Expression of interferon genes in trophoblast: its impact on pregnancy

Claude La Bonnardière
F. Lefèvre
Institut National de la Recherche Agronomique
Unite de Virologie et d'Immunologie Moléculaires I.R.N.A.
Jouy-en-Josas, France

1. Introduction

Interferons (IFN) are cytokines gifted with multiple biological activities. A characteristic property of IFNs is their potent antiviral activity, which was at the origin of their discovery (Isaac, Lindenmann, 1957). They were subsequently shown to elicit numerous other functions, among which cell growth regulation, stimulation of cellular antigen expression (e.g. MHC, attachment antigens), and modulation of immune functions (e.g. natural killer cells, macrophages). IFNs are therefore viewed, in the adult organism, as the effectors of primary and non specific line of defense of the animal organism against pathogens and also tumors.

IFNs are proteins and glycoproteins classified according to their primary sequence and antigenicity, into six species, that belong to two types: Type I IFNs (alpha-, beta, omega, tau and porcine spI) are encoded by intronless genes, all clustered on the same region of a chromosome (chr 9 in humans). IFN-alpha, omega and tau belong to multigenic families, each with 10 or more members very close in sequence to each other. Type II or IFN-gamma is a unique gene with introns (Table 1). Despite an antiviral effect shared with type I IFNs, IFN-gamma, by its main sources (T lymphocytes and NK cells), its immunomodulatory effects on lymphocytes and macrophages is a lymphokine. In the immune response (in a wide sense), IFN-alpha, omega and gamma are produced by known inducers. The two formers are induced mainly by virus infection, whereas IFN-gamma results from activation by antigens of sensitized T lymphocytes (De Maeyer, De Maeyer-Guignard, 1988).

One important discovery came since 1989, when molecular physiologists (Imakawa et al., 1987; Stewart et al., 1987; Charpigny et al., 1988) found that a major embryonic protein, produced by the trophectoderm of the sheep

at the time of implantation, and previously named trophoblast protein 1 (oTP-1) or trophoblastin (Martal et al., 1979), was in fact a type I interferon. The same was found in cattle. This showed for the first time that an IFN was involved in a physiological process, and moreover that its expression was developmentally programmed. This paper briefly reviews present knowledge of IFNs such as involved in pregnancy, with an emphasis on ruminant and pig trophoblast IFNs, whose molecular gene structure has been determined and recombinant proteins obtained.

TABLE 1
MAIN CHARACTERISTICS OF INTERFERONS (HUMAN)

Type	IFN species	Gene structure (chromosome)	Known inducers	Producing cell	Size of mature protein (amino acids)	Specific cell receptor
I	IFN-α	20 intronless loci (4 pseudogenes) (chr 9)	Viruses infected cells Bacteria	Leucocytes Macrophages	165-166	I
	IFN-β	1 intronless locus (chr 9)	Viruses DS RNA	Fibroblasts	166	I
	IFN-ω	7 intronless loci (6 pseudogenes) (chr 9)	Viruses (other?)	Leucocytes (other?)	172	I
	(IFN-τ*)	4-5 loci	Programmed (ruminants)	Trophoblast	172	I
	(IFN-Spl*)	2 loci	Programmed (pig)	Trophoblast	149	I
II	IFN-γ	1 locus with 3 introns (chr 12)	Antigens Mitogens	T lymphocytes Large granular lymphocytes	143	II

* These IFNs species, whose names are in parentheses, have not been found expressed in humans.

2. Trophoblastic IFNs: structure and regulations

2.1. In humans and rodents: weak IFN expression in mid and late pregnancy

Man and mouse are two species with hemochorial placentation, in which extraembryonic trophoblast undergoes complex differentiation while deeply invading the maternal uterus. In these two species (and others) it is relevant to consider the foetus as a semi-allogenic graft, which raises the question of how the maternal immune system tolerates this "transplant". The demon-

stration, in the placental blood and tissues, of cytokines known to modulate the immune system, can make sense in this regards (Table 2).

TABLE 2
INTERFERONS IN PREGNANCY IN SPECIES WITH HAEMOCHORIAL PLACENTATION

Species	Period	Source	IFN Species	References
Human	37 wk → term	Placenta	IFNα + atypical IFNs	Duc-Goirand et al., (1985)
Murine	$>D_{10}$	Placenta	nd*	Fowler et al., (1980)
	term	Placenta	atypical IFNs to (27-70 RD)	Weislow et al., (1983)
	D_4	Blastocyst	nonα, nonβ	Cross et al., (1990)
	$>D_9$	Placenta	nonα, nonβ	Cross et al., (1990)

* not determined

In humans, since the first trimester of pregnancy, types I and II IFNs are found at the fetal-maternal interface, which appear to be constitutively expressed. In 1982, Lebon et al., consistently found IFN-alpha activity in amniotic fluids of pregnancies with no signs of viral infection. Later, low but consistent IFN activity was detected in placental blood from the 35^{th} week of pregnancy onwards, at a higher frequency than in amniotic fluid and membranes, suggesting that amniotic IFN must derive from the placenta. The same group showed that IFN-alpha and -beta species were co-expressed, which seemed somewhat atypical (Duc-Goiran et al., 1985; Chard et al., 1986). Subsequently, antigens reactive with antibodies to the three main IFN species (alpha, beta and gamma) could be detected in extravillous syncytiotrophoblast throughout pregnancy (Bulmer et al., 1990). Paulesu et al, using monoclonal antibodies, also reported on the expression of the three IFN species, but mainly in villous syncytiotrophoblast, which decreased with gestation age, and which became almost imperceptible at term (Paulesu et al., 1991). The proof that immunoreactive IFNs were indeed synthesized by trophoblast was not made. Attempts at molecular cloning of one of these human placental IFNs apparently failed, most probably due to their low level of expression. However, one human cDNA with IFN-related sequence was cloned from a cytotrophoblast cDNA library, which appeared most related to IFN-omega (Whaley et al., 1994). This might indicate that most IFN species are expressed in the placenta at mid and late pregnancy, that might exert paracrine, or autocrine effects, since IFN receptors are present in the placenta (Branca, 1986).

In the mouse, IFN activity was found in the placenta by several groups, mostly in late gestation (Fowler et al., 1980; Weislow et al., 1983; Yamada

et al., 1985). The precise nature of IFN species involved could not be determined, but like in humans, term placenta might express IFNs that are atypical: in particular, one protein shown antigenically related to IFN alpha/beta, appeared to be much higher in MW than either of these two IFNs (Weislow et al., 1983). Only Cross et al., reported an IFN-like activity from implantation onwards, namely as soon as Day 4 of gestation (Cross et al., 1990). This result was not confirmed (La Bonnardière, 1993).

Thus, in both human and mouse species, after implantation, the placenta is the seat of a physiologic synthesis of several IFNs, maintained throughout pregnancy. They could belong to three main IFN species, but precise molecules in play were not characterized. Their function is unknown.

Homozygous mice were independently obtained, in which genes encoding either the receptor chains for type I IFNs (Müller et al., 1994) or for IFN-gamma (Huang et al., 1993) or the IFN-gamma itself (Dalton et al., 1993) were "knocked out". Mice deficient for type II receptor do not differ from their normal counterparts in terms of pregnancy and fecundity scores (Huang et al., 1993), implying that IFN-gamma *per se* may not play an important role, if any, in development. It is not well established whether or not type I IFN receptor deficient mice suffer of reproductive failures. But one must notice that all experiments on gene knock-out utilize inbred mice, somewhat irrelevant models for the study of immune mechanisms related to histocompatibility, unless proper crosses are not performed and analyzed.

2.2. Intense IFN expression by ungulate trophoblast around implantation

In several ungulate species (ruminants, pig, horse), the conceptus (inner cell mass + trophoblast) presents some common characteristics, among which a typical elongation of the trophoblast, and a delayed implantation: thus the conceptus remains "free" in the uterus for several days before getting apposed to the endometrium. Another common trait between ruminants and pigs, is the mode of placentation, which is not hemochorial, but rather of the "epichorial" type, in which the trophoblast does not invade the uterine mucosa. In pigs, the most extreme type of epitheliochorial placentation, the integrity of fetal and maternal epithelia in the placenta is maintained, with no connection between fetal and maternal bloods (see Weitlauf, 1988, for a review). Another common characteristics of ruminant and pig that will be developed here is the appearance of transient and elevated synthesis of IFNs at the time of implantation.

2.2.1. In ruminants: expression of a trophoblast specific type I IFN

Gene(s) and protein(s)

Reproductive physiologists had for many years focused their attention upon a major protein secreted by sheep conceptus between day 12 and 20 of pregnancy. Secreted by most cells of the trophectoderm, this hormone-like

protein (named oTP for ovine trophoblast Protein-1, or Trophoblastin) plays an important role on the maintenance of corpus luteum and hence synthesis of progesterone, a key steroid hormone for successful pregnancy (reviewed by Roberts, Bazer).

Molecular cloning and sequencing of oTP from a trophoblast cDNA library revealed a 195 aminoacid long IFN-like sequence, including a 23 AA signal peptide (Imakawa et al., 1987). By length and sequence, oTP/IFN appeared related to IFN-omega gene family, previously known in humans and cattle (Capon et al., 1985). Despite 70% homology with bovine IFN-omega, further analysis indicated that this IFN could constitute a new species of type I IFN (reviewed by Roberts et al., 1992). IFN-Tau (evoking trophoblast) was proposed for this IFN species.

It had been shown that natural oTP protein was in fact a mixture of several isoforms (Martal et al., 1990), which could explain the high amount of protein found.

This was confirmed by analysis of bovine genome, where at least 5 highly homologous genes were found (Hansen et al., 1991). Southern blot analysis of DNAs from different species showed that the IFN-Tau subfamily is widely represented in ruminants, but absent in humans, pigs and horse (Leaman,

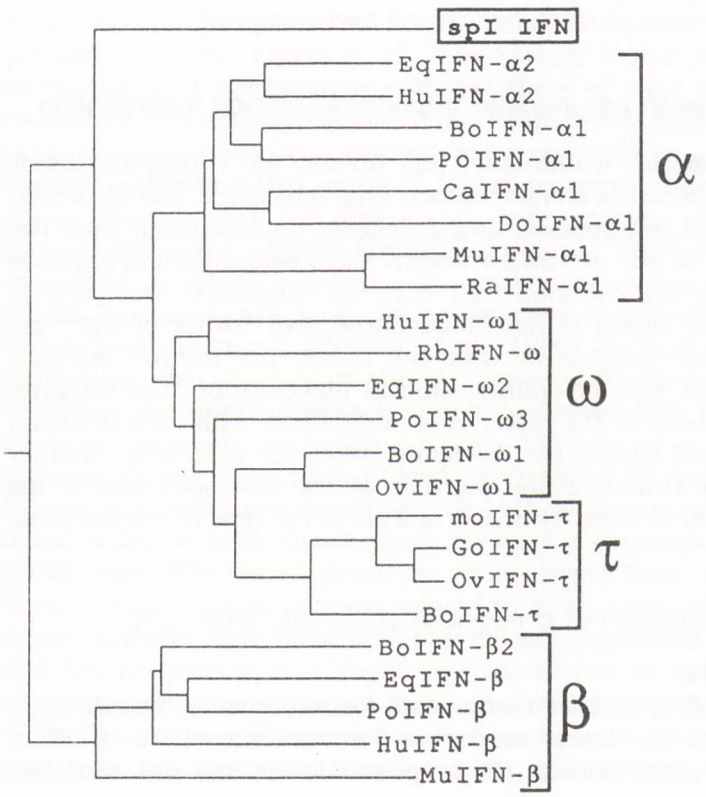

Fig. 1. Phylogenetic tree of 24 type I IFN mature protein sequences including spI IFN and selected members of type I IFN familiers from 12 mammalian species (one subtype per species per family). This tree was established using the Fitch-Margoliash distance matrix method. Horizontal branch lenght is proportional to the amount of sequence divergence. The branch linking the IFN-β family to all other type I IFNs was divided at midlength in order to root the tree. The first two letters of sequence names refer to the species (*Hu*, human; *Bo*, bovine; *Ov*, ovine; *Go*, goat; *mo*, musk ox; *Po*, porcine; *Mu*, murine; *Do*, dog; *Ca*, cat; *Eq*, equine; *Rb*, rabbit). (Reproduced from Lefèvre and Boulay, 1993).

Roberts, 1992). Thus IFN-Tau, although probably diverged from a common IFN ancestor gene (Fig. 1), seem restricted to ruminant species within the artiodactyla order.

The trophoblastic oTP1/IFN Tau, by structural and functional criteria, belong to type I IFNs. Indeed i) their primary sequence is clearly homologous to other type I (alpha, and particularly omega subfamilies), and like those is encoded by intronless gene(s); ii) they bind to the same cellular receptor as IFN-alpha, -beta, and -omega (Stewart et al., 1987; Flores et al., 1991); iii) they can trigger in susceptible cells the well known biological effects of IFNs: induction of a potent antiviral activity (Pontzer et al., 1988; Klemann et al., 1990) and stimulation of the enzyme (2'-5')oligo-A synthetase (Short et al., 1991). However IFN-Tau has no obvious antigenic relationship with other type I IFNs (Cross, Roberts, 1991).

Regulation

All available data argue for a genetic programmation of IFN-Tau (TP-1) in the course of trophoblast development. In particular, initiation of IFN-Tau gene expression could be demonstrated by RT-PCR in bovine blastocysts obtained by IVM/IVF, showing that gene induction does not depend on uterine factor(s) (Hernandez-Ledezma et al., 1992).

Analysis of 5' non-coding sequence of different ovine and bovine IFN-Tau revealed a remarkable conservation within the upstream promoter region, and only limited similarity to other virus inducible type I IFNs. Hexamer motifs known to bind factors (IRF1) implicated in the regulation of type I IFN gene expression are present in the IFN-Tau promoter, and were shown to effectively bind this factor. Also present are GAAANN motifs that have been implicated in virus inducibility of IFN-alpha and -beta, although they are organized in the IFN-Tau promoter very differently than the other IFN genes (Hansen et al., 1991; Leaman et al., 1994). In fact, IFN-Tau can be induced at low rate in leucocytes by Sendaï Virus (Cross, Roberts, 1991). On the other hand IFN-Tau is not constitutively produced by bovine cells, but transfection of various cell lines with promoter-reporter gene constructs indicated that only JAR cells (a human choriocarcinoma cell line) could direct IFN-Tau promoter dependent transcription in the absence of viral induction (Cross, Roberts, 1991; Leaman et al., 1994).

Thus, IFN-Tau genes probably diverged in the ruminant from more widely distributed IFN-omega, and although they retained some of the regulatory sequences common to type I IFNs (virus inducible elements), their massive and transient expression in ruminant trophoblast is controlled by very specific elements allowing their tissue specific expression during a critical phase of pregnancy. They hereby differ completely from "adult" or leucocytic IFNs, known to be punctually induced in response to pathogens and during subsequent immune response.

2.2.2. Porcine trophoblast co-expresses type I and type II IFNs

Genes and proteins

In 1989, it was shown that elongating pig conceptus (between days 11th and 17th) synthesizes and secretes an antiviral activity, both in uterine flushings and in supernatants of conceptus-conditioned culture medium (Cross, Roberts, 1989). Initially thought related to IFN-alpha, this activity was subsequently shown to be a mixture of two IFN species: one was IFN-gamma as a predominant component, and the other was a type I IFN (Lefèvre et al., 1990; La Bonnardière et al., 1991).

The cDNAs encoding both IFNs were cloned from a day 15th trophoblast cDNA library, and sequenced. The relative frequency of IFN-gamma related cDNA clones in the library amounted to 1-2%, indicative of an abundant mRNA (Lefèvre personal communication). The type I IFN cDNA was cloned from the same library by low stringency hybridization with a porcine IFN-omega probe. 100-fold less abundant than IFN-gamma, the type I cDNA appeared to be a new sequence, distantly related to the three main IFNs species, and encoding a mature protein 149 residues in length (Lefèvre, Boulay, 1993). This IFN was named spI IFN for short porcine type I IFN. It is very atypical IFN with 7 cysteins in its coding sequence. The alignment of spI preprotein sequence with several members of the four known families of type I IFN and its phylogenetic analysis revealed that IFN spI is clearly distinct and divergent from the cluster defined by IFN-beta sequences on the one hand, and that defined by IFN-alpha, -omega and -Tau on the other hand (Fig. 1). Moreover, sequences related to spI IFN cDNA where found in the genome of several ungulate pieces like ruminants and equidae (Lefèvre, unpublished result), indicating that spI IFN represents the first member of a new type I IFN family more widely distributed in mammals than IFN-Tau.

A recombinant spI IFN protein was expressed in insect cells, and biochemical and biological properties were determined. spI IFN was found to possess high antiviral activity on porcine and bovine cells, very low if any on human cells (Niu et al., 1995a). Data of competitive binding experiments on porcine cells showed that spI IFN binds to a major component of type I IFN receptor (Niu et al., 1995b).

Regulation

Northern analysis of mRNAs for these two IFNs revealed that their time-related transcriptions are quite similar, with maximal amount of mRNA around days 14-16, and almost complete arrest at day 20 (Fig. 2). Another peculiarity of this transcription is the presence of equimolar amounts of two IFN-gamma mRNAs, differing by only 100 nucleotides, suggesting a trophoblast specific regulation since in induced pig lymphocytes only one IFN-gamma mRNA is present (Lefèvre et al., 1990).

Using spI IFN cDNA as a probe, two homologous, non-allelic loci were found in pig genomic DNA and sequenced, one of which is most likely a

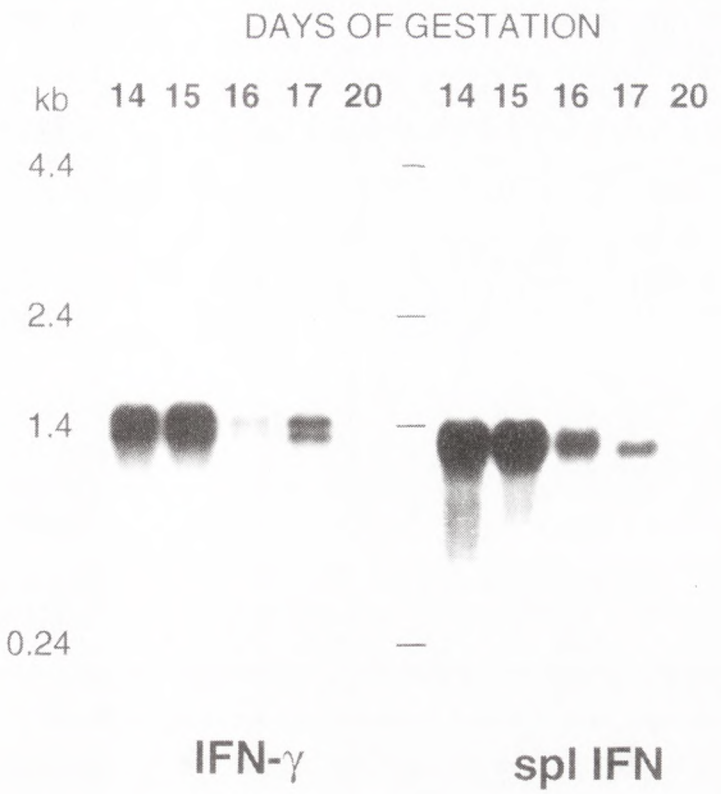

Fig. 2. Northern Blot analysis of Poly (A)+ mRNAs from pig conceptus sampled at different days of gestations. Left panel: hybridization was performed at low stringency with a human IFN-γ cDNA probe. Note the presence of two RNA transcripts (1.3 and 1.4 kb). Right panel: the probe was spI IFN cDNA.

pseudogene (Lefèvre, Boulay, 1993). Detailed analysis of about 1 kb upstream of the functional spI coding sequence revealed a very specific promoter region, with no homology with other type I IFN promoters, including those of IFN-Tau.

Several permanent cell lines have been derived from porcine embryonic trophectoderm, which may constitute a relevant model for studies on trophoblast-specific regulation of IFN genes (La Bonnardière et al., 1993). In conditions of culture that allow cell polarization (two-sided microporous filters), at least endogenous IFN-gamma is spontaneously expressed and secreted by trophoblast cell lines. Interestingly, in this model, the onset of IFN expression is correlative of the rise of cell polarity, as measured by trans-epithelial resistance (Fig. 3).

The simultaneous secretion of spI IFN has not been shown, but this might reflect a threshold effect since IFN-gamma, which is supposed far more abundent in native trophoblasts, is detected in the range 1 – 40 ng/ml.

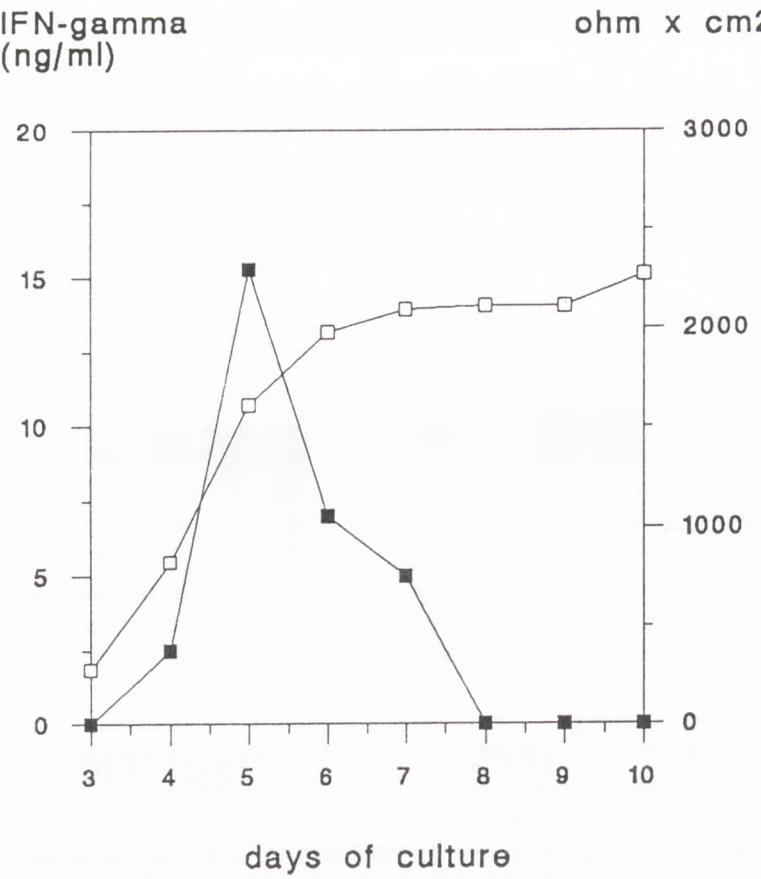

Fig. 3. Secretion of IFN-gamma at the apical side of a pig trophoblast cell line (TBA) cultured on two-sided, collagen-treated, microporons membranes (TRANSWELL-COLL, COSTAR). IFN-gamma (–■–) was assayed by ELISA. Transepithelial electrical resistance (–□–) was measured by "Millicel ERS" ohmmeter (Millipore).

3. Functions of trophoblastic interferons

3.1. In ruminants, IFN-Tau is antiluteolytic

The molecular identification of cloned trophoblastin/oTP1 as an IFN (Imakawa et al., 1987) was unexpected, because: i) oTP1 secretion is developmentally regulated; ii) the protein secretion is intense, up to several hundredths of µg per conceptus per day; iii) oTP-1 was known as a paracrine hormone. None of these properties had been previously found for "adult" interferons. There is no doubt that this discovery has brought a new and important function to those already known for IFN family. But reciprocally,

the identification of oTP/bTP as interferons did not help much to elucidate (at the cellular level) the precise mechanism of antiluteolysis, whose major pathway was known.

In nonpregnant sheep and cows, near the end of the cycle, the uterus releases pulses of prostaglandin (PG) F2, itself triggered by pulsatile synthesis of follicular oxytocin. These PGF2 pulses, by the endocrine route, reach the ovary where they provoke the regression of CL (luteolysis), subsequent drop of blood progesterone, and return to cyclicity (reviewed by Niswender, Nett, 1988). The TP-1/IFN Tau secreted by the trophoblast prevents the uterus-derived PG pulses and subsequent CL lysis. A strong argument in favor of this role comes from repeated experiments showing that injection of high doses of natural or recombinant oTP during 5-6 days to cyclic ewes, starting at day 10, can significantly delay blood progesterone drop and simultaneous return to estrus (reviewed by Roberts et al., 1992). The target of trophoblast IFN is most probably the endometrial epithelium, which expresses high levels of type I IFN receptors (Hansen et al., 1989; Knickerbocker et al., 1989).

According to this model, ruminant IFN-Tau produced at the time of attachment of conceptus to the uterus, is a paracrine signal sent by one epithelium (the embryonic trophectoderm) to another (the endometrial epithelium), the consequence of which is the triggering of so-called "maternal recognition of pregnancy". The possibility exists of yet unknown effects in addition to this.

3.2. In pigs, paracrine effect of IFN-gamma on the endometrial epithelium

In pigs, it has been shown since many years that the hormonal control of CL in pregnancy differs from that found in ruminants: Prostaglandin F2, like in ruminants, is the luteolytic factor, but its hormonal control by implanting conceptus is mediated by estrogens, produced by pig conceptus since day 11 (Bazer et al., 1989). As a confirmation, we performed repeated injections of recombinant IFN-gamma + spI to cyclic gilts, without significant effect upon the ovarian cycle (unpublished results). Therefore, in pigs, the role played by trophoblast IFNs, whose amount and time kinetics much resemble IFN-Tau in ruminants, is presently unknown. Our group has tried to identify what was the target(s) of trophoblast IFNs. Like for sheep and cows, the endometrium is the most likely tissue, since we showed that i) IFNs gamma and spI are absolutely confined in the uterine lumen (unpublished results); ii) the endometrial epithelium expresses type I and II IFN receptors and responds to antiviral effect and (2-5)A synthetase inducibility of both IFNs; iii) by these two criteria, the trophectoderm is not responsive to any IFN (D'Andrea et al., 1994).

The latter result means that, by one way or another, the trophoblast escapes the biological effect of IFNs (no autocrine effect). It would be interesting to perform comparative studies on the ovine and/or bovine trophoblast.

3.3. A common role for IFNs in early pregnancy?

Concerning the function of pig trophoblast IFNs, it is risky to propose an hypothesis among many possibilities. IFNs in early pregnancy might be anti-infectious (antiviral), growth suppressor, factors of differentiation (on the embryo), adjuvant for implantation, and others. But whatever the function, it is conceivable that it could also be effective in other species with early IFN expression. In other words, IFN-Tau in sheep and cows migh exert other functions as that shown on CL maintenance. It was found that ovine IFN-Tau, like IFN-alpha, is able to suppress PHA-driven lymphocyte proliferation, suggesting a possible modulation of the (maternal) immune system (Fillion et al., 1991). To us, this function is unlikely, since in ruminants like in the pig, trophoblastic IFNs are extremely confined in the uterine lumen: no evidence was made that they cross the epithelial barrier. Another indirect argument in disfavour of an immune function is the fact that MHC antigens (Swine Leucocyte Antigens) do not seem to be expressed on the pig trophoblast (unpublished results), which obviate any immune "rejection".

Since the endometrium is the most probable target for IFNs, experiments are under way in the pig model to determine which genes are specifically induced by the mixture IFN-gamma and spI in endometrial cells from non-pregnant gilts. It is hoped that identification of known functions and/or of new IFN-inducible genes will make it possible to access to a common function for these intriguing interferons.

4. As a conclusion: possible applications in animal breeding and health

As it is known that many pregnancies terminate at the critical period when the conceptus signals its presence to the mother (Wilmut et al., 1986), it was reasonned that providing a supplement of IFN might rescue some conceptuses. In fact, this was made possible very soon atfter the identification of trophoblastin as an IFN, since i) it was shown that IFN-Tau possesses all major biological activities of other IFNs; ii) bovine IFN-alpha had already made available in high amounts by recombinant technology. The effect of rboIFN-alpha on pregnancy success was then tested in ewes and cows, of course by the general route, in order not to perturb gestation. In ewes, intramuscular injection of IFN-alpha significantly increased pregnancy rates when compared to placebo; in one study, the number of ewes carrying a lamb to term was increased by almost 20% in the IFN-treated group (Nephew et al., 1990; Schalue-Francis et al., 1991; Martinod et al., 1991) (Table 3).

TABLE 3

EFFECTS OF INTRAMUSCULAR INJECTION OF rbo IFN-α|1 ON PREGNANCY RATE IN EWES

(Reproduced with Permission from Schalue-Francis et al., 1991)

Experiment and year	% of ewes pregnant		% of ewes which lambed		No. of lambs born	
	rboIFN-a|1	Placebo	rboIFN-a|1	Placebo	rboIFN-a|1	Placebo
2; 1988-89	79% (27/34)	58% (21/36)	71% (24/34)	50% (18/36)	35	27
	P=0.05		P=0.07			
3; 1989-90	89% (58/65)	78% (48/61)	84% (55/65)	74% (45/61)	98	80
	P=0.08		P=0.10			
Combined	86% (85/99)	71% (69/97)	80% (79/99)	65% (63/97)	133	107
			P=0.01			

P — values were not calculated for the combined data on ewes pregnant since two different methods were used to assess pregnancy states (detection of oestrous behaviour and ultrasound in Exps 2 and 3, respectively).

In cattle, however, for which the practical impact would be considerably more, there was no report to-date of success in improving reproductive efficiency. On the contrary, it was found that the administration of IFN-alpha to heifers provokes a lowering of pregnancy rate, that could be related to an observed toxic effect of IFN, as measured by fever (Roberts et al., 1992). Now recombinant IFN-Tau could be produced at high rate in different cell systems, no doubt that the same type of experiments will have to be carried out with this molecule. To us, the foregoing data obtained in sheep are certainly promising, although there is no evidence that the mode of action of exogenous bovine IFN-alpha was through improvement of maternal recognition of pregnancy rate. Other possibilities exist, among which an improvement by IFN of antiviral/antiinfectious defences at the foeto-maternal interface cannot be ruled out.

In pigs, where trophoblast IFNs are unlikely to effect luteolysis (*vide supra*), it will be interesting to test, in pregnant sows, the effect of IFN administration on embryonic survival; it is well known indeed that embryonic mortality, very early in pregnancy, is high in pigs. In this regards, the example of hyperprolific breeds (e.g. Chinese Meishan) proves that the relative poor fecundity scores of European breeds could be improved by a significant factor.

Other perspectives could be opened by the advent of these new IFN proteins (IFN-Tau, spI IFN) in veterinary medicine, and possibly in human clinics. IFN-Tau for instance, while active in cell culture against viruses (including HIV) was shown to be less toxic than IFN-alpha (reviewed by Bazer, Johnson, 1991); the possibility that IFN-Tau might advantageously substitute to IFN-alpha for instance, the toxicity of which is well established, should be considered.

Literature

1. Isaac A., Lindenmann J., (1957), *Virus interference I: the interferon*, Proceedings of the Royal Society (B), 147, 258.
2. De Mayer E, De Mayer-Guignard J., (1988), *Interferon and Other Regulatory Cytokines*, Wyleg, New York.
3. Imakawa K., Anthony R. V., Kazemi M., Marotti I. C. R., Polites H. G., Robert R. M., (1987), *Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm*, Nature, 330, 377-379.
4. Stewart H. J., MacCann S. H. E., Northrop A. J., Lamming G. E., Flint A. P. F., (1989), *Sheep antiluteolytic interferon : cDNA sequence and analysis of mRNA levels*, Journal of Molecular Endocrinology, 2, 65-70.
5. Charpigny G., Reinaud P., Hue D., Guillomot M., Charlier M., Pernollet J. C., Martal J., (1988), *High homology between a trophoblastin isolated from ovine embryo and α-interferons*, FEBS Letters, 228, 12-16.
6. Lebon P., Girard S., Thepot F., Chany C., (1982), *The presence of α–interferon* in human amniotic fluid, J. Gen. Virol., 59, 393-396.
7. Duc-Goiran P., Robert-Galliot B., Lopez J., Chany C., (1985), *Unusual apparently constitutive interferons and antagonists in human placental blood*, Proc. Natl. Acad. Sci. USA, 82, 5010-5014.
8. Chard T., Craig P. H., Menabawey M., Lee C., (1986), *Alpha interferon in human pregnancy*, Br. J. Obstet. Gynecol., 93, 1145-1149.
9. Bulmer J. N., Morrison L., Johnson P. M., Meager A., (1990), *Immunohistochemical localization of interferons in human placental tissues in normal, ectopic, and molar pregnancy*, American Journal of Reproductive Immunology, 22, 109-116.
10. Paulesu L., Bocci V., King A., Loke Y. M., (1991), *Immunocytochemical localization of interferons in human trophoblast populations*, J. Biol. Reg. Homeo Ag., 5, 81-85.
11. Whaley A. E., Reddy Meka C. S., Harbison L. A., Hunt J. S., Imakawa K., (1994), *Identification and cellular localization of unique interferon mRNA from human placenta*, Journal of Biological Chemistry, 269(14), 10864-10868.
12. Branca A. A., (1986), *High-affinity receptors for human interferon in bovine lung and human placenta*, Journal of Interferon Research, 6, 305-311.
13. Fowler A. K., Reed C. D., Giron D. J., (1980), *Identification of an interferon in murine placentas*, Nature, 286, 266-267.
14. Weislow O. S., Kiser R., Allen P. T., Fowler A. K., (1983), *Partial purification of a placental interferon with atypical characteristics*, J. Interferon Res., 3, 291-298.
15. Yamada K., Shimizu Y., Okamura K., Kumagai K., Suzuki M., (1985), *Study of interferon production during pregnancy in mice and antiviral activity in the placenta*, Am. J. Obstet. Gynecol., 153, 335-341.
16. Cross J. C., Farin C. E., Sharif S. F., Roberts R. M., (1990), *Characterization of the antiviral activity constitutively produced by murine conceptuses: Absence of placental mRNAs for interferon alpha and beta*, Mol. Reprod. Dev., 122-128.
17. La Bonnardière C., (1993), *No detection of interferon-γ activity during early pregnancy in the mouse*, American Journal of Reproductive Immunology, 30, 26-31.
18. Müller U., Steinhoff U., Reis L. F. L., Hemmi S., Pavlovic J., Zinkernagel R. M., Aguet M., (1994), *Functional role of type I and type II interferons in antiviral defense*, Science, 264, 1918-1921.
19. Huang S., Hendricks W., Athage A., Hemmi S., Bluethsmann H., Kamijo R., Vilcek J., Zinkernagel R. M., Aguet M., (1993), *Immune response in mice that lack the interferon-γ receptor*, Science, 259, 1742-1745.
20. Dalton D. K., Pitts-Meek S., Keshav S., Figari I. S., Bradley A., Stewart T. A., (1993), *Multiple defects in mice with disrupted interferon-γ genes*, Science, 259, 1739-1742.
21. Weitlauf H. M., (1988), *Biology of Implantation*, Eds. E. Knobil, J. Neil et al., *The Physiology of Reproduction*, Raven Press Ltd., chap. 7, 231-262.

22. Bazer F. W., Vallet J. L., Roberts R. M., Sharp D. C., Thatcher W. W., (1986), *Role of conceptus secretory products in establishment of pregnancy*, Journal of Reproduction and Fertility, 76, 841-850.
23. Bazer F. W., Vallet J. L., Gross T. S., Thatcher W. W., (1989), *Comparative aspects of maternal recognition of pregnancy between sheep and pigs*, Journal of Reproduction and Fertility Supplement, 37, 85-89.
24. Capon D. J., Shepard H. M., Goeddel D. V., (1985), *Two distinct families of human and bovine interferon α genes are coordinately expressed and encode functional polypeptides*, Molecular and Cell Biology, 5, 768-779.
25. Roberts R. M., Leaman D. W., Cross J. C., (1992), *Role of interferons in maternal recognition of pregnancy in ruminants*, Society for Experimental Biology and Medicine, 200, 7-18.
26. Martal J., Chene N., Charlier M., Guillomot M., Reinaud P., Bertin J., Danef G., Zouari K., Charpigny G., (1990), *Trophobastin o TP, embryonic interferons*, in: Eds. L. Cedard, E. Alsat, J. C. Challier, G. Chaouat, A. Malassine, *Placental Communications : Biochemical, Morphological and Cellular Aspects*, London: Colloque INSERM/John Libbey Eurotext Ltd., vol. 199, 125-131.
27. Hansen T. R., Leaman D. W., Cross J. C., Mathialagan N., Bixby J. A., Robert R. M., (1991), *The genes for the trophoblast interferons and the related interferon-α II possess distinct 5'-promoter and 3'-flanking sequences*, Journal of Biological Chemistry, 266, 3060-3067.
28. Leaman D. W., Roberts R. M., (1992), *Genes for the trophoblast interferons in sheep goat and musk ox, and distribution of related genes among mammals*, J. Interferon Res., 12, 1-11.
29. Stewart H. J., MacCann S. H., Barker P. J., Lee K. E., Lamming G. E., Flint A. P. F., (1987), *Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein*, Journal of Endocrinology, 115, R13-R15.
30. Flores I., Mariano T. M., Petska S., (1991), *Human interferon omega (ω) binds to the α/β receptor*, Journal of Biological Chemistry, 226(19), 875-877.
31. Pontzer C. H., Torres B. A., Vallet J. L., Bazer F. W., Johnson H. M., (1988), *Antiviral activity of the pregnancy recognition hormone ovine trophoblast protein 1*, Biochemical and Biophysical Research Communications, 152, 801-807.
32. Klemann S. W., Li J., Imakawa K., Cross J. C., Frances H., Roberts R. M., (1990), *The production purification and bioactivity of bovine trophoblast protein-1 (bovine trophoblast interferon)*, Molecular Endocrinology, 4, 1506-1514.
33. Short E. C., Geisert R. D., Helmer S. D., Zavy M. T., Fulton R. W., (1991), *Expression of antiviral activity and induction of 2'-5' oligoadenylate synthetase by conceptus secretory proteins enriched in bovine trophoblast protein-1*, Biology of Reproduction, 44, 261-268.
34. Cross J. C., Roberts R. M., (1991), *Constitutive and trophoblast-specific expression of a class of bovine interferon genes*, Proc. Natl. Acad. Sci. USA, Cell Biology, 88, 3817-3821.
35. Hernandez-Ledezma J. J., Sikes J. D., Murphy C. N., Watson A. J., Schultz G. A., Roberts R. M., (1992), *Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques*, Biology of Reproduction, 47, 374-380.
36. Leaman D. W., Cross J. C., Roberts R. M., (1994), *Multiple regulatory elements are required to direct trophoblast interferon gene expression in choriocarcinoma cells and trophectoderm*, Molecular Endocrinology, 94, 456-468.
37. Cross J. C., Roberts R. M., (1989), *Porcine conceptuses secrete an interferon during the preattachment period of early pregnancy*, Biol. Reprod., 40, 1109-1118.
38. Lefèvre F., Martinat-Botte F., Guillomot M., Zouari K., Charley B., La Bonnardière C., (1990), *Interferon-gamma gene and protein are spontaneously expressed by the porcine trophectoderm early in gestation*, European Journal of Immunology, 20, 2485-2490.
39. La Bonnardière C., Martinat-Botte F., Terqui M., Lefèvre F., Zouari K., Martal J.,

Bazer F. W., (1991), *Production of two species of interferon by Large White and Meishan pig conceptuses during the peri-attachment period,*, Journal of Reproduction and Fertility, 91, 469-478.
40. Lefèvre F., Boulay V., (1993), *A novel and atypical type one interferon gene expressed by trophoblast during early pregnancy*, The Journal of Biological Chemistry, 268(26), 19760-19768.
41. Niu P. D., Lefèvre F., Mege D., La Bonnardière C., (1995), *Atypical porcine type I interferon: Biochemical and biological characterization of the recombinant protein expressed in insects cells*, FEBS Eur. J. Biochem., 230, 200-206.
42. Niu P. D., Lefèvre F., La Bonnardière C., (1995b), *Atypical spI interferon binds on porcine cells to a major component of type I interferon receptor*, Journal of Interferon and Cytokine Research, 15, 769-775.
43. La Bonnardière C., Flèchon J. E., Flèchon B., D'Andréa S., de Vaureix C., (1993), *Development of trophoblast cell lines from pig conceptus : a tool for studies on embryonic IFN gene regulations*, in: *Control of Pig Reproduction IV*, J. Reprod. Fertil., Supl. 48.
44. Hansen T. R., Kazemi M., Keisler D. H., Malathy P. V., Imakawa K., Roberts R. M., (1989), *Complex binding of the embryonic interferon, ovine trophoblast protein-1, to endometrial receptors*, J. Interferon Res., 9, 215-225.
45. Knickerbocker J. J., Niswender G. D., (1989), *Characterization of endometrial receptors for ovine trophoblast protein-1 during the estrous cycle and early pregnancy in sheep*, Biol. Reprod., 40, 361-369.
46. D'Andréa S., Chousterman S., Flèchon J. E., La Bonnardière C., (1994), *Paracrine activities of porcine trophoblastic interferons*, J. Reprod. Fertil., 102, 185-194.
47. Fillion C., Chaouat G., Reinaud P., Charpigny J. C., Martal J., (1991), *Immunoregulatory effects of ovine trophoblastin protein (oTP): All five isoforms suppress PHA-induced lymphocyte proliferation*, J. Reprod. Immunol., 19, 237-249.
48. Wilmut I, Sales D. I, Ashworth C. J., (1986), *Maternal and embryonic factors associated with prenatal losses in mammals*, J. Reprod. Fertil., 76, 851-864.
49. Nephew K. P., McLure K. E., Day M. L., Xie S., Roberts R. M., (1990), *Effects of intramuscular administration of recombinant bovine interferon-alpha1 during the period of maternal recognition of pregnancy*, J. Animal Sci., 68, 2766-2770.
50. Schalue-Francis T. K., Farin P. W., Cross J. C., Keisler D., Roberts R. M., (1991), *Effect of injected bovine interferon-alpha1 on oestrous cycle length and pregnancy success in sheep*, J. Reprod. Fertil., 91, 347-356.
51. Martinod S., Maurer R. P., Siegenthaler B., Gerber C., Hansen P. J., (1991), *The effect of recombinant bovine interferon-alpha on fertility in ewes*, Theriogenology, 36, 231-239.
52. Bazer F. W., Johnson H. M., (1991), *Type I conceptus interferons: maternal recognition of pregnancy signals and potential therapeutic agents*, American Journal of Reproductive Immunology, 26, 19-22.

Expression on interferon genes in trophoblast: its impact on pregnancy

Summary

In mammals, successful implantation of semi-allogenic conceptus (embryo + trophoblast) in the maternal uterus is still an enigma. Its understanding will require better knowledge of the complex hormonal and immunological "cross-talk" between implanting conceptus and mother.

In several ungulate species, around the time of implantation, the trophoblast synthesizes and secretes interferons (IFNs), known as antiviral and immunomodulatory cytokines. The IFN induction is transient (6-7 days long), and reaches high levels in ruminants and pigs. This IFN synthesis in early pregnancy addresses two main questions:

1) Is there a specific IFN gene regulation in trophoblast, and what are the molecular determinants of this specificity (coding and regulatory sequences)? Among the many known IFN gene subspecies, the embryonic trophoblast either "selects" specific ones: IFN-tau in ruminants, spI in pigs, or expresses previously described IFNs (in adults): IFN-gamma in pigs. On the other hand, trophoblastic IFN genes differ from those of "adult", anti-infectious IFNs, in that they are developementally induced. Their promoter sequences seem quite distinct from those of virus-induced IFN genes.

2) What is (are) the effect(s) of IFN synthesis on the physiological and/or immune maternal response ? So far, the only known effect is that of ovine and bovine IFN-tau on corpus luteum maintainance in pregnant females. However, results obtained in pigs suggest that this effect is not a general one, since in suidae the maternal recognition of pregnancy is governed by embryonic estrogens. Other effects of trophoblastic IFNs are being searched for, in particular by analyzing gene induction in the endometrial epithelium following IFN treatment.

This area of research might find applications in animal breeding, in particular through improvement of embryo transfer and increase in fecondity, in several animal species.

Key words:
interferon, trophoblast, pregnancy.

Address for correspondence:
Claude La Bonnardière, Institut National de la Recherche Agronomique, Unite de Virologie et d'Immunologie Moléculaires I.N.R.A., 78350 Jouy-en-Josas, France.