

## Interactions between the immune system and the ruminant conceptus

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Interactions of the conceptus with the immune system can involve either anti-sperm or anti-conceptus immune responses that limit the success of pregnancy or beneficial effects of cytokines released from lymphoid cells on embryonic growth and gene expression. The immune system is functional in the uterus and therefore there is the potential for anti-conceptus immune responses. However, endometrial lymphocytes are distinct in many respects from lymphoid cells at peripheral sites; one major subpopulation expresses the  $\gamma\delta$  T-cell receptor and may not recognize major histocompatibility antigens. There are also several control systems to limit anti-conceptus immune responses. In particular, expression of major histocompatibility antigens on the trophoblast is either absent or of limited distribution. In addition, activation of anti-conceptus immune responses leading to cytolytic responses is further limited by the presence of molecules that can inhibit lymphocyte transformation. The most well-characterized of these are prostaglandin  $E_2$  from placental and endometrial tissues, interferon- $\tau$  from the trophoblast during early pregnancy, and two endometrial proteins called the uterine milk proteins (UTMP). Progesterone plays a central role in inhibition of immune responses in actions that are mediated at least in part through endometrial secretion of UTMP. Cytokines play important roles as autocrine and paracrine regulators in many tissues including the reproductive tract. In ruminants, the best described example is interferon- $\tau$ . Other cytokines found in the reproductive tract or produced by the conceptus include interleukin-1, leukaemia inhibitory factor, granulocyte-macrophage colony stimulating factor and interleukin-6. It is possible that the major source of cytokines in the reproductive tract is non-lymphoid cells of the endometrium and trophoblast. It is not known to what extent endometrial lymphocytes contribute to the cytokine milieu because no cytokine has been identified as a product of endometrial lymphocytes. However, there is a population of granulated lymphocytes that increase in number and granularity in the luminal epithelium of the late-pregnant ewe that is a potential source of cytokines.

### Introduction

The possibility that interactions with the maternal immune system affect development and growth of the conceptus has been recognized since Sir Peter Medawar's description of the fetus in 1953 as an "antigenically-foreign body". Several mechanisms by which the fetal allograft might survive or prevent immunological attack have been delineated. They include: (1) lack of expression of histocompatibility antigens on the placenta; (2) separation of maternal and fetal systems; (3) immunosuppression at the interface between conceptus and mother; and (4) the existence of the uterus as an immunologically

privileged site. Each of these mechanisms has been examined experimentally and, except for the last hypothesis, there is good evidence that all are operative to some degree. A second major impetus directing research in the field of reproductive immunology was provided by Thomas Wegmann's iconoclastic interpretation of the relationship between the conceptus and the maternal immune system. Wegmann (1988) argued that recognition of conceptus antigens by the maternal immune system could be beneficial to the conceptus through provision of growth-promoting cytokines at the fetal-maternal interface. This concept, called the immunotrophism theory, has resulted in a new understanding of the role of immune cytokines in the establishment and maintenance of pregnancy. It is now known that lymphocyte-mediated interactions with the conceptus are not required for pregnancy, since normal pregnancy rates can occur in mice deficient in B- and T-cells (Croy and Chapeau, 1990). However, such interactions could modify the likelihood of a successful pregnancy. In addition, macrophages may be important for pregnancy (Pollard *et al.*, 1991), and the endometrium and placenta produce a variety of immune cytokines that may also be important for regulation of placental function.

The study of immunological relationships between the conceptus and dam of domestic animals has lagged behind studies in rodents and humans. In part, this is because reagents and laboratory procedures required to make a detailed study of domestic animal immunology are only now becoming available. More importantly, it has not been established that aberrations in conceptus-immune interactions lead to reproductive problems in domestic animals. Notwithstanding, cells and soluble products of the immune system do participate in establishment and maintenance of pregnancy. This participation includes surveillance and removal of microorganisms from the reproductive tract and could also involve anti-sperm or anti-conceptus immune responses that limit the success of pregnancy as well as beneficial effects of cytokines released from leucocytes on embryonic growth and gene expression. Possible strategies for manipulating immune responses to improve reproductive function are emerging. For example, products of activated lymphocytes can stimulate growth of cultured bovine placental cells (Low *et al.*, 1991a) and the incidence of retained fetal membranes is related to histocompatibility between conceptus and dam (Joosten *et al.*, 1991). Development of management schemes to enhance reproductive function of domestic animals through manipulation of immunological function will require a good knowledge of the immunological relationship between conceptus and dam in domestic animals. The purpose of this review is to detail what is known about this relationship in ruminants.

## Lymphocyte Populations in the Uterus

### Characteristics

The uterus is by no means an immunologically privileged site – tissue graft rejection can occur *in utero* (Hansen *et al.*, 1986) and local antibody production can be induced by intrauterine immunization (Watson *et al.*, 1990). Functional immunological defences are probably a prerequisite to reproduction because the reproductive tract is repeatedly exposed to microorganisms as a result of breeding, parturition and other causes. The lymphocyte subpopulations in the endometrium have been best described in the sheep (Table 1). These cells are distinct in many regards from peripheral blood lymphocytes and resemble more closely the lymphocytes in the gut. Intraepithelial lymphocytes fall into three major subclasses, based on their reactivity to three differentiation antigens (Meeusen *et al.*, 1993). The most abundant population in nonpregnant animals (about 50% of the lymphocyte population) is a cell having the phenotype  $CD8^+ CD45R^- \gamma\delta$  T-cell receptor (TCR)-negative. The remainder of the intraepithelial lymphocytes are about equally represented in two classes, a  $CD8^+ CD45R^+ \gamma\delta$  TCR<sup>-</sup> cell, and a  $CD8^+ CD45R^+ \gamma\delta$  TCR<sup>+</sup> cell. None of these cells reacts strongly with the T-cell marker CD5 (Lee *et al.*, 1988; Gogolin-Ewens *et al.*, 1989; Meeusen *et al.*, 1993). Similarly, expression of the CD8 marker is weak for the  $CD45R^+$  lymphocytes (Meeusen *et al.*, 1993). Moreover, expression of B-cell markers on intraepithelial lymphocytes is rare (Meussen *et al.*, 1993).

Few lymphocytes are present in the stroma except in lymphoid follicles that contain primarily B cells (Lee *et al.*, 1988). Other cells located in the stroma include a few  $CD4^+$  cells (T cells that recognize antigen in association with MHC class II molecules, classically defined as helper T cells) and  $CD8^+$  cells (T cells that recognize antigen in association with MHC class I molecules, classically defined as

suppressor/cytotoxic T cells), as well as cells positive for MHC class II antigen that represent B cells and macrophages (Lee *et al.*, 1988; Gogolin-Ewens *et al.*, 1989; Gottshall and Hansen, 1992). The predominant immunoglobulin in uterine secretions, at least in cattle, is IgG derived from serum transudation and local synthesis (Curtain *et al.*, 1971; Lander Chacin *et al.*, 1990). IgA is an immunoglobulin characteristically produced by mucosal epithelia and is also present in uterine secretions (Curtain *et al.*, 1971; Lander Chacin *et al.*, 1990).

#### Regulation of numbers of endometrial lymphocytes

There is a decline in the number of intraepithelial lymphocytes when attachment of the conceptus to the endometrium occurs. This reduction has been described as a local effect at the site of attachment, as reported at days 22–24 in sheep (Staples *et al.*, 1983), or as a general effect throughout both uterine horns, as reported to occur in the bovine uterus from day 19 to day 27 of pregnancy (Vander Wielen and King, 1984). Nothing is known about changes in specific lymphocyte subpopulations in the endometrium associated with attachment of the conceptus. It is possible that only certain types of lymphocyte undergo a reduction in number and that some subpopulations become activated as a result of attachment. Study of regulation of endometrial lymphocytes at this stage of pregnancy, which ensues coincident with production of interferon- $\tau$  (IFN- $\tau$ ), is warranted because IFN- $\tau$  can regulate lymphocyte function (Newton *et al.*, 1989; Tuo *et al.*, 1993) and products of lymphocytes such as granulocyte-macrophage colony-stimulating factor-1 (GM-CSF) have the potential for altering conceptus function (Imakawa *et al.*, 1993).

Once placentation is complete, there are few lymphocytes in the placental regions of the endometrium in sheep (Gogolin-Ewens *et al.*, 1989) and cattle (Low *et al.*, 1990). As pregnancy progresses, there is also a decrease in numbers of lymphocytes in the glandular epithelium (especially non-granulated lymphocytes) and stromal areas (especially MHC class II<sup>+</sup> cells and T19<sup>+</sup> cells) of the interplacental region of the sheep endometrium (Gogolin-Ewens *et al.*, 1989; Lee *et al.*, 1992). In contrast, the number of granulated lymphocytes in the luminal epithelium of the interplacental sheep endometrium increases markedly during late pregnancy, by which time they comprise an estimated 10% of cells in the epithelium (Lee *et al.*, 1992). The increase in the number of these cells is accompanied by a concomitant increase in the size and number of granules (Gogolin-Ewens *et al.*, 1989; Lee *et al.*, 1992; Meeusen *et al.*, 1993) (Fig. 1). The granulated lymphocyte that is activated during pregnancy has been identified as the CD8<sup>+</sup> CD45R<sup>+</sup>  $\gamma\delta$  TCR<sup>+</sup> lymphocyte subpopulation (Meeusen *et al.*, 1993).

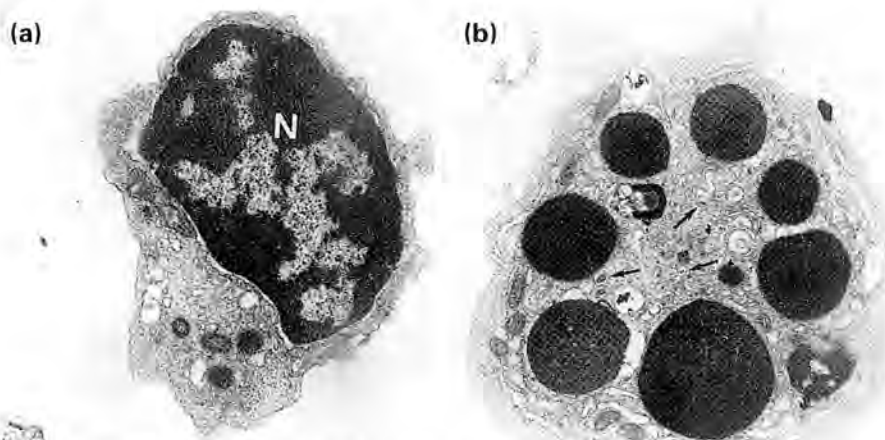
#### Possible functions

Plasma cells in stromal follicles are presumably responsible for most local immunoglobulin synthesis but functions of other endometrial lymphocyte populations are unclear. The CD8<sup>+</sup> CD45R<sup>-</sup>  $\gamma\delta$  TCR<sup>-</sup> cell is presumably an  $\alpha\beta$  TCR<sup>+</sup> cell and may function like other CD8<sup>+</sup> lymphocytes in MHC class-I-restricted cytotoxicity of cells infected with viruses and protozoa. The remaining two subsets of lymphocytes in the endometrial epithelium both express CD45R antigen, which in peripheral blood reacts with B cells and a small fraction of circulating T cells (Mackay *et al.*, 1987). Endometrial CD45R<sup>+</sup> cells do not express B-cell markers and express certain T-cell markers weakly (CD8) or not at all (CD5) (Lee *et al.*, 1988; Gogolin-Ewens *et al.*, 1989; Meeusen *et al.*, 1993). The high degree of granularity of the CD8<sup>+</sup> CD45R<sup>+</sup>  $\gamma\delta$  TCR<sup>+</sup> lymphocyte population as well as the fact that they are apparently activated during late pregnancy has led to the speculation that one of their functions is to secrete cytokines that promote conceptus development (Meeusen *et al.*, 1993). Another proposed function for lymphocytes of the  $\gamma\delta$  TCR lineage include recognition of microbial antigens and tumour cells (Haas *et al.*, 1993). Unlike T-cells of  $\alpha\beta$  TCR lineage,  $\gamma\delta$  TCR<sup>+</sup> lineage do not readily recognize MHC antigens or participate in tissue rejection (Haas *et al.*, 1993). Thus, these cells may not recognize conceptus alloantigens. There are also reports that  $\gamma\delta$  TCR<sup>+</sup> lymphocytes can downregulate immune responses (Howard *et al.*, 1989). The function of the endometrial CD45R<sup>+</sup>  $\gamma\delta$  TCR<sup>-</sup> cells is unknown; they could be analogous to the natural killer (NK) like cells reported in mice and humans (King and Loke, 1991), but there is no evidence for this.

Table 1. Major lymphocyte subpopulations in the endometrium of sheep

Phenotype	Major location	Relative abundance	Possible function
CD8 <sup>+</sup> CD45R <sup>-</sup> $\gamma\delta$ TCR <sup>-</sup>	Interplacentomal epithelium	Major lymphocyte ( $\approx$ 50%) in luminal epithelium of nonpregnant ewes	Probably an $\alpha\beta$ -TCR <sup>+</sup> lymphocyte; may therefore function as a cytotoxic cell directed towards viral-infected cells or to MHC antigens
CD8 <sup>+</sup> CD45R <sup>+</sup> $\gamma\delta$ TCR <sup>-</sup>	Interplacentomal epithelium	About 25% of lymphocytes in luminal epithelium of nonpregnant ewes	Unknown
CD8 <sup>+</sup> CD45R <sup>+</sup> $\gamma\delta$ TCR <sup>+</sup>	Interplacentomal epithelium	About 25% of lymphocytes in luminal epithelium of nonpregnant ewes; during late pregnancy, numbers increase ( $\approx$ 10% of all cells in the luminal epithelium)	Could be directed towards conceptus or bacterial antigens; large granules may be a source of cytokines
MHC class II <sup>+</sup>	Stroma	Scattered throughout stroma; predominant cell in lymphoid follicles located in stroma	Macrophages and B cells (antibody production)
CD4 <sup>+</sup>	Stroma	Present in low amounts	Probably T helper cells
T19 <sup>+</sup>	Stroma	Only a few present	T19 identifies a $\gamma\delta$ TCR <sup>+</sup> subset

Data from Lee *et al.* (1988, 1992), Gogolin-Ewens *et al.* (1989), Gottshall and Hansen (1992) and Meusen *et al.* (1993).



**Fig. 1.** Characteristics of granulated lymphocytes recovered from epithelium of ewes: (a) a granulated cell recovered from a nonpregnant uterus; and (b) a granulated cell recovered from the uterus of a pregnant ewe. Note the increase in number and size of granules associated with pregnancy. N: nucleus. The arrows in (b) represent small vesicles that are postulated to be involved in granule formation. Magnification: (a)  $\times 10\,145$  (b)  $\times 16\,286$ . Reproduced with permission from the *European Journal of Immunology* (Meeusen *et al.*, 1993).

Natural killer cells could potentially be detrimental to the conceptus because lymphokine-activated killer cells can lyse peri-attachment sheep conceptuses (Segerson and Gunsett, 1994).

### Antigenicity of Spermatozoa and the Conceptus

Spermatozoa can be antigenic in females (Menge, 1969; Awad *et al.*, 1984; Lander *et al.*, 1990), but there are only a few reports of antibody responses to spermatozoa associated with reduced fertility (Awad *et al.*, 1984; Erski-Biljic and Varadin, 1985). Deposition of semen in the reproductive tract results in migration of leucocytes (primarily polymorphonuclear leucocytes) into the uterus (Mattner, 1968). Accordingly, many spermatozoa are probably lost by phagocytosis and not accessible to cellular components causing specific immunity. However, seminal components inhibiting phagocytosis of spermatozoa have been reported in cattle (Strzemienski, 1989). Specific immunity mediated by lymphocytes is probably also inhibited by an RNase and an antibacterial protein found in seminal plasma that inhibits T- and B-cell proliferative responses (Derwenskus *et al.*, 1989). Immunization of heifers with spermatozoa up to 21 days after insemination was reported to cause early embryonic death (Menge, 1969). This finding suggests that embryos share antigens with spermatozoa and that embryonic loss could result from immunological recognition of these antigens. The study involved small numbers of animals and has implications important enough to warrant replication of the experiment.

The bovine blastocyst expresses paternally and maternally inherited polymorphic MHC class I antigens (Templeton *et al.*, 1987). After placentation, the antigenicity of the placenta is diminished. In sheep, there was no detectable expression of MHC class I or class II antigens on fetal placental tissues in contact with endometrium (Gogolin-Ewens *et al.*, 1989). In cows, placentomal chorion was also negative for MHC antigens (Low *et al.*, 1990) but there was expression of MHC class I antigen on limited regions of the interplacentomal chorion. The antibody used by Low *et al.* (1990) recognizes a monomorphic determinant of MHC class I molecules and it is not certain whether molecules detected were polymorphic. In humans, MHC class I molecules are of a generally monomorphic class (Schmidt and Orr, 1993). In spite of the reduced antigenicity of the placenta, anti-fetal leucocyte antibodies can be detected in the blood of parous sheep (Ford and Elves, 1974; Stear and Spooner, 1983), cattle (Newman and Hines, 1980) and goats (van Dam *et al.*, 1976), possibly because females become exposed to fetal cells during pregnancy or after parturition (Newman and Hines, 1980). Maternal responses to



Table 2. Endometrium and trophoblast-derived substances capable of inhibiting lymphocyte proliferation in ruminants

Substance	Species	Tissue source	Comments	Reference
Progesterone	Mammals	Corpus luteum, placenta	Concentrations to inhibit may be pharmacological; does not act through classical receptor; induces synthesis of other inhibitors	Low and Hansen, 1988; Monterroso and Hansen, 1993
Prostaglandin E <sub>2</sub>	Sheep, cow, goat, other mammals	Preattachment embryos, endometrium, placenta	—	Low and Hansen, 1988
Interferon- $\tau$	Sheep, goat, cow, other ruminants	Trophoctoderm	Transient synthesis coincident with expected time of luteolysis	Newton <i>et al.</i> , 1989
High molecular weight glycoprotein <sup>a</sup>	Cow, sheep, pig	Periattachment conceptus	$M_r \approx 800\text{--}900\ 000$ ; contains lactosaminoglycans	Newton <i>et al.</i> , 1989
Pronase-sensitive protein <sup>a</sup>	Sheep	Placentomal chorion, day 100	$M_r \approx 46 \times 10^3\text{--}162 \times 10^3$	Low <i>et al.</i> , 1991b
Periodate-sensitive protein <sup>a</sup>	Sheep	Placentomal chorion, day 100	$M_r > 4 \times 10^6$ , pronase-resistant, requires carbohydrate	Low <i>et al.</i> , 1991b
Uterine milk proteins	Sheep, cow, pig	Endometrial epithelial cells (induced by progesterone)	Pair of 55 and 57 kDa glycoproteins; related to serine protease inhibitors; major inhibitory proteins in uterine fluid	Skopets and Hansen, 1993; Hansen and Liu, 1994
Megasuppressin <sup>a</sup>	Sheep, cow	Pregnant endometrium (induced by progesterone)	$M_r > 4 \times 10^6$ , $\approx 35\%$ carbohydrate; minor inhibitory molecule in uterine fluid	Stephenson <i>et al.</i> , 1989; Segerson and Bazer, 1989; Skopets and Hansen, 1993

<sup>a</sup>Molecule not purified to homogeneity.

placental tissues may be beneficial to normal processes associated with parturition; it has been reported that cows that are MHC class-I-compatible with their fetuses have a higher rate of retained placenta (Joosten *et al.*, 1991).

### Immunosuppression

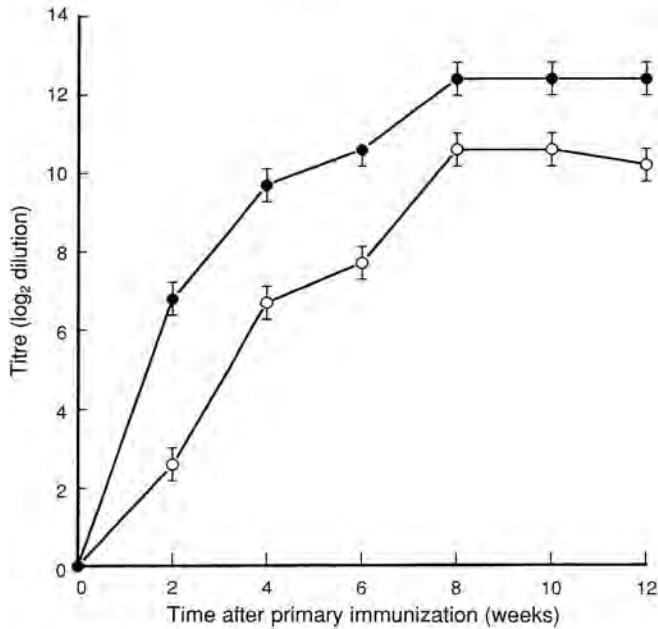
Although there is conflicting evidence (Rai-El-Balhaa *et al.*, 1987), most of the evidence suggests that pregnant females do not experience systemic immunosuppression (Billingham and Lampkin, 1957; Outteridge and Dufty, 1973; Monterroso and Hansen, 1993). However, the conceptus-maternal interface is rich in molecules that can inhibit immune responses (Table 2) and the existence of so many locally produced lymphocyte-inhibitory molecules support Billingham's hypothesis (1964) that the placenta itself is in an immunosuppressed environment. However, caution is necessary when interpreting this evidence. Most immunoregulatory molecules produced by the uterus or trophoblast have been identified on the basis of inhibition of peripheral blood lymphocyte proliferative responses induced by T-cell mitogens or mixed lymphocyte reactions. It is likely that some molecules inhibit activity of cultured lymphocytes through mechanisms that would not affect immune responses *in vivo*. Molecules might also exert more complex immunoregulation *in vivo* than indicated by lymphocyte proliferation tests. The uterine milk proteins (UTMP), for example, block T-cell proliferative responses *in vitro* (Skopets and Hansen, 1993) and antibody production *in vivo* (Skopets *et al.*, in press) but stimulate phytohaemagglutinin (PHA)-stimulated delayed hypersensitivity reactions in skin (Skopets *et al.*, 1995). Clearly, there is a need to evaluate the effects of putative immunosuppressants on endometrial lymphocytes and to determine whether immune responses *in utero* are actually suppressed during pregnancy.

### Role of Progesterone in Regulating Uterine Immune Function

The best evidence for alteration of uterine immune responses during pregnancy is indirect and involves the actions of progesterone, one of the major pregnancy hormones. It has long been known that females treated with progesterone are more likely to develop infection after intrauterine inoculation of bacteria (Rowson *et al.*, 1953). Some of this effect of progesterone may be related to uterine drainage or the function of phagocytic cells. However, long-term (60 day) treatment of ovariectomized ewes also results in a decline in numbers of CD45R<sup>+</sup> lymphocytes and MHC class II<sup>+</sup> cells in the endometrium (Gottshall and Hansen, 1992) and increased survival of intrauterine skin grafts (Hansen *et al.*, 1986). Progesterone can inhibit lymphocyte proliferation directly at concentrations of 10<sup>-6</sup> to 10<sup>-5</sup> mol l<sup>-1</sup> (Low and Hansen, 1988; Monterroso and Hansen, 1993). These concentrations are only approached in the utero-ovarian lymph ipsilateral to the corpus luteum (Staples *et al.*, 1982) and perhaps in the placenta of species such as sheep and goats in which there is significant placental progesterone synthesis. It is likely that many inhibitory effects of progesterone on uterine immune function are mediated indirectly through induction of other immunoregulatory molecules. Treatment of animals with doses of progesterone too low to affect lymphocyte proliferation directly can induce the presence of molecules that inhibit lymphocyte proliferation *in vitro* in uterine secretions of ovariectomized ewes (Hansen *et al.*, 1986; Stephenson and Hansen, 1990) and cows (Lander Chacin *et al.*, 1990). Uterine fluid from progesterone-treated ewes can also reduce antibody responses *in vivo* (Stephenson and Hansen, 1990).

### The Uterine Milk Proteins – Putative Mediators of Effects of Progesterone on Uterine Immune Function

Uterine fluid from pregnant ewes and cows also inhibits lymphocytes (Segerson and Bazer, 1989; Stephenson *et al.*, 1989). The molecules responsible for most of the non-dialysable lymphocyte-inhibitory activity in uterine fluid from pregnant ewes are a pair of progesterone-induced proteins called the uterine milk proteins (Skopets and Hansen, 1993). The biochemical properties and endocrine regulation of the UTMP have been reviewed recently (Hansen and Liu, 1994). This pair of progesterone-induced, basic glycoproteins, having molecular weights of 55 000 and 57 000, makes up the majority of protein in uterine fluids of pregnant and progesterone-treated ewes and they are present



**Fig. 2.** Antibody titres in ewes immunized with ovalbumin that had been emulsified in adjuvant containing either (●) uterine milk proteins (UTMP) or the negative control (○) ovine serum albumin (OSA). Note the reduced antibody response in ewes in which UTMP were co-administered; at 8 weeks, titres averaged 1:5404 for ewes receiving OSA and 1:1552 for ewes receiving UTMP. Reproduced with permission from *American Journal of Reproductive Immunology* (Skopets *et al.*, in press).

in lower quantities within the bovine uterus. The two UTMP are related, having an identical amino-terminal amino acid sequence, and there is evidence that they are formed from a common precursor. The inferred amino acid sequence of UTMP indicates that the proteins are members of the serpin superfamily of serine protease inhibitors (Ing and Roberts, 1989). Although no antiprotease activity characteristic of serpins has been described for the proteins, the UTMP share two properties with another serpin,  $\alpha 1$ -antitrypsin, namely the ability to bind selectively to IgA (Hansen and Newton, 1988) and inhibit lymphocyte blastogenic responses (Skopets and Hansen, 1993).

The uterine milk proteins can inhibit proliferation of cultured peripheral blood lymphocytes induced by several T-cell activators including phytohaemagglutinin, concanavalin A, mixed lymphocyte reactions and *Candida albicans* antigen (Skopets and Hansen, 1993; Skopets *et al.*, in press). The concentrations of UTMP required to inhibit lymphocyte proliferation (about 50–500  $\mu\text{g ml}^{-1}$ ) are high but well within the range of concentrations present in uterine fluid after about day 30 of pregnancy. The UTMP can also inhibit immune responses of the whole animal including antibody production in sheep (Fig. 2; Skopets *et al.*, in press), NK cell activity (Liu and Hansen, 1993) and NK-cell mediated abortion in mice (Liu and Hansen, 1993). However, not all lymphocyte subpopulations are inhibited by UTMP. Skopets and Hansen (1993) reported that UTMP did not inhibit proliferation of sheep peripheral blood lymphocytes stimulated with the T- and B-cell activator pokeweed mitogen. UTMP also enhanced delayed hypersensitivity responses to subcutaneous administration of PHA (Skopets *et al.*, in press). The selectiveness of actions of UTMP make it important to test whether UTMP can inhibit the action of endometrial lymphocytes before the significance of the proteins can be established.

The mechanism by which UTMP inhibits lymphocyte proliferation is incompletely understood. One possible mechanism could involve inhibition of proteases such as dipeptidyl peptidase IV involved in lymphocyte activation. However, UTMP do not block dipeptidyl peptidase IV coactivation of



lymphocytes or enzyme activity (Liu and Hansen, in press). One of the properties of serpins is their propensity to interact with other proteins and it is possible that UTMP bind to a cell surface receptor or soluble product of activated lymphocytes that are required for proliferation.

### Involvement of Cytokines in Regulation of Endometrial and Placental Function

Many of the cytokines that were described originally as being products of the immune system have since been shown to play important roles as autocrine and paracrine regulators in many tissues including the reproductive tract. In rodents, several cytokines have been implicated in reproductive processes and the greatest evidence is for colony-stimulating factor-1 (Pollard *et al.*, 1991), leukaemia inhibitory factor (Stewart *et al.*, 1992), and interleukin 1 (Simón *et al.*, 1994). A summary of cytokines that have been associated with pregnancy in ruminants is presented in Table 3. Of these cytokines, IFN- $\tau$  is the only one for which an important role in pregnancy (regulation of luteal lifespan) has been established (Roberts *et al.*, 1992). Interferon- $\tau$  is a pleiotrophic molecule and, like other interferons, has anti-viral (Pontzer *et al.*, 1988) and immunosuppressive activity (Newton *et al.*, 1989), induces synthesis of 2',5'-oligoadenylate synthetase (Short *et al.*, 1991) and activates NK cells (Tuo *et al.*, 1993). It is not known to what extent these other actions of IFN- $\tau$  are important to the course of pregnancy. Perhaps the decrease in the number of intraepithelial lymphocytes that occurs following IFN- $\tau$  secretion (Staples *et al.*, 1983; Vander Wielen and King, 1984) is a result of immunoregulatory actions of IFN- $\tau$ . The antiproliferative actions of interferons could also alter endometrial function, but recent experiments indicate that endometrial cell growth is only slightly affected by IFN- $\tau$  (Davidson *et al.*, 1994).

Other cytokines may also play a role in pregnancy; GM-CSF has been localized to glandular epithelial cells of the ovine endometrium and it has been reported that it enhances IFN- $\tau$  synthesis by the ovine conceptus (Imakawa *et al.*, 1993). In cattle, interleukin 1 has been detected in uterine flushings. It increases endometrial secretion of prostaglandins and inhibits growth of endometrial stromal cells (Davidson, 1994). In addition, leukaemia inhibitory factor (LIF) has been immunolocalized to stroma and epithelium of ovine endometrium (Vogiagis *et al.*, 1994) and receptors for colony-stimulating factor 1 have been identified on bovine trophoblast (Beauchamp and Croy, 1991). Knowledge of the roles of these and other cytokines in pregnancy is incomplete because it is not known which cytokines are present in the ruminant reproductive tract, how their secretion is regulated, and whether the functions exerted by the cytokines are of critical importance to pregnancy. One major question is the degree to which cytokines at the conceptus-maternal interface are derived from cells of the immune system. According to Wegmann's immunotrophism hypothesis (1988), stimulation of lymphocytes by conceptus antigens should increase the cytokines available at the conceptus-maternal interface and promote conceptus growth and endocrine function. Consistent with this theory is the observation that bovine placental cells are sensitive to growth promoting effects of supernatants from activated lymphocytes (Low *et al.*, 1991a). However, the fact that the fetal placenta has reduced antigenicity and exists in an environment bathed in immunosuppressive substances makes the development of strong immune responses towards the conceptus unlikely. Cytokine secretion from endometrial leucocytes has not been described in ruminants, although granulated lymphocytes located in the endometrial epithelium of sheep are a potential source of lymphocyte-derived cytokines (Meeusen *et al.*, 1993). Immune cytokines, identified in the ruminant uterus, that have a known origin are produced by trophoblast or endometrial epithelial cells (Table 3). Thus, it is possible that the major source of immune cytokines at the conceptus-maternal interface are these tissues rather than leucocytes. Cytokines from these sources could affect the function of both the conceptus and endometrial leucocytes.

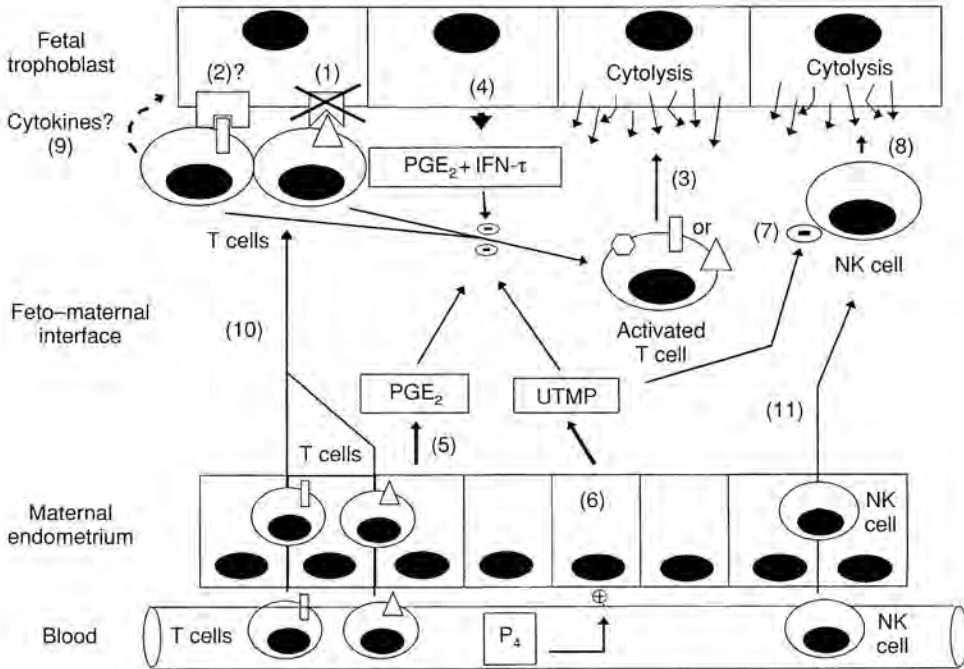
### Conclusions: A Model of Conceptus-Immune Interactions

On the basis of results presented in this paper, a model of conceptus-immune interactions in ruminants can be developed (Fig. 3). After early pregnancy, the placenta of the ruminant conceptus is of limited antigenicity, and expression of MHC antigens is absent or greatly reduced. Moreover, unlike most grafts, the contact between conceptus and the maternal system is limited to the outer layers of trophoblast and endometrium. Thus, lymphocyte trafficking into more antigenic regions of the

Table 3. Cytokines implicated in establishment and maintenance of pregnancy in ruminants

Cytokine	Tissue source	Species	Control of secretion	Actions in the reproductive tract	Reference
Interferon- $\tau$	Trophoblast	Ruminants	Transient secretion peaks coincident with expected time of luteolysis	Inhibits prostaglandin $F_{2\alpha}$ secretion; blocks lymphocyte proliferation; induces antiviral state and synthesis of several proteins; activates NK cells	Newton <i>et al.</i> , 1989; Roberts <i>et al.</i> , 1992; Tuo <i>et al.</i> , 1993
Interleukin 6	Conceptus	Sheep, cow, pig	mRNA identified at time of conceptus elongation/attachment	Unknown	Mathialagan <i>et al.</i> , 1992
GM-CSF	Endometrial epithelium	Sheep	Found at day 17 by <i>in situ</i> hybridization; secretion and endocrine control unknown	Enhances synthesis and secretion of interferon- $\tau$	Imakawa <i>et al.</i> , 1993
Interleukin 1	Uterine flushings	Cow	Detected by ELISA, day 11-17 after oestrus in cyclic and pregnant cows; not detected at days 25-30 of pregnancy	Stimulates prostaglandin $E_2$ secretion from endometrial cells; inhibits proliferation of endometrial stroma but not epithelium	Davidson, 1994
LIF	Endometrial epithelium and stroma	Sheep	Detected by immunohistochemistry and northern blotting; tends to peak at day 12 after oestrus in pregnant and cyclic ewes	Unknown	Vogiagis <i>et al.</i> , 1994

GM-CSF: Granulocyte-macrophage colony-stimulating factor; LIF: leukaemia inhibitory factor; ELISA: enzyme-linked immunosorbent assay; NK cells: natural killer cells.



**Fig. 3.** Model of conceptus-immune interactions in ruminants. After early pregnancy, the trophoblast is of limited antigenicity, with expression of major histocompatibility antigens (□) being absent or greatly reduced (1). Thus, T cells expressing the  $\alpha\beta$  T-cell receptor (TCR) ( $\Delta$ ) are unlikely to recognize trophoblast as foreign. In addition, many of the lymphocytes present in the endometrium are  $\gamma\delta$  TCR<sup>+</sup> lymphocytes ( $\square$ ) which probably have only limited ability to recognize MHC antigens. It is not known whether antigen-presenting molecules for these cells (□) are present on the trophoblast (2). Activation of anti-conceptus lymphocytes (indicated schematically by acquisition of interleukin-2 receptor (□)) could lead to cytolytic responses towards the trophoblast (3: cytolysis indicated by streams of arrows from the trophoblast cell) but these responses are probably blocked by molecules that can inhibit lymphocyte transformation. The most well-characterized of these are prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) from placental and endometrial tissues (4, 5), interferon- $\tau$  (IFN- $\tau$ ) from the trophoblast (4), and the progesterone-induced uterine milk proteins (UTMP) (6). The uterine milk proteins can also inhibit natural killer (NK) cells (7). This may be important because activated killer cells can lyse trophoblast (8). It is not known whether leucocytes in the uterus produce cytokines beneficial to the developing conceptus, but one possible source of these cytokines are the granulated CD45R<sup>+</sup>  $\gamma\delta$  TCR<sup>+</sup> lymphocytes that increase in number and granularity in the luminal epithelium of the late-pregnant ewe (9). Note that while the source of endometrial lymphocytes is indicated as the blood (10), this has not been established. Similarly, the presence and source of NK cells in the endometrium of ruminants is conjectural (11). P<sub>4</sub>: progesterone.

conceptus is blocked. In addition, many of the lymphocytes present in the endometrium are  $\gamma\delta$  TCR<sup>+</sup> lymphocytes that probably have only limited ability to recognize MHC antigens. Activation of anti-conceptus immune responses leading to cytolytic responses towards the trophoblast is further limited by the presence of molecules that can inhibit lymphocyte transformation. The most well-characterized of these are PGE<sub>2</sub> from placental and endometrial tissues, IFN- $\tau$  from the trophoblast during early pregnancy, and the UTMP. Progesterone plays a central role in inhibition of immune responses in actions that are mediated through endometrial secretion of UTMP but which could also include other unknown effects.

The possibility that leucocytes in the uterus produce cytokines beneficial to the developing conceptus is a largely untested hypothesis in ruminants. One possible source of these cytokines is the granulated lymphocytes that increase in number and granularity in the luminal epithelium of the ewe at

late pregnancy. This increase in number and activity suggests that these cells are largely unresponsive to the inhibitory effects of the many lymphocyte-inhibitory molecules present at the conceptus-maternal interface. Production of cytokines by these lymphocytes, or by tissues such as endometrium and trophoblast, may be important for regulating growth, gene expression and hormonal secretion of placental, fetal and endometrial tissues.

Our understanding of conceptus-immune interactions in ruminants is fragmentary. This is mainly because little is known of the functions of endometrial lymphocytes with respect to activation status, responsiveness to putative immunoregulatory molecules and production of cytokines. Elucidation of the functions of these cells should clarify their role in the uterus, while simultaneously providing a more complex view of the cellular interactions between placenta and the diverse cellular populations in the endometrium that are required for a successful pregnancy.

This is Journal Series Number R-04068 of the Florida Agricultural Experiment Station. The author gratefully recognizes the students, postdoctoral scientists and visiting scholars who contributed to the work from his laboratory that was reported in this paper as well as the sponsors of the research: NIH (Grant HD 20671 and HD 26421), USDA (Grant 89-37240), the Florida Dairy Milk Checkoff Program and CIBA-GEIGY. Appreciation is also extended to M. J. Fields, for providing the author with the collection of early papers in the field compiled by A. C. Warnick, and to W. W. Thatcher, for providing a thorough review of the paper.

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