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# IL-10 Is an Autocrine Inhibitor of Human Placental Cytotrophoblast MMP-9 Production and Invasion

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**During human placentation, fetal cytotrophoblast stem cells differentiate and then invade the uterine wall and its associated spiral arteries. This process anchors the placenta to the uterus and supplies maternal blood to the fetus. Cytotrophoblast invasion *in vitro* requires the expression of matrix metalloproteinase-9 (MMP-9). Recently, we showed that cytotrophoblasts produce interleukin-10 (IL-10), a potent immunomodulatory cytokine that could have paracrine effects on the maternal immune system. IL-10 synthesis is dramatically downregulated after the first 12 h of culture, while MMP-9 secretion is rapidly upregulated and the cells acquire an invasive phenotype. These observations prompted us to investigate whether IL-10 is an autocrine regulator of cytotrophoblast MMP-9 production. We found that the cells expressed IL-10 receptor mRNA, suggesting that autocrine effects are possible. Adding recombinant IL-10 to cytotrophoblast cultures significantly decreased the cells' MMP-9 expression at both protein and mRNA levels, but did not affect mRNA levels of the tissue inhibitor of metalloproteinase-3. Thus, IL-10 may alter the proteinase/inhibitor balance. IL-10 treatment further caused a net decrease in MMP activity, thereby reducing cytotrophoblast invasiveness. An antibody that neutralized endogenous IL-10 function had the opposite effect in all experiments. Together, these data suggest that IL-10 is an autocrine inhibitor of cytotrophoblast MMP-9 activity and invasiveness.** 

***Key Words:*** **placenta; cytotrophoblast; IL-10; MMP-9; invasion.**

## INTRODUCTION

Successful formation of the human placenta requires the correct differentiation of specialized fetal cells termed cytotrophoblasts (reviewed in Cross *et al.*, 1994; Rinkenberger *et al.*, 1997). Cytotrophoblast stem cells are attached to an extensive basement membrane that surrounds the stromal core of two types of chorionic villi. In floating villi, cytotrophoblasts differentiate by fusing to form an overlying layer of multinucleate syncytiotrophoblasts. These cells are in contact with maternal arterial blood that bathes the floating villi. The primary function of the syncytium is to perform nutrient, waste, and gas exchange between the maternal and fetal circulations. In anchoring villi, a subset of cytotrophoblasts at the distal tips of the villi differentiate by leaving their basement membrane and invading the uterus. These invasive cytotrophoblasts populate the decidualized endometrium and the first third of the myometrium, replacing the walls of uterine spiral arterioles in these regions (Brosens *et al.*, 1978; Damsky *et al.*, 1992). This unusual process anchors the placenta to the uterus and supplies the fetus with maternal blood. Cytotrophoblast invasion must be tightly regulated because errors in the depth of uterine penetration can have severe negative effects on pregnancy outcome. Disorders such as preeclampsia (Zhou *et al.*, 1993) and approximately half the cases of intrauterine growth retardation (Gerretsen *et al.*, 1981) are associated with abnormally shallow cytotrophoblast invasion. Conversely, invasion that is overly extensive can result in both benign and malignant trophoblast tumors (Berkowitz and Goldstein, 1996).

Control of cytotrophoblast invasion requires the developmental regulation of several classes of molecules, including those involved in adhesion and proteolysis. Normally, the

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expression of cell–cell (e.g., cadherins, Ig superfamily) and cell–extracellular matrix (ECM) (e.g., integrin receptors) adhesion molecules is extensively modulated as cytotrophoblasts invade the uterine wall (Fisher *et al.*, 1989; Aplin, 1991; Damsky *et al.*, 1992). In preeclampsia, where placentation is shallow, differentiating/invading cytotrophoblasts fail to correctly modulate members of all three adhesion molecule families (Zhou *et al.*, 1993, 1997; Lim *et al.*, 1997). With regard to proteinases, human cytotrophoblasts produce both the urokinase-type plasminogen activator (Queenan *et al.*, 1987) and matrix metalloproteinase-9 (MMP-9; Fisher *et al.*, 1989). Although the urokinase-type plasminogen activator does not appear to directly mediate cytotrophoblast invasion *in vitro*, MMP-9 activity is absolutely required for cytotrophoblast degradation of and invasion through Matrigel (Librach *et al.*, 1991). As with the adhesion molecules that are critical to invasion, MMP-9 expression is misregulated in preeclampsia (Lim *et al.*, 1997).

MMPs are a family of ECM-degrading enzymes that share common structural domains (reviewed in Matrisian, 1990; Sang and Douglas, 1996). These proteinases are secreted as inactive zymogens and are activated by proteolytic cleavage. MMPs require $Ca^{2+}$ binding for structural integrity and $Zn^{2+}$ for catalysis. Consequently, their activity can be inhibited by chelating agents *in vitro* (Murphy and Docherty, 1992). *In vivo*, MMP induction in areas of matrix remodeling is under the tight control of several regulatory mechanisms. Proteinase abundance is regulated at the transcriptional level, as well as posttranscriptionally, by changes in mRNA stability. After secretion, MMP enzymatic activity is controlled by interactions with proteolytic activators (e.g., membrane-type MMP) and inhibitors (e.g., tissue inhibitors of metalloproteinases or TIMPs). This high degree of regulation may reflect the important roles MMPs play in normal tissue remodeling processes such as organogenesis and ovulation. The abundance and activity of family members is also increased in a variety of pathological processes such as inflammation and tumor metastasis (Ries and Petrides, 1995).

Cytokines and growth factors involved in the regulation of these normal and pathological events have been shown to modulate MMP expression and proteolytic activity (Birkedal-Hansen, 1993; Ries and Petrides, 1995). Interleukin (IL)-1$\beta$ upregulates MMP-9 production in chondrocytes (Ogata *et al.*, 1992), synovial fibroblasts (Unemori *et al.*, 1991), and keratinocytes (Lyons *et al.*, 1993). Transforming growth factor-$\beta$ also increases MMP-9 activity in keratinocytes (Salo *et al.*, 1991), and in monocytes as well (Wahl *et al.*, 1993). Tumor necrosis factor-$\alpha$ upregulates MMP-9 in both monocytes (Watanabe *et al.*, 1993) and cervical fibroblasts (Sato *et al.*, 1996), whereas epidermal growth factor and transforming growth factor-$\alpha$ upregulate keratinocyte MMP-9 (Lyons *et al.*, 1993). The localized secretion of proinflammatory cytokines can thus lead to tissue destruction in disorders such as rheumatoid arthritis and periodontal disease (Ries and Petrides, 1995). Among the cytokines that act to downregulate proteinase activity, both IL-4 (Lacraz *et al.*, 1992) and IL-10 (Lacraz *et al.*, 1995; Mertz *et al.*, 1994) have been shown to inhibit MMP-9 production in monocytes and macrophages. Thus, cytokines play an important role in the control of MMP-9 activity in a variety of cell types.

Several of these cytokines and growth factors are also found at the maternal–fetal interface, making them likely candidates for regulating cytotrophoblast MMP-9 expression and/or activity. We have previously reported that human cytotrophoblasts produce IL-1$\beta$ in culture and that this cytokine is an autocrine stimulator of MMP-9 secretion and invasion (Librach *et al.*, 1994). We hypothesized that the highly aggressive, yet precisely regulated nature of cytotrophoblast invasion was evidence that the cells had analogous mechanisms for inhibiting MMP activity. Of the cytokines known to downregulate MMP-9 in other cell types, we recently showed that IL-10 is spontaneously produced in substantial quantities by human cytotrophoblasts *in vitro* (Roth *et al.*, 1996). IL-10 in cytotrophoblast conditioned medium can inhibit an allogeneic immune reaction, which suggests that IL-10 may be important in protecting the fetus from a potentially deleterious maternal immune response *in vivo*. IL-10 production is significantly downregulated after 12 h in culture, at the time when cytotrophoblasts upregulate MMP-9 and differentiate into invasive cells. Because the secretion patterns of IL-10 and MMP-9 are inversely related, we undertook the present study to determine whether IL-10 is involved in the control of cytotrophoblast MMP-9 production and, consequently, invasion.

## MATERIALS AND METHODS

### *Cell Isolation and Culture*

Cytotrophoblasts were isolated by published methods from first-, second- (Fisher *et al.*, 1989; Librach *et al.*, 1991), and third-trimester (Kliman *et al.*, 1986) human placentas. In all cases, contaminating leukocytes were removed by using an antibody to CD-45, a protein tyrosine phosphatase found on bone marrow-derived cells (Charbonneau *et al.*, 1988), coupled to magnetic beads. Cytotrophoblast populations prepared in this way are 95–99% free of nontrophoblast cells as shown by the absence of classical HLA class I antigens (Kovats *et al.*, 1990). Cytotrophoblasts ($1 \times 10^6$) were cultured in 1 ml serum-free medium [DME H-21 containing 2% Nutridoma (Boehringer-Mannheim)] in 16-mm culture wells precoated with a basement membrane substrate (Matrigel; Collaborative Research Inc., Bedford, MA) diluted 2:1 in serum-free medium as described (Librach *et al.*, 1991). Where specified, 1–100 ng/ml of recombinant IL-10 (rIL-10; R & D Systems), 1–100 $\mu$g/ml of a rat neutralizing monoclonal antibody (mAb) ($IgG_{2a}$) to IL-10 [anti-IL-10; 19F1(Bejarano *et al.*, 1992; Gastl *et al.*, 1993)], or 1–100 $\mu$g/ml of a rat $IgG_{2a}$ isotype control (IgG; GL117, anti-*Escherichia coli* $\beta$-galactosidase) was added to the cytotrophoblast culture medium. Medium containing these additives was replaced every 24 h.

Placental fibroblasts were isolated from first-trimester placentas and cultured as previously described (Fisher *et al.*, 1989). Peripheral

blood leukocytes were prepared by centrifugation of blood from normal donors on a Ficoll–Hypaque 1077 (Sigma) density gradient according to the manufacturer's recommendation. The leukocytes were cultured in RPMI 1640 supplemented with 10% (v/v) heat-inactivated human AB serum at a density of $1 \times 10^6$ cells/ml of culture medium.

U937 cells were maintained in RPMI 1640 supplemented with 10% (v/v) fetal calf serum. To induce differentiation and MMP-9 secretion, control cells were cultured at a concentration of $1 \times 10^6$/ml in RPMI containing 50 ng/ml phorbol 12-myristate 13-acetate (Sigma) and 0.2% lactalbumin hydrolysate (Gibco BRL). As described under Results, 10 ng/ml rIL-10, 10 $\mu$g/ml 19F1, or 10 $\mu$g/ml control IgG was added to the cultures, and medium containing these additives was replaced daily.

## *Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) for Detection of IL-10 Receptor mRNA*

Total RNA was isolated from $10^6$ cells by using RNAzol B (Biotecx Laboratories, Inc., Houston, TX) according to the manufacturer's recommendations. RNA was reverse transcribed by using murine Moloney leukemia virus reverse transcriptase (Gibco BRL) and random hexamer primers (Sigma) as previously described (Reiner *et al.*, 1993). PCR was performed using primers for either the constitutively expressed housekeeping gene, hypoxanthine-guanine phosphoribosyltransferase (HPRT), as a control for RNA integrity or the IL-10 receptor. The following primer sets were used: HPRT forward primer, 5′-CCTGCTGGATTACATCAAAG-CACTG-3′; HPRT reverse primer, 5′-TCCAACACTTCGT-GGGGTCCT-3′; IL-10 receptor forward primer, 5′-AAA-ACTTCAGCCTCCTAACCTCTGG-3′ and Il-10 receptor reverse primer, 5′-CGGGTGGATGGTGTCTTGGGTCTCT-3′. Amplification products (HPRT, 288 bp; IL-10 receptor, 317 bp) were visualized by electrophoresis on 3% ethidium bromide-stained agarose gels and photographed.

## *Immunoblotting*

Cytotrophoblasts were cultured for 72 h as described. Conditioned medium (CM) was collected and centrifuged at 300*g* for 10 min and then stored at −80°C until analysis by immunoblotting, zymography, or a soluble assay of metalloproteinase activity (described below). For immunoblot analysis, CM [75% (v/v)] was solubilized in loading buffer, subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and transferred by blotting to nitrocellulose as described (Librach *et al.*, 1991). Nonspecific reactivity was blocked by incubating the blots for 1 h at 25°C in phosphate-buffered saline (PBS) containing 0.05% Tween 20 (T-PBS) and 5% nonfat dried milk (Carnation). The nitrocellulose blots were then incubated for 2 h at 25°C with 5 $\mu$g/ml anti-MMP-9 antibody (7-11C; Oncogene Research Products, Cambridge, MA) suspended in T-PBS containing 5% nonfat dry milk. The blots were washed six times (10 min each time) in T-PBS and then incubated for 1 h at 25°C with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Jackson Immunoresearch) diluted 1:3000 in T-PBS containing 5% nonfat dry milk. Blots were then washed six times (10 min each time) in T-PBS and rinsed once in PBS. An enhanced chemiluminescence system (Amersham Corp.) was used to detect antigen–antibody complexes, and the blot was exposed to film.

## *Northern Hybridization*

Total RNA (10 $\mu$g) was separated by formaldehyde–agarose gel electrophoresis and transferred to Nytran membranes (Schleicher and Schuell, Keene, NH). In all experiments, gels were stained with acridine orange before transfer to ensure integrity of the RNA samples and to confirm that equal amounts of RNA had been loaded onto each lane. A TIMP-3-specific cDNA probe was synthesized by random priming of a 515-bp fragment of the cDNA [nucleotides 369–884 (Silbiger *et al.*, 1994)]. An MMP-9-specific probe was synthesized by PCR amplification of U937 cell cDNA to generate a 382-bp fragment [nucleotides 15–397 (Wilhelm *et al.*, 1989)]. Nytran membranes were analyzed by Northern blot hybridization as previously described (McMaster *et al.*, 1995).

## *Substrate Gel Zymography*

Cytotrophoblast or U937 cell CM [75% (v/v)] was solubilized in nonreducing sample buffer and separated by SDS–PAGE, using 10% acrylamide gels containing 5 mg/ml gelatin (Sigma). After electrophoresis, proteins were renatured by incubating the gel in 50 mM Tris–HCl, pH 7.6, containing 2.5% Triton X-100 for 1 h at 25°C to remove SDS. Metalloproteinases were then activated by incubating the gel overnight at 37°C in 50 mM Tris–HCl, pH 7.8, containing 150 mM NaCl and 5 mM $CaCl_2$. To visualize proteinase activity, the gel was stained with Coomassie blue. Clear bands corresponded to areas where gelatin digestion took place. The gels were dried onto filter supports and photographed.

## *Soluble Assay of Metalloproteinase Activity*

Metalloproteinase activity in cytotrophoblast CM was measured using the thiopeptolide substrate Ac-Pro-Leu-Gly-SCH[$CH_2$-$CH(CH_3)_2$]CO-Leu-Gly-$OC_2H_5$ (Bachem, Philadelphia, PA) in a spectrophotometric assay (Weingarten *et al.*, 1985). CM [25% (v/v)] was added to a reaction mixture containing 40 $\mu$M thiopeptolide and 800 $\mu$M chromophore 4,4′-dithiodipyridine (Sigma) in 50 mM Hepes buffer, pH 7.0, with 10 mM $CaCl_2$. The reaction mixture was incubated at 25°C, and absorbance ($A_{324}$) was read at intervals of 5 min for 30 min. The rate of substrate degradation is a measure of total MMP activity. To verify that the activity measured reflected that of metalloproteinases, other CM samples were incubated with 1 mM 1,10-phenanthroline (Sigma) for 10 min at 25°C prior to addition of substrate. In all cases, this abolished the observed activity.

## *Invasion Assay*

To quantify cytotrophoblast invasion, Transwell polycarbonate filter inserts (6.5 mm; Costar) with 8-$\mu$m pores were coated with 10 $\mu$l Matrigel, and second-trimester cells ($2.5 \times 10^5$ cells/250 $\mu$l serum-free medium) were plated on top of each filter. Where specified, 10 ng/ml rIL-10, 10 $\mu$g/ml 19F1, or 10 $\mu$g/ml control IgG was added to the culture medium both above and below filter inserts and replaced every 24 h. At 60 or 72 h, filters were rinsed with PBS and the cells were fixed in 3% paraformaldehyde for 1 h at 25°C. After three 10-min washes in PBS, cell membranes were permeabilized with methanol for 5 min at −20°C. The filters were washed in PBS and then incubated in PBS containing 1% bovine serum albumin (PBS–BSA) for 1 h at 25°C to block nonspecific antibody reactivity. Cytotrophoblasts were exposed to an antibody to cytokeratin [1:100 (v/v) in PBS–BSA; 7D3 (Fisher *et al.*, 1989)] for

2 h at 25°C and then washed thoroughly with PBS. To detect cytotrophoblast cell bodies and processes, the filters were incubated with a rhodamine-conjugated anti-rat IgG [1:2000 (v/v) in PBS–BSA; Jackson Immunoresearch] for 1 h at 25°C and then washed again in PBS. Each of triplicate filters was cut from the Transwell inserts with a scalpel and mounted on a slide with the underside of the filter facing up. Cell bodies and processes that had migrated through the filter pores and had reached the underside were visualized by fluorescence microscopy, counted, and then photographed.

### *Statistical Analyses*

The statistical significance of the data was analyzed by using analysis of variance (ANOVA) followed by pairwise comparison using Fisher's PLSD. The significance level was set at $P < 0.05$ for differences from control.

## RESULTS

### *Human Cytotrophoblasts Express IL-10 Receptor mRNA*

Our recent findings that cytotrophoblasts secrete substantial amounts of IL-10 *in vitro* and that production patterns of IL-10 and MMP-9 are inversely related (Roth *et al.*, 1996) suggested that IL-10 may function as an autocrine regulator of cytotrophoblast MMP-9 expression and invasive activity. We began to test this hypothesis by using RT-PCR to determine whether cytotrophoblasts express the IL-10 receptor and thus have the potential to respond to the IL-10 they produce. Amplification of HPRT cDNA verified RNA integrity in each of the samples (Fig. 1). As positive and negative controls, cDNA for the IL-10 receptor was amplified from peripheral blood leukocytes and placental fibroblasts, respectively. After culture for 12 h, cytotrophoblasts of all gestational ages expressed IL-10 receptor mRNA (Fig. 1).

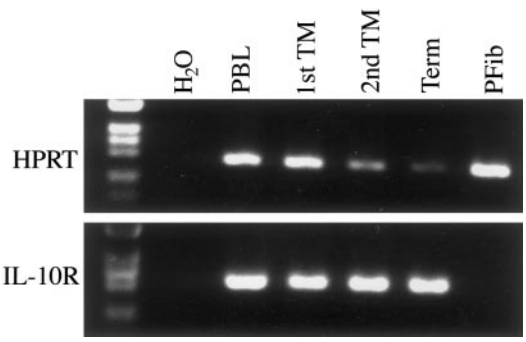


**FIG. 1.** Human cytotrophoblasts express IL-10 receptor mRNA *in vitro.* RNA was extracted from peripheral blood leukocytes (PBL), first- and second-trimester (TM) and term placental cytotrophoblasts, and first-trimester placental fibroblasts (PFib) and reverse transcribed. The cDNA for HPRT or the IL-10 receptor (IL-10R) was amplified by PCR. The products (and a standard 1-kb DNA ladder) were separated by electrophoresis in agarose gels, visualized by ethidium bromide staining, and photographed. Amplification with the HPRT primer set produces a 288-base-pair PCR product, while IL-10 receptor primers amplify a 317-base-pair fragment. RNA integrity was assured by the amplification of HPRT in all samples.

### *IL-10 Downregulates Cytotrophoblast MMP-9 Secretion in Vitro*

Next, we investigated whether maintaining cytotrophoblasts in high levels of IL-10 after endogenous cytokine secretion has ceased affects their MMP-9 secretion. In these experiments, the cells were plated in medium that contained 10 ng/ml rIL-10, and this medium was replaced every 24 h. Cytotrophoblasts were also incubated in medium that contained 10 $\mu$g/ml of either a function-perturbing anti-IL-10 mAb or an irrelevant mAb (IgG) of the same isotype. Control cells were cultured in medium alone. The amount of MMP-9 in the conditioned medium of the variously treated cells was estimated at 24, 48, and 72 h by immunoblotting. Figure 2A shows the results of a typical experiment. The effects of cytokine and anti-IL-10 treatment became more pronounced over time. By 72 h, we consistently found that cytotrophoblasts cultured in rIL-10 secreted significantly less MMP-9 than cells incubated in medium alone. Conversely, cytotrophoblasts incubated in anti-IL-10 secreted significantly more MMP-9 than control cells, whereas adding an irrelevant IgG to the medium had no effect. Figure 2B summarizes the results of five different experiments in which we analyzed, by immunoblotting, the effects of these various treatments on cytotrophoblast MMP-9 secretion. Compared with the controls (medium alone, irrelevant IgG), adding rIL-10 decreased cytotrophoblast MMP-9 secretion to 41 ± 10% of control levels ($P < 0.05$), whereas anti-IL-10 treatment increased MMP-9 levels to 119 ± 7% ($P < 0.05$).

We then determined, by immunoblotting, whether the cytokine and antibody effects we observed after 72 h of culture were dose dependent. Figure 3 shows the results of a typical experiment. The addition of 1–100 ng/ml rIL-10 to cultured cytotrophoblasts resulted in a dose-dependent inhibition of MMP-9 secretion; the lowest dose had no effect and the highest dose had the greatest effect. Conversely, 1–100 $\mu$g/ml of anti-IL-10 resulted in a dose-dependent increase in MMP secretion. As expected, cells treated with 1–100 $\mu$g/ml of an irrelevant IgG secreted the same amount of MMP-9 as control cytotrophoblasts incubated in medium alone. Together, the data in Figs. 2 and 3 demonstrate that IL-10 is an autocrine inhibitor of cytotrophoblast MMP-9 production *in vitro.*

### *IL-10 Downregulates MMP-9 Production at a Pretranslational Level, but Does Not Affect TIMP-3 mRNA Expression*

To better understand the regulatory mechanisms involved, we determined whether the addition of exogenous

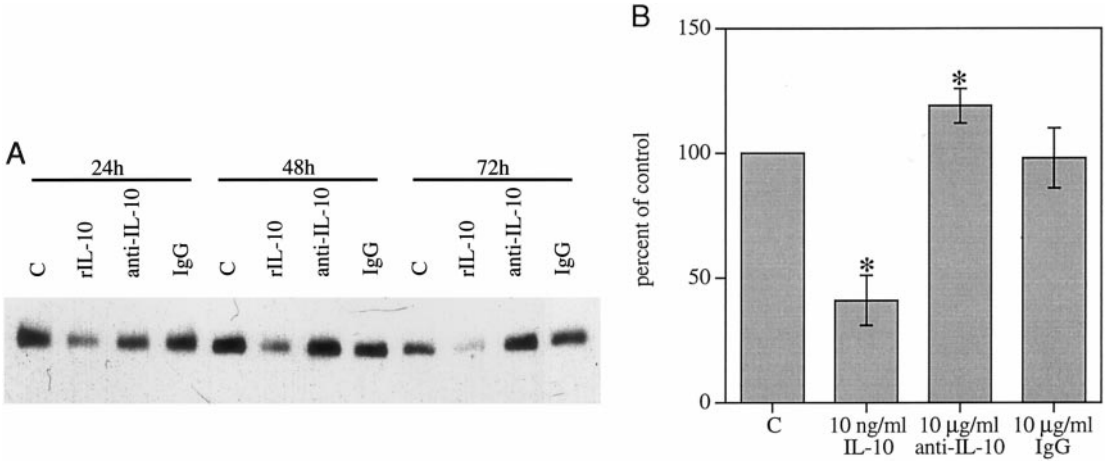


**FIG. 2.** Cytotrophoblast IL-10 is a negative regulator of MMP-9 secretion. Second-trimester cytotrophoblasts were cultured for the indicated times in culture medium alone (C), 10 ng/ml recombinant IL-10 (rIL-10), 10 $\mu$g/ml of a function-perturbing mAb to IL-10 (anti-IL-10), or 10 $\mu$g/ml of an isotype-matched irrelevant antibody (IgG). (A) Conditioned media (CM) were analyzed by immunoblotting using an antibody specific for MMP-9. (B) The relative intensities of MMP-9 bands from five experiments were measured after 72 h by densitometric analysis. Data are expressed as percentages of control. Values represent means ± SEM; $*P < 0.05$ compared with control by analysis of variance (ANOVA) followed by pairwise comparison.

IL-10 to cytotrophoblasts affects their levels of MMP-9 mRNA. Three different preparations of cells were cultured in medium alone (control) or in medium containing 10 ng/ml rIL-10, 10 $\mu$g/ml anti-IL-10 mAb, or 10 $\mu$g/ml of an irrelevant mAb (IgG). Medium containing these additives was replaced daily, and total RNA was extracted after 36 and 72 h. The RNA samples from each cell preparation were pooled, and MMP-9 mRNA levels were assessed by Northern blot hybridization (Fig. 4). In accord with the regulation we observed at the protein level, rIL-10 treatment decreased MMP-9 mRNA levels, whereas anti-IL-10 treatment increased them, and the observed effects became more pronounced over time. Normalized for RNA loading differences, densitometric analysis showed that exposing cytotrophoblasts to rIL-10 for 36 and 72 h caused 15 and 50% decreases in MMP-9 mRNA levels, respectively. Conversely, exposing cytotrophoblasts to anti-IL-10 for 36 and

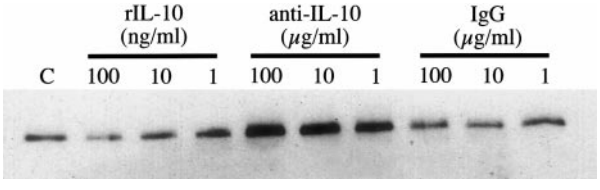


**FIG. 3.** The effect of IL-10 on MMP-9 secretion is dose dependent. Second-trimester cytotrophoblasts were cultured in culture medium alone (C) or with decreasing concentrations of rIL-10 (100–1 ng/ml), anti-IL-10 (100–1 $\mu$g/ml), or IgG (100–1 $\mu$g/ml). CM were collected after 72 h in culture and analyzed by immunoblotting.

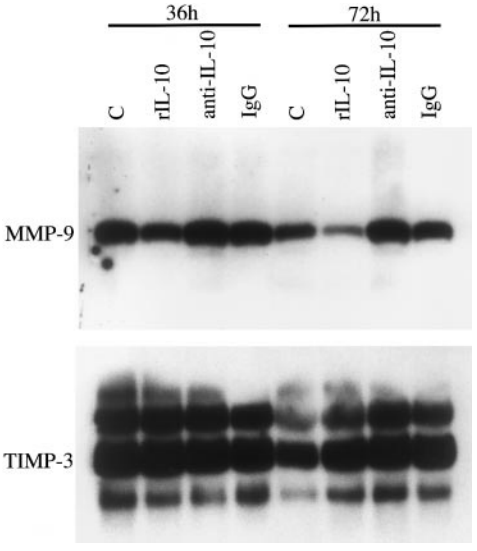


**FIG. 4.** IL-10 downregulates cytotrophoblast MMP-9 mRNA expression, but does not affect TIMP-3 mRNA levels. Second-trimester cytotrophoblasts were cultured in medium alone (C), 10 ng/ml rIL-10, 10 $\mu$g/ml anti-IL-10, or 10 $\mu$g/ml IgG. Total RNA was extracted from cytotrophoblasts after 36 and 72 h in culture. RNA from three different preparations of cells was pooled and subjected to Northern hybridization using a cDNA probe for MMP-9, which hybridized to a single 2.4-kb transcript. This blot was then stripped and probed for TIMP-3 as described under Materials and Methods. The TIMP-3 probe hybridized to multiple cytotrophoblast transcripts, 1.1, 2.4, 2.7, and 4.4 kb in size.

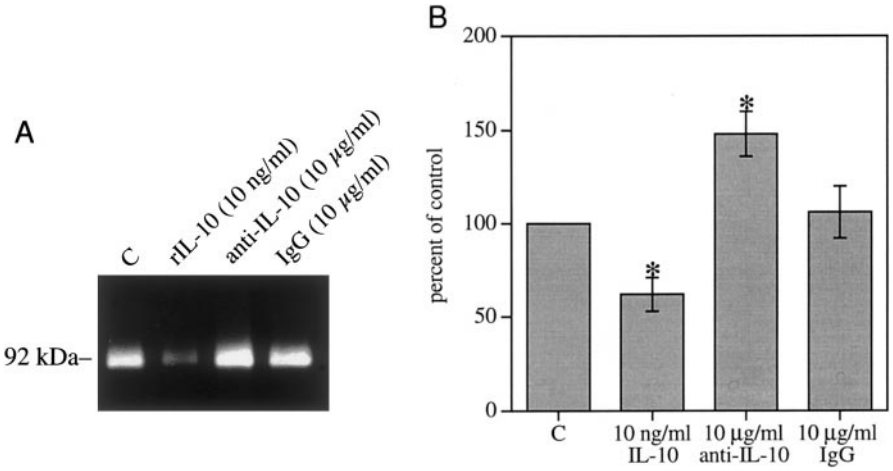


**FIG. 5.** Cytotrophoblast IL-10 is a negative regulator of MMP-9 activity. Second-trimester cytotrophoblasts were cultured in culture medium alone (C), 10 ng/ml rIL-10, 10 $\mu$g/ml anti-IL-10, or 10 $\mu$g/ml IgG. CM was collected after 72 h and analyzed by zymography as described under Materials and Methods. (A) Bands show areas of gelatinolysis. (B) The relative intensities of bands from five experiments were measured by densitometric analysis. Data are expressed as percentages of control. Values represent means ± SEM; $*P < 0.05$ compared with control by analysis of variance (ANOVA) followed by pairwise comparison.

72 h caused 16 and 46% increases in MMP-9 mRNA levels, respectively. MMP-9 mRNA levels in cells treated for the same length of time with an irrelevant IgG were no different from those of control cells (Fig. 4). These results suggest that IL-10 can regulate cytotrophoblast MMP-9 production at the pretranslational level.

We were also interested in whether IL-10 has similar effects on MMP-9 mRNA in other cell types. We chose to study U937 cells, a line of human mononuclear phagocytes that produce MMP-9 (Morodomi *et al.*, 1992) and express the IL-10 receptor (Takeshita *et al.*, 1996). U937 cells were cultured in medium alone (control) or in medium containing 10 ng/ml rIL-10, 10 $\mu$g/ml anti-IL-10 mAb, or 10 $\mu$g/ml of an irrelevant IgG for 72 h. Northern blot hybridization showed that neither IL-10 nor antibody treatment significantly affected MMP-9 mRNA levels (data not shown). This was in contrast to previous studies showing that the same amount of IL-10 downregulates MMP-9 production by primary monocytes and macrophages (Lacraz *et al.*, 1995). Taken together, our results and those of other investigators suggest that the effects of IL-10 on MMP-9 production are cell type specific.

Next, we determined whether IL-10 regulates cytotrophoblast TIMP production. These cells make very little TIMP-1 and -2. However, expression of TIMP-3 protein and mRNA, as that of MMP-9, is dramatically upregulated in the first 12 h in culture (Bass *et al.*, 1997). Since TIMP-3 can inhibit MMP-9 activity (Apte *et al.*, 1995), changes in its levels could affect cytotrophoblast proteolytic capacity. To determine whether IL-10 regulates cytotrophoblast TIMP-3 mRNA expression, the filter shown in Fig. 4 was stripped and hybridized with a TIMP-3-specific probe. When normalized for differences in RNA loading, densitometric analysis showed that neither IL-10 nor anti-IL-10 treatment for 72 h changed TIMP-3 mRNA levels. These data suggest that IL-10 changes the MMP/TIMP balance in favor of the inhibitor. As a consequence, net cytotrophoblast MMP activity should decrease.

### *IL-10 Downregulates Cytotrophoblast MMP-9 Activity*

We used two methods to assess the effects of IL-10 on cytotrophoblast MMP activity, beginning with zymography for an initial estimate of changes. Consistent with our previous observations (Fisher *et al.*, 1989; Librach *et al.*, 1991), the major gelatinolytic activity was in the 92-kDa region, and none of the cytokine or antibody treatments induced other activities (data not shown). The effects of rIL-10 and anti-IL-10 on cytotrophoblast MMP-9 activity paralleled their effects on MMP-9 protein expression, as shown by immunoblotting (Fig. 2A). Again, these changes were somewhat variable during the first 2 days of culture (data not shown), but at 72 h we found consistent differences. Figure 5A shows the results of a typical experiment. Cytotrophoblasts cultured in rIL-10 secreted considerably less 92-kDa gelatinolytic activity than control cells, and cytotrophoblasts incubated in anti-IL-10 secreted considerably more 92-kDa gelatinolytic activity. Figure 5B summarizes the results of five experiments. After cytokine treatment for 72 h, MMP-9 activity declined to 62 ± 9% of control levels ($P < 0.05$), whereas anti-IL-10 treatment caused MMP-9 activity to increase by 48 ± 12% ($P < 0.05$). Addition of the control, isotype-matched IgG did not affect gelatin degradation. Finally, these effects on cytotrophoblast MMP-9 activity depended on the dose of IL-10 or

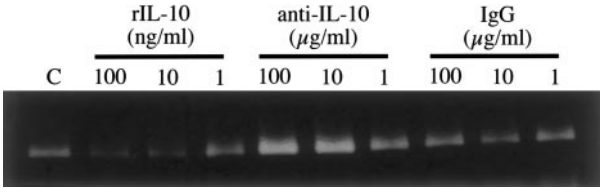


**FIG. 6.** The effect of IL-10 on MMP-9 activity is dose dependent. Second-trimester cytotrophoblasts were cultured in culture medium alone (C) or with decreasing concentrations of rIL-10 (100–1 ng/ml), anti-IL-10 (100–1 μg/ml), or IgG (100–1 μg/ml). CM was collected after 72 h and analyzed by zymography (see Materials and Methods). Bands show areas of gelatinolysis.

anti-IL-10 that was added to the cells. Figure 6 shows results typical of the three experiments we performed. The addition of 1–100 ng/ml rIL-10 to cultured cytotrophoblasts resulted in a dose-dependent inhibition of MMP-9 gelatinolytic activity; the lowest dose had no effect and the higher doses had the greatest effects. Conversely, 1–100 μg/ml anti-IL-10 resulted in a dose-dependent increase in MMP gelatinolytic activity. Again, the control IgG had no effect.

Because zymography is not rigorously quantitative, we also used a very sensitive chromogenic assay to quantify the effects of IL-10 and anti-IL-10 on cytotrophoblast MMP activity (Weingarten *et al.*, 1985). Figure 7 shows the results of five such experiments. After 72 h in culture, control conditioned medium and medium that contained an irrelevant IgG contained the same levels of MMP activity. As predicted from the zymogram data, rIL-10 treatment resulted in a 33 ± 9.7% decrease in MMP activity from control levels ($P < 0.05$). Conversely, anti-IL-10 stimulated MMP activity by 43 ± 15% ($P < 0.05$). Together, the results of the zymogram and chromogenic assays suggest that IL-10 is a negative regulator of cytotrophoblast MMP-9 activity.

### *IL-10 Inhibits Cytotrophoblast Invasive Capacity in Vitro*

Our lab has shown that cytotrophoblast MMP-9 activity is required for one of the cells' most important and unusual functions, namely invasion (Librach *et al.*, 1991). To test the functional relevance of IL-10 regulation of MMP-9 expression and activity, we quantified the invasive capacity of cytotrophoblasts treated with cytokine, anti-IL-10, or control IgG *in vitro*. Specifically, we assayed the cells' ability to invade Matrigel-coated, porous polycarbonate filters. Initially, the cells send long processes through the filter pores (48–72 h). Soon thereafter, entire cell bodies emerge on the filter underside (72 h onward).

Figure 8A summarizes the results of three experiments in which we assayed the effects of IL-10 on the appearance of cytotrophoblast cell processes on the bottom of the filter. When the cells were cultured for 60 h in the presence of rIL-10, 64 ± 8% fewer cytotrophoblasts traversed the filter pores compared with cells cultured in medium alone ($P < 0.05$). Conversely, neutralizing IL-10 function caused a 33 ± 7% increase in the number of cell processes that emerged on the filter underside (anti-IL-10), whereas the irrelevant mAb (IgG) had no effect ($P < 0.05$). Later in the culture period, we found that these same treatments also affected the number of cell bodies that emerged on the bottom of the filters. After 72 h, cytokeratin staining of control cultures and those treated with irrelevant IgG showed that most of the pores contained cell processes (Fig. 8B). A few cell bodies were also visible on the bottom of the filter. In the presence of rIL-10, many fewer processes reached the pores and no cell bodies were visible. With anti-IL-10 treatment, invasion was enhanced such that many cell bodies were present on the filter underside. Together, these data show that IL-10 is an autocrine inhibitor of cytotrophoblast invasion *in vitro*.

## DISCUSSION

IL-10 is a pleiotropic cytokine whose known functions include the regulation of both immune responses and proteinase production. Among its potent immunosuppressive effects are the downregulation of proinflammatory cytokine synthesis and the inhibition of alloreactive T-cell proliferation (Moore *et al.*, 1993). IL-10 has also been shown to regulate the expression of both serine proteinases (Ghildyal *et al.*, 1992a,b) and matrix metalloproteinases

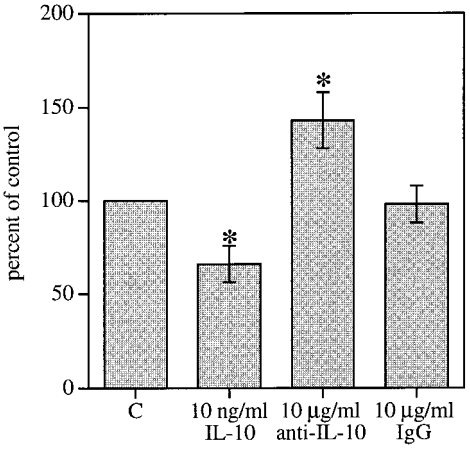


**FIG. 7.** IL-10 downregulates cytotrophoblast MMP-9 activity. Second-trimester cytotrophoblasts were cultured in culture medium alone (C) or medium containing 10 ng/ml rIL-10, 10 μg/ml anti-IL-10, or 10 μg/ml IgG. CM was collected after 72 h and added [25% (v/v)] to a synthetic thiopeptolide MMP substrate as described under Materials and Methods. Hydrolysis of this substrate was measured over 30 min. Data are expressed as percentages of control. Values represent means ± SEM of five experiments; *$P <$ 0.05 compared with control by analysis of variance (ANOVA) followed by pairwise comparison.

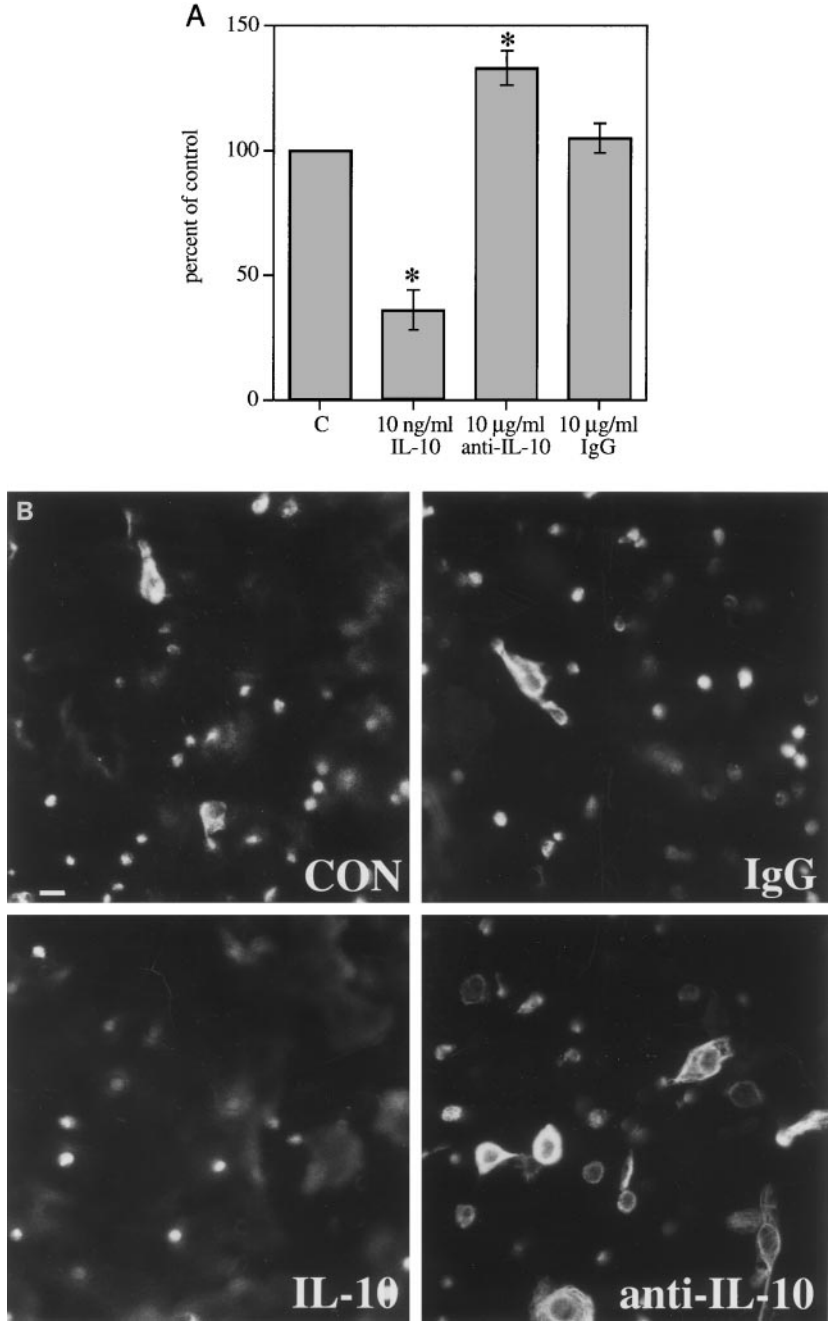


**FIG. 8.** IL-10 inhibits cytotrophoblast invasion *in vitro*. Second-trimester cytotrophoblasts were cultured on top of Matrigel-coated porous polycarbonate filter supports in culture medium alone (C) or medium containing 10 ng/ml rIL-10, 10 $\mu$g/ml anti-IL-10, or 10 $\mu$g/ml IgG (see Materials and Methods). (A) Some cultures were fixed after 60 h and stained with cytokeratin antibodies and cytotrophoblasts on the filter underside were counted. Data are expressed as percentages of control. Values represent means ± SEM from three experiments; $*P < 0.05$ compared with control by analysis of variance (ANOVA) followed by pairwise comparison. (B) In other cultures, cytotrophoblasts were fixed after 72 h and then subjected to immunohistochemistry and photographed. The photomicrograph shown in B is typical of the results of the three experiments. Bar = 15 $\mu$m.

(Mertz *et al.*, 1994; Reitamo *et al.*, 1994; Lacraz *et al.*, 1995) in a variety of cell types. Proteinase activity is required for cytotrophoblast invasion of the uterine wall during placentation. At the same time, these allogeneic fetal cells must avoid maternal immune rejection. This suggested to us that cytotrophoblast IL-10 could play a role in the regulation of

both of these biological processes. We previously described cytotrophoblast IL-10 synthesis *in vitro* and showed that this cytokine, present in the cells' conditioned medium, can suppress allogeneic T-lymphocyte reactivity (Roth *et al.*, 1996). We now demonstrate that IL-10 acts via an autoregulatory mechanism to specifically inhibit cytotrophoblast MMP-9 production at the pretranslational level. The subsequent downregulation of proteinase secretion results in a decrease in MMP-9 activity; as a consequence, cytotrophoblast invasion is significantly compromised.

IL-10 thus represents an important link between placental immune protection of the fetus and cytotrophoblast invasion of the uterus. We find that cytotrophoblast stem cells produce IL-10 *in vivo* (I. Roth and D. Figueroa, unpublished observations). Our data show that IL-10 is downregulated as these cells differentiate, thus permitting the production of MMP-9 necessary for uterine invasion. In the absence of IL-10 secretion, the expression of HLA-G by invasive cytotrophoblasts in the uterine wall may protect these cells from maternal immune attack (Kovats *et al.*, 1990; McMaster *et al.*, 1995; Pazmany *et al.*, 1996). However, if invading cytotrophoblasts encounter an immunologically hostile environment, IL-10 production could be upregulated to serve two important functions: high cytokine levels will suppress a harmful immune reaction, and invasion will be restricted to minimize the number of cytotrophoblasts in contact with maternal leukocytes. In support of this hypothesis, preliminary observations suggest that IL-10 levels are markedly elevated in severe preeclampsia *in vivo* (I. Roth and S. Fisher, unpublished observations). This disorder is believed to have an immunological basis and is characterized by abnormally shallow cytotrophoblast invasion of the uterine wall (Friedman *et al.*, 1991).

Here we demonstrate that cytotrophoblast IL-10 acts via an autocrine mechanism to downregulate MMP-9 secretion without affecting TIMP-3 mRNA expression. Cytokine treatment thus increases the effective ratio of inhibitor-to-proteinase levels, resulting in a net decrease in proteolytic activity and invasion. Hence, the consequences of suppressing proteinase production alone are amplified. These observations suggest that cytotrophoblast expression of MMP and TIMP mRNAs is regulated by disparate mechanisms. A similar phenomenon has been described in monocytes and macrophages. In these cells, treatment with interferon-$\gamma$ (Shapiro *et al.*, 1990), IL-4 (Lacraz *et al.*, 1992), or IL-10 (Lacraz *et al.*, 1995) has different effects on expression of MMP and TIMP mRNA. For example, IL-10 downregulates monocyte/macrophage MMP-9 mRNA but stimulates TIMP-1 expression (Lacraz *et al.*, 1995). Together, these findings highlight the complex, cell-type-specific nature of cytokine regulation of ECM degradation.

Finally, cytotrophoblast invasion of the uterine wall is one of the critical first steps for successful human pregnancy. Because of its importance, this process is undoubtedly influenced by many redundant regulatory mechanisms. We have identified several molecular targets for both the spatial and temporal control of cytotrophoblast invasion (Damsky *et al.*, 1993; Cross *et al.*, 1994). In addition to MMP-9, cytotrophoblast expression of adhesion molecules, including integrins, cadherins, and Ig superfamily members, is modulated in normal pregnancy (Damsky *et al.*, 1993; Zhou *et al.*, 1997). We have also shown that adhesion molecule expression is disregulated in preeclampsia, in which cytotrophoblast invasion is shallow (Zhou *et al.*, 1993, 1997). Thus, the interesting possibility exists that IL-10 also affects the production of these important molecules.

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## REFERENCES

Aplin, J. D. (1991). Implantation, trophoblast differentiation and haemochorial placentation: Mechanistic evidence *in vivo* and *in vitro*. *J. Cell Sci.* **99**(Pt. 4), 681–692.

Apte, S., Olsen, B. R., and Murphy, G. (1995). The gene structure of tissue inhibitor of metalloproteinases (TIMP)-3 and its inhibitory activities define the distinct TIMP gene family. *J. Biol. Chem.* **270,** 14313–14318.

Bass, K. E., Li, H., Hawkes, S., Howard, E., Bullen, E., Vu, T.-K. H., McMaster, M., Janatpour, M., and Fisher, S. J. (1997). TIMP-3 expression is upregulated during human cytotrophoblast invasion *in vitro*. *Dev. Genet.* **21,** 61–67.

Bejarano, M. T., de Waal Malefyt, R., Abrams, J. S., Bigler, M., Bacchetta, R., de Vries, J., and Roncarolo, M. G. (1992). Interleukin 10 inhibits allogeneic proliferative and cytotoxic T cell responses generated in primary mixed lymphocyte cultures. *Int. Immunol.* **4,** 1389–1397.

Berkowitz, R. S., and Goldstein, D. P. (1996). Chorionic tumors. *New Engl. J. Med.* **335,** 1740–1748.

Birkedal-Hansen, H. (1993). Role of cytokines and inflammatory mediators in tissue destruction. *J. Periodont. Res.* **28**(6, Pt. 2), 500–510.

Brosens, I., Robertson, W. B., and Dixon, H. G. (1978). Morphologic changes in uteroplacental arteries during pregnancy. *Am. J. Obstet. Gynecol.* **132,** 233.

Charbonneau, H., Tonks, N. K., Walsh, K. A., and Fischer, E. H. (1988). The leukocyte common antigen (CD45): A putative receptor-linked protein tyrosine phosphatase. *Proc. Natl. Acad. Sci. USA* **85,** 7182–7186.

Cross, J. C., Werb, Z., and Fisher, S. J. (1994). Implantation and the placenta: Key pieces of the development puzzle. *Science* **266,** 1508–1518.

Damsky, C., Sutherland, A., and Fisher, S. (1993). Extracellular matrix 5: Adhesive interactions in early mammalian embryogenesis, implantation, and placentation. *FASEB J.* **7,** 1320–1329.

Damsky, C. H., Fitzgerald, M. L., and Fisher, S. J. (1992). Distribution patterns of extracellular matrix components and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the invasive pathway, *in vivo*. *J. Clin. Invest.* **89,** 210–222.

Fisher, S. J., Cui, T. Y., Zhang, L., Hartman, L., Grahl, K., Zhang, G. Y., Tarpey, J., and Damsky, C. H. (1989). Adhesive and degradative properties of human placental cytotrophoblast cells *in vitro*. *J. Cell Biol.* **109,** 891–902.

Friedman, S. A., Taylor, R. N., and Roberts, J. M. (1991). Pathophysiology of preeclampsia. *Clin. Perinatol.* **18,** 661–682.

Gastl, G. A., Abrams, J. S., Nanus, D. M., Oosterkamp, R., Silver, J., Liu, F., Chen, M., Albino, A. P., and Bander, N. H. (1993). Interleukin-10 production by human carcinoma cell lines and its relationship to interleukin-6 expression. *Int. J. Cancer* **55,** 96–101.

Gerretsen, G., Huisjes, H. J., and Elema, J. D. (1981). Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation. *Br. J. Obstet. Gynaecol.* **88,** 8768–8781.

Ghildyal, N., McNeil, H. P., Gurish, M. F., Austen, K. F., and Stevens, R. L. (1992a). Transcriptional regulation of the mucosal mast cell-specific protease gene, MMCP-2, by interleukin 10 and interleukin 3. *J. Biol. Chem.* **267,** 8473–8477.

Ghildyal, N., McNeil, H. P., Stechschulte, S., Austen, K. F., Silberstein, D., Gurish, M. F., Somerville, L. L., and Stevens, R. L. (1992b). IL-10 induces transcription of the gene for mouse mast cell protease-1, a serine protease preferentially expressed in mucosal mast cells of *Trichinella spiralis*-infected mice. *J. Immunol.* **149,** 2123–2129.

Kliman, H. J., Nestler, J. E., Sermasi, E., Sanger, J. M., and Strauss, J. D. (1986). Purification, characterization, and *in vitro* differentiation of cytotrophoblasts from human term placentae. *Endocrinology* **118,** 1567–1582.

Kovats, S., Main, E. K., Librach, C., Stubblebine, M., Fisher, S. J., and DeMars, R. (1990). A class I antigen, HLA-G, expressed in human trophoblasts. *Science* **248,** 220–223.

Lacraz, S., Nicod, L., Galve-de Rochemonteix, B., Baumberger, C., Dayer, J. M., and Welgus, H. G. (1992). Suppression of metalloproteinase biosynthesis in human alveolar macrophages by interleukin-4. *J. Clin. Invest.* **90,** 382–388.

Lacraz, S., Nicod, L. P., Chicheportiche, R., Welgus, H. G., and Dayer, J. M. (1995). IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J. Clin. Invest.* **96,** 2304–2310.

Librach, C. L., Feigenbaum, S. L., Bass, K. E., Cui, T. Y., Verastas, N., Sadovsky, Y., Quigley, J. P., French, D. L., and Fisher, S. J. (1994). Interleukin-1 beta regulates human cytotrophoblast metalloproteinase activity and invasion *in vitro*. *J. Biol. Chem.* **269,** 17125–17131.

Librach, C. L., Werb, Z., Fitzgerald, M. L., Chiu, K., Corwin, N. M., Esteves, R. A., Grobelny, D., Galardy, R., Damsky, C. H., and Fisher, S. J. (1991). 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. *J. Cell Biol.* **113,** 437–449.

Lim, K.-H., Zhou, Y., Janatpour, M., McMaster, M., Bass, K., Chun, S.-H., and Fisher, S. J. (1997). Human cytotrophoblast differentiation/invasion is abnormal in preeclampsia. *Am. J. Pathol.* **151,** 1809–1818.

Lyons, J. G., Birkedal, H. B., Pierson, M. C., Whitelock, J. M., and Birkedal, H. H. (1993). Interleukin-1 beta and transforming growth factor-alpha/epidermal growth factor induce expression of M(r) 95,000 type IV collagenase/gelatinase and interstitial fibroblast-type collagenase by rat mucosal keratinocytes. *J. Biol. Chem.* **268,** 19143–19151.

Matrisian, L. M. (1990). Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet.* **6,** 121–125.

McMaster, M. T., Librach, C. L., Zhou, Y., Lim, K. H., Janatpour, M. J., DeMars, R., Kovats, S., Damsky, C., and Fisher, S. J. (1995). Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J. Immunol.* **154,** 3771–3778.

Mertz, P. M., DeWitt, D. L., Stetler, S. W., and Wahl, L. M. (1994). Interleukin 10 suppression of monocyte prostaglandin H synthase-2. Mechanism of inhibition of prostaglandin-dependent matrix metalloproteinase production. *J. Biol. Chem.* **269,** 21322–21329.

Moore, K. W., O'Garra, A., de Waal Malefyt, R., Vieira, P., and Mosmann, T. R. (1993). Interleukin-10. *Annu. Rev. Immunol.* **11,** 165–190.

Morodomi, T., Ogata, Y., Sasaguri, Y., Morimatsu, M., and Nagase, H. (1992). Purification and characterization of matrix metalloproteinase 9 from U937 monocytic leukaemia and HT1080 fibrosarcoma cells. *Biochem. J.* **285,** 603–611.

Murphy, G., and Docherty, A. J. (1992). The matrix metalloproteinases and their inhibitors. *Am. J. Respir. Cell Mol. Biol.* **7,** 120–125.

Ogata, Y., Pratta, M. A., Nagase, H., and Arner, E. C. (1992). Matrix metalloproteinase 9 (92-kDa gelatinase/type IV collagenase) is induced in rabbit articular chondrocytes by cotreatment with interleukin 1 beta and a protein kinase C activator. *Exp. Cell Res.* **201,** 245–249.

Pazmany, L., Mandelboim, O., Vales, G. M., Davis, D. M., Reyburn, H. T., and Strominger, J. L. (1996). Protection from natural killer cell-mediated lysis by HLA-G expression on target cells. *Science* **274,** 792–795.

Queenan, J. J., Kao, L. C., Arboleda, C. E., Ulloa, A. A., Golos, T. G., Cines, D. B., and Strauss, J. D. (1987). Regulation of urokinase-type plasminogen activator production by cultured human cytotrophoblasts. *J. Biol. Chem.* **262,** 10903–10906.

Reiner, S. L., Zheng, S., Corry, D. B., and Locksley, R. M. (1993). Constructing polycompetitor cDNAs for quantitative PCR. *J. Immunol. Methods* **165,** 37–46.

Reitamo, S., Remitz, A., Tamai, K., and Uitto, J. (1994). Interleukin-10 modulates type I collagen and matrix metalloprotease gene expression in cultured human skin fibroblasts. *J. Clin. Invest.* **94,** 2489–2492.

Ries, C., and Petrides, P. E. (1995). Cytokine regulation of matrix metalloproteinase activity and its regulatory dysfunction in disease. *Biol. Chem. Hoppe Seyler* **376,** 345–355.

Rinkenberger, J. L., Cross, J. C., and Werb, Z. (1997). Molecular genetics of implantation in the mouse. *Dev. Genet.* **21,** 6–20.

Roth, I., Corry, D. B., Locksley, R. M., Abrams, J. S., Litton, M. J., and Fisher, S. J. (1996). Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10. *J. Exp. Med.* **184,** 539–548.

Salo, T., Lyons, J. G., Rahemtulla, F., Birkedal, H. H., and Larjava, H. (1991). Transforming growth factor-beta 1 up-regulates type IV collagenase expression in cultured human keratinocytes. *J. Biol. Chem.* **266,** 11436–11441.

Sang, Q. A., and Douglas, D. A. (1996). Computational sequence analysis of matrix metalloproteinases. *J. Protein Chem.* **15,** 137–159.

Sato, T., Ito, A., Ogata, Y., Nagase, H., and Mori, Y. (1996). Tumor necrosis factor alpha (TNFalpha) induces pro-matrix metalloproteinase 9 production in human uterine cervical fibroblasts but interleukin 1alpha antagonizes the inductive effect of TNFalpha. *FEBS Lett.* **392,** 175–178.

Shapiro, S. D., Campbell, E. J., Kobayashi, D. K., and Welgus, H. G. (1990). Immune modulation of metalloproteinase production in

human macrophages. Selective pretranslational suppression of interstitial collagenase and stromelysin biosynthesis by interferon-gamma. *J. Clin. Invest.* **86,** 1204–1210.

Silbiger, S. M., Jacobsen, V. L., Cupples, R. L., and Koski, R. A. (1994). Cloning of cDNAs encoding human TIMP-3, a novel member of the tissue inhibitor of metalloproteinase family. *Gene* **141,** 293–297.

Takeshita, S., Gage, J. R., Kishimoto, T., Vredevoe, D. L., and Martinez, M. O. (1996). Differential regulation of IL-6 gene transcription and expression by IL-4 and IL-10 in human monocytic cell lines. *J. Immunol.* **156,** 2591–2598.

Unemori, E. N., Hibbs, M. S., and Amento, E. P. (1991). Constitutive expression of a 92-kD gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. *J. Clin. Invest.* **88,** 1656–1662.

Wahl, S. M., Allen, J. B., Weeks, B. S., Wong, H. L., and Klotman, P. E. (1993). Transforming growth factor beta enhances integrin expression and type IV collagenase secretion in human monocytes. *Proc. Natl. Acad. Sci. USA* **90,** 4577–4581.

Watanabe, H., Nakanishi, I., Yamashita, K., Hayakawa, T., and Okada, Y. (1993). Matrix metalloproteinase-9 (92 kDa gelatinase/type IV collagenase) from U937 monoblastoid cells: Correlation with cellular invasion. *J. Cell Sci.* **104**(Pt. 4), 991–999.

Weingarten, H., Martin, R., and Feder, J. (1985). Synthetic substrates of vertebrate collagenase. *Biochemistry* **24,** 6730–6734.

Wilhelm, S. M., Collier, I. E., Marmer, B. L., Eisen, A. Z., Grant, G. A., and Goldberg, G. I. (1989). SV40-transformed human lung fibroblasts secrete a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophages. *J. Biol. Chem.* **264,** 17213–17221.

Zhou, Y., Damsky, C. H., Chiu, K., Roberts, J. M., and Fisher, S. J. (1993). Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. *J. Clin. Invest.* **91,** 950–960.

Zhou, Y., Damsky, C. H., and Fisher, S. J. (1997). Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype—One cause of defective endovascular invasion in this syndrome? *J. Clin. Invest.* **99,** 2152–2164.

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