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Mini-Review

Human trophoblast interferons

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Summary

The human placental trophoblasts which constitute the first fetal cells and form the major cell layer of the feto-maternal interface are potent producers of interferons (IFNs). The IFN production is dependent on the gestational age of the trophoblast, type of inducer and the stage of differentiation of the trophoblasts. First trimester trophoblast populations produce higher levels (5–6 times) of IFN than the third trimester trophoblasts when stimulated with viruses. Non-viral inducers, such as poly(rI).poly(rC), induce exclusively IFN-β whereas viruses such as Sendai and Newcastle Disease Virus (NDV) induce mixtures of IFN-α subtypes and IFN-β. Differentiation of mononuclear cytotrophoblasts into syncytiotrophoblasts in vitro increase the IFN production. High-performance and immunoaffinity chromatography of the virus-induced trophoblast IFN preparations resulted in the isolation of three antigenically distinct IFNs, namely, α_I, $\alpha_{II}1$ ($\omega 1$), and β with molecular masses of 16, 22 and 24 kDa, respectively, on SDS-PAGE. The human trophoblast IFNs have physical and antiviral activities characteristic of the Type 1 IFNs. The possible roles of the trophoblast IFNs in human placental and fetal development are also discussed in this review.

Interferon; Trophoblast interferon

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Introduction

Interferons (IFNs) are a family of proteins produced by vertebrate cells after exposure to certain inducers, e.g., viruses, synthetic dsRNA or mitogens. They exert a broad spectrum of biological activities, the most prominent being the ability to impair virus replication. In humans, four functionally related but antigenically distinct IFNs (α_I, α_{II} (ω), β and γ) have been identified and are recognized by the Committee on the Nomenclature of Interferons. However, it is also recognized that there are a variety of different IFNs, and probably there are others that have not yet been discovered (Kirchner, 1986).

The human placenta is formed in the early stage of gestation as cytotrophoblastic cells, the progenitor cells for all trophoblast subpopulations (Hsi et al., 1991). The cytotrophoblast is derived from the first differentiated cell type to arise during embryogenesis, the trophophectoderm layer of the blastocyst. In the first trimester, the cytotrophoblasts are highly proliferative and invasive and undergo a series of differentiation to form a multinuclear syncytiotrophoblast which is continuously exposed to maternal blood and displays little potential to proliferate. The balance between proliferation and differentiation determines the structure and functions of the trophoblast and its "pseudo-malignant" properties.

Trophoblasts have many unique properties related to its roles in providing nutrition, allowing exchange of nutrients and removal of waste products, secreting hormones, and providing immunological protection for the antigenically foreign fetus (Loke and Whyte, 1983). Furthermore, the trophoblasts are the first cell layer that an invading agent such as viruses, bacteria and protozoa, have to transverse from mother to fetus. Therefore, due to their biologic niche, it was hypothesized (Dr. Peter Ebbesen, unpublished data) that the trophoblasts may have a specialized IFN system of great interest to medicine. Thus, the present review will summarize our knowledge on viral and non-viral production of human placental trophoblast IFNs from different stages of gestation, their purification, biochemical and biological characterization. Furthermore, the possible roles of the trophoblast IFNs will be discussed in the light of fetal and placental protection and development.

Production of placental IFNs

It is now known that IFN production is characteristic of the placenta and/or fetus in man and other species (Chard, 1986). In the human, IFNs are not detected in maternal blood and tissues but are detected in the fetus (fetal blood and organs) and in its surroundings such as amniotic fluids (Lebon et al., 1982), feto-placental unit (Howatson et al., 1988) and in the placental blood (Duc-Goiran et al., 1985) but it is not known whether the production is the result of natural developmental pattern or is induced by virus and mitogens from the maternal blood or nonviral inducers such as growth factors and cytokines

(DeMaeyer and DeMaeyer-Guignard, 1988) produced locally by the maternal endometrium. There is considerable evidence that IFN production is characteristic of the cells forming the feto-placental unit in both the human and other species. Our data (Aboagye-Mathiesen et al., 1993) show that human first and third trimester trophoblast and syncytiotrophoblast cultures in vitro do not or produce undetectable amounts of IFN in the absence of an inducer. When trophoblast cultures are stimulated with IFN inducers they produce large amount of IFNs. The trophoblast IFNs therefore have to be induced in vitro. However, in vivo conditions may be different and the production may be constitutive or unrelated to viral inducers. This can be documented by the work of others (Howatson et al., 1988; Duc-Goiran, 1985) who have detected IFN-α, -β and -γ on trophoblasts and in placental blood in vivo in the absence of viral inducers. In species such as sheep and cattle, it has been reported (Roberts, 1991; Pontzer et al., 1988) that IFN is produced transiently by the trophoblast in the early stage (13 to 21 days) of pregnancy. However, the induction mechanisms of these embryonic IFNs is still not well understood.

Induction of human trophoblast IFNs by non-viral inducers

Human trophoblast cultures, established from term placentae, stimulated with poly(rI).poly(rC) in vitro produce exclusively IFN-β (Toth et al., 1990a; Aboagye-Mathiesen et al., 1990). The IFN production increases when the normal trophoblast and trophoblast-derived malignant cells (BeWo) are superinduced with metabolic inhibitors such as actinomycin D and cyclohexamide (Table 1). However, combined superinduction and priming of the normal trophoblast and the trophoblast-derived malignant cells do not produce higher yields than when superinduced alone. This suggests that the mechanism of IFN induction in trophoblast cultures may be different from that of other cell lines such as fibroblast and macrophages since combined priming and superinduction significantly increase the IFN production (Toth et al., 1990b). In other species it has been reported (Nephew et al., 1993) that distinct mRNAs for ovine trophoblast and related Type 1 IFNs are differentially expressed suggesting the existence of distinct trophoblast IFN and related Type 1 IFN

TABLE 1

Effect of priming and superinduction on poly(rI).poly(rC)-stimulated IFN production

Treatment	Total IFN yield (IU/10^6 cells)			
	Trophoblast	Fibroblast	Macrophage	BeWo*
None	820 ± 160	256 ± 24	128 ± 24	32 ± 6
Priming	3200 ± 480	1800 ± 200	3400 ± 520	32 ± 6
Superinduction	8100 ± 1600	8600 ± 1800	3600 ± 540	1024 ± 180
Priming + superinduction	7800 ± 1400	25000 ± 6200	6600 ± 1400	1024 ± 180

*BeWo is a human trophoblast-derived malignant (choriocarcinoma) cell line.

genes. These data further suggests that the trophoblast and related Type 1 IFN genes display differential, tissue-specific expression and developmental regulation during pregnancy.

Induction of human trophoblast IFNs by viruses

When isolated trophoblast cultures are infected with viruses, such as Sendai and Newcastle Disease Virus (NDV) mixtures of IFN-α subtypes and -β are produced (Aboagye-Mathiesen et al., 1993). Table 2 shows the differential IFN yields and compositions from first and third trimester trophoblast and syncytiotrophoblast cultures stimulated with Sendai virus and NDV. The data demonstrate that first trimester trophoblast produce higher levels (about 5–6-fold) of IFNs than the third trimester trophoblast. The high level of IFN production in the first trimester as compared to term trophoblast, and the diverse biological effects of IFNs together suggest a role of trophoblast IFNs in a complex series of events in the early pregnancy.

The syncytiotrophoblast cultures established by culturing mononuclear cytotrophoblast for 3 to 4 days produce twice as much (in the case of NDV) IFN than mononuclear trophoblast at term (Table 2). Most of the physiological and biochemical functions of the human placenta are carried out by the syncytiotrophoblast which is the first fetally derived cell layer that an invading agent, such as viruses from the mother, has to transverse. This enhanced IFN production, as a result of the syncytia formation (differentiation), support the notion of IFNs being important for placental and fetal

TABLE 2

IFN yields and compositions of Sendai- and NDV-stimulated first and third trimester trophoblast and syncytiotrophoblast cultures

			Composition*	
	Inducer	IFN yield (IU 10^6 cells)	IFN-α (%)	IFN-β (%)
First trimester trophoblast	Sendai virus	42440 ± 4220 (9.9%)	25	75
Third trimester trophoblast	Sendai virus	7400 ± 880 (11.9%)	25	75
First trimester trophoblast	NDV	81200 ± 6340 (7.8%)	35	65
Third trimester trophoblast	NDV	16000 ± 1480 (9.3%)	35	65
Syncytiotrophoblast (first trimester)	Sendai virus	64240 ± 4820(7.5%)	25	75
Syncytiotrophoblast (third trimester)	Sendai virus	12800 ± 240(9.7%)	25	75
Syncytiotrophoblast (first trimester)	NDV	86680 ± 5560(6.4%)	35	65
Syncytiotrophoblast (third trimester)	NDV	32860 ± 2400(7.3%)	35	65

Data are the mean ± S.D. and the coefficient of variation of IFN antiviral activities in parenthesis; number of IFN preparations (N) is 9 and the replication of assay (n) is 6 ($P < 0.05$). *Determined by IFN neutralization test.

protection.

The magnitude of the IFN production in trophoblast cultures is dependent on the type of inducer (Table 2). Different viruses induce varying compositions of IFN-α and -β in trophoblast cultures. NDV induced higher levels of IFN than Sendai virus in both first and third trimester trophoblast and syncytiotrophoblast cultures. Table 2 also shows the compositions of the different trophoblast IFN preparations determined by antiviral neutralization assay using specific human IFN antisera. The data demonstrate that Sendai virus and NDV produce mixtures of IFN-α and -β. Sendai virus induce 75% IFN-β and 25% IFN-α whereas NDV induce 65% IFN-β and 35% IFN-α in trophoblast cultures. Although the compositions of the produced IFNs vary with the two inducers the levels of IFN-α and -β are the same for each inducer in the first and third trimester trophoblast and syncytiotrophoblast cultures.

Purification of human trophoblast IFN species

High-performance dye-ligand (HP-DLAC) and immunoaffinity chromatography (HP-IAC) of virus-induced trophoblast IFN preparations permitted the isolation of trophoblast IFN species (Aboagye-Mathiesen et al., 1991). The trophobast IFN species bound completely to a cibacron blue column, HEMA-BIO 1000 VS 3GA, when applied in 0.02 M sodium phosphate buffer (pH 7.2) at low ionic strength (0.2 M NaCl). The interactions of the trophoblast IFN

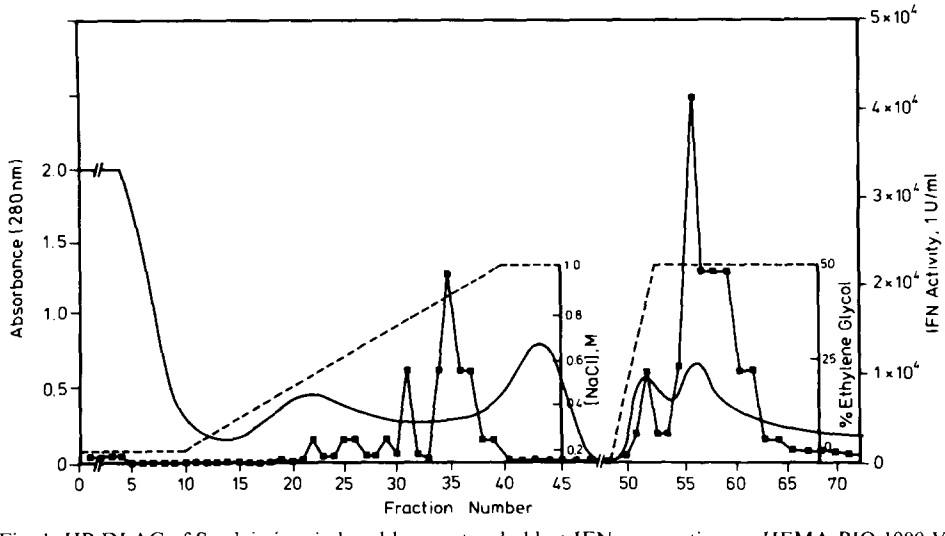

Fig. 1. HP-DLAC of Sendai virus-induced human trophoblast IFN preparation on HEMA-BIO 1000 VS 36A. The crude IFN preparation was applied at 0.2 M NaCl concentration. IFN activity (■) was eluted with a linear gradient of 0.2 M to 1.0 M NaCl (- - - - -). The column was further eluted with increasing concentration of ethylene glycol (- - - - -) from 0 to 50% for 10 min and 50% for 35 min. The A_{280} is shown by the continuous line.

components to the cibacron blue column were different. Elution of the column with a linear concentration gradient of NaCl (from 0.2 to 1.0 M) separated five IFN peaks (fractions 21 to 40 eluted between 0.6 to 0.8 M NaCl). This demonstrated that some fraction of the trophoblast IFNs binds electrostatically to the cibacron blue. Further development of the column with a linear concentration gradient of the hydrophobic solute ethylene glycol produced two IFN peaks (fractions 50 to 53 and 54 to 70), eluted from the column at ethylene glycol concentrations of 40 to 50% and 50% (Fig. 1) demonstrating the two IFN fractions to bind to the cibacron blue column by hydrophobic interaction. A combination of HP-DLAC and HP-IAC results in highly purified trophoblast IFN-α and -β species with specific activities ranging between 0.7–2.7×10^8 IU/mg of protein. A summary of a typical tandem HP-DLAC and HP-IAC of the trophoblast IFNs is presented in Table 3.

Characterization of purified trophoblast IFNs

Antigenic characterization

HP-DLAC and HP-IAC-purified trophoblast IFN components were classified by antiviral neutralization tests using polyclonal and monoclonal antisera to human IFN-α, -β and -γ and anti-IFN-α_{II}1 antibodies. A fraction of HP-DLAC-purified trophoblast IFN did not bind to polyclonal anti-IFN-α or -β column, and further the antiviral activity was not neutralized by polyclonal anti-IFN-α or -β. This showed that trophoblast cultures infected with Sendai virus produce mixtures of IFN-α subtypes, -β, and -α_{II} (Aboagye-Mathiesen et al., 1991) and probably a unique type of IFN whose antiviral activity is not neutralized by the already known human IFNs. Antiserum to recombinant IFN-α_{II}1 could not neutralize purifed trophoblast IFN-α_I and -β components but completely neutralised a fraction isolated by HP-IAC (Aboagye-Mathiesen et al., 1991). Since the trophoblast IFNs show distinct antigenicity and differences in their interactions with cibacron blue columns, this may suggest that they are structurally different.

TABLE 3

Purification of Sendai- and NDV-induced tro-IFNs by tandem HP-DLAC and HP-IAC

	Total activity (IU)	Specific activity (IU/mg)	Purification (-fold)	Recovery (%)
Crude tro-IFN (Sendai)	4.25×10^6	9.05×10^3	1	100
HPAC (tro-IFN-β)	2.99×10^6	1.15×10^8	12707.1	70.3
HPAC (tro-IFN-α)	9.80×10^5	7.01×10^7	7745.8	23
Crude tro-IFN (NDV)	5.50×10^6	9.98×10^3	1	100
HPAC tro-IFN-β	3.18×10^6	2.70×10^8	27054.1	57.8
HPAC tro-IFN-α	1.60×10^6	9.10×10^7	9118.2	29

tro-IFN = trophoblast IFN; HPAC = HP-DLAC and HP-IAC.

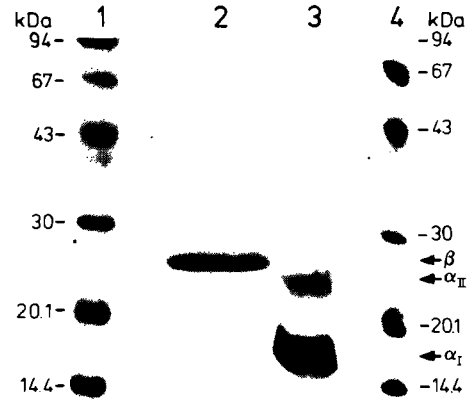

Fig. 2. Silver-stained SDS-polyacrylamide gel of tandem HP-DLAC and HP-IAC-purified trophoblast IFNs. Lanes 1 and 4, standard protein markers; lanes 2 and 3 are trophoblast IFN-β and $\alpha_I + \alpha_{II}$, respectively.

Chemical characterization

Silver-stained SDS-PAGE of purified trophoblast IFN-α, -α_{II}, and -β showed the molecular masses to be 16, 22 and 24 kDa, respectively (Fig. 2). Con A affinity chromatography of purified trophoblast IFN species revealed trophoblast IFN-α_{II} and -β to be glycoproteins (Aboagye-Mathiesen et al., 1992). The antiviral activities of purified trophoblast IFN components are stable at acidic conditions (pH 2.0) for 2 weeks at 4°C and for several months at −20°C. The trophoblast IFNs are therefore classified as human Type 1 IFN due to their stability at acidic conditions. Purified trophoblast IFN-β at concentration of 30 μg/ml is stable for 1 month in 0.02 M sodium phosphate buffer at pH 7.2 containing 50% ethylene glycol at 4°C. The antiviral activities of the trophoblast IFNs are stabilized to different degrees by SDS under reducing and non-reducing conditions at 37°C and 100°C. As shown in Table 4,

TABLE 4

Chemical stability of tro-IFN-α_I + -α_{II} and tro-IFN-β

Treatment	Incubation	tro-IFN-α_I + -α_{II}	tro-IFN-β
None	37°C, 1 h	2560 (100%)	4000 (100%)
1% SDS		2816 (110%)	640 (16%)
1% SDS + 1% β-ME + 5 M urea		640 (25%)	2560 (64%)
1% β-ME + 5 M urea		24 (0.93%)	64 (1.6%)
None	100°C, 1 min	22 (0.86%)	32 (0.8%)
1% SDS		2400 (93.7%)	640 (16%)
1% SDS + 1% β-ME + 5 M urea		256 (10%)	2560 (64%)
1% β-ME + 5 M urea		16 (0.63%)	768 (19.2%)

β-ME = β-mercaptoethanol, tro-IFN = trophoblast IFN.

the antiviral activities of trophoblast IFN-$\alpha_I + \alpha_{II}$ are stable in 1 per cent SDS at 37°C and 100°C but less stable under reducing (1% SDS, 1% β-mercaptoethanol and 5 M urea) conditions whereas trophoblast IFN-β is more stable under reducing conditions at 37°C and 100°C.

Biologic characterization of trophoblast IFNs

Antiviral activities of trophoblast IFNs

The human trophoblast IFNs (α, -α_{II}, and -β) have potent antiviral activities (Aboagye-Mathiesen et al., 1991–1992) with specific activities between 0.7–2.7×10^8/mg of protein (see Table 3). Table 5 shows the antiviral activities of the trophoblast components on different human and bovine cell lines. They exhibit a broad spectrum of antiviral activities on the human cells (WISH, GM 2504 and GM 2767) tested, but trophoblast IFN-α_I and IFN-α_{II} protected bovine MDBK cells better than human cells (2-fold and 4-fold, respectively, relative to human WISH cells). However, the protection of MDBK cells by trophoblast IFN-α_{II} was twice that conferred by trophoblast IFN-α_I. In contrast, trophoblast IFN-β does not protect bovine MDBK cells but protect all the human cell lines tested.

Inhibition of cell proliferation

The trophoblast IFNs inhibit or slow cell proliferation in vitro. Such effects on cellular function are apparent on both normal and malignant cells. The biologic characterization of trophoblast IFNs as an inhibitor of cell proliferation has been examined by [^{3}H]thymidine incorporation in normal trophoblast (Fig. 3A) and trophoblast-derived malignant cell line JAR (Fig. 3B). The inhibition of cell proliferation as measured by [^{3}H]thymidine incorporation was up to 52% in the choriocarcinoma cell line compared to the 4 to 17% inhibition in the normal first trimester trophoblast cultures (Fig. 3).

The biochemical mechanisms for the regulation of cell proliferation by trophoblast IFNs remains to be studied. Furthermore, it remains to be studied

TABLE 5

Protection of human and bovine cell lines against VSV infection

		Antiviral activity*		
Species	Cell line	tro-IFN-α_I	tro-IFN-α_{II}	tro-IFN-β
Human	WISH	64	64	256
Human	GM 2504	192	224	768
Human	GM 2767	192	224	768
Bovine	MDBK	128	256	2

*Antiviral activity is expressed as the highest dilution giving 50% protection against VSV. tro-IFN = trophoblast IFN.

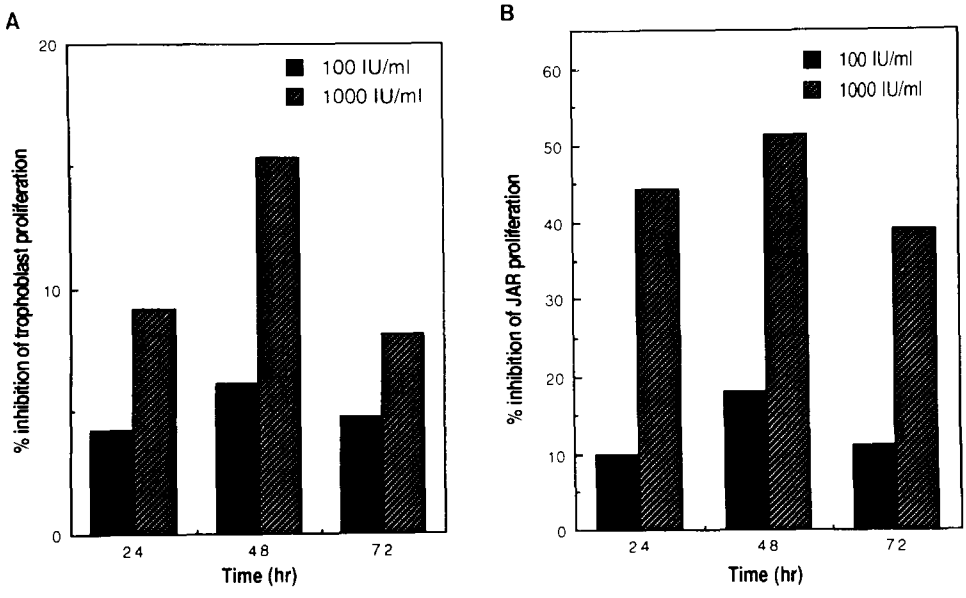

Fig. 3. Antiproliferative effect of human trophoblast IFN-β on first trimester trophoblast (Fig. 3A) and trophoblast-derived malignant cell line JAR (Fig. 3B) in vitro. First trimester trophoblast and trophoblast-derived malignant cell line JAR cultures were cultured with (100 and 1000 IU ml) and without trophoblast IFN-β. At different time intervals the cultures were pulsed with [^{3}H]thymidine (1 μg ml with specific activity of 2 Ci mmol, Amersham International) for 4 h. The radioactive incorporated was acessed by scintillation counter and the per cent inhibition of [^{3}H]thymidine incorporation in IFN-treated cells were calculated with respect to untreated cultures.

whether all the trophoblast IFNs (α, -β and α_{II}) alter cell proliferation via a similar mechanism.

Immunosuppressive properties

Trophoblast cells forming the reactive interface between the mother and the semiallogeneic fetus risk attack by the maternal immune system. The success of pregnancy in the face of potential maternal immune reactions has therefore been attributed to the placenta, which serve as an immunological barrier between the mother and the fetus. One of the factors hypothesized for the trophoblast to survive conditions of allograft rejection is the local immunosuppression surrounding the placenta. We (Zdravkovic et al., 1993) have therefore investigated the immunosuppressive effects of the human trophoblast IFN on the immune response in vitro. High-performance immunoaffinity-purified trophoblast IFN-β suppresses mitogen-induced proliferation of human T- (Fig. 4A) and B-lymphocytes (Fig. 4B) in vitro in a dose-dependent manner. These results indicate that local immunosuppression mediated by trophoblast IFNs may contribute to the absence of consistently cellular immunity against the fetus and thereby may prevent the maternal rejection of the embryo.

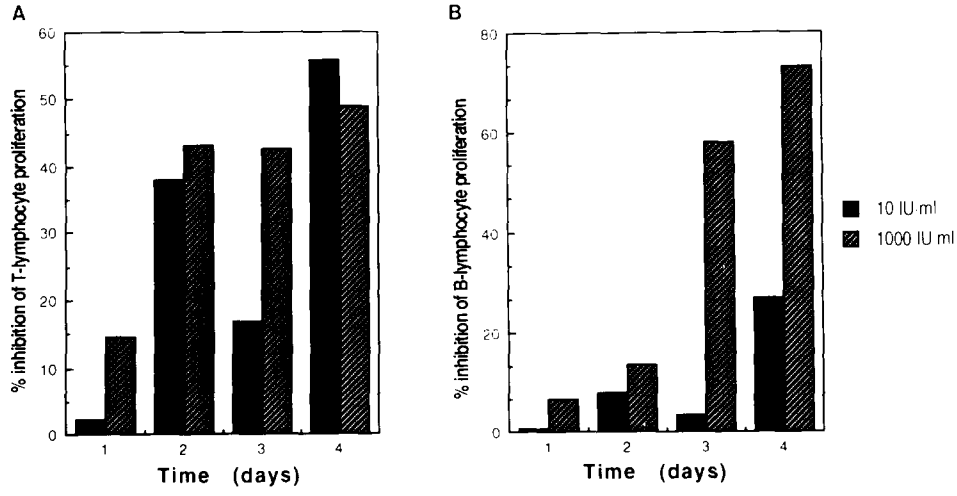

Fig. 4. Inhibition of mitogen-stimulated lymphocyte proliferation by trophoblast IFN-β. PHA-stimulated T lymphocyte (Fig. 4A) and pockweed-stimulated B-lymphocyte (Fig. 4B) cultures treated with (10 and 1000 IU ml) and without trophoblast IFN-β. At different time intervals the cultures were pulsed with [^{3}H]thymidine for 4 h. The per cent inhibition of proliferation in IFN treated cultures were calculated as the per cent inhibition of [^{3}H]thymidine incorporation with respect to untreated cultures.

Possible functions of trophoblast IFNs in fetal and placental protection and development in vivo

The data obtained on the human trophoblast IFNs and the reported biological functions of IFNs produced from other cells types are used here to hypothesize the possible functions of the trophoblast IFNs in the fetal and placental development. We speculate that the trophoblast IFNs may (i) protect the fetus from virus infections (ii) regulate trophoblast proliferation and differentiation (iii) regulate cellular differentiation of the fetus (iv) regulate the expression of a variety of trophoblast cell surface antigen expression and (v) have immunoregulatory functions in the feto-placental unit. At present, there is no evidence implicating the human trophoblast IFNs in maternal recognition of pregnancy as postulated for trophoblast IFNs of sheep and cattle. However, this is not to say that a possible role for human trophoblast IFNs during pregnancy can be ruled out. In humans, chorionic gonadotrophin (hCG) is thought to be responsible for the maintenance and stimulation of steriodogenesis in corpus luteum. IFN-α has been demonstrated to increase (2–3-fold) production of hCG (Iles and Chard, 1989) in experimental systems in vitro. This may suggest the possibility of an indirect involvement of the trophoblast IFNs in pregnancy.

Protection of the foetus from viral infections

The trophoblast layer of the human placenta acts as a barrier to the transmission of infection from mother to fetus. The ability of the trophoblast

layer to produce IFNs with potent antiviral activities, as demonstrated on different human cell lines, may represent a system for the protection of the foetus from viral infection. Human trophoblast IFNs and IFNs produced from other cell types have been shown to increase the expression of MHC-I antigen. This increased expression of MHC-I antigens has been implicated in the antiviral activity of IFNs, since a successful specific antiviral cell-mediated immune response depends on the ability of target cells to present the viral antigens in conjunction with MHC-I antigens. For example, it has been demonstrated that virus-induced IFNs restrict infection not only by inducing the antiviral state but also by conditioning infected cells (e.g., vaccinia or lymphocytic choriomeningitis virus-infected fibroblasts) for the destruction by cytotoxic T cells (Bukowski and Welsh, 1985). The possible antiviral functions of the trophoblast IFNs in the placenta during pregnancy may be documented by the fact that, in some cases, maternal infections may spread to the placenta but fail to progress to the foetus (Yamauchi et al., 1974; Klein et al., 1976; Remington and Desmonts, 1976).

Regulation of trophoblast proliferation and differentiation

IFNs are known to induce differentiation in a number of cell types. There is evidence that IFNs may function in a negative feed-back regulation of cell proliferation (Zullo et al., 1985). Cytotrophoblast cells have been demonstrated to be highly proliferative as measured by the presence of mitoses or by the proliferative marker Ki67 (Bulmer et al., 1988). These cells loose their proliferative activity and differentiate as they migrate into decidual during implantation. The factors that control these different characteristics of trophoblast behaviour are not known, but the possibility that trophoblast IFNs may play a regulatory role is worthy of consideration. IFN-α and -β have been shown to inhibit the proliferation of three human trophoblast-derived malignant (choriocarcinoma) cell lines (BeWo, HCCM-$_5$ and NUC-$_1$) in vitro in a dose-dependent manner as measured by [^{3}H]thymidine incorporation (Sekiya et al., 1986). Our preliminary data have shown trophoblast IFNs to inhibit the proliferation of extravillous and villous trophoblasts in vitro (unpublished data).

IFN-α and -β have been reported to down-regulate receptors for colony stimulating factor-1 (CSF-1) on murine peritoneal macrophages (Chen, 1986). This is encoded by *fms* cellular oncogene which has been identified in both murine (Adamson, 1987) and human trophoblasts (Hoshina et al., 1985). CSF-1 is thought to play a role in placental development (Arceci et al., 1989), and is an important component of the "immunotropism" concept of placental growth postulated by Wegmann (1988). Since CSF-1 also induce IFN-α/β production by cells its ability to induce trophoblast IFNs may provide a negative feed-back for growth regulation.

Regulation of cellular differentiation in the fetus

IFN-α has been demonstrated to up-regulate the production of ε-globin, a

component of embryonic hemoglobin, in K-562 cells (Friedman et al., 1992). Such an increase in ε-globin production has been equated by some with cellular differentiation towards an erythroid phenotype. This ε-globin is present in the hemoglobins Gower 1 and Gower 2 of the early human embryo during first trimester of pregnancy (Cioe et al., 1983). Since trophoblast IFNs are produced at high levels during the first trimester it is possible that it might up-regulate the production of ε-globin in the early human embryo and therefore may play a role in cellular differentiation in the fetus.

Regulation of trophoblast and other placental cell surface antigen expression

The different trophoblast subpopulations have different antigenic characteristic profile (Loke, 1988). The villous trophoblast is devoid of both MHC Class I and Class II antigens whereas the extravillous expresses a Class I-like molecule. Very little is known about the factors that regulate the MHC expression by human trophoblast.

IFNs have been reported to be a powerful inducer of both MHC Class I and Class II antigens on a number of cell lines (Hokland et al., 1988). Our recent results have demonstrated trophoblast IFNs to induce the expression of MHC Class I antigen on cytotrophoblast cells in vitro (Hager et al., unpublished data). This possible regulatory effects of trophoblast IFNs on MHC expression on trophoblast cell surface will be of considerable interest to immunologists.

IFNs are known to have different effects on different cells. Although our results have demonstrated the different trophoblasts to produce IFN-α and -β their ability to regulate MHC-I or/and MHC-II antigens on different trophoblast subpopulations remains to be determined. It is interesting to note that, while villous syncytiotrophoblast have been demonstrated to contain appreciable amounts of IFN-α in vivo (Howatson et al., 1988) and our data showing large amounts of IFN-α and -β production in vitro, this trophoblast layer has been reported to be devoid of Class I and Class II antigens. Furthermore, the syncytiotrophoblast is refractory to the inductive effects of IFN. It will therefore be of great interest to determine the effects of the trophoblast IFNs (α and β) on the expression of MHC Class I and Class II antigens on the different trophoblast subpopulations.

In addition, the trophoblast IFNs may modulate trophoblast and other placental cell surface antigens. For example, IFNs have been shown to increase both expression and shedding of surface antigens, including tumor-associated antigens (Marth et al., 1989). One of such antigens, Sialyl-Le^x, have been described to be localized to extravillous trophoblast (King and Loke, 1988). Increased expression or shedding of this antigen may affect many cellular interactions, for example, sensitivity to NK cell lysis (Bergelson et al., 1989).

Immunoregulatory effects

The trophoblast IFNs may have immunoregulatory functions in the feto-placental unit. The human placenta serves as an immunologic barrier between the maternal and fetal circulations, preventing the potentially destructive

maternal immune response from damaging the semiallogeneic foetus. The manner in which this barrier functions is, however, not well understood. One hypothesis advanced to explain the fetal survival is that local immunosuppression prevents the sensitization of the maternal immune system to paternal alloantigens and development of subsequent effector functions. Our present data (Zdravkovic et al., 1993) have demonstrated the trophoblast IFN-β to have immunosuppressive properties, that is, suppress the proliferation of stimulated B- and T-lymphocytes in vitro. This property of the trophoblast IFN can contribute to the immunosuppression in the feto-placental unit so that the local maternal immune response does not destroy pregnancy. Furthermore, IFN-α has been shown to be involved in prolongation of allograft survival (Hirsch et al., 1974; Mobraaten et al., 1973) and suppression of graft vs. host disease (Corettini et al., 1973). Such modulation of the immune response by trophoblast IFNs may also be important in preventing the rejection of the allogenic foetus.

Conclusion

The present results on the production and biochemical characterization of the human trophoblast IFNs have raised several questions. For example, apart from viruses are there natural inducers of the trophoblast IFNs? If so, can they be used pharmacologically to manipulate placental function during pregnancy? How many trophoblast IFNs can be expected to exist and are there any structural dissimilarities of the molecules? How do the trophoblast IFNs prevent the transmission of some viruses but not all from mother to foetus? Do the trophoblast IFNs have any involvement in controlling the maternal response to the fetal allograft? Do the trophoblast IFNs have specialized biological and chemical properties with respect to their unique site and time (only during pregnancy) of expression? How does the trophoblast IFNs regulate the specialized functions (proliferation, differentiation, invasive and the malignant-like properties) characteristic of the trophoblast? These questions are likely to have impact on the directions of future work in the areas of virology, immunology, biochemistry, oncology and reproductive biology.

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