MINIREVIEW

Role of Interferons in Maternal Recognition of Pregnancy in Ruminants (43387A)

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Abstract. **It has recently become evident that a type I interferon (IFN) subtype signals the presence of a viable conceptus to the mother during early pregnancy in cattle, sheep, and related mammalian species. This IFN, which is a product of the epithelium (trophectoderm) of the expanding trophoblast, is expressed in extremely large quantities for a few days just prior to implantation. It appears to be involved in modulating the release of the luteolytic hormone, prostaglandin $F_{2\alpha}$, from the uterine endometrium and, hence, preventing the destruction of the corpus luteum that normally occurs at the end of an estrous cycle if an egg has not been fertilized. These trophoblast IFN have antiviral, antiproliferative, and immunomodulatory properties quite similar to other type I IFN, such as IFN-α, -β, and -ω. However, they constitute a structurally and serologically distinct subtype. In addition, they are poorly inducible by virus, and the promoter regions of their genes are organized differently than other type I IFN. The genes for these trophoblast IFN are confined to ruminant species in the Artiodactyla order and probably evolved from IFN-ω less than 55 million years ago. There is no evidence for comparable production of type I IFN by trophoblast and placental tissues of mammals outside this ruminant group. Recent experiments have indicated that IFN treatment may have value in improving reproductive performance of sheep when provided during the period of maternal recognition of pregnancy, when much embryonic loss is believed to occur.**

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The term maternal recognition of pregnancy was coined to describe the biochemical and physiological processes that allow the pregnant mother to adjust to the presence of an embryo in her reproductive tract. It has also been used more narrowly to describe those events that allow the lifespan of the corpus luteum to be extended when an embryo is present in the uterus, so avoiding a return to ovarian cyclicity and a loss of progesterone production at a crucial time in pregnancy when placentation is in its early stages (see Ref. 1). In many species, the requirement for ovarian progesterone may only be short lived and is soon superseded when the placenta itself begins to synthesize steroids in quantity (2). Nevertheless, in all mammalian species where the length of the pregnancy exceeds that of the normal ovarian cycle, the corpus luteum must be "rescued" in some manner.

In at least some species of primates, chorionic gonadotropin produced by the implanting trophoblast has a direct luteotrophic role on the corpus luteum and is thought to be primarily responsible for luteal maintenance (3). Nevertheless, chorionic gonadotropin is almost certainly not the sole bioactive compound released by the implanting primate embryo (4). On the other hand, there is no strong evidence for production of a chorionic gonadotropin in early pregnancy of most other mammals, including domestic ruminant species (1, 3).

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It is generally agreed that in cattle and sheep, the corpus luteum regresses toward the end of a normal estrous cycle because the uterus releases a luteolytic hormone, prostaglandin (PG) $F_{2\alpha}$, in a pulsatile manner (1, 5) (Fig. 1). In pregnancy, even though $PGF_{2\alpha}$ may continue to be secreted, pulsatility is dampened (6), and the corpus luteum survives throughout pregnancy. By about 1980 it became evident that the antiluteolytic substance released by the conceptuses of sheep and cattle was most probably a protein that was produced maximally for only a few days of pregnancy (roughly between Days 13 and 20 in sheep and for a week or so after Day 15 in cattle) at a time when the trophoblast is only loosely attached to the uterine wall (see Ref. 7 for review). The ovine factor present in conceptus extracts was highly effective in extending the estrous cycle of nonpregnant ewes if it was introduced directly into the uterine lumen. This observation suggested that its action was probably on the uterine lining (the endometrium) and not on the ovary itself. In 1982, a product secreted by the ovine conceptuses was purified that appeared to bear all the hallmarks of the so-called antiluteolysin (8). This protein consisted of several isoforms with mol wt of about 18,000 and was the major secretory product of conceptus trophoblast tissue during the critical Day 13–15 period, when maternal recognition of pregnancy occurs in ewes. It became known as ovine trophoblast protein-1 (oTP-1), but was probably identical to the factor "trophoblastin" that had been extracted from homogenized conceptuses by earlier workers (9).

Purified oTP-1 is able to extend the interestrous interval of nonpregnant ewes when injected into the uterine lumen during the period of maternal recognition of pregnancy (10) and reduces the pulsatile release of $PGF_{2\alpha}$ from the uterus (11, 12). The protein itself does not appear to escape the uterus and has not been detected in the peripheral circulation of pregnant sheep (13, 14). It is not suspected, therefore, of having any direct luteotrophic action on the corpus luteum. A protein with very similar properties (known as bTP-1) was subsequently characterized as a secretory product of bovine conceptus (15). It too has been shown to be antiluteolytic (16, 17) and to block pulsatile $PGF_{2\alpha}$ output (18). More recently, a caprine TP-1 has been identified in goats (19) that seems to be very similar to the ovine and bovine proteins.

In all these ruminant species, the production of the trophoblastic antiluteolysin coincides with a dramatic change in the morphology of the conceptus (see Ref. 20). In sheep, for example, the conceptus at Day 12 is usually still spherical, unattached to the uterine wall, and no more than about 2 mm in diameter. On Day 13, this sphere begins to elongate and can become several centimeters long within 24 hr. By Day 17, a conceptus may reach 15 cm or longer, and, where only a single ovulation has occurred, stretch into both uterine horns. At this stage, the trophectoderm (the outer epithelial layer of the trophoblast) is definitively attached to the uterine epithelium (though the conceptus can still be removed by simply flushing the uterine lumen) and implantation has begun at discrete sites that will eventually become placentomes, which are the main foci of blood flow and gas and nutrient exchange in the uterus (21). In cattle, events are delayed by about 2 days, relative to sheep, but proceed in much the same manner. Thus, the major phase of trophoblast protein synthesis begins at about the stage of blastocyst expansion and is in decline by the time that the maternal uterine epithelium is extensively eroded by the intrusion of trophoblast binucleate cells (22).

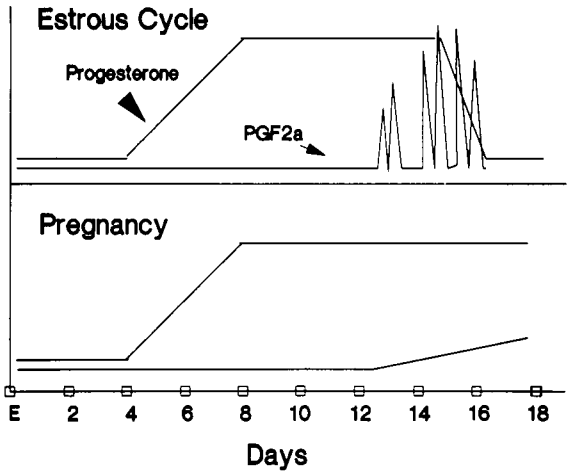

Figure 1. A diagram illustrating changes in plasma progesterone and prostaglandin $F_{2\alpha}$ concentrations in the utero-ovarian veins of a ewe during the estrous cycle or pregnancy. Note that during pregnancy, although prostaglandin $F_{2\alpha}$ may rise, pulsatile release from the uterus is diminished. Reproduced from BioEssays **13**:121, with permission.

Molecular Cloning of oTP-1 and bTP-1 cDNA and Identification of the Proteins as Type I Interferons

Molecular cloning of the cDNA for oTP-1 (23–26) and later bTP-1 (27–29), as well as N-terminal protein sequencing of purified polypeptides (30, 31), revealed that both trophoblast proteins were related structurally to the alpha-interferon (IFN) family of proteins. The mRNA themselves were about 1 kb in length and possessed a 585-base open reading frame that coded for a polypeptide of 195 amino acids, of which the first 23 residues were a signal peptide. The length of the mature polypeptide (172 amino acids), however, clearly distinguished the trophoblast proteins from the typical 165- or 166-amino acid IFN-α. In addition, their predicted amino acid sequences showed only about 50% identity to various bovine IFN-α whose cDNA sequences were known. On the other hand, they were considerably more similar (about 70% sequence identity) to a little-studied group of longer (172–174 residues long) IFN

that had been named either IFN-α_{II} or IFN-ω (see Ref. 28), although the latter term is now recommended. Both IFN-α and IFN-ω, along with the more distantly related IFN-β, are known as type I IFN because they are likely derived from a common ancestral gene, share a common receptor, and are coded by genes that are clustered together on the same chromosome (32). The properties of these different IFN are compared in Table I. The trophoblast IFN showed no similarity to type II (of γ) IFN, which is also known as immune interferon because of its production by T cells.

That oTP-1 and bTP-1 are indeed functional IFN has been confirmed in a number of biological tests. For example, their antiviral activity is broad and of a magnitude comparable to that of other type I IFN (about 10^8 IV/mg) (32–36). They also can inhibit the proliferation of lymphocytes and other cells with a potency comparable to IFN-α (34, 37, 38). Different isoforms of oTP-1 show comparable antiviral (39) and immunomodulatory (40) properties and have not so far displayed subtly different biological activities that would distinguish one from another. Finally, the trophoblast proteins can compete with IFN-α for binding to Type I IFN receptors (30, 41, 42) and induce a comparable set of proteins in endometrial tissue (43).

The trophoblast proteins are probably best referred to, therefore, as trophoblast IFN. For reasons that will become clear, it is presently not recommended that they simply be included in the IFN-ω that they most resemble.

Expression of the Trophoblast IFN during Development

Trophoblast IFN are products of the trophectoderm, which constitutes the outer epithelial layer of the conceptus (13, 44–47) (Fig. 2). High rates of ovine trophoblast IFN synthesis occur between Days 13 and 21 of pregnancy (8, 48), with production increasing over three orders of magnitude from Day 12, when synthesis is low, to Day 15–16, when total synthesis is maximal (49, 50). However, demonstrable amounts of ovine trophoblast IFN are detectable as early as Days 8–10 of pregnancy (49), approximately 1 week before peak production at the time of conceptus elongation. The amounts of IFN produced around Days 14–16 are extremely high, with some individual conceptuses able to release as much as 0.5 mg in a 24-hr culture period. Bovine conceptuses appear to have a roughly equivalent ability to synthesize bovine trophoblast IFN for a few days after they begin the morphological transition from spherical to elongated forms (47, 51). More than 99% of the IFN released during this period can be neutralized by antiserum to bTP-1, and the antiviral activity has been estimated to be up to two orders of magnitude greater than from a population of Sendai virus-activated bovine leukocytes when calculated in relation to the RNA content of the tissues (51). Significantly, none of the leukocyte activity is neutralizable with anti-bTP-1 antiserum, but instead appears to be largely IFN-α.

Estimates of trophoblast IFN mRNA content of both ovine and bovine conceptuses by either Northern or dot blot analysis has correlated well with the data on protein production discussed above (23–25, 47, 52). *In situ* hybridization analysis has shown that the amount of mRNA per trophoblast cell increases most markedly as the spherical blastocysts begin to elongate, rather than strictly correlating with day of pregnancy (46, 47, 53). Such measurements have also confirmed that mRNA is detectable in low concentrations several days before this stage of elongation is reached. Thus, a period of demonstrable, but nevertheless low, expression is followed by a more massive induction at the time of maternal recognition of pregnancy.

IFN Effects on the Uterus

The uterus is the site of production of the uterine luteolysin $PGF_{2\alpha}$ (5), and it is the pulsatile output of this prostanoid that is believed to be modulated in some manner by the trophoblast IFN during early pregnancy (1). Consistent with such a mechanism, the ovine en-

Table I. A Comparison of the Properties of Type I Interferons

Property	IFN-α	IFN-β	IFN-ω	Trophoblast IFN
Type I bioactivity				
Antiviral	Yes	Yes	Yes	Yes
Antiproliferative	Yes	Yes	Yes	Yes
Mature protein (amino acid)	165–166	166	172–174	172
Receptor	Type I	Type I	Type I	Type I
Virus inducibility	++++	++++	++++	(+)
Conceptus expression (ruminants)	(+)	?	(+)	++++
Tissue distribution	Leukocytes + others	Fibroblasts + others	T cells + others	Trophoblast
Species distribution	All mammals	All mammals	Most mammals	Ruminants
Number of genes (sheep and cattle)	>10	5–6	>10	4–5

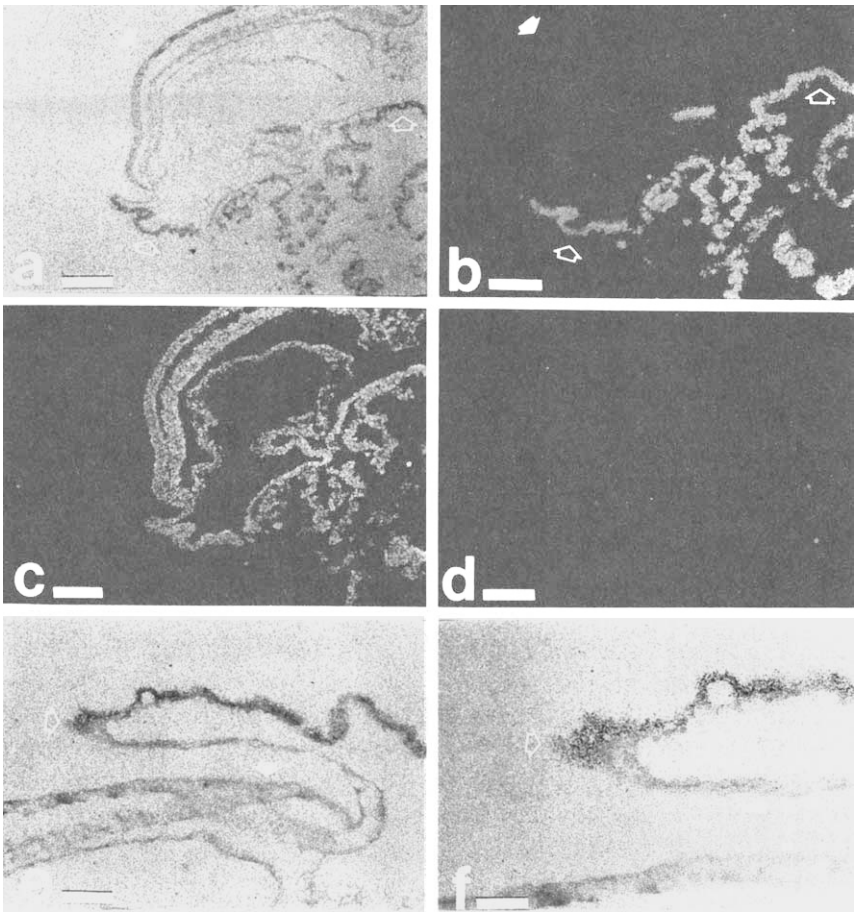

Figure 2. Localization of ovine trophoblast IFN in Day 15 ovine conceptus tissue by *in situ* hybridization. (a) Brightfield micrograph of a section from a Day 15 ovine conceptus hybridized with an ^{35}S-labeled probe to ovine trophoblast IFN. Portions of both embryonic disc (closed arrow) and trophoblast (open arrows) are illustrated. Section was lightly stained with toluidine blue. (b) Darkfield micrograph of the same section shown in (a). Note the strong hybridization to trophoblast (open arrows) but not to embryonic disc (closed arrow). (c) Adjacent section hybridized with an ^{35}S-actin cDNA probe showing positive hybridization in all tissues. (d) Adjacent section (control) hybridized with an [^{35}S]pUC probe demonstrating low background hybridization signal. Scale bar (a–d) = 36 μm. (e) and (f) Micrographs illustrating the abrupt transition of trophoblast mRNA expression between cells of the trophoblast (open arrows) and those of the embryonic epiblast (closed arrows). Scale bars (e) = 18 μm and (f) = 9 μm. Reproduced from Ref. 46 with permission.

dometrium has a high content of type I IFN receptors (41, 42). However, the means whereby $PGF_{2\alpha}$ production or output is altered remains unclear.

Experiments to determine whether type I IFN can interfere with prostaglandin synthesis in endometrial tissue have yielded inconsistent results. Both human IFN-α and ovine trophoblast IFN had a slight inhibitory effect on production of $PGF_{2\alpha}$ and a related but nonluteolytic prostanoid, PGE, by cultured ovine endometrial cells (54). Bovine trophoblast IFN also inhibited $PGF_{2\alpha}$ secretion by tissue explants from bovine endometrium (15, 54, 55) and appeared to induce the buildup of an intracellular inhibitor of $PGF_{2\alpha}$ synthesis (56). More recent studies (57) have shown that bTP-1, oTP-1, bovine IFN-α, and human IFN-α were all equally effective in inhibiting $PGF_{2\alpha}$ synthesis by bovine endometrial explants obtained at Day 17 of the estrous cycle.

It should be emphasized, however, that in sheep, if not in cattle, total output of $PGF_{2\alpha}$ is not necessarily reduced during early pregnancy. Rather, it is the frequency and magnitude of pulses of hormone that determine whether luteolysis occurs (1, 6) (Fig. 1). Because oxytocin is considered to have a role in mediating $PGF_{2\alpha}$ release from endometrium during luteolysis, attention has recently been paid to whether IFN might inhibit the formation of oxytocin receptors immediately preceding the time that normal pulsatile output of $PGF_{2\alpha}$ is initiated (see Refs. 58 and 59). Although some evidence for an inhibitory effect has been noted, it is unclear how such a selective block to receptor formation may be accomplished by IFN.

IFN has been noted to have additional effects on endometrial protein synthesis, presumably by way of its binding to the abundant type I receptors present. For example, endometrial explants from Day 12 nonpregnant ewes (13, 43, 60) and primary uterine epithelial cells (54) have shown increased synthesis of a

selective group of secretory proteins that include a prominent acidic protein of M_r 70,000 (70 kDa protein) and a 15,000-M_r component. The latter has recently been tentatively identified as β_2-microglobulin, a component of the type I histocompatibility complex, which is known to be upregulated by IFN-α in other cell types (61). Production of the 70-kDa protein, in particular, is diagnostic of endometrium that has been in local contact with the conceptus (62). It has also been noted as a major product of the endometrium when ewes are injected intramuscularly with bovine IFN-α (43). Although the function and identity of this 70-kDa pregnancy-associated protein is unknown, it appears to be a highly sensitive marker of IFN action on the uterus and is evident during the peri-implantation period of pregnancy (62). Trophoblast IFN can also induce at least one component, the 2′,5′-oligo(A)adenylate synthetase, of the antiviral response pathway in endometrium (57, 63).

Effects of Administering Type I IFN on Estrous Cycle Length and Pregnancy

The trophoblast IFN purified from conceptus culture medium are, as discussed earlier, able to extend estrous cycle length when provided to sheep or cattle within the critical "window" of maternal recognition of pregnancy (10, 12, 15–17). These results raised the interesting possibility that IFN administration might be useful for manipulating fertility in these farm species. However, it became obvious that even pilot studies of this kind would require relatively large amounts of material that could only be purified in limited quantities and at considerable expense from natural sources. It is for this reason that a more widely available homolog of the trophoblast IFN, recombinant bovine IFN-α1, has been tested quite extensively. The use of such a recombinant IFN seemed reasonable, because the properties of the recombinant bovine IFN-α1 and the trophoblast IFN were almost indistinguishable by most *in vitro* tests. Additional studies have also demonstrated that some of the effects of trophoblast IFN on estrous cycle length can be mimicked by using recombinant bovine IFN-α1. For example, infusion of milligram quantities of the latter into the uteri of cyclic ewes (64), or intrauterine infusion or intramuscular injection of the protein into nonpregnant cows (15, 65, 66) during the period of maternal recognition of pregnancy extended luteal lifespan of the treated animals. Nevertheless, it has become evident from these experiments that considerably more IFN-α1 had to be introduced into the uterus to achieve an extension of interestrous interval than if "natural" trophoblast IFN had been used instead.

Despite the differences noted between trophoblast IFN and IFN-α treatments, the results with recombinant bovine IFN-α were sufficiently encouraging to prompt a series of studies to test whether this reagent could be used pharmacologically to improve pregnancy success in sheep and cattle. The rationale for these experiments was that a major cause of embryonic loss may be failure of the conceptus to signal its presence to the mother. Indeed, it is well known that many pregnancies terminate during this critical period (67). Conceivably, conceptuses lagging in their development produce insufficient trophoblast IFN to prevent the corpus luteum from regressing. Therefore, it was argued that providing supplemental IFN might "rescue" such conceptuses. It was necessary to administer the IFN by intramuscular injection, since any disturbance to the pregnant uterus at this time causes abortion. That such injected IFN reached the uterine lining has been proved by its effects on protein synthesis (43). Despite this "unnatural" mode of administration, the injected IFN significantly increased pregnancy rate (but not average litter size) in treated ewes (14, 68). In one study (69), the number of ewes carrying a lamb to term was increased by almost 20% in the IFN-treated group. In none of the experiments was there any increase in stillbirths, abnormal progeny, or postpartum deaths. This considerable success in rescuing normal embryos that might otherwise have been lost was somewhat surprising, since it has been suggested that pregnancy losses in livestock (70) as well as in humans (71) are associated with chromosomal abnormalities that would presumably have been evident in the sheep studies as early abortions, stillbirths, or malformed young.

As a result of the sheep work, it seemed likely that IFN-α treatment for a few days in early pregnancy might also be useful as a means for improving reproductive efficiency in cattle, where the therapy would have considerably more commercial impact. To date, however, there have been no reports of increased fertility in cattle treated with IFN. Indeed, fever and lowering of serum progesterone concentrations (72), and even a lowering of pregnancy rate (73), have been noted. IFN therapy in humans for treatment of cancer and certain viral diseases also causes fever and a number of other serious side effects (74). Now that both recombinant ovine (75) and bovine trophoblast IFN (35) have become available, it will be of considerable interest if these "natural" reagents can be used effectively at lower doses to improve pregnancy success in livestock. Recently, a recombinant ovine trophoblast IFN, prepared in yeast, was shown to be highly effective at increasing estrous cycle length of ewes when introduced in low amounts into the uteri between Days 10 and 18 of the estrous cycle (75). These experiments lend support to the hypothesis that the trophoblast IFN, on an equivalent dose basis, may be much more potent at modulating reproductive parameters than IFN-α. In this regard, it should be noted that Pontzer *et al.* (76) have used C-terminal and N-terminal peptides as competitors of

trophoblast IFN and IFN-α binding to endometrial receptors. The former competed well with both IFN subtypes and presumably bound to a common site on the receptor. The N-terminal peptide, however, competed only with the trophoblast IFN and had no effect on IFN-α binding. Conceivably, the trophoblast IFN interacts with a unique site on the receptor, and its binding to that domain triggers a distinct intracellular signaling pathway, so far undefined. It should be emphasized that there is considerable disagreement over the nature of the second messenger system for all types of IFN (77).

IFN Expression in Trophoblast and Placenta of Nonruminant Mammals and the Distribution of Trophoblast IFN Genes

There have been several reports of constitutive IFN production by trophoblast and placental tissues from a range of species other than cattle, sheep, and goats, raising the intriguing possibility that the process has broad general significance to pregnancy. In particular, it has been suggested that IFN might play a role in protecting the fetal allograft from immune rejection (see Refs. 78 and 79). However, it would be premature to regard trophoblast IFN production as a universal phenomenon among mammals. Here we shall briefly review the evidence in three very different species.

In the pig, conceptuses older than about 10 to 11 days release antiviral activity as measured in a cytopathic reduction assay (80, 81), although the amounts produced are considerably less than noted for cattle and sheep. In addition, most of this activity appears to be due to IFN-γ rather than to a type I IFN of the trophoblast IFN subtype (82, 83). There is no evidence to implicate IFN-γ or any other secretory protein product of the pig conceptus as having an antiluteolytic role in swine. However, it is intriguing that, like the trophoblast IFN, this potent cytokine is produced at the time of blastocyst elongation in the period immediately preceding firm attachment of the trophoblast to the uterine wall.

Considerable effort has been made to detect constitutive (rather than virally induced) IFN production by murine placental tissue during pregnancy (84–86). Although antiviral activity can be detected, even in the preimplantation blastocyst (87), the nature of the substance responsible for this activity at this and later stages remains equivocal. In some instances, partial neutralization has been noted with anti-IFN-α/β antiserum, while in others, anomalous molecular weights upon gel filtration and a failure to neutralize activity with antiserum have suggested that the substances may not be typical type I IFN. Yet other investigators have failed to note any constitutive production of antiviral activity in mouse tissue (88, 89).

A placental IFN was first suggested for humans by demonstrating that the antiviral activity in amniotic fluid from normal pregnancies was capable of being neutralized with anti-IFN-α antiserum (90). There have been several subsequent papers confirming such activity in placenta (91–93), though where reported, the molecular weights of the active factors again appeared anomalous and serological analysis was not invariably clear-cut. Immunocytochemical analysis has been employed to localize constitutive IFN-α, -β, and -γ activity to cells of the syncytiotrophoblast (94, 95). However, no evidence for constitutive IFN production by first trimester cytotrophoblast cells (96) or placental tissue (97) was found by this laboratory. Recently, production of IFN-β has been described from full-term cytotrophoblast and choriocarcinoma cells that had been induced by virus or double-stranded RNA (98). Although touted as a "trophoblast IFN" and undoubtedly having potential importance in protecting the fetus against viral infection, it seems unlikely that this IFN has a role in the normal physiology of pregnancy, since noninduced tissue produced little or no activity.

Attempts to demonstrate expression of mRNA structurally similar to those of ovine or bovine trophoblast IFN in nonruminant species such as human, pig, and horse (96), as well as mouse (87), have generally failed. Such negative results, though not conclusive, suggest that the uncharacterized IFN produced by placental and conceptus tissues of such species may not be the structural or even the functional equivalents of the trophoblast IFN. Conceivably, type I IFN has no role in conceptus signaling to the mother in these nonruminant species or is required in much smaller amounts to exert an effect.

Southern blotting with bovine IFN-ω cDNA probes has shown that the IFN-ω are widely distributed among mammals (99). Individual IFN-ω genes have also been cloned from species as different as the human (see Ref. 28), horse (100), and pig (101). Available evidence indicates that the IFN-ω must have split from the IFN-α at around the time of mammalian divergence 200 million years ago (99), although it is unclear why the genes have not been retained universally.

The distribution of the trophoblast IFN as judged by blotting with a cDNA probe derived from the 3′-end of a bovine cDNA is much narrower than the IFN-ω and appears to be restricted to ruminant species within the Artiodactyla order (99). These genes are present in deer and antelope species and giraffe as well as bovidae, but are absent in the pig and horse. On this basis, it seems likely that the trophoblast IFN diverged from the IFN-ω less than 55 million years ago.

Number and Organization of Trophoblast IFN Genes

The diversity of cloned cDNA that has been noted for the trophoblast IFN in sheep and cattle has strongly suggested the presence of multiple genes for the tropho-

blast IFN (23–27, 99, 102). A probe derived from the 3′-untranslated region of the trophoblast IFN cDNA was employed to identify trophoblast IFN genes selectively (Fig. 3). With this probe, only about five genes were identified from the 15 or so that bound a full-length cDNA, and these were distinct from the four genes that hybridized to a comparable 3′-specific IFN-ω probe.

Several bovine and ovine trophoblast IFN genes have been cloned from genomic libraries and their sequences reported (28, 29, 103). In addition, the promoter regions of the goat and musk ox genes have been isolated by use of the polymerase chain reaction procedure with primers that had been constructed on the basis of sequences derived from a bovine gene (99). The genes from all species show remarkable conservation within the upstream promoter region (Fig. 4), yet have

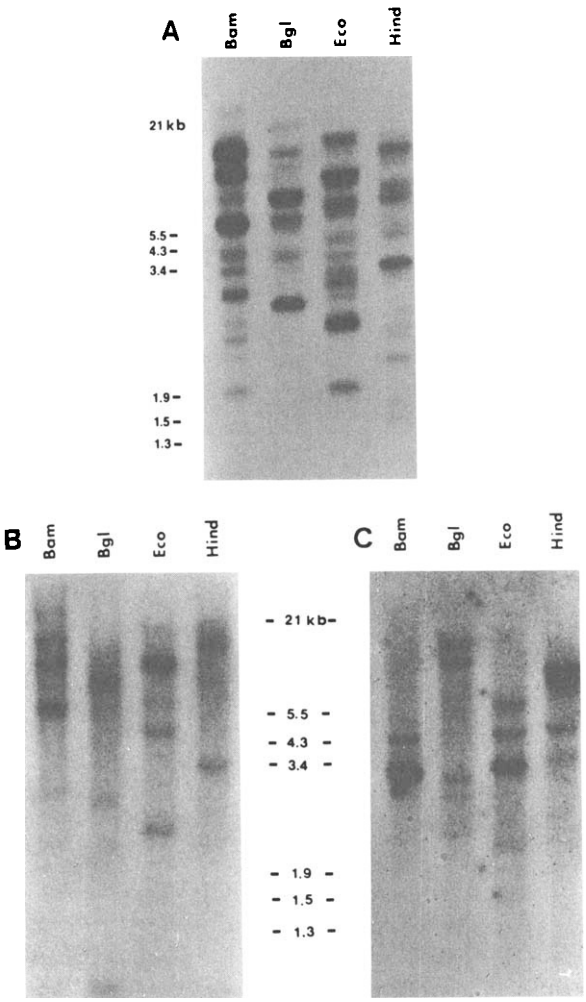

Figure 3. Southern genomic blotting of DNA from restriction enzyme digests of bovine DNA. (A) Hybridized to a full-length bovine trophoblast IFN cDNA probe; (B) hybridized to a probe derived from the 3′-end of such a cDNA; (C) a probe derived from the 3′-end of a bovine IFN-ω (αII) probe. The restriction enzymes used were *Bam*HI, *Bgl*II, *Eco*RI, and *Hind*III. The positions of molecular weight standards (kb × 10^{-3}) are shown adjacent to each blot. From Ref. 28, with permission.

only limited similarity to other type I IFN, including the closely related IFN-ω. A number of sequence motifs that have been implicated in viral inducibility such as GAAANN (where N is any nucleotide) (104, 105) and hexamers that can potentially bind the transcription factor, interferon-regulatory factor-1 (106), are present and retained at identical positions in all of the trophoblast genes (Fig. 4). However, these motifs are arranged very differently than in the type I IFN genes and are not clustered as they are in the virally responsive IFN genes (see Ref. 28). Possibly, this may be the reason that the trophoblast IFN genes are so poorly inducible by virus in leukocytes (51) and by double-stranded RNA in Day 11 conceptuses (53). Also interesting in this regard is that the majority of studied type I IFN genes depend upon only about 150 bases upstream of the transcription start site for providing full virus inducibility, and even closely related genes diverge in sequence beyond this point. In contrast, the trophoblast IFN genes are highly conserved, even across species, up to at least base position −400 (Fig. 4). While this conservation may be, in part, the result of the recent emergence of theses genes as a distinct functional group, promoter deletion studies in human choriocarcinoma cell lines permissive for trophoblast IFN expression (see below) have shown that sequences as far upstream as base position −450 may be important for transcriptional regulation (51).

Cell-Specific Expression of the Trophoblast IFN

In addition to their massive production prior to the time of implantation, the other most striking feature of the trophoblast IFN genes is that this expression is confined to the trophectoderm layer of cells that forms the first epithelium of the conceptus and is the precursor lineage of the placenta. The trophoblast IFN account for virtually all of the antiviral activity produced by conceptuses at the stage of blastocyst elongation, although trace levels of transcripts for both IFN-α and IFN-ω are also detectable, an observation that suggests that common induction mechanisms for all three IFN subtypes may be at play (51). Furthermore, although trophoblast IFN expression is extremely low or absent in cells other than trophectoderm, bTP-1 mRNA is detectable in bovine leukocytes after they have been exposed to Sendai virus (51). Immunoneutralization studies have suggested that bTP-1, however, contributes insignificantly (<0.1%) to the total IFN produced by these cells. Therefore, while the trophoblast IFN genes may share some regulatory features with other type I IFN, there must also be tissue-specific factors that ensure their high transcriptional rates in trophectoderm for a few days in early pregnancy.

This apparent confinement of trophoblast IFN production to trophectoderm led to a search for cell lines that might be useful in studying transcriptional regula-

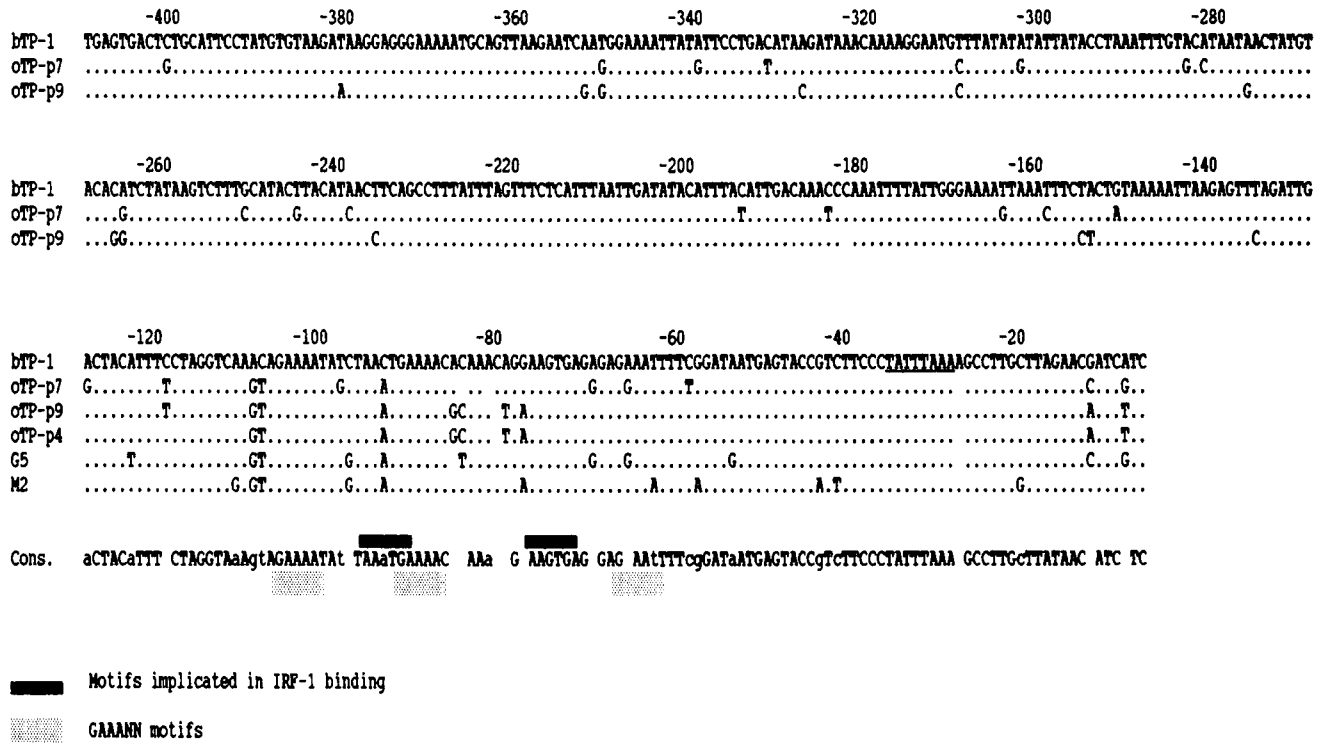

Figure 4. Promoter sequence alignment of a bTP-1 gene (bTP-1), three different oTP-1 genes (oTP-p7, oTP-p9, and oTP-p4), and a goat (G5) and a musk ox (M2) trophoblast IFN gene. The bases that are conserved between all promoters in the first 120 base pairs upstream of the putative transcript start site are indicated in capital letters of the consensus sequence (cons). These bases that are conserved in all but one gene promoter are indicated as lower case letters. Blank spaces in the consensus sequence are positions where there is no clearly preferred nucleotide. Regions that resemble sequences implicated in viral responsiveness such as GAAANN (□) and potential interferon regulatory factor-1 (IRF-1) binding sites (■) are indicated. The figure is adapted from Ref. 96 with permission.

tion of these genes. Transfection of a bovine trophoblast IFN gene or promoter constructs coupled to a reporter gene into several nontrophoblast cells such as CHO, L929, and GBK-2 showed that transcription was not supported in such cells. Constitutive expression did occur, however, in human choriocarcinoma cell lines such as JAR (51) and other similar lines such as BeWo and Jeg-3 (unpublished results from this laboratory). All these cell lines are derived from human trophoblast tumors. Promoter sequences as distal as −450 relative to the transcription start site were necessary to provide maximal expression in such cells, whereas a comparable bovine IFN-ω promoter was inactive (51; unpublished). Again, this observation demonstrates that the trophoblast IFN are distinct from the related IFN-ω in the manner whereby their respective genes are regulated. However, whether the transcription factors that permitted the low level of trophoblast IFN gene expression in transfected choriocarcinoma cells are the same or approximate those present in bovine or ovine trophoblast tissue is unclear. Unfortunately, no well-defined trophoblast cell lines have been established from cattle and sheep. Attempts to maintain primary cultures in this laboratory have invariably led to rapid loss of trophoblast IFN expression. Moreover, such primary cell lines do not permit constitutive expression of genes from transfected bovine trophoblast IFN promoters.

Concluding Remarks

The discovery of the trophoblast IFN has led to the surprising realization that a potent cytokine regulates a crucial physiological event in early pregnancy, namely rescue of the corpus luteum. Even though the biochemical and physiological details of how this conceptus-derived IFN intervenes to prevent prostaglandin $F_{2\alpha}$-mediated luteolysis in sheep, cattle, and other ruminants remain unclear, this "new" role for IFN is intriguing, particularly since it has become evident that the trophoblast IFN share many structural and functional properties with other type I IFN and would presumably have far-ranging effects on the maternal immune system and other maternal tissues carrying type I IFN receptors in the immediate zone of trophoblast contact. What remains unclear is whether the trophoblast IFN are unique merely by virtue of the location, magnitude, and temporal nature of their expression, or whether they have some unusual properties that equip them particularly well for a role in early pregnancy.

Although they possess many properties in common with other type I IFN, there is good reason to warrant separation of the trophoblast IFN from other type I subtypes on the basis of their unusual primary sequences, their serological distinctiveness, the manner

in which their promoter regions are organized, and their unique type of tissue expression. The divergence of the trophoblast IFN from the IFN-ω, which probably occurred only about 55 million years ago, may have gone hand in hand with the evolution of the syndesmochorial type of placenta, where the sustained production of large amounts of IFN in early pregnancy was incorporated into a novel mechanism for signaling the presence of a viable embryo to the maternal system. Other species seem to have evolved rather different means for ensuring the maintenance of the corpus luteum of pregnancy, and the fact that humans lack trophoblast IFN genes and do not appear to make use of this signaling device most likely will preclude any adoption of IFN for prevention of early embryonic loss in women, though such therapy clearly has potential value for improving fertility in ruminant livestock.

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