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Production of Interferons in Human Placental Trophoblast Subpopulations and Their Possible Roles in Pregnancy

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The human cytotrophoblasts are the first fetal cells to arise during embryogenesis and are the progenitor cells to villous (noninvasive), syncytiotrophoblast (noninvasive), "intermediate" extravillous (invasive), and "anchoring" extravillous (invasive) trophoblast subpopulations. These trophoblast subpopulations were isolated from first- and third-trimester placentae and were stimulated with Sendai virus, granulocyte-macrophage colony-stimulating factors (GM-CSF), and platelet-derived growth factor (PDGF) to produce interferons (IFNs). GM-CSF and PDGF induced very low levels of IFN in first-trimester extravillous and villous trophoblast subpopulations. Highly proliferating and invasive intermediate extravillous trophoblast cultures produced five- to eightfold more IFNs than villous trophoblast cultures and two- to fivefold more IFN than the syncytiotrophoblast cultures when stimulated with Sendai virus. Syncytiotrophoblast cultures produced higher levels of IFNs (up to twofold) than villous trophoblast cultures when stimulated with the same virus. Pretreatment of first-trimester extravillous and villous trophoblast cultures with GM-CSF and PDGF followed by infection with Sendai virus resulted in greater IFN production than when the cultures were stimulated with virus alone. The levels of IFN produced were dependent on the type of trophoblast, the type of inducer, and the stage of differentiation of the trophoblasts. The purified trophoblast IFNs have potent antiviral activities when assayed on human amniotic WISH cells, and they inhibited proliferation of normal trophoblasts and trophoblast-derived malignant cells in vitro without any toxicity. Furthermore, the trophoblast IFNs activated NK cell activity and suppressed mitogen-stimulated lymphocyte proliferation at concentrations of between 10 and 1,000 IU/ml. The possible functions of the trophoblast IFNs during pregnancy are discussed with respect to human placental and fetal protection and development.

The human placental cytotrophoblast is the first fetal cell type to arise during embryogenesis. It is derived from the trophectoderm layer of the blastocyst (25) and is the progenitor cell type for all trophoblast subpopulations (17). The cytotrophoblast cells undergo two morphologically distinct pathways of differentiation leading to villous (noninvasive) and extravillous (invasive) trophoblast subpopulations (10). The extravillous trophoblasts are highly proliferative and invade into the maternal tissues, where they intermingle with decidual cells, while the villous trophoblast cell population forms the outermost layer of the placental villi. Furthermore, the villous and extravillous trophoblasts have different phenotypic and functional characteristics in the placenta (24). The syncytiotrophoblast cell layer is formed via fusion of cytotrophoblast cells and is continuously washed in the maternal blood. Cytotrophoblasts and syncytiotrophoblasts differ from each other not only in morphology but also in numerous endocrine properties (14, 15, 21).

Trophoblasts have many unique properties related to their role in providing nutrition, allowing exchange of nutrients and removal of waste products, secreting hormones, and providing immunological protection for the antigenically foreign fetus (26). Furthermore, the trophoblast layer is the first fetal cell layer that an invading agent, such as a virus, bacterium, or protozoan, has to transverse from mother to fetus. In humans, interferons (IFNs) have been detected in the amniotic fluid (22), feto-placental unit (16), and placental blood (7), but it is not known whether their production is the result of a natural developmental pattern or is induced by virus and mitogens from the maternal blood or by nonviral inducers such as growth factors and cytokines (5) produced locally by the maternal endometrium.

A growing body of evidence indicates that trophoblast cells from several species produce unique IFNs different from the classical type 1 IFNs (18, 23). For ruminants, it has been reported that trophoblasts of ovine and bovine conceptuses constitutively produce a new class of type 1 IFN, now called IFN-τ, in the early stages of pregnancy (9, 18, 34), and they are thought to initiate maternal recognition of pregnancy by maintaining the function of the corpus luteum. In nonruminant mammalian species (for example, in pigs, where maternal recognition is not mediated), a new type 1 trophoblast-specific and virus-inducible IFN has been identified and sequenced (23). Serological characterization of the trophoblast IFNs from both ruminants and nonruminants has shown their cross-reactivity with antibodies to virus-induced leukocyte IFNs. However, antibodies raised against the trophoblast IFNs failed to neutralize leukocyte IFNs. We have earlier reported IFN production from human term cytotrophoblast cultures stimulated with Sendai virus and have characterized these IFNs (1, 36). The inducers and the exact roles of the trophoblast IFNs in vivo during pregnancy are not known. In this paper, we report IFN production in human placental trophoblast subpopulations stimulated with Sendai virus, granulocyte-macrophage colony-stimulating factor (GM-CSF), and platelet-derived growth factor (PDGF). Furthermore, the possible roles of the trophoblast IFNs in the feto-placental unit are

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discussed with respect to fetal and placental protection and development during pregnancy.

MATERIALS AND METHODS

Viruses. Sendai virus (parainfluenza virus type 1) and vesicular stomatitis virus (VSV) (Indiana strain) were obtained from the American Type Culture Collection (Rockville, Md.). Sendai virus was propagated in 9- to 10-day-old embryonated eggs, and VSV was grown in monolayers of human amnion (WISH) cells.

Isolation and characterization of trophoblast subpopulations. First-trimester trophoblast populations were isolated from placental tissues (5 to 12 weeks) obtained after legal termination of pregnancy. Briefly, placental tissues were washed in sterile phosphate-buffered saline (s-PBS) and were subjected to three sequential 10-min treatments with 0.125% trypsin–10 U of DNase I per ml in s-PBS containing 5 mM $MgCl_2$. At every 10-min step the cells released were pooled and filtered through two layers of muslin. The filtrates were centrifuged, and the cell pellets were resuspended in s-PBS. Cell pellets from each 10-min step were resuspended in s-PBS and were subjected to 25 to 70% Percoll gradient centrifugation. Cells from the middle band (density, 1.048 to 1.062 g/ml) of the gradient were pooled, washed, and seeded at a density of 10^6 cells per ml of keratinocyte growth medium (KGM) (CA 92123; Clonetics Corporation) supplemented with 10% fetal calf serum (FCS). After 12 to 20 h of incubation in humidified 5% CO_2–95% air at 37°C, the cultures were washed twice with s-PBS and refed with fresh KGM supplemented with 10% FCS. After 3 to 4 days, the "intermediate" extravillous trophoblasts (cells released from the 10-min trypsinization) that detach from the culture bottles and form cell clumps were removed and transferred into a new culture bottle. The medium was changed every 3 days until the cell clumps differentiated to "anchoring" extravillous trophoblasts at 21 days after plating. Third-trimester villous trophoblasts were isolated from placentae from uncomplicated deliveries by the method described by Douglas and King (6). Syncytiotrophoblast cultures were prepared by culturing isolated first-trimester villous trophoblasts (cells from the 30-min trypsinization) and third-trimester villous trophoblasts for 3 to 5 days in KGM supplemented with 10% FCS.

The isolated first- and third-trimester trophoblast cells were analyzed by flow cytometry and immunohistochemistry staining by using a mouse monoclonal antibody to human cytokeratin, which within the placental villi stains only trophoblast cells, anti-CD9, which stains CD9 antigens expressed on all placental mesenchymal cells, placental macrophages, and blood monocytes but not expressed on trophoblast cells (29, 39); antibody to porcine vimentin; anti-CD68 (a macrophage marker); and anti-human HLA class 1 antibodies (W6/32; Dakopatts A/S, Glostrup, Denmark), which react with extravillous trophoblast but not villous trophoblast cells. The trophoblast cultures were further characterized by their secretion of human chorionic gonadotropin (hCG) hormone.

Assay of hCG. hCG was analyzed by using a two-site immunometric method. The capture antibody was a monoclonal anti-α-chain antibody, and the detecting antibody was biotinylated anti-β-chain antibody. The biotinylated antibody was detected by using peroxidase-labeled avidin, which was measured by a colorimetric reaction with 3, 3′, 5, 5′-tetramethylbenzidin. The results were standardized against the World Health Organization standard, 1. I.R.P. (75/537).

Induction of IFN in trophoblast subpopulations. First- and third-trimester mononuclear trophoblast cells were seeded in KGM supplemented with 10% FCS at 37°C and 5% CO_2 in air. Trophoblast cultures were infected with Sendai virus (100 hemagglutinating units per 10^6 cells) for 1 h in serum-free KGM. The unadsorbed virus was removed by washing the cells three times in serum-free medium. The virus-infected cells were cultured in KGM containing 5 or 1% FCS (for immunoaffinity purification) for 18 h, and the supernatants which contained the produced IFNs were harvested and acidified with HCl to pH 2.0 for 48 h at 4°C to inactivate virus and then were neutralized with NaOH to pH 7.2. To test the effects of GM-CSF and PDGF on the production of trophoblast IFNs, cultures were stimulated with GM-CSF (200 IU/ml with or without PDGF [5 ng/ml]) for 24 h and were then infected with Sendai virus as described above. GM-CSF at doses of 150 to 300 IU/ml has been demonstrated to enhance trophoblast IFN production (19). Control trophoblast cultures were established similarly, except that no virus, GM-CSF, or PDGF was added.

IFN bioassay. IFN antiviral activity was determined in a cytopathic effect inhibition assay with human amniotic WISH cells (American Type Culture Collection) with VSV as the challenge virus. In brief, 20 to 25,000 cells were seeded in wells of microtiter plates (Costar) in minimal essential medium with 5% FCS. After 24 h of incubation at 37°C and 5% CO_2 in air, the medium was removed and the cells were incubated with 100 μl of medium or serial two- or fourfold dilutions of various IFN preparations. After incubation for 18 to 24 h, the cells were infected with VSV (50 PFU per well). The IFN titers were scored microscopically 24 h after virus incubation. The highest dilution giving 50% protection was taken as the end point. All IFN titers were determined in duplicate on at least two occasions. IFN titers were standardized by comparison with the National Institutes of Health standard for human IFN-α (GA 239-025-30) and -β (G-023-902-527).

Typing of IFNs. IFN species present after induction or purification were determined by antibody neutralization assay. Aliquots of IFN samples were independently mixed with specific antisera. Quantities of antisera were selected to neutralize 100 U of IFN bioactivity, and IFN samples were adjusted to contain 100 IU. Incubations were carried out for 1 h at 37°C with the specific antisera (specific anti-human IFN-α, anti-human IFN-β, and anti-human IFN-γ) or with normal control serum, and then the samples were assayed for presence of IFN antiviral activity.

Tandem immunoaffinity chromatography. IFNs were purified by tandem immunoaffinity chromatography with anti-IFN-α and anti-IFN-β columns connected in series. Polyclonal anti-IFN-α and anti-IFN-β were coupled to CNBr-activated Sepharose 4B by in situ immobolization as recommended by the supplier (Pharmacia, Uppsala, Sweden). Culture supernatants were applied to 5-ml columns at a flow rate of 1 ml/min. The columns were washed with 100 mM sodium phosphate buffer (pH 7.4) and eluted with 100 mM glycine-HCl (pH 2.6) by step elution or by a pH gradient from pH 7.4 to 2.6. Eluate fractions were neutralized with 1 M NaOH and were assayed for IFN activity. Fractions containing IFN antiviral activity were pooled and stored frozen at −20°C.

Antiproliferative responses. The antiproliferative effect of the trophoblast IFNs was measured on normal trophoblasts and the trophoblast-derived malignant cell line BeWo, using the tetrazolium salts XTT cell proliferation kit II (catalog no. 1465015; Boehringer Mannheim Biochemical) according to the manufacturer's instructions. The assay is based on the cleavage of the yellow tetrazolium salt XTT to formazan dye by dehydrogenase activity in active mitochondria. This conversion occurs only in living and proliferating cells. The formazan dye formed was directly quantified by using a multiwell spectro-

photometer (enzyme-linked immunosorbent assay [ELISA] reader).

Briefly, 10^5 cells per well in a final volume of 100 μl were grown in a 96-well microtiter plate with or without IFN treatment. At 24-h intervals cell cultures were incubated with XTT solution (final concentration of 0.3 mg/ml) for 6 h, and the orange formazan color formed was quantified at 490 and 650 nm with an ELISA reader. After spectrophotometric measurement, the medium was removed and the cells were stained with trypan blue for cell viability. The percent inhibition of proliferation was calculated as $[(C_n - I_n)/C_n] \times 100$, where $C_n = A_{490}/A_{650}$ for control cultures at day n and $In = A_{490}/A_{650}$ for IFN-treated cultures.

Suppression of lymphocyte proliferation by trophoblast IFN. Human peripheral blood was obtained from Aarhus University Hospital (Aarhus, Denmark), and mononuclear lymphocytes were isolated by gradient centrifugation with Ficoll-Paque (Pharmacia) (38). The intermediate band containing the peripheral blood mononuclear cells (PBMC) was harvested and washed in PBS. The cells were resuspended to a final concentration of 10^6 viable cells per ml in RPMI 1640 supplemented with 10% FCS. B- and T-lymphocyte proliferation was obtained by stimulating PBMC with pokeweed mitogen (PWM) (5 μg/ml) and phytohemagglutinin (PHA) (1 μg/ml), respectively.

PHA- or PWM-stimulated PBMC at a concentration of 1×10^6 cells per ml were seeded in 96-well microtiter plates (Nunc, Roskilde, Denmark) with 50×10^3 cells per well. Triplicate wells were stimulated with various concentrations of trophoblast IFN-β or recombinant IFN-β (0, 10, 100, and 1,000 IU/ml). Proliferation was measured by incorporation of [^{3}H]thymidine (1 μg/ml; specific activity, 2 Ci/mmol; Amersham International). Briefly, cells were pulsed with [^{3}H]thymidine for 4 h at 24, 48, 72, 96, and 128 h after being seeded. Following incorporation of the radioactive precursor, the cells were harvested and absorbed on filter discs. The filters were washed in 10% trichloroacetic acid (Sigma, St. Louis, Mo.) and then in 5% trichloroacetic acid and finally dried in 99.9% ethanol. The filters were transferred to plastic beta-vials (Sarstedt, Nümbrecht-Rommelsdorf, Germany), and 5 ml of scintillation fluid (Filter-Count; Packard) was added to the vials. The radioactivity was assessed with a scintillation counter (LKB 1203; Wallac Finland). The inhibition of proliferation in cells supplemented with IFN was calculated relative to proliferation in cells not stimulated with IFN by the following equation:

$$\{1 - [(\text{cpm}_{\text{cells without IFN}} - \text{cpm}_{\text{cells with IFN}})/\text{cpm}_{\text{cells without IFN}}]\} \times 100 \quad (1)$$

Cell viability. In all experiments the viability of the cells was determined by the trypan blue dye exclusion test.

Effect of trophoblast IFNs on NK cell activity. Natural killer (NK) activity was determined by the spontaneous release of radioactivity from ^{51}Cr-labeled K562 (ATCC CCL 243) target cells placed in contact with different concentrations of effector PBMC in a 3-h assay. The K562 cells were maintained continuously in RPMI 1640 supplemented with 10% FCS. After two washes with PBS, 100 μl of sodium chromate-51 and 100 μl of complete medium were added, and the cells were incubated for 3 h. The labeled K562 cells were washed three times in PBS and resuspended at density of 10^5 cells per ml in RPMI 1640 supplemented with 10% FCS.

The effector cells were prepared from fresh buffy coats of healthy donors. The PBMC were fractionated by Ficoll-Paque (Pharmacia) gradient centrifugation. The cells in the intermediate band were harvested, washed, and seeded at a density of 5×10^6 cells per ml in medium alone or medium containing various concentrations of trophoblast IFNs (α and β) for 18 h at 37°C.

Treated effector and ^{51}Cr-labeled target cells were seeded into 96-well plates with effector cell-to-target ratios of 100:1, 50:1, 25:1, and 12.5:1. Spontaneous-release wells contained target cells only, and maximum-release wells contained target cells plus 100 μl of 5% Triton X-100. The plates were centrifuged at $50 \times g$ for 5 min to facilitate contact between effector and target cells before incubation at 37°C. The assay was terminated at 3 h by centrifuging the plates at $400 \times g$ for 10 min at room temperature, and then 100 μl of the supernatant was removed from each well and a gamma counter was used to determine the amount of released ^{51}Cr. Results of triplicate assays were expressed as percent specific release (NK activity) by using the following formula: percent specific release = (sample dpm − spontaneous-release dpm)/(maximum-release dpm-spontaneous-release dpm) × 100.

RESULTS

Culture and characterization of trophoblast subpopulations. Figure 1 shows the cultures of villous trophoblast (noninvasive), villous syncytiotrophoblast, intermediate extravillous trophoblast (invasive), and anchoring extravillous trophoblast (invasive) cultures used for the IFN production. The syncytiotrophoblast and the differentiated extravillous trophoblast cultures were established from mononuclear villous and intermediate extravillous trophoblast cultures 4 and 21 days after seeding, respectively. The extravillous trophoblast populations (Fig. 1E to G) were HLA positive, whereas the villous trophoblast populations (Fig. 1A to D) were HLA negative. Immunohistochemical staining of the trophoblast cultures revealed the cultures to be negative to anti-CD68 (macrophage marker) and anti-CD9, indicating that the cultures were free of macrophages and possible contamination by placental fibroblast cells. Cytokeratin staining of the differentiated trophoblast populations, showing their epithelial characteristics, is shown in Fig. 2.

The secretions of the pregnancy hormone hCG by the trophoblast populations were different and increased with time in culture (Fig. 3). However, intermediate extravillous trophoblast cells secreted higher levels of hCG than the villous trophoblast and syncytiotrophoblast (from 96 to 144 h; Fig. 3) cells from the first and third trimesters. When the intermediate extravillous trophoblast cells were cultured for 21 days after seeding, they differentiated (Fig. 1E to G) to anchoring extravillous trophoblasts, and the hCG secretion reduced significantly, to below 5% (data not shown).

Differential IFN production in trophoblast subpopulations. Table 1 illustrates the differential IFN production in the trophoblast populations stimulated with Sendai virus, GM-CSF, and PDGF. GM-CSF induced very low levels of IFN in the extravillous trophoblast populations. However, villous trophoblast cells at term produced undetectable levels in most cases, whereas villous trophoblast and syncytiotrophoblast cells at the first trimester produced lower levels of IFN than the extravillous trophoblasts when treated with GM-CSF and PDGF. Pretreatment of the extravillous trophoblast populations and the first-trimester villous trophoblast cells with GM-CSF and PDGF followed by infection with Sendai virus resulted in a much higher IFN production than when the cells were infected with virus alone (Table 1).

First-trimester intermediate extravillous trophoblasts pro-

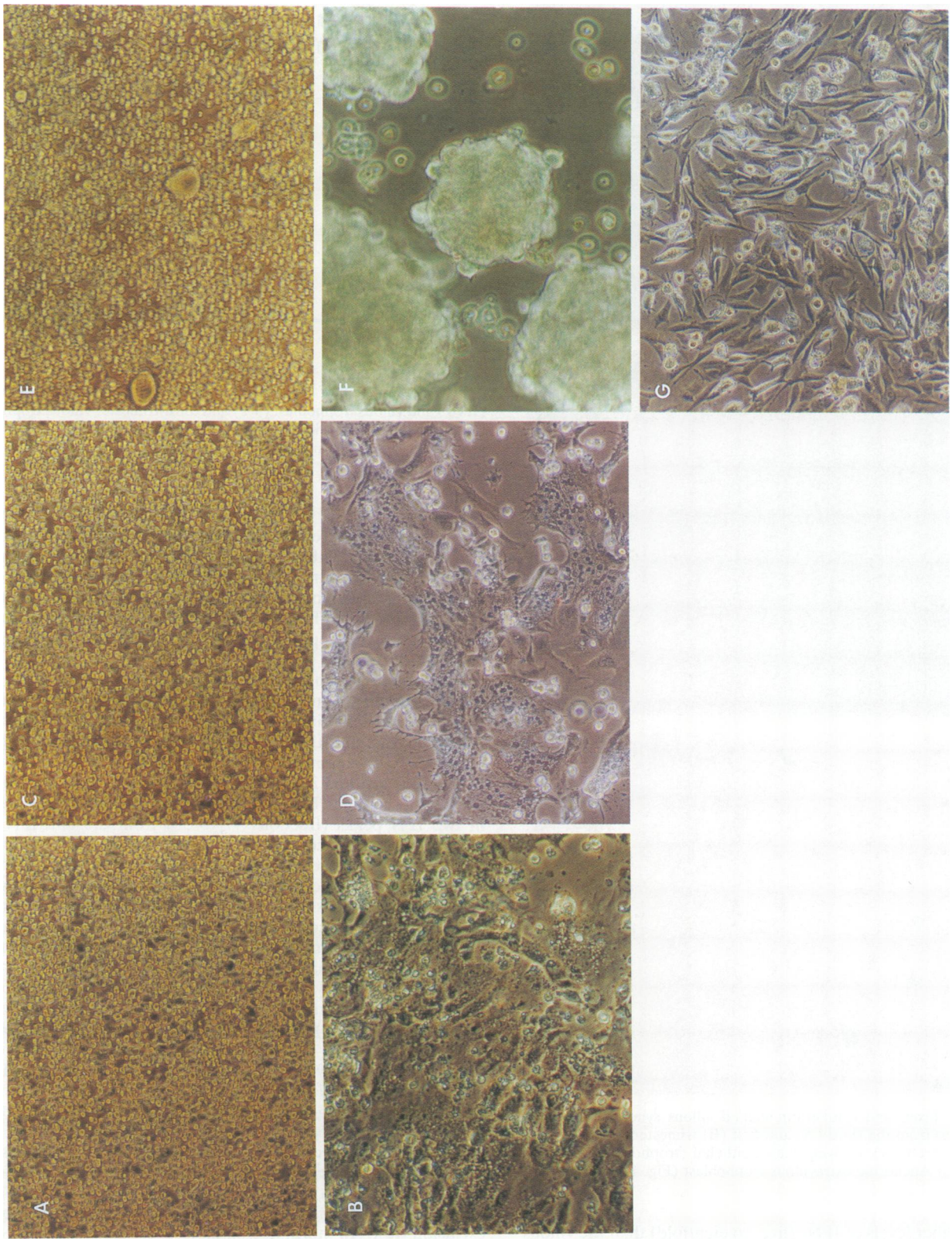

FIG. 1. Isolated human trophoblast populations in culture (phase-contrast microscopy; unfixed and unstained). (A and B) Third-trimester villous trophoblast populations 6 h and 4 days after plating, respectively. (C and D) First-trimester villous trophoblast populations 6 h and 4 days after plating, respectively. Panels B and D show the villous syncytiotrophoblast populations differentiated from the populations shown in panels A and C, respectively. (E to G) First-trimester extravillous trophoblast populations cultured for 6 h, 4 days, and 21 days, respectively. Panels F and G show the intermediate and anchoring extravillous trophoblast populations, respectively.

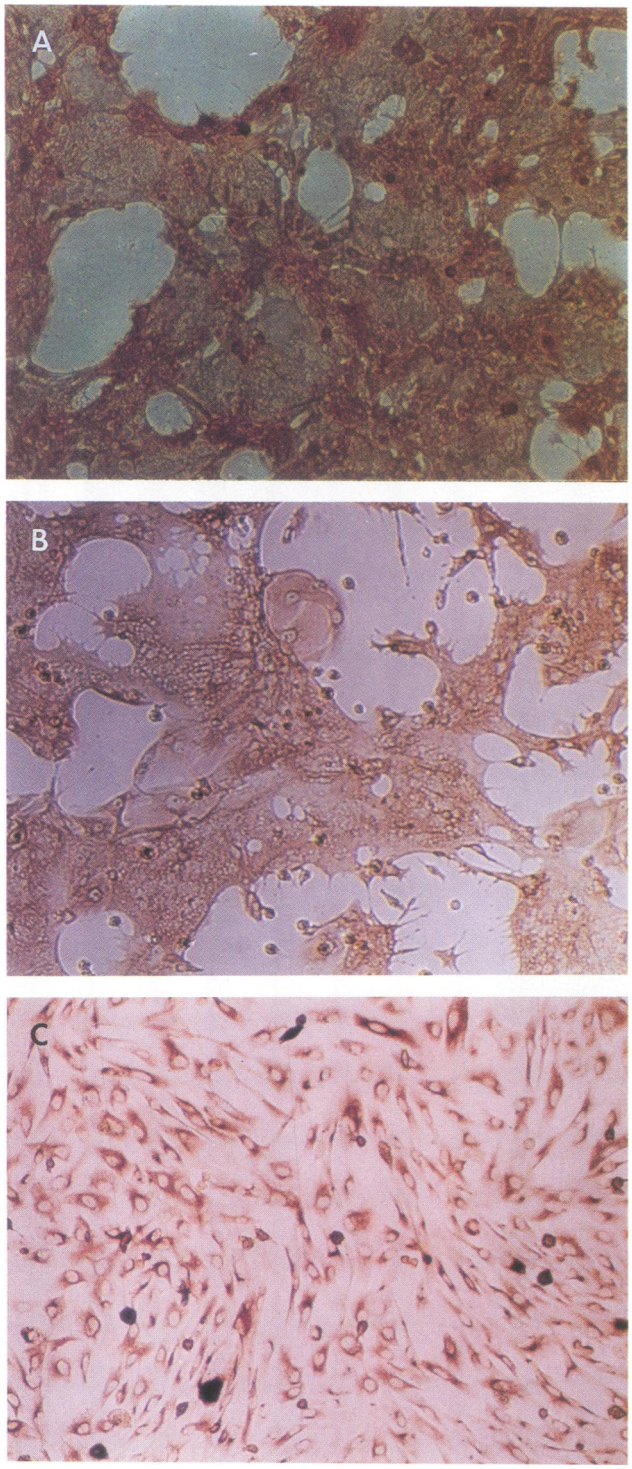

FIG. 2. Fixed and cytokeratin-stained villous syncytiotrophoblast populations from the third (A) and first (B) trimesters (from Fig. 1B and D, respectively), showing their epithelial (trophoblastic) characteristic. (C) Anchoring extravillous trophoblast (Fig. 1G).

duced higher levels of IFNs (five- to eightfold) than the villous trophoblasts from the first and third trimesters (Table 1). Differentiation of the mononuclear villous trophoblast cells to syncytiotrophoblast cells increased the IFN production. Syncytiotrophoblast cultures produced up to twofold more IFN than the mononuclear villous trophoblast cultures. However, anchoring extravillous trophoblast cells produced a lower level of IFN on a per-cell basis than the intermediate extravillous trophoblast cells. Thus, the level of IFN production was dependent on the trophoblast population, differentiation stage, and type of inducer.

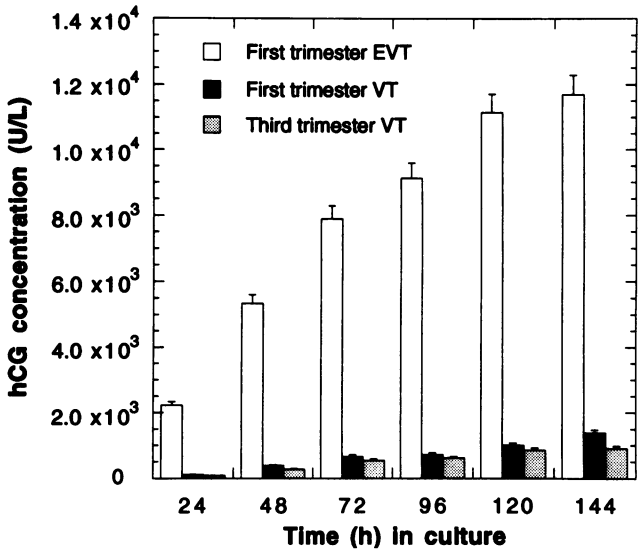

FIG. 3. Secretion of hCG by first-trimester extravillous trophoblast (EVT) and villous trophoblast (VT) and third-trimester villous trophoblast populations. Results are means (n = 3), and error bars represent standard errors of the mean.

Purification of trophoblast IFNs. Figure 4 shows tandem immunoaffinity chromatography of virus-induced trophoblast IFN preparations. Ninety-two percent of the total IFN activity bound to the anti-IFN-β and -α columns. Elution of the immunoaffinity columns with a step gradient or a pH gradient (7.4 to 2.6) resulted in the elution of one IFN peak from the anti-IFN-β column (Fig. 4A), whereas a pH gradient resulted in two IFN peaks (fractions 47 to 56) from the anti-IFN-α column (Fig. 4B). The pooled fractions 12 to 24 (Fig. 4A) and 47 to 56 (Fig. 4B) had specific activities of 1.13×10^8 and 9.87×10^7 IU/mg of protein, respectively, when assayed on human amniotic WISH cells. The antiviral activities eluted from the anti-IFN-β and -α columns were neutralized by monoclonal anti-IFN-β (100 IU/ml initially; 0 IU/ml after antibody treatment) and polyclonal anti-IFN-α (100 IU/ml initially; <2 IU/ml after antibody treatment). However, monoclonal anti-IFN-α could not completely neutralize the IFN-α preparation (fractions 47 to 56), suggesting that the preparation may consist of mixtures of IFN-α subtypes that are devoid of antigen cross-reactivity with monoclonal antibodies to the classical IFN-α species.

Antiproliferative activity of trophoblast IFNs. The antiproliferative effect of trophoblast IFN-α and -β was determined in normal trophoblast cultures and cultures of the trophoblast-derived malignant cell line BeWo (Fig. 5 and 6). The trophoblast IFNs showed a dose-dependent inhibition of cell proliferation without toxicity as determined by the trypan blue dye exclusion test. In normal trophoblast cultures, 100 IU of trophoblast IFN-α and -β per ml caused inhibition of proliferation by 4.5 to 12% ($P < 0.005$) and 6 to 13% ($P < 0.005$), respectively, in 72 h, whereas at 1,000 IU/ml the inhibition was

TABLE 1. IFN production in trophoblast subpopulations stimulated with GM-CSF, PDGF, and/or Sendai virus

Inducer	IFN (IU/10^6 cells) in[a]:					
	First-trimester trophoblasts				Third-trimester trophoblasts	
	Intermediate extravillous	Anchoring extravillous	Villous	Syncytiotrophoblast	Villous	Syncytiotrophoblast
GM-CSF	36 ± 8	24 ± 4	12 ± 6	16 ± 4	UD[b]	12 ± 4
GM-CSF + PDGF	64 ± 16	36 ± 9	24 ± 4	32 ± 6	UD	16 ± 5
Sendai virus	62,500 ± 7,860	28,400 ± 4,620	12,140 ± 1,640	22,800 ± 2,200	7,400 ± 480	12,800 ± 1,760
Sendai virus + GM-CSF + PDGF	115,625 ± 8,440	56,880 ± 9,640	31,250 ± 5,460	42,700 ± 8,640	12,690 ± 1,630	24,420 ± 3,200

[a] Data represent means ± standard deviations (n = 5).
[b] UD, undetectable.

12 to 17% ($P < 0.001$) and 12 to 19% ($P < 0.001$), respectively, relative to the control.

The trophoblast IFN-α and -β also inhibited proliferation of the trophoblast-derived malignant cell line BeWo (Fig. 5B and 6B). The inhibition at 100 IU of trophoblast IFN-α and -β per ml was 4 to 9% ($P < 0.001$) and 15 to 19% ($P < 0.001$), respectively, in 72 h relative to the control. At 1,000 IU/ml, trophoblast IFN-α and -β were much more effective in inhibiting BeWo cell proliferation. Trophoblast IFN-α and -β inhibited the proliferation of BeWo cells by 31 to 47% ($P < 0.001$) (Fig. 5B) and 40 to 52.5% ($P < 0.001$) (Fig. 6B), respectively, in 72 h after IFN treatment.

Suppression of mitogen-stimulated lymphocyte proliferation by trophoblast IFNs. Stimulation of T- and B-lymphocyte proliferation caused by PHA and PWM stimulation of PBMC, respectively, was inhibited by trophoblast IFN-β (Fig. 7). The inhibition by trophoblast IFN-β was higher for PHA-stimulated lymphocytes (Fig. 7A) than for PWM-stimulated B lymphocytes (Fig. 7B). The inhibition of T-lymphocyte proliferation by trophoblast IFN-β was dose dependent at IFN activities of between 10 and 1,000 IU/ml. The maximum average inhibition caused by trophoblast IFN-β was 56% with 1,000 IU/ml for PHA-stimulated T lymphocytes at day 4 and 31% with 1,000 IU/ml for PWM-stimulated B lymphocytes at day 2.

Activation of NK cell activity. Trophoblast IFN-α and -β activated NK cell activity in vitro (Fig. 8). The increased NK cell activity was observed at all tested effector/target cell ratios at concentrations of between 10 and 1,000 IU/ml. However, the NK cell activity was much higher at effector/target cell ratios of 50 when activated with 10 IU/ml than it was when activated with 1,000 IU/ml ($P < 0.05$).

DISCUSSION

IFN production during pregnancy is now known to be characteristic of the human placenta. Isolated cells and in vitro culture of human unstimulated trophoblast cells produce undetectable levels of IFN. Our earlier reports and the present data demonstrate that trophoblast cells can produce IFNs when stimulated with CSF, PDGF, or viruses. Trophoblast cells have been demonstrated to express receptors for CSFs (37), and CSFs have been shown to be produced by decidual cells (8), including large granular lymphocytes (LGL) and macrophages. Therefore, the interaction between trophoblast cells and soluble factors, including CSF and PDGF secreted by

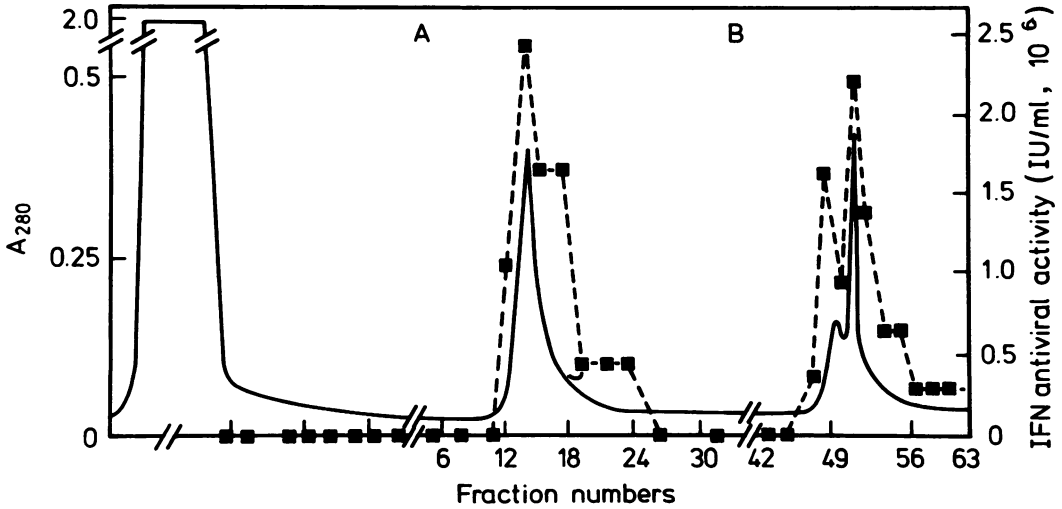

FIG. 4. Tandem immunoaffinity chromatography of human trophoblast IFNs. A concentrated crude trophoblast IFN preparation (15.5 × 10^6 IU) was filtered through a 0.22-μm-pore-size Millipore filter and applied to tandem anti-IFN-β and anti-IFN-α columns. The columns were washed with 100 mM sodium phosphate buffer, pH 7.4. IFN activity (– – –) was eluted from the anti-IFN-β column (A) and anti-IFN-α column (B) by a step elution (0.1 M glycine-HCl, pH 2.6) and a pH gradient elution (pH 7.4 to 2.6), respectively. ——, A_{280}.

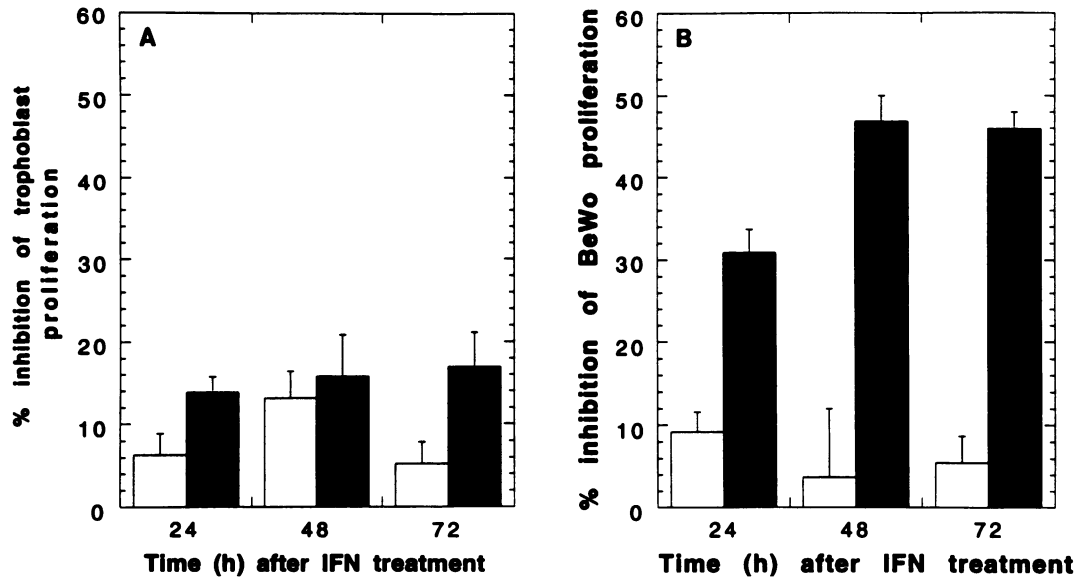

FIG. 5. Antiproliferative effect of human trophoblast IFN-α on first-trimester trophoblast cells (A) and the trophoblast-derived malignant cell line BeWo (B) with 100 (□) and 1,000 (■) IU/ml. Results are means (n = 3), and error bars represent standard errors of the mean.

maternal tissues, may be one of the mechanisms of induction of trophoblast IFNs in vivo during pregnancy. The feto-placental unit is known to be a site of viral, bacterial, and protozoal infections. All of these infectious agents are known to induce IFNs (13). Furthermore, the feto-maternal tissue yields a graft-versus-host reaction, and for species such as the mouse it has been reported (32) that both acute and chronic graft-versus-host disease potentiate IFN-α/β production. The topographical locations of the trophoblast populations in vivo and the varied IFN production may suggest their specific local production and their functions in the complex series of events during pregnancy.

IFN has long been recognized to induce a variety of cells to express antiviral activity and numerous biological functions. The potent antiviral, antiproliferative, and immunosuppressive effects, as well as increased NK cell activity, induced by the trophoblast IFNs may have important physiological and pathological roles in human pregnancy.

The antiviral activity of trophoblast IFNs may serve as the first line of defense against hematogenous infections. IFNs,

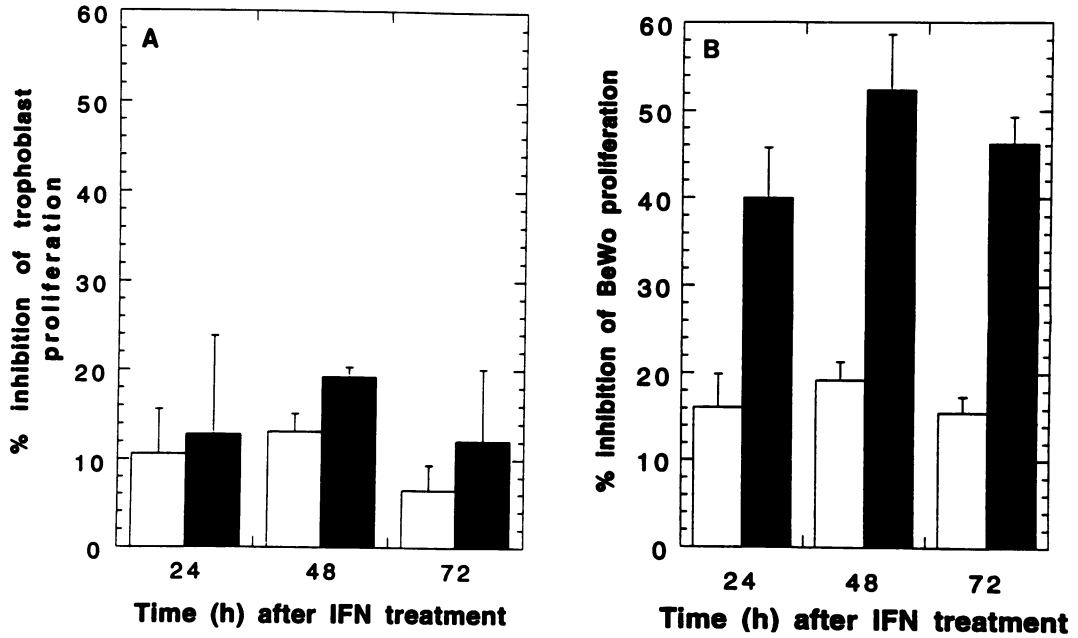

FIG. 6. Antiproliferative effect of human trophoblast IFN-β on first-trimester trophoblast (A) and BeWo (B) cell lines at 100 (□) and 1,000 (■) IU/ml. Results are means (n = 3), and error bars represent standard errors of the mean.

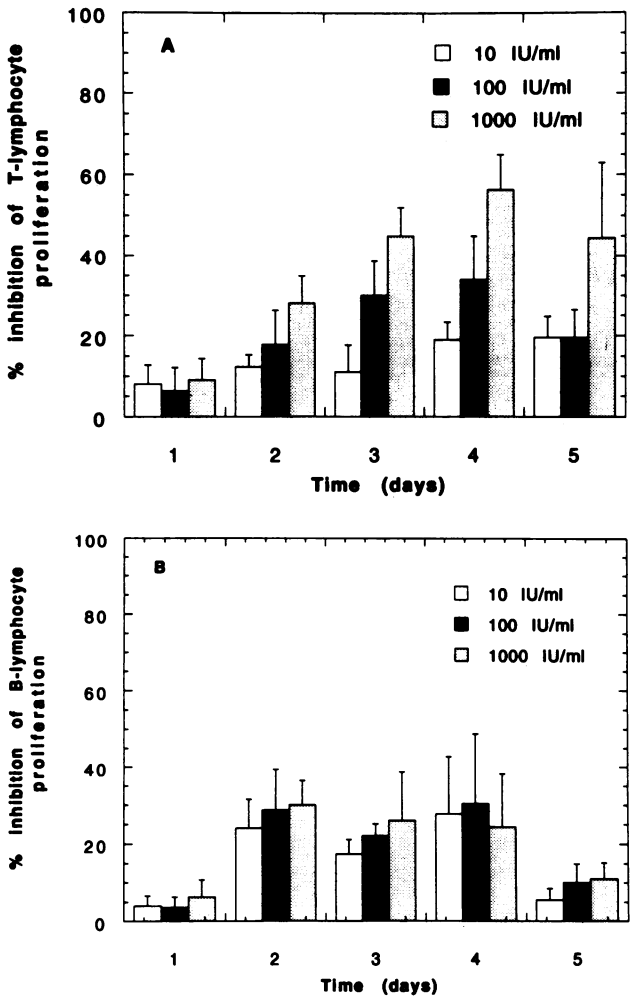

FIG. 7. Inhibition of mitogen-stimulated lymphocyte proliferation by trophoblast IFN-β, showing suppression of PHA-stimulated T-lymphocyte (A) and PWM-stimulated B-lymphocyte (B) proliferation at different IFN concentrations. Results are means of triplicate determinations for each treatment, and error bars represent standard errors of the mean.

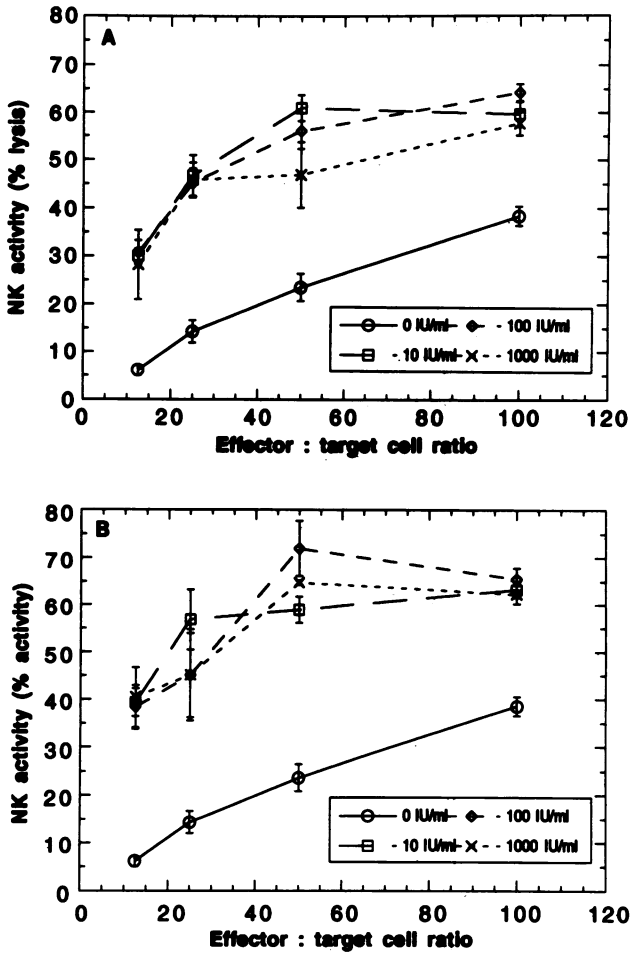

FIG. 8. Effect of different concentrations of trophoblast IFN-α (A) and -β (B) on NK cell activity (percent lysis) towards K562. Each value represents the mean of triplicate determinations for each treatment, and error bars represent standard errors of the mean.

apart from their antiviral activity, are also known to have antibacterial and antiprotozoal activities. Both IFN-α and -β have been reported (30) to play a role in host defense against nonviral pathogens and in mononuclear phagocyte activity against both intra- and extracellular parasites, bacteria, and fungi. For example, IFN-β has been reported to activate macrophages in vitro to kill the protozoan *Toxoplasma gondii* (31, 35). Both clinical evidence and experimental evidence suggest that on some occasions infectious agents may infect the placenta without involving the fetus (2). For example, in maternal rubella the placenta is more commonly infected than is the fetus (27); placenta malaria occurs frequently, while fetal malaria is uncommon (4); and tuberculosis of the placenta is uncommonly associated with any evidence of fetal involvement. Placental trophoblast IFN production therefore is probably an important factor in limiting transplacental viral and possibly bacterial spread from mother to fetus. This concept may be supported by the ability of different trophoblast populations with different topographical functions to produce different levels of IFNs when stimulated with different inducers. Furthermore, the ability of soluble factors such as CSFs and PDGF secreted by the maternal cells to regulate trophoblast IFN production may also suggest the notion that the protective function of the trophoblasts may be partly regulated by maternal factors.

The antiproliferative activity of the trophoblast IFNs may function to down-regulate the proliferation of trophoblast cell populations during their interaction with maternal cells. Furthermore, the cystostatic activity of the trophoblast IFNs in normal and neoplastic trophoblast cells without toxicity may suggest its possible clinical application as an antineoplastic agent.

The human placenta also functions as an immunologic barrier between the maternal and fetal circulations, preventing the potentially destructive maternal immune response from damaging the semiallogeneic fetus. Local immunosuppression has been one of the means hypothesized to prevent the sensitization of the maternal immune system to paternal alloantigens and development of subsequent effector functions, possibly leading to the survival of the fetus. The ability of trophoblast IFNs to suppress T- and B-lymphocyte proliferation may be one of the factors contributing to the immunosuppression at the feto-placental unit so that maternal immune responses do not destroy pregnancy. Furthermore, IFN-α has

been demonstrated to be involved in prolongation of allograft survival (12, 28) and suppression of graft-versus-host disease (3). Such regulation of the immune response by trophoblast IFNs may also be important in preventing the rejection of the allogenic fetus.

NK cell activity is known to be associated with the ability of lymphocyte cells to mediate spontaneous lysis of virus-infected and tumor cells in humans (11, 20). In the decidual tissue, the large granular lymphocytes, which are positive for the human NK cell marker NKH-1 (Leu 19), are often concentrated in areas of trophoblast invasion (33). Furthermore, NK cells are known to be recruited to the uterus and contribute to the paraimmunotrophic effects in the feto-maternal interface. These facts may suggest that recruited NK cells and large granular lymphocytes present in the decidual tissue during pregnancy may interact with trophoblast IFNs. Apart from their direct antiviral properties, the trophoblast IFNs may prevent virus infection and spreading by activating immunoreactive NK cells to lyse virus-infected cells during pregnancy. This may be significant, since viral infection could occur during pregnancy when the maternal immune system is suppressed.

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