Modulation of production of matrix metalloproteinases from ovine endometrial cells by ovine trophoblast interferon

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Ovine trophoblast interferon modulates the secretion of a number of proteins by ovine endometrium, but only one of these proteins has so far been identified. We examined the effects of trophoblast interferon on the secretion of matrix metalloproteinase-1, -2 and -3 by cultured ovine endometrial cells and determined whether they are mediated via effects on prostaglandin synthesis. Both ovine trophoblast interferon (30 ng ml⁻¹) and human recombinant interferon α (50 U ml⁻¹) inhibited the production of latent matrix metalloproteinase-1 and -3 ($P < 0.05$), as measured by enzyme assays, but had no effect on the secretion of latent matrix metalloproteinase-2. These inhibitory effects were not overcome by PGE₂ or PGF_{2 α} (each 10 μ mol l⁻¹) either alone or in combination. Indomethacin (12 μ mol l⁻¹) similarly inhibited the production of latent matrix metalloproteinase-1 and -3, but production was partially restored by adding the prostaglandins either singly or in combination. PGE₂ and PGF_{2 α} together had no effect on enzyme production. These data were confirmed by gelatin and casein zymography. Northern analysis showed a 4.5-fold increase in the abundance of specific mRNA for latent matrix metalloproteinase-1 following treatment of cells with phorbol myristate acetate, but a marked decrease following interferon treatment. Thus, ovine trophoblast interferon inhibits the production of the latent forms of matrix metalloproteinase-1 and -3 by ovine endometrial cells, and this is independent of its effect on prostaglandin production.

Introduction

The sheep trophoblast secretes a number of proteins into the uterine lumen (Godkin *et al.*, 1982; Salamonsen *et al.*, 1984) while undergoing extensive elongation during the peri-implantation period (12–16 days after ovulation; Bindon, 1971). The dominant secreted protein is a 19 kDa protein (Godkin *et al.*, 1982), now identified as a type I interferon (Imakawa *et al.*, 1987; Stewart *et al.*, 1987; Charpigny *et al.*, 1988), for which, along with other ruminant trophoblast interferons, a nomenclature of IFN τ has recently been suggested (Roberts *et al.*, 1992). It has profound paracrine effects on the endometrium, attenuating the production of uterine prostaglandins (PG; Salamonsen *et al.*, 1988; Vallet *et al.*, 1988) and particularly of PGF_{2 α} , which in the sheep is the luteolytic agent, but also the production of PGE (Salamonsen *et al.*, 1988, 1989). Furthermore it modifies the pattern of protein secretion by both endometrial explants and cultured endometrial cells *in vitro* (Godkin *et al.*, 1984; Salamonsen *et al.*, 1988; Sharif *et al.*, 1989). However, only one of these interferon-responsive proteins has been identified (Vallet *et al.*, 1991). The actions of IFN τ *in vitro* can be mimicked by recombinant human or bovine IFN α (Salamonsen *et al.*, 1988,

1989; Francis *et al.*, 1991), which share common IFN α / β receptors. These are present in the ovine endometrium (Stewart *et al.*, 1987; Hansen *et al.*, 1989; Knickerbocker and Niswender, 1989; Russell *et al.*, 1993) in greater numbers than on any other ovine tissue tested (corpus luteum, spleen, adrenal cortex, kidney, skeletal muscle, cerebral cortex; Knickerbocker and Niswender, 1989) or on bovine spleen or human placenta and endometrium (Russell *et al.*, 1993).

In the sheep and other ungulates that have similar epitheliochorial placentation, early stages of implantation involve the fusion of binucleate cells of trophoblast origin with caruncular epithelial cells, and some penetration of the basal lamina occurs at defined sites near areas of formation of minisyncytia (Wooding and Morgan, 1989). However, little is known of events in the endometrial stroma at this time, although reorganization of the vasculature and associated structural changes in the interstitial matrix would be required in association with a greatly enhanced blood supply to the implantation site (Weitlauf, 1988).

Sheep endometrial cells in primary culture secrete a number of matrix metalloproteinases (MMPs): interstitial collagenase (MMP-1), gelatinase A (MMP-2) and stromelysin 1 (MMP-3) (Salamonsen *et al.*, 1991b, 1993). Our previous studies have demonstrated the presence of matrix metalloproteinases in uterine flushings from pregnant ewes on day 16 of gestation

and in medium following the culture of conceptuses isolated on day 16 (Salamonsen *et al.*, in press). This family of enzymes together have the potential to degrade most components of the extracellular matrix and are therefore likely to play an important role in the tissue remodelling associated with implantation. Regulation of MMP activity can occur at a number of levels: gene transcription; activation of latent enzyme; or inactivation of the active enzyme by its binding to specific tissue inhibitors of MMPs (Woessner, 1991; Birkedal-Hansen *et al.*, 1993). A wide range of regulatory molecules can act as either inducers (Brenner *et al.*, 1989; Auble and Brinkerhoff, 1991) or inhibitors (Jonat *et al.*, 1990; Yang-Yen *et al.*, 1990) of MMP production. Bioregulators of MMP genes include cytokines, growth factors, prostaglandins and steroid hormones, their actions varying with cell type. It seems likely that at the time of implantation, IFN τ , the dominant product of the trophoblast cells, could be an important regulator of MMP production either directly or via its known actions on prostaglandin activity.

The aim of the present study was to examine the effects of IFN τ and human recombinant interferon α (IFN α) on the production of proMMP-1, proMMP-2 and proMMP-3 by ovine endometrial cells in culture, and to establish whether any changes observed were mediated via the known actions of the interferons on prostaglandin synthesis.

Materials and Methods

Animals

Ten parous Corriedale ewes were ovariectomized and maintained for at least 1 month before treating them with implants of oestradiol (days 1–12) plus intravaginal inserts containing progesterone (CIDR-G: Riverina Artificial Breeders Pty Ltd, Albury) from day 3 to day 12 to mimic the oestrous cycle (Salamonsen *et al.*, 1991b). On day 12 the ewes were initially anaesthetized i.v. with a mixture of 6 ml 10% sodium pentobarbitone (May and Baker, Melbourne) and 3 ml Nembutal (60 mg ml⁻¹; Bomac Laboratories, Asquith), maintained on halothane/oxygen and then hysterectomized. The endometrium was dissected from the myometrium.

Cell isolation and culture

A mixture of stromal fibroblasts and epithelial cells was prepared from the endometrium using techniques described by Salamonsen *et al.* (1991b). In brief, tissue was chopped finely with scissors, digested with 150 U bacterial collagenase ml⁻¹ (type 111; Worthington Biochemical Corporation, Freehold, NJ), incubated in a shaking water bath at 37°C for 75 min and filtered through two grades of nylon mesh. The cell mixture was centrifuged (200 g for 5 min) at room temperature through a Ficoll gradient (2–20%; Pharmacia Ltd, Uppsala) and the cells lying at the interfaces 6–8%, 8–10% and 10–20% were pooled and counted. Cells (2 × 10⁶ per well) were plated into 24-well culture dishes in 2 ml medium 199 with 10% fetal calf serum and 100 U penicillin/100 U streptomycin/250 ng fungisone ml⁻¹ (PSF; all from Flow Laboratories, Sydney), and left for 24–48 h until nearly confluent. They were then washed three times with PBS and the medium was replaced with 1 ml

medium 199 containing antibiotics only. Treatments were added into triplicate wells as 10 μ l aliquots of 100 $\times$ solutions and included 12 μ g indomethacin ml⁻¹, 40 nmol phorbol myristate acetate (PMA) l⁻¹, 10 μ mol PGE₂ l⁻¹ and 10 μ mol PGF_{2 α} l⁻¹ (all from Sigma Chemical Co., St Louis, MO). These concentrations of prostaglandins represent the upper range secreted from similar cultures in 48 h (Salamonsen *et al.*, 1989). Doses of 30 ng IFN τ ml⁻¹ and 50 U IFN α ml⁻¹ (recombinant human α _{2a}; Hoffman La Roche & Co., Basel) maximally modulate prostaglandin and protein synthesis by similar cell cultures, and do not affect the proliferation of such cultures (Salamonsen *et al.*, 1989). Cultures were maintained for a further 48 h, and the medium was collected, centrifuged (200 g for 5 min at room temperature) and stored at -20°C until analysis. Each experiment was performed separately in cell cultures from four or six ewes. For mRNA analyses, cells were cultured in flasks in medium 199 plus PSF either alone or with PMA or IFN α for 48 h, and then harvested with 0.5 ml 0.1% (w/v) trypsin 0.02% (w/v) versene (Flow Laboratories).

Enzyme assays

MMP-1 (interstitial collagenase) activity was measured by the diffuse fibril method of Cawston and Barrett (1979), using ¹⁴C-acetylated type-1 collagen prepared by salt extraction from guinea-pig skin and incubation at 37°C for 20 h. Gelatinolytic activity (predominantly MMP-2; gelatinase A) was measured using heat-denatured ¹⁴C-acetylated type-1 collagen incubated at 37°C for 4 h according to the method of Harris and Krane (1972). MMP-3 (stromelysin 1) activity was measured using reduced ³H-carboxymethylated transferrin (CmTf), as described by Okada *et al.* (1986). All samples were assayed in duplicate for enzyme activity in the activated form following preincubation of the samples with 1.5 mmol (4-aminophenyl)mercuric acetate (APMA) l⁻¹. In each case, 1 U enzyme activity was defined as that amount of enzyme capable of degrading 1 μ g substrate min⁻¹ at 37°C. Sensitivities of the assays are 0.05 U ml⁻¹ for MMP-1 and 0.02 U ml⁻¹ for MMP-3. The mean intra-assay coefficient of variation for the MMP-1 assay was 3.7% and 5.4% for the MMP-3 assay. Aliquots of a single sample were included in every assay, and the interassay variability was calculated as 70.7% for the MMP-1 assay and 35.8% for the MMP-3 assay. All samples from a single experiment were assayed in single assays and data were expressed as percentages of control values (no added treatments: 100%).

Zymography

MMP-1, -2 and -3 activity in the culture medium was also analysed by zymography. Media samples were subjected to SDS-PAGE on a 10% acrylamide slab gel containing 1 mg gelatin or casein ml⁻¹ (Sigma Chemical Co.) under non-reducing conditions. After washing the gels in a buffer containing 50 mmol Tris-HCl l⁻¹, 5 mmol ZnCl₂ l⁻¹, 0.02% (w/v) NaN₃ (pH 7.5) and 1% Triton X-100, the gels were incubated in the same buffer without Triton X-100 at 37°C for 20 h and stained with Coomassie Brilliant Blue G250. Enzyme activities were visualized by negative staining. Individual

MMPs were identified using molecular weight standards run on the same gels, with laboratory standards for proMMP-2 activity (conditioned medium from a culture of human synovial cells) or with purified human proMMP-3 and proMMP-1 (on the casein gels). All MMP activity was inhibited by including 10 mmol EDTA l^{-1} in the incubation buffer.

Probes

Human proMMP-1 cDNA (1.6 kb) was isolated from a human colon cancer Lo-Vo cell cDNA library. Sequencing indicated that this cDNA is identical to human fibroblast proMMP-1 cDNA except for two bases (B. Xie and H. Nagase, unpublished). The probe was labelled with [$\alpha^{32}P$]CTP (Bresatec, Adelaide) to a specific activity of $1\text{--}3 \times 10^9$ c.p.m. μg^{-1} . The rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cRNA probe used was complementary to nucleotides 96–660 of the cDNA clone (Tso *et al.*, 1985) and was labelled with [^{32}P]UTP to a specific activity of 10^8 c.p.m. μg^{-1} .

Northern analyses

Total RNA was isolated from harvested cells by a single-step extraction with guanidinium isothiocyanate, phenol and chloroform (Chomczynski and Sacchi, 1987). For northern blotting, 20 μg total RNA was denatured in 1 mol glyoxal l^{-1} containing 50% dimethyl sulfoxide, and subjected to electrophoresis in a 1.2% agarose gel, and passively transferred to Hybond (Amersham Australia, Melbourne) nylon membranes by capillary blotting. An RNA ladder (Bethesda Research Laboratories, Bethesda, MD) was used to determine the size of the bands. The nylon membrane was baked at 80°C for 2 h, crosslinked with uv for 10 min and prehybridized for 4 h at 42°C in the following hybridization buffer: 50% formamide, 1 $\times$ Denhardt's solution (50 $\times$ 0.02% each of Ficoll, polyvinylpyrrolidone and BSA), 5 $\times$ SSPE (0.75 mol NaCl l^{-1} , 50 mmol $NaH_2PO_4 \cdot 2H_2O$ l^{-1} , and 5 mmol EDTA l^{-1} , pH 7.4) and 100 mg pre-boiled herring spermatozoa DNA ml^{-1} . The blot was hybridized with the ^{32}P -labelled proMMP-1 probe (2×10^6 c.p.m. ml^{-1}) for 17 h at 42°C, and washed in 2 $\times$ SSC (1 $\times$ SSC is 0.15 mol sodium chloride l^{-1} and 0.15 mol sodium citrate l^{-1} , pH 7.4) containing 0.1% SDS at room temperature and then in 2 $\times$ SSC and 0.1% SDS at 42°C. For the GAPDH probe (2×10^6 c.p.m. ml^{-1}), pre-hybridization was for 4 h at 42°C in a solution containing 50% formamide, 5 $\times$ SSPE, 0.15 mol Tris-HCl l^{-1} (pH 8), 1% SDS and 125 iu heparin ml^{-1} . Hybridization was for 17 h in the same solution at 60°C and the blot was washed to its highest stringency with 0.2 $\times$ SSC and 0.1% SDS at 70°C. Autoradiography was performed with Fuji RX film and an intensifying screen at $-70^\circ C$ for 11 days and 22 h (proMMP-1 and GAPDH, respectively) and densitometric analysis was performed using an Olympus Cue 2 image analyser.

Statistical analyses

The absolute concentration of each enzyme measured by enzyme assays varied between batches of cells derived from different animals; for this reason, data were normalized within

animals and expressed as percentages of enzyme activities in control wells (no treatment: 100%). Normal distribution of data was confirmed using Bartlett's test. Data from enzyme assays were analysed by one-way analysis of variance and Duncan's multiple range *F* test. Differences were considered significant when $P < 0.05$.

Results

Modulation of MMP-1, MMP-2 and MMP-3 production by IFN τ and IFN α

MMP-1, -2 and -3 activities were observed in conditioned medium from ovine endometrial cells; much of the enzyme present was in the latent form and required activation *in vitro* with APMA, as demonstrated by Salomonsen *et al.* (1991b, 1993; data not shown). Mean MMP activities (U $ml^{-1} \pm$ SEM, $n = 4$ ewes) following activation were: 0.80 ± 0.52 for MMP-1; 1.3 ± 0.51 for MMP-2; and 0.80 ± 0.52 for MMP-3. PMA significantly increased the activity of MMP-1 and -3 ($P < 0.05$) but not that of MMP-2, confirming the potential of the cells to respond to a known stimulus. Both IFN τ and IFN α attenuated the production of MMP-1 and MMP-3 but not of MMP-2 (Fig. 1). The inhibitory effect was greatest on MMP-1, with activities reduced below the level of detection in all cultures by both IFN τ and IFN α . Inhibition of MMP-3 activity was only partial (mean inhibition 47% of control) using the same treatment doses.

Effect of indomethacin treatment on MMP production

Addition of exogenous prostaglandins (PGE $_2$ and PGF $_{2\alpha}$) to the cell cultures had no effect on the activity of MMP-1 or MMP-3 in the culture medium (Fig. 2), whereas the activity of both were significantly decreased ($P < 0.05$) by treating the cells with indomethacin. These activities were partially restored by PGE $_2$ or PGF $_{2\alpha}$ added separately or together to the cultures. These data were confirmed by casein zymography (shown in part in Fig. 3a), which detected both proMMP-1 (52 kDa), proMMP-3 (57 kDa) and the active forms of these enzymes. No effects of either indomethacin or prostaglandins on either proMMP-2 (72 kDa) or active MMP-2 (67 kDa) production were demonstrated using gelatin zymography (Fig. 3b).

MMP production following treatment of cells with a combination of interferons and prostaglandins

Mean MMP-1 and MMP-3 activities were significantly decreased by IFN α , as before. However, enzyme activity was not restored by adding PGE $_2$ or PGF $_{2\alpha}$ to the cell cultures, either separately or in combination (Figs 3a and 4), unlike the restoration seen following indomethacin treatment. ProMMP-2 activity detected by zymography was not altered by treatment with IFN α (Fig. 3b).

Fig. 1. Activities of (a) matrix metalloproteinase-1 (MMP-1), (b) MMP-2 and (c) MMP-3 in medium from ovine endometrial cells cultured without treatments (Con) or with 40 nmol phorbol myristate acetate (PMA) l^{-1} , 30 ng trophoblast interferon (IFN τ) ml^{-1} or 50 U human recombinant interferon (IFN α) ml^{-1} . Activities were measured following the activation of latent enzyme with (4-aminophenyl)mercuric acetate. All cultures were in triplicate and assays were performed in duplicate. Data from four ewes are expressed as mean percentage of control value $\pm$ SEM. * $P < 0.05$ compared with control; $^{\dagger}0.06 > P > 0.05$.

Effect of combined treatments with PMA, indomethacin and interferons

PMA significantly stimulated MMP-1 ($P < 0.05$) production by ovine endometrial cells (Fig. 5). MMP-3 production was also increased, but not significantly owing to variability among animals. This stimulation was not diminished when PMA was administered in combination with either indomethacin, IFN α or

Fig. 2. Activities of (a) matrix metalloproteinase-1 (MMP-1) and (b) MMP-3 in medium from ovine endometrial cells cultured alone (Con), with added 10 μ mol prostaglandin E_2 (E) l^{-1} and 10 μ mol prostaglandin $F_{2\alpha}$ (F) l^{-1} , 12 μ mol indomethacin (Indo) l^{-1} , and indomethacin with added E, F or E plus F. Activities were measured following the activation of latent enzyme with (4-aminophenyl)mercuric acetate. Data are expressed as mean percentage of control value $\pm$ SEM ($n = 6$ ewes). * $P < 0.05$ compared with control.

PGE $_2$ plus PGF $_{2\alpha}$ (Figs 5 and 3a). PMA had little effect on the production of proMMP-2, as detected by zymography (Fig. 3b).

Effect of PMA and interferons on the steady-state concentrations of mRNA encoding proMMP-1 in ovine endometrial cells

Northern analysis of total RNA derived from ovine endometrial cells and hybridized with the ^{32}P -labelled cDNA probe for human proMMP-1 detected an mRNA of 2.2 kb (using the standard RNA ladder). Analysis of mRNA from either untreated cells or those subjected to PMA or IFN α treatment demonstrated a marked increase in the abundance of mRNA encoding proMMP-1 following PMA treatment. The abundance of mRNA encoding proMMP-1 was decreased to concentrations that could not be measured by densitometry following incubation with doses of IFN α that resulted in lowered MMP-1 activity in the culture medium (Fig. 6). A second, much smaller band of hybridization could be seen at 1.8 kb, the size of the mRNA encoding proMMP-3, and presumably is a result of crosshybridization of the probe. This

Fig. 3. (a) Casein and (b) gelatin zymography of culture medium from ovine endometrial cells, untreated (C) or treated *in vitro* with prostaglandin E_2 and prostaglandin $F_{2\alpha}$ (PG), indomethacin (I), interferon (IF), or phorbol myristate acetate (P) alone or in combination. Human synovial cell culture medium (SCM) is used as a standard for matrix metalloproteinase-2 (MMP-2). Latent matrix metalloproteinases-1 and -3 (proMMP-1 and proMMP-3) were identified by comparison with purified human standards.

result is similar to that obtained for proMMP-1 with the treatments *in vitro* (Fig. 1). Hybridization of the same northern blot with the oligonucleotide probe for GAPDH demonstrated equivalent loading of total RNA in each lane.

Discussion

IFN τ , the major product of the sheep blastocyst at the time of implantation, has an inhibitory effect *in vitro* on the release of both proMMP-1 and proMMP-3 by ovine endometrial cells, but does not alter the production of proMMP-2. Northern analysis demonstrated that IFN α reduced the steady-state concentration of mRNA encoding proMMP-1 in the cell cultures in contrast to the stimulation seen with PMA. The actions of interferons on these MMPs were not mediated by attenuating the production of endometrial prostaglandins (Salamonsen *et al.*, 1988, 1989), as addition of both PGE_2 and $PGF_{2\alpha}$ to the cell cultures in the presence of human IFN α did not restore MMP production, even in part. This is in contrast to the situation in which indomethacin (an inhibitor of prostaglandin synthase and hence of prostaglandin synthesis) similarly inhibited MMP production in an action reversed by exogenous prostaglandins. The stimulation of both proMMP-1 and proMMP-3 production by PMA (an agonist of protein kinase C and hence a stimulator of prostaglandin release) in this study confirms our previous findings (Salamonsen *et al.*, 1991b, 1993). Since none of the other regulators examined (interferons, indomethacin or prostaglandins) modified MMP production in the presence of PMA, it appears that while proMMP-1 and proMMP-3 production by ovine endometrial cells can be regulated by IFN τ and IFN α , by prostaglandins and

Fig. 4. Activities of (a) matrix metalloproteinase-1 (MMP-1) and (b) MMP-3 in medium from ovine endometrial cells cultured alone (Con), or with human recombinant interferon (IFN α) alone or in combination with 10 μ mol prostaglandin E_2 (E) l^{-1} , 10 μ mol prostaglandin $F_{2\alpha}$ (F) l^{-1} or E plus F. Activities were measured following the activation of latent enzyme with (4-aminophenyl)mercuric acetate. Data are expressed as mean percentage of control $\pm$ SEM ($n = 6$ ewes). * $P < 0.05$ compared with control.

by PMA, the mechanisms of action of these regulatory molecules are different.

α -Interferons have been implicated in the regulation of MMP production in a variety of cells. MMP-1 is stimulated by IFN α in cultured human dermal fibroblasts (Duncan and Berman, 1989), in keloidal fibroblasts (Berman and Duncan, 1989) and in Ewing sarcoma cells (Siegal *et al.*, 1982). In addition, the invasive potential of human melanoma can be modulated *in vitro* by IFN α (Hujanen and Turpeenniemi-Hujanen, 1991), suggesting that it inhibits MMP synthesis or action. Whether trophoblast interferons have properties uniquely different from IFN α has not been fully established but in most test systems *in vitro* their biological activities are essentially indistinguishable; it is therefore likely that IFN τ acts as a classical type-1 interferon (Roberts *et al.*, 1992) via IFN α / β receptors. In the ovine endometrium, ^{125}I -labelled IFN τ binds to the epithelium (Godkin *et al.*, 1984), although binding to stromal cells was not precluded. It is primarily the stromal rather than the epithelial cells that are the endometrial source of MMP in the sheep (Salamonsen *et al.*, 1993, in press), and it is impossible in the present studies of mixed cells to define whether the action of IFN τ on stromal-cell MMP production is

Fig. 5. Activities of (a) matrix metalloproteinase-1 (MMP-1) and (b) MMP-3 in medium from ovine endometrial cells cultured alone (Con), with added phorbol myristate acetate (PMA) alone or in combination with indomethacin (Indo), human recombinant interferon (IFN α) or prostaglandin E $_2$ plus prostaglandin F $_2\alpha$ (E + F). Activities were measured following the activation of latent enzyme with (4-aminophenyl)mercuric acetate. Data represent combined data from six ewes and are expressed as mean percentage of control value $\pm$ SEM ($n = 6$ ewes). * $P < 0.05$ compared with control values.

direct or mediated via interactions with epithelial cells. Furthermore, it is possible that interferons from cellular sources other than the trophoblast modulate MMP production in the uterus and thus are of general importance in the remodelling of this tissue.

Stimulatory effects of PGE $_2$ on MMP-1 production have been shown in a number of experimental models (Dayer *et al.*, 1984; Krane *et al.*, 1985; Goshowaki *et al.*, 1988) and in the present study prostaglandins stimulated both MMP-1 and MMP-3 secretion from indomethacin-treated cells, although they had no effect on untreated cells. Ovine endometrial cells in culture under these conditions and without specific stimuli secrete both PGE and PGF $_{2\alpha}$, the PGE being derived primarily from the stromal cells (Salamonsen *et al.*, 1991a) that are also the major source of MMPs in these cell cultures (Salamonsen *et al.*, 1993, in press). It is therefore likely that endogenously produced prostaglandins acting in an autocrine or paracrine manner are exerting their maximal effect on MMP synthesis and masking any potential actions of exogenous prostaglandins. The actions of prostaglandins in MMP regulation in a

Fig. 6. Northern blot analysis of total RNA (20 μ g per lane) from ovine endometrial cells hybridized with a 32 P-labelled cDNA probe for latent matrix metalloproteinase-1 (proMMP-1) and a cRNA probe for glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Lane 1: control cells (untreated); lane 2: cells treated with phorbol myristate acetate (PMA) for 48 h; lane 3: cells treated with human recombinant interferon (IFN α) for 48 h. Kb values were assigned by reference to an RNA ladder run on the same gel. Autoradiography was for 11 days for proMMP-1 and 22 h for GAPDH with Fuji RX film.

range of situations involve cAMP-dependent pathways (Case *et al.*, 1990; Corcoran *et al.*, 1992). Although indomethacin inhibited prostaglandin synthesis in rabbit uterine cervical fibroblasts, it did not alter MMP-1 production that had been enhanced by interleukin 1, which, like PMA, is dependent on protein kinase C (Goshowaki *et al.*, 1988). These studies are consistent with the findings reported here.

Thus, IFN τ acts either directly or indirectly to ensure local inhibition of proMMP-1 and proMMP-3 production. Whether the action of IFN τ on stromal-cell MMP production is direct or mediated via interactions with epithelial cells is not defined by these studies in mixed cells. Such paracrine interactions between the trophoblast, endometrial epithelium or endometrial stroma could act to limit invasion of the stroma by the trophoblast and to modulate remodelling within the endometrium. Although trophoblast cells of species such as humans are highly invasive, the use of a trophoblast protein to restrain such invasiveness may be a special adaptation in ruminants. Further studies are in progress to establish the sites of production of MMP around implantation sites, and their spatial and temporal relationships with trophoblast development and IFN τ production.

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