensitization of the maternal immune system in the first place. The expression of high levels of MHC class I antigens by the invasive trophoblast cells of the chorionic girdle and the loss of this expression by the descendent gonadotrophin-secreting cells of the endometrial cups is, to our knowledge, a unique example of the regulation of class I MHC genes.

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References


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