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Pregnancy-Associated Immunoregulatory Molecules Discovered in Ruminants and Their Possible Relevance to Other Species

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Abstract

Several distinct molecules involved in maternal-conceptus interactions have been discovered in ruminants. Among these are two families of immunoregulatory molecules that represent genes that have undergone evolution to perform a function distinct from that of the ancestral gene. Interferon-τ (IFN-τ) is a product of the trophoblast that retains its antiviral activity and other functions characteristic of interferons but whose primary role is the inhibition of luteolysis. The uterine serpins are progesterone-induced members of the serpin superfamily of serine proteinase inhibitors. The uterine serpin has only a weak antiproteinase activity and, at least in the sheep, appears to function as an inhibitor of lymphocyte proliferation to mediate the immunosuppressive effects of progesterone on uterine immune function. The IFN-τ are not present in mammals that diverged from ancestors of ruminants before 36 million years ago, the approximate origin of IFN-τ, but uterine serpins apparently arose before the divergence of mammals and the proteins are present in at least one nonruminant species, the pig. The clinical value of these molecules can extend beyond use in ruminants. IFN-τ has been used to treat experimental allergic encephalomyelitis in mice and can inhibit human immunodeficiency virus replication. Ovine uterine serpin is also active against murine and human cells but a clinical use has not yet been identified.

Introduction

The pecoran ruminants diverged as a separate infraorder during the Middle Eocene epoch about 33–46 million years ago and radiated into 5 separate families during the Early/Late Olicene transition about 20–29 million years ago

[1]. While the basic pattern of reproduction in pecoran ruminants is similar to other mammals, there are distinct features of reproduction including, most prominently, characteristics of placental anatomy [2]. There are also several molecules involved in endocrine and paracrine signaling processes during pregnancy that have been described in pecoran ruminants only.

Despite the unique features of pecoran reproduction, the immunological relationship between the conceptus and mother is similar in many ways to that of other mammals. The uterus of ruminants contains components of the immune system capable of tissue graft rejection responses [3, 4]. Like for other species, expression of MHC antigens on the trophoblast is largely downregulated [5, 6] although, at least in the cow, there is limited expression of class I MHC molecules on trophoblast in later pregnancy and the degree of fetal-maternal MHC compatibility affects placentomal lymphocyte populations and cytokine expression [6]. That extensive regulation of lymphocyte populations in the uterus takes place is indicated by the dynamic changes in uterine lymphocyte populations that occur during pregnancy. In the sheep, for example, macrophages accumulate in large numbers in the endometrial stroma during pregnancy [7] and a population of granulated γδ T cells becomes abundant in luminal epithelium of the interplacentomal regions during mid and late pregnancy [8, 9]. In contrast, numbers of nongranulated T cells in the glandular epithelium decline during pregnancy while numbers of these cells in luminal epithelium first decline and then return to levels seen in nonpregnant ewes [8, 9]. T cells are nearly absent in the placentomes [5].

The nature of the regulatory signals controlling changes in lymphocyte function during pregnancy is incompletely understood. Studies in which pregnancy is confined to one uterine horn have provided evidence for both systemic and local regulation of endometrial leukocyte populations [7, 9]. Both placental and endometrial tissues also produce a variety of cytokines and growth factors. For the purposes of this review, focus is placed on the properties of two immunoregulatory molecules that were initially discovered in ruminants and which appear to represent genes that have undergone evolution to perform a function distinct from that of the ancestral gene. Interferon-τ (IFN-τ) is a product of the trophoblast whose synthesis peaks around the time when placental attachment begins. While it retains its antiviral activity and other functions characteristic of interferons, the IFN-τ gene has evolved to encode for a protein whose primary role is the inhibition of luteolysis to maintain sustained progesterone secretion [10]. The other protein, uterine serpin, is a progesterone-induced member of the serpin superfamily of serine proteinase inhibitors. Uterine serpin has only weak antiproteinase activity and appears to function as an inhibitor of lymphocyte proliferation to mediate the immunosuppressive effects of progesterone on uterine immune function [11].

Interferon-τ

The IFN-τ gene arose via gene duplication from IFN-ω about 36 million years ago [12], a time before the radiation of the pecoran ruminants about 20–29 million years ago [1]. Thus, IFN-τ genes have been found in a variety of pecoran ruminants including the cow, sheep, goat, musk ox, and giraffe. The genes continued to evolve after the initial gene duplication – 12 genes have been identified in sheep and 9 in cattle.

As pointed out by Roberts et al. [12], it is not its activity that makes IFN-τ such an important molecule in maintaining corpus luteum function because other interferons can also block luteolysis [13] and because IFN-τ retains the antiviral, antiproliferative, natural killer (NK) cell-activating, and immunosuppressive properties of type I interferons [14–18]. Rather it is its unique tissue-specific and temporal expression pattern. In particular, IFN-τ gene expression is limited to the trophoblast for only a few days at a time coincident with the time when the uterus is poised to produce prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) pulses that lead to luteolysis. In the cow, for example, the trophoblast produces low amounts of IFN-τ beginning at the expanded blastocyst stage at ~day 8 of pregnancy [19]. Synthesis becomes prominent by days 12–13, peaks at days 15–17, and continues to be produced in decreasing amounts until it ceases between day 25 and 29 [20–22]. These times correspond to the programmed time for luteolytic release of $PGF_{2\alpha}$ at days 16–19 after estrus [23]. The evolutionary changes that lead to this pattern of gene expression must therefore have involved changes in the transcriptional control of IFN-τ gene expression. IFN-τ genes have no apparent viral control elements in the promoter region but do contain an Ets-2 binding site that is likely required for expression in the trophoblast [12]. Signals from the uterus are important for induction of IFN-τ gene expression; expression can be induced by placing in vitro derived embryos in the uterus for several days [19]. The specific uterine signal involved is not known. There is evidence supporting the induction of IFN-τ gene expression by maternal granulocyte colony-stimulating factor (GM-CSF) and interleukin-3 [24] although effects of GM-CSF have not always been observed [25].

The mechanism by which IFN-τ blocks luteolysis involves disruption of the control of $PGF_{2\alpha}$ synthesis in the uterus. In the cow, IFN-τ induces synthesis of linoleic acid which, in turn, acts as a competitive inhibitor to cyclooxygenase 2 (COX-2) that is the rate-limiting enzyme for $PGF_{2\alpha}$ synthesis [26]. In addition, IFN-τ acts through a transcriptional-dependent mechanism to decrease the stability of COX-2 mRNA [27]. Induction of $PGF_{2\alpha}$ synthesis by endometrial epithelial cells requires actions of both estradiol and oxytocin. In sheep, IFN-τ reduces estrogen receptor-α gene expression, which in turn leads to reduction in amounts of oxytocin receptor [10].

The degree to which immunoregulatory functions of IFN-τ are important for pregnancy is unclear. In fact, the endometrium appears resistant to some of the actions of IFN-τ. Thus, although like other interferons, IFN-τ inhibits lymphocyte proliferation and causes activation of NK cells [17, 18], there was no noticeable effect of the conceptus on endometrial lymphocyte populations at day 16 of pregnancy in cattle [28]. A possible amplifying effect of IFN-τ on presentation of placental antigens through upregulation of class I MHC molecules is limited by the fact that uterine luminal epithelial cells (although not stroma or glandular epithelium) are resistant to this action of IFN-τ [29]. Similarly, endometrial epithelium and stroma are resistant to the antiproliferative action of IFN-τ [30]. There are a large number of endometrial genes whose expression is changed by IFN-τ, however, and some of these could conceivably alter the immune status of the conceptus-maternal interaction. Among these are monocyte chemotactic protein-1 and 2 in endometrial eosinophils [31] and GM-CSF in the luminal epithelium [32].

The Uterine Serpins

The uterine serpins are members of a large superfamily of proteins that, prototypically, fold into a conserved structure and inhibit serine proteinases through a unique suicide-like mechanism [33, 34]. Uterine serpins have been identified in sheep [35], cattle [36], goats [37], and pigs [38] and are therefore more widely distributed phylogenetically than the IFN-τ genes. It has been estimated that uterine serpins diverged at some point before the divergence of mammals [39] and it is possible that uterine serpins are widely distributed among mammalian orders. Depending on analytical methods used, the uterine serpins are classified either as a distinct serpin clade or as a highly diverged group of the α_1-antitrypsin clade [33, 39].

Many serpins have evolved to have functions distinct from proteinase inhibition and this appears true for uterine serpins. The best studied of these, the ovine uterine serpin (OvUS), can inhibit proliferation of αβ T lymphocytes [11] and has been proposed to protect the conceptus from destruction by the maternal immune system [40]. The porcine uterine serpins form complexes with the iron-containing protein, uteroferrin [41], and may be involved in metabolism of that protein. In addition, OvUS exhibits weak pepsin inhibitory activity [36, 42] and can bind to IgA and IgM [43] and activin [44].

All uterine serpins are products of endometrial epithelium [predominately glandular epithelium for most of pregnancy; see 45, 46]. The major signal for induction of synthesis for the ovine and bovine proteins (and presumably caprine

and porcine proteins) is progesterone [47] although placental lactogen or growth hormone can also increase OvUS mRNA in ovariectomized ewes treated with progesterone and IFN-τ [48].

The diversity of effects of OvUS on cells of the immune system and its potential mechanism of action have been reviewed [11]. This protein can inhibit activation of T cells induced by a variety of stimuli, NK cell activation in culture, NK cell-mediated abortion in vivo, and antibody production in response to T cell-dependent antigen, ovalbumin. The protein appears not to inhibit γδ T cells and the resistance of these cells to OvUS may explain in part why their numbers in the luminal epithelium of the endometrium rise in mid and late pregnancy [8]. What is unusual about OvUS as an immunoregulator is that it is not very potent – concentrations in the 100–500 μg/ml range are required to inhibit T cells and NK cells in vitro. These concentrations are well within the physiological ranges of OvUS in uterine fluid, however, and the inhibitory effects of OvUS are likely to be physiologically relevant. Nonetheless, the fact that the protein is only active at high concentrations suggests that it acts to inhibit lymphocytes through some unusual mechanism. Perhaps, the protein has a low affinity for an immunosuppressive receptor or competes with the native ligand for receptors required for lymphocyte activation.

Relevance to Other Species

Whether or not IFN-τ or uterine serpins play an important role in species other than the ones mentioned in this review depends upon their phylogeny. IFN-τ did not arise until soon before pecoran ruminants emerged [12] and therefore one would not find IFN-τ genes in orders of mammals that diverged before this time. Conversely, the early emergence of uterine serpins may mean that these proteins are produced in the uterus of a wide range of mammalian species. It may, however, be that the uterine serpin genes were not retained as functional genes in all species or exhibit expression in tissues other than the uterus.

Regardless of their role in species other than ruminants, there is clinical value in using the ruminants as a source of gene products with therapeutic potential. IFN-τ is less cytotoxic than other type I interferons [15] and has been used to treat experimental allergic encephalomyelitis in mice [49]. IFN-τ also inhibits human immunodeficiency virus replication [50]. Ovine uterine serpin is also active against murine [51, 52] and human cells [53] and either the uterine serpins or a more potent derivative of the serpins may prove useful in regulating lymphocyte proliferative responses.

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References

1 Hassanin A, Douzery EJ: Molecular and morphological phylogenies of ruminantia and the alternative position of the moschidae. Syst Biol 2003;52:206–228.
2 Carter AM, Enders AC: Comparative aspects of trophoblast development and placentation. Reprod Biol Endocrinol 2004;2:46.
3 Hansen PJ, Bazer FW, Segerson EC: Skin graft survival in the uterine lumen of ewes treated with progesterone. Am J Reprod Immunol Microbiol 1986;12:48–54.
4 Majewski AC, Hansen PJ: Progesterone inhibits rejection of xenogeneic transplants in the sheep uterus. Horm Res 2002;58:128–135.
5 Gogolin-Ewens KJ, Lee CS, Mercer WR, Brandon MR: Site-directed differences in the immune response to the fetus. Immunology 1989;66:312–317.
6 Davies CJ, Hill JR, Edwards JL, Schrick FN, Fisher PJ, Eldridge JA, Schlafer DH: Major histocompatibility antigen expression on the bovine placenta: Its relationship to abnormal pregnancies and retained placenta. Anim Reprod Sci 2004;82–83:267–280.
7 Tekin S, Hansen PJ: Regulation of numbers of macrophages in the endometrium of the sheep by systemic effects of pregnancy, local presence of the conceptus, and progesterone. Am J Reprod Immunol 2004;51:56–62.
8 Lee CS, Meeusen E, Gogolin-Ewens K, Brandon MR: Quantitative and qualitative changes in the intraepithelial lymphocyte population in the uterus of nonpregnant and pregnant sheep. Am J Reprod Immunol 1992;28:90–96.
9 Majewski AM, Tekin S, Hansen PJ: Local versus systemic control of numbers of endometrial T-cells during pregnancy in sheep. Immunology 2001;102:317–322.
10 Spencer TE, Burghardt RC, Johnson GA, Bazer FW: Conceptus signals for establishment and maintenance of pregnancy. Anim Reprod Sci 2004;82–83:537–550.
11 Peltier MR, Hansen PJ: Immunoregulatory activity, biochemistry, and phylogeny of ovine uterine serpin. Am J Reprod Immunol 2001;45:266–272.
12 Roberts RM, Ezashi T, Rosenfeld CS, Ealy AD, Kubisch HM: Evolution of the interferon τ genes and their promoters, and maternal-trophoblast interactions in control of their expression. Reprod Suppl 2003;61:239–251.
13 Plante C, Hansen PJ, Thatcher WW: Prolongation of luteal lifespan in cows by intrauterine infusion of recombinant alpha-interferon. Endocrinology 1988;122:2342–2344.
14 Pontzer CH, Bazer FW, Johnson HM: Antiproliferative activity of a pregnancy recognition hormone, ovine trophoblast protein-1. Cancer Res 1991;51:5304–5307.
15 Pontzer CH, Yamamoto JK, Bazer FW, Ott TL, Johnson HM: Potent anti-feline immunodeficiency virus and anti-human immunodeficiency virus effect of IFN-τ. J Immunol 1997;158:4351–4357.
16 Alexenko AP, Ealy AD, Roberts RM: The cross-species antiviral activities of different IFN-τ subtypes on bovine, murine, and human cells: Contradictory evidence for therapeutic potential. J Interferon Cytokine Res 1999;19:1335–1341.
17 Tekin S, Ealy AD, Wang S-Z, Hansen PJ: Differences in lymphocyte-regulatory activity among variants of ovine interferon-τ. J Interferon Cytokine Res 2000;20:1001–1005.
18 Tekin S, Hansen PJ: Natural-killer like cells in the sheep: Functional characterization and regulation by pregnancy-associated proteins. Exp Biol Med 2002;227:803–811.
19 Hernandez-Ledezma JJ, Sikes JD, Murphy CN, Watson AJ, Schultz GA, Roberts RM: Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. Biol Reprod 1992; 47:374–380.

20 Bartol FF, Roberts RM, Bazer FW, Lewis GS, Godkin JD, Thatcher WW: Characterization of proteins produced in vitro by periattachment bovine conceptuses. Biol Reprod 1985;32:681–693.
21 Helmer SD, Hansen PJ, Anthony RV, Thatcher WW, Bazer FW, Roberts RM: Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1. J Reprod Fertil 1987;79:83–91.
22 Farin CE, Imakawa K, Hansen TR, McDonnell JJ, Murphy CN, Farin PW, Roberts RM: Expression of trophoblastic interferon genes in sheep and cattle. Biol Reprod 1990;43:210–218.
23 Kotwica J, Skarzynski D, Jaroszewski J, Williams GL, Bogacki M: Uterine secretion of prostaglandin $F_{2\alpha}$ stimulated by different doses of oxytocin and released spontaneously during luteolysis in cattle. Reprod Nutr Dev 1998;38:217–326.
24 Imakawa K, Carlson KD, McGuire WJ, Christenson RK, Taylor A: Enhancement of ovine trophoblast interferon by granulocyte macrophage-colony stimulating factor: Possible involvement of protein kinase C. J Mol Endocrinol 1997;19:121–130.
25 de Moraes AAS, Davidson JA, Fleming JGW, Bazer FW, Edwards JL, Betts JG, Hansen PJ: Lack of effect of granulocyte-macrophage colony-stimulating factor on secretion of interferon-τ, other proteins and prostaglandin E_2 by the bovine and ovine conceptus. Domest Anim Endocrinol 1997; 14:193–197.
26 Thatcher WW, Meyer MD, Danet-Desnoyers G: Maternal recognition of pregnancy. J Reprod Fertil Suppl 1995;49:15–28.
27 Guzeloglu A, Subramaniam P, Michel F, Thatcher WW: Interferon-τ induces degradation of prostaglandin H synthase-2 messenger RNA in bovine endometrial cells through a transcription-dependent mechanism. Biol Reprod 2004;71:170–176.
28 Leung ST, Derecka K, Mann GE, Flint AP, Wathes DC: Uterine lymphocyte distribution and interleukin expression during early pregnancy in cows. J Reprod Fertil 2000;119:25–33.
29 Choi Y, Johnson GA, Spencer TE, Bazer FW: Pregnancy and interferon tau regulate major histocompatibility complex class I and β_2-microglobulin expression in the ovine uterus. Biol Reprod 2003;68:1703–1710.
30 Davidson JA, Betts JG, Tiemann U, Kamwanja LA, Monterroso VH, Hansen PJ: Effects of interferon-τ and interferon-α on proliferation of bovine endometrial cells. Biol Reprod 1994; 51:700–705.
31 Asselin E, Johnson GA, Spencer TE, Bazer FW: Monocyte chemotactic protein-1 and -2 messenger ribonucleic acids in the ovine uterus: Regulation by pregnancy, progesterone, and interferon-τ. Biol Reprod 2001;64:992–1000.
32 Emond V, MacLaren LA, Kimmins S, Arosh JA, Fortier MA, Lambert RD: Expression of cyclooxygenase-2 and granulocyte-macrophage colony-stimulating factor in the endometrial epithelium of the cow is up-regulated during early pregnancy and in response to intrauterine infusions of interferon-τ. Biol Reprod 2004;70:54–64.
33 Irving JA, Pike RN, Lesk AM, Whisstock JC: Phylogeny of the serpin superfamily: Implications of patterns of amino acid conservation for structure and function. Genome Res 2000;10: 1845–1864.
34 Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG, Irving JA, Lomas DA, Luke CJ, Moyer RW, Pemberton PA, Remold-O'Donnell E, Salvesen GS, Travis J, Whisstock JC: The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. J Biol Chem 2001;276:33293–33296.
35 Ing NH, Roberts RM: The major progesterone-induced proteins secreted into the sheep uterus are members of the serpin superfamily of protease inhibitors. J Biol Chem 1989;264:3372–3379.
36 Mathialagan N, Hansen TR: Pepsin-inhibitory activity of the uterine serpins. Proc Natl Acad Sci USA 1996;93:13653–13658.
37 Tekin S, Padua MB, Newton GR, Hansen PJ: Identification and cloning or caprine uterine serpin. Mol Reprod Dev 70:262–270.
38 Malathy PV, Imakawa K, Simmen RC, Roberts RM: Molecular cloning of the uteroferrin-associated protein, a major progesterone-induced serpin secreted by the porcine uterus, and the expression of its mRNA during pregnancy. Mol Endocrinol 1990;4:428–440.

39 Peltier MR, Raley LC, Liberles DA, Benner SA, Hansen PJ: Evolutionary history of the uterine serpins. J Exp Zool 2000;288:165–174.
40 Hansen PJ: Regulation of uterine immune function by progesterone – lessons from the sheep. J Reprod Immunol 1998;40:63–79.
41 Baumbach GA, Ketcham CM, Richardson DE, Bazer FW, Roberts RM: Isolation and characterization of a high molecular weight stable pink form of uteroferrin from uterine secretions and allantoic fluid of pigs. J Biol Chem 1986;261:12869–12878.
42 Peltier MR, Grant TR, Hansen PJ: Distinct physical and structural properties of the ovine uterine serpin. Biochim Biophys Acta 2000;1479:37–51.
43 Hansen PJ, Newton GR: Binding of immunoglobulins to the major progesterone-induced proteins of the sheep uterus. Arch Biochem Biophys 1988;260:208–217.
44 McFarlane JR, Foulds LM, O'Connor AE, Phillips DJ, Jenkin G, Hearn MT, de Kretser DM: Uterine milk protein, a novel activin-binding protein, is present in ovine allantoic fluid. Endocrinology 1999;140:4745–4752.
45 Stephenson DC, Leslie MV, Low BG, Newton GR, Hansen PJ, Bazer FW: Secretion of the major progesterone-induced proteins of the sheep uterus by caruncular and intercaruncular endometrium of the pregnant ewe from days 20–140 of gestation. Domest Anim Endocrinol 1989;6:349–362.
46 Stewart MD, Johnson GA, Gray CA, Burghardt RC, Schuler LA, Joyce MM, Bazer FW, Spencer TE: Prolactin receptor and uterine milk protein expression in the ovine endometrium during the estrous cycle and pregnancy. Biol Reprod 2000;62:1779–1789.
47 Leslie MV, Hansen PJ: Progesterone-regulated secretion of the serpin-like proteins of the ovine and bovine uterus. Steroids 1991;56:589–597.
48 Spencer TE, Gray CA, Johnson GA, Taylor KM, Gertler A, Gootwine E, Ott TL, Bazer FW: Effects of recombinant ovine interferon tau, placental lactogen and growth hormone in the ovine uterus. Biol Reprod 1999;61:1409–1418.
49 Soos JM, Subramaniam PS, Hobeika AC, Schiffenbauer J, Johnson HM: The IFN pregnancy recognition hormone IFN-τ blocks both development and superantigen reactivation of experimental allergic encephalomyelitis without associated toxicity. J Immunol 1995;155:2747–2753.
50 Rogez C, Martin M, Dereuddre-Bosquet N, Martal J, Dormont D, Clayette P: Anti-human immunodeficiency virus activity of tau interferon in human macrophages: Involvement of cellular factors and β-chemokines. J Virol 2003;77:12914–12920.
51 Stephenson DC, Hansen PJ, Isaak DD: Inhibition of antigen- and mitogen-induced activation of mouse splenocytes by uterine secretions from pregnant and progesterone-treated ewes. Am J Reprod Immunol 1991;26:178–185.
52 Liu W-J, Hansen PJ: Effect of the progesterone-induced serpin-like proteins of the sheep endometrium on natural-killer cell activity in sheep and mice. Biol Reprod 1993;49:1008–1014.
53 Fitzgerald JS, Tekin S, Markert UR, Hansen PJ: Effects of ovine uterine serpin (OvUS) on interleukin production in human lymphocytes (abstract). Am J Reprod Immunol 2001;45:350.

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