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Insulin-Like Growth Factor (IGF)-II Inhibition of Endometrial Stromal Cell Tissue Inhibitor of Metalloproteinase-3 and IGF-Binding Protein-1 Suggests Paracrine Interactions at the Decidua:Trophoblast Interface during Human Implantation*

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ABSTRACT

In human pregnancy, insulin-like growth factor (IGF)-II messenger RNA (mRNA) is expressed at the maternal-fetal interface exclusively by the placental trophoblast. Highest levels are expressed by the invading extravillous trophoblasts, which also secrete matrix metalloproteinases as they degrade the decidual extracellular matrix. In contrast, the maternal decidua expresses high levels of IGF-binding protein (IGFBP)-1 and tissue inhibitors of matrix metalloproteinase (TIMPs), both of which inhibit trophoblast invasiveness *in vitro*. The present study investigated the hypothesis that IGF-II may serve as a paracrine modulator of maternal restraints on invasion, by examining its effects on TIMP-3 and IGFBP-1 expression by decidualized endometrial stromal cells. Human endometrial stromal cells were decidualized *in vitro* with progesterone (P), after which 0–130 nM IGF-II and IGF analogs were added. IGFBP-1 in conditioned medium was assayed by immunoradiometric assay. In addition, Northern analyses were conducted using a PCR-generated 421-bp complementary DNA (cDNA) fragment corresponding to nucleotides 132–553 of the human TIMP-3 cDNA, and a 934-bp *Eco*RI fragment of the human IGFBP-1 cDNA. TIMP-3 mRNA transcripts of 2.2, 2.5, and 4.4 kilobases were detected in decidualized stromal cells not treated with IGF-II, but not detected in nondecidualized stromal cells, consistent with its known induction upon decidualization and in response to P. In decidualized stromal cells, IGF-II and Des(1-6) IGF-II, an analog with reduced affinity for IGFBPs, caused a dose-dependent inhibition of TIMP-3 mRNA expression. Long R^3 IGF-I, an IGF analog with minimal affinity for IGFBPs, also significantly inhibited (79 ± 0.3%) TIMP-3 mRNA expression in these cells at 6 nM. Decidualized stromal cells secreted IGFBP-1 and expressed a 1.5-kilobase IGFBP-1 transcript, which was not detected in nondecidualized cells, consistent with its known induction upon decidualization and in response to P. IGF-II caused a dose-dependent inhibition of IGFBP-1 mRNA expression and protein secretion in decidualized stromal cells when added in molar excess of endogenous IGFBP-1 levels, with virtually complete inhibition at higher concentrations of IGF-II (65 and 130 nM). By comparison, Long R^3 IGF-I inhibited IGFBP-1 expression with a 50% effective dose of 0.2–0.4 nM. These data suggest that the invading trophoblast has the capacity, via IGF-II, to inhibit maternal restraints on trophoblast invasiveness by regulating decidual TIMP-3 and IGFBP-1. (*J Clin Endocrinol Metab* **86:** 2060–2064, 2001)

DURING THE DEVELOPMENT of the human placenta, placental trophoblasts invade the gestational uterine tissues to gain access to the maternal blood supply. Trophoblast invasion requires endometrial extracellular matrix proteolysis, as well as cellular migration through the maternal decidua (1–3). In normal placental development, these processes are precisely regulated, in that they are both spatially and temporally limited. Such a precise and orderly process requires a balanced interplay between the factors that promote and restrain trophoblast invasion. Existing evidence suggests that trophoblast invasion is controlled by the uterine microenvironment and supports a role of the maternal decidual tissue in, primarily, restraining trophoblast invasion (4, 5). This implies that factors that promote invasion are, primarily, trophoblast-derived. Consistent with this paradigm, the invading trophoblast secretes matrix metalloproteinases (MMPs), which degrade the decidual extracellular matrix (1–3), and the maternal decidua expresses high levels of the tissue inhibitors of MMP (TIMPs) (6, 7), as well as insulin-like growth factor-binding protein (IGFBP)-1 (8–10), both of which inhibit trophoblast invasiveness *in vitro* (3, 11). However, the precise autocrine/paracrine interplay of signaling and effector molecules that ultimately regulates trophoblast invasion remains to be fully elucidated. For example, cytokines present at the placental-decidual interface such as interleukin-1β and transforming growth factor β (12, 13) have been shown to modulate trophoblast invasion *in vitro* through their effects on MMP and TIMP production, respectively (5, 3). However, because of the widespread distribution of these cytokines at the maternal-fetal interface (12), the precise contributions of placental and/or decidual cells to these regulatory loops are unclear. The IGF system is

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also prominent in human pregnancy. IGF-II is the main IGF peptide expressed at the maternal-fetal interface, where *in situ* hybridization studies have shown it to be expressed exclusively, and in high abundance, by the invading extravillous trophoblast (14). The pattern of expression of IGF-II further suggests its involvement in trophoblast invasion, because it shows a gradient of IGF-II messenger RNA (mRNA) abundance that is highest at the invading front (14). On the maternal side, IGFBP-1 is the most abundant protein and the most abundant IGFBP at the maternal-fetal interface, and it is expressed exclusively by the decidual cells. Such clear and discrete patterns of expression of IGF-II and IGFBP-1 within the utero-placental unit provides a unique opportunity to gain further insights into the paracrine interactions of signaling and effector molecules that regulate trophoblast invasion. To this end, the current study investigated the effects of IGF-II, a trophoblast-derived regulator, upon decidual TIMP-3 and IGFBP-1, effector molecules that mediate maternal restraints on invasion.

Materials and Methods

IGF peptides and analogs

Recombinant human IGF-II was from Bachem (Torrance, CA). The following IGF analogs were obtained from GroPep Pty. Ltd. (Adelaide, Australia). Long R^3 IGF-I, a recombinant IGF-I analog, has an arginine for glutamate substitution at position 3 and a 13-amino-acid extension peptide at the N terminus. This analog does not bind to the type 2 IGF receptor, and its affinity for the type 1 IGF receptor is similar to native IGF-I, but it has three orders of magnitude lower affinity for IGFBPs (15). Consequently, it has approximately 10-fold increased potency, compared with IGF-I, as a result of its increased bioavailability. The IGF-II analog Des(1-6) IGF-II lacks the N-terminal hexapeptide and binds with 2.6-fold lower affinity to the type 2 IGF receptor and with 2-fold lower affinity to the type 1 IGF receptor, compared with native IGF-II. However, its affinity for the IGFBPs is reduced more than 300-fold overall, resulting in a biological potency similar to IGF-II (16).

Tissue specimens

Tissues used in the present study were obtained in accordance with the guidelines of The Declaration of Helsinki. Informed consent was obtained from patients and the study was approved by the Stanford University Human Subjects Committee (Stanford, CA). Some tissues were also obtained through the Cooperative Human Tissue Network (Cleveland, OH). Histologically normal endometrial tissue samples were obtained from cycling premenopausal patients (27–34 yr old) undergoing endometrial biopsy or hysterectomy for benign reasons in the secretory phase of natural cycles. Samples were collected at room temperature in DMEM, transported to the laboratory, and processed as described below.

Cell cultures

Endometrial tissue was subjected to collagenase digestion and stromal cells were separated from epithelium, cultured, and passaged as previously described (17). Cells isolated from 3 different subjects were used at passages 1–4 for these studies. Stromal cells were grown to confluence in 6-cm diameter tissue culture plates (Costar, Cambridge, MA) in DMEM supplemented with 5 μg/ml insulin and 10% heat-inactivated charcoal-stripped FBS. Confluent cultures were then decidualized *in vitro* with 10 nM estradiol, 1 μM progesterone, and 20 ng/ml epidermal growth factor in serum-free medium (75% DMEM, 25% MCDB-104, 50 μg/ml ascorbic acid, 1 mg/ml BSA, 5 μg/ml transferrin) for 11 days in the absence of IGF peptides, and then cultured for 6 days in the same decidualizing medium without added IGF peptides, or with the addition of various concentrations of IGF-II (13, 27, 67, 134 nM), or Des(1-6) IGF-II (7, 30, 74 nM), or with Long R^3 IGF-I (6 nM). Cells cultured in serum-free-medium with epidermal growth factor but without estradiol and progesterone were used as nondecidualized endometrial stromal cells controls. Every 2 days the culture medium was renewed, the conditioned media were collected and centrifuged, and the supernatant was stored at −80 C for further analysis. Cells were harvested for RNA analysis after 6 days of treatment.

Northern analysis

Northern analysis was conducted using as probes a PCR-generated 421-bp complementary DNA (cDNA) fragment corresponding to nucleotides 132–553 of the human TIMP-3 cDNA, and a 934-bp *Eco*RI fragment of the human IGFBP-1 cDNA (18), kindly provided by David Powell (Baylor College of Medicine, Houston, TX). Total RNA was isolated using a modified acid guanidine-phenol extraction with TRIzol (Life Technologies, Inc., Rockville, MD). RNA samples (20 μg) were size fractionated by 1.2% agarose/formaldehyde gel electrophoresis and transferred to nitrocellulose. Membranes were hybridized with the TIMP-3 cDNA probe labeled with ^{32}P by random priming. Following exposure to radiographic film, the TIMP-3 probe was stripped and blots were hybridized with the ^{32}P-labeled IGFBP-1 cDNA probe. Autoradiograms were analyzed on a PDI desktop scanner (Protein DNA ImageWare Systems, Huntington Station, NY). For each sample, densities of TIMP-3 and IGFBP-1 bands were normalized for loading according to the respective density of 18s and 28s bands in the ethidium bromide stained gels. Examination of agarose gels by ethidium bromide staining after RNA transfer to nitrocellulose membranes revealed approximately 95% efficiency of RNA transfer.

IGFBP-1 immunoradiometric assay (IRMA)

IGFBP-1 levels were assayed in duplicate in 2-day conditioned media from duplicate or triplicate cultures for each treatment group. IGFBP-1 IRMA kits from Diagnostics Systems Laboratories, Inc. (Webster, TX) were used. Intraassay coefficients of variation were 5.2, 4.6, and 2.7% for IGFBP-1 concentrations of 5.2, 50.2, and 144.6 ng/ml, respectively. Interassay coefficients of variation were 3.5, 6.0, and 3.6% for IGFBP-1 concentrations of 5.2, 47.1, and 142.0 ng/ml, respectively.

Statistical analysis

Experimental variables were tested in duplicate or triplicate cultures, and statistical analysis was carried out by one-way ANOVA. For TIMP-3 Northern blot densitometry, the variable analyzed was the hybridization signal normalized for loading against the density of the respective 18s and 28s bands, *i.e.* the sum of the densities corresponding to the three TIMP-3 bands [2.2, 2.5, 4.4 kilobases (kb)], divided by the sum of the densities of the respective 18s and 28s rRNA bands for each sample. In the case of IGFBP-1 production, the variable analyzed was the IGFBP-1 protein output normalized against micrograms of RNA in the cellular component, *i.e.* the total micrograms of IGFBP-1 protein produced, divided by the total micrograms of cellular RNA for each sample. *Post hoc* testing for the significance of the differences between treatment group means was done using Scheffé's test, with significance level at P less than 0.05.

Results

Experiments were conducted to investigate the effects of IGF-II on TIMP-3 and IGFBP-1 mRNA expression by human endometrial stromal cells decidualized *in vitro*. Confluent endometrial stromal cultures were treated with or without 1 μM progesterone and 10 nM estradiol in serum-free medium. Cells began to decidualize after about 6–8 days of hormonal treatment, as indicated by the secretion of classic decidual markers such as PRL and IGFBP-1. After 11 days of hormonal therapy, experimental treatments were started by adding IGF peptides in various concentrations to the decidualized cultures. Following 6 days of IGF treatment, cells were harvested for Northern analysis. Figure 1 shows a representative

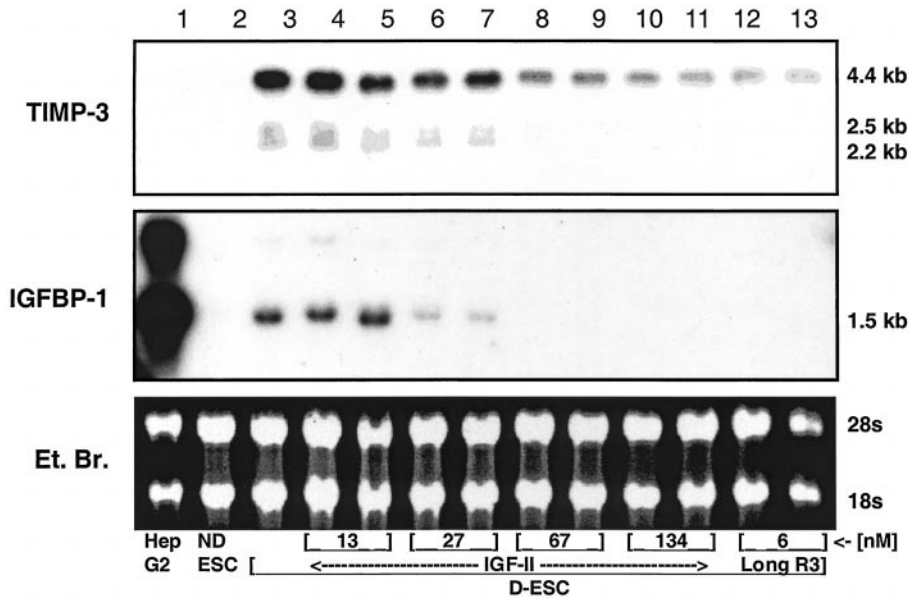

FIG. 1. IGF-II inhibits TIMP-3 and IGFBP-1 mRNA expression in decidualized endometrial stromal cells. Northern analysis of total RNA from cultures of human endometrial stromal cells first decidualized *in vitro* for 11 days without IGF peptides (*lanes 3–13*), and then cultured for an additional 6 days without IGFs (*lane 3*), with IGF-II concentrations of 13 nM (*lanes 4* and *5*), 27 nM (*lanes 6* and *7*), 67 nM (*lanes 8* and *9*), or 134 nM (*lanes 10* and *11*), or with 6 nM Long R^3 IGF-I (*lanes 12* and *13*). Total RNA from Hep-G2 cells (*lane 1*) was included as positive control for IGFBP-1 expression. RNA from nondecidualized endometrial stromal cells (*lane 2*) served as negative control for TIMP-3 and IGFBP-1 expression. The *top* and *middle panels* show hybridization with the TIMP-3 and IGFBP-1 probes respectively, with the size of the transcripts being shown on the right margin expressed in kb. The *bottom panel* shows the ethidium bromide staining, with the position of the ribosomal RNA 18s and 28s bands indicated on the right margin.

Northern blot, hybridized with the TIMP-3 (*top panel*) or IGFBP-1 (*middle panel*) probes. TIMP-3 mRNA transcripts of 4.4, 2.5 and, minimally, 2.2 kb, and a major 1.5-kb IGFBP-1 transcript, were detected in decidualized endometrial stromal cells not treated with IGF-II (*lane 3*), but were not detected in nondecidualized stromal cells (*lane 2*). With decidualized stromal cells, IGF-II (*lanes 4–11*) caused a dose-dependent inhibition of TIMP-3 and IGFBP-1 mRNA expression. Long R^3 IGF-I (*lanes 12* and *13*) also inhibited TIMP-3 and IGFBP-1 mRNA expression in decidualized stromal cells. Densitometric analysis of relative hybridization intensities (Fig. 2) showed a dose-dependent inhibition of TIMP-3 mRNA expression by IGF-II, which was significant ($P < 0.05$) at a peptide concentration of 134 nM (59%). Des(1-6) IGF-II, an IGF-II analog with reduced affinity for IGFBPs, also caused a dose-dependent inhibition of TIMP-3 expression with maximal inhibition of TIMP-3 mRNA expression ($P < 0.05$) at a concentration of 74 nM (73%). By comparison, Long R^3 IGF-I, an IGF analog that has minimal affinity for IGFBPs, could significantly inhibit ($P < 0.05$) TIMP-3 mRNA expression in these cells to a similar degree (79%), but at a much lower concentration (6 nM).

IGF peptides also had pronounced effects on IGFBP-1 mRNA expression in decidualized endometrial stromal cells. Northern blot densitometry showed a dose-dependent inhibition of IGFBP-1 mRNA expression by IGF-II. However, compared with the maximal 59% inhibition observed for TIMP-3 expression, IGF-II inhibited IGFBP-1 expression more effectively, causing more than 96% reduction ($P < 0.05$) of steady-state mRNA levels at peptide concentrations of 67 and 134 nM (Fig. 3). The IGF-II analog Des(1-6) IGF-II had a similar effect to the native peptide, showing a dose-dependent inhibition of IGFBP-1 expression, with a maximal effect (97% inhibition; $P < 0.05$) at 74 nM (Fig. 3). The higher

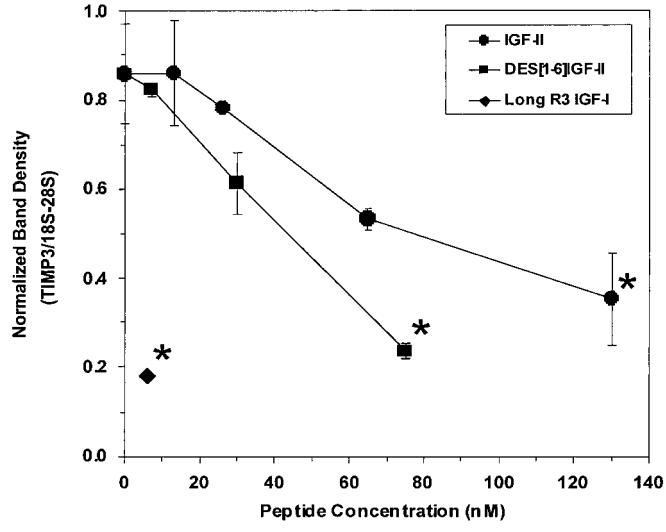

FIG. 2. Dose-dependent inhibition of TIMP-3 mRNA expression in decidualized endometrial stromal cell by IGF peptides. Densitometric analysis of Northern blots of human endometrial stromal cultures decidualized *in vitro* for 11 days without IGF peptides, and then treated for 6 additional days without IGFs (0 nM), or with various concentrations of IGF-II (●) or Des(1-6) IGF-II (■), or with 6 nM Long R^3 IGF-I (◆). Values represent hybridization signals, normalized according to the respective density of 18s and 28s bands. Each point is the mean ± SEM (*error bars*) from duplicate cultures. *, Significantly different from no IGF ($P < 0.05$).

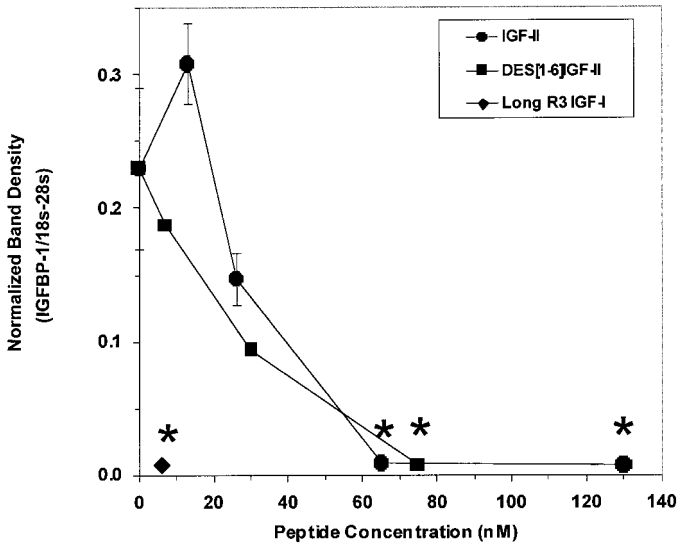

FIG. 3. Dose-dependent inhibition of IGFBP-1 mRNA expression in decidualized endometrial stromal cells by IGF peptides. Densitometric analysis of Northern blots of human endometrial stromal cultures decidualized *in vitro* for 11 days without IGF peptides, and then cultured for 6 additional days without IGFs (0 nM), or with various concentrations of IGF-II (●) or Des(1-6) IGF-II (■), or with 6 nM Long R^3 IGF-I (◆). Values represent hybridization signals, normalized according to the respective density of 18s and 28s bands. Each point is the mean ± SEM (*error bars*) from duplicate cultures. *, Significantly different from no IGF ($P < 0.05$).

potency of Long R^3 IGF-I was also manifest in its inhibition of IGFBP-1 expression. This analog inhibited IGFBP-1 mRNA expression by 97% ($P < 0.05$) a concentration of 6 nM (Fig. 3).

Analysis of secreted IGFBP-1 protein (Fig. 4) showed levels of 200–300 ng/ml in decidualized stromal cultures at the time of starting IGF treatment. In the absence of IGFs, IGFBP-1 protein levels increased 4-fold in decidualized stromal cultures over the following 4 days (Fig. 4). Treatment of decidualized cultures with IGF-II or Des(1-6) IGF-II had a dose-dependent inhibitory effect on IGFBP-1 protein secretion. As a result, over the same 4-day period, cultures treated with lower concentrations (7–30 nM) of IGF-II (Fig. 4A) or its analog (Fig. 4B) showed a slowing of the rise of IGFBP-1 levels, whereas cultures treated with higher concentrations of these peptides (67–134 nM), or with 6 nM Long R^3 IGF-I (Fig. 4B), showed a frank decline of IGFBP-1 protein concentration. After 6 days of IGF treatment, net IGFBP-1 production by decidualized cells was reduced in a dose-dependent fashion (Fig. 5). The inhibitory effect of IGF-II resulted in significant reduction (>97%; $P < 0.05$) of IGFBP-1 protein production at peptide concentrations of 65 and 130 nM, whereas similar inhibition(>99%; $P < 0.05$) was obtained with 67-nM Des(1-6) IGF-II, or 6 nM Long R^3 IGF-I (Fig. 5). The effects of both IGF-II and its analog on IGFBP-1 protein production were consistent with their effects on IGFBP-1 mRNA expression (compare Figs. 3 and 5), both showing a dose-dependent inhibition that reached significance levels at the higher peptide concentrations (>30 nM).

Discussion

During the process of implantation in humans, the placental extravillous trophoblast invades into the maternal decidua (12). The extravillous trophoblast expresses IGF-II mRNA, with the highest levels expressed at the invading front, and also expresses matrix degrading enzymes, primarily MMP-2 and MMP-9 (13, 14). On the maternal side, the uterine decidua expresses high levels of basement membrane type extracellular matrix and also IGFBP-1 and TIMP-3, known inhibitors of trophoblast invasiveness in *in vitro* models (6, 7). The juxtaposition of the decidua and the trophoblast and their known secreted products suggest control of invasion by paracrine (and perhaps autocrine) modulators at the decidua:trophoblast interface. The present study has demonstrated that IGF-II inhibits TIMP-3 and IGFBP-1 mRNA expression by decidualized human endometrial stromal cells. Thus, these findings suggest that a trophoblast-derived growth factor, such as IGF-II, can act at the maternal-placental interface to modulate effector molecules that mediate maternal restraints on invasion, such as decidual TIMP-3 and IGFBP-1.

In the present study, strong TIMP-3 mRNA expression

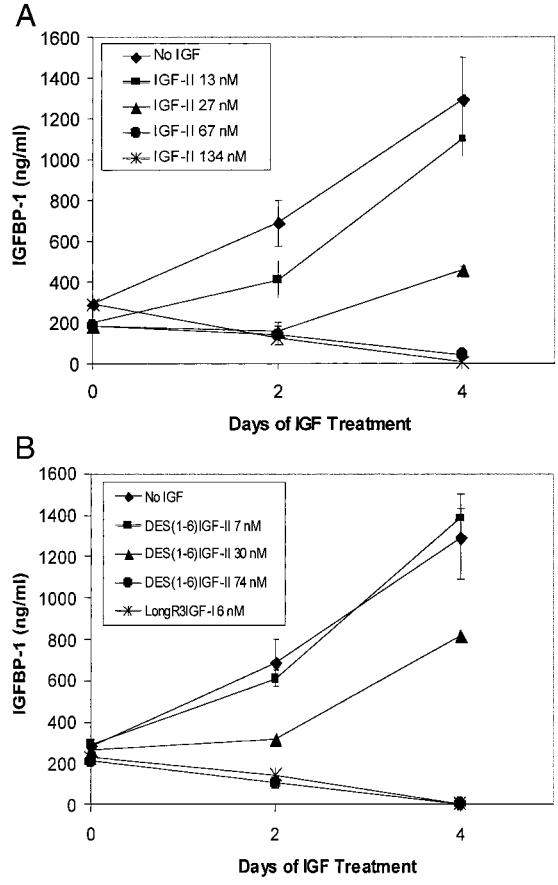

FIG. 4. Dose-dependent effects of IGF peptides on IGFBP-1 protein levels in decidualized endometrial stromal cell cultures. Human endometrial stromal cultures were decidualized *in vitro* for 11 days without IGF peptides, and then cultured for 4 additional days without IGFs (No IGF), or with the indicated concentrations of IGF-II (A), Des(1-6) IGF-II (B), or Long R^3 IGF-I (B). IGFBP-1 levels were measured in 2-day conditioned media collected immediately before starting IGF treatment (day 0), or after 2 and 4 days of IGF treatment. IGFBP-1 in the conditioned medium of each culture was assayed in duplicate by IRMA. Values represent the IGFBP-1 concentration in the culture medium expressed in ng/ml. Each point is the mean ± SEM (*error bars*) from duplicate cultures.

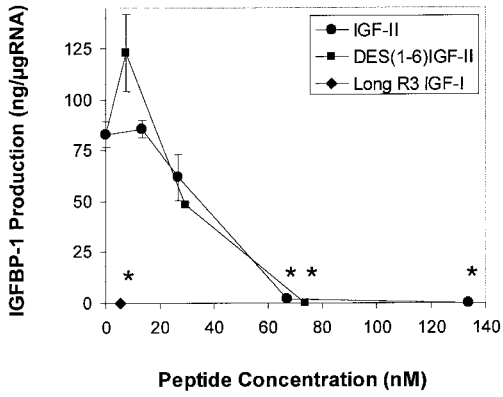

FIG. 5. Dose-dependent effects of IGF peptides on IGFBP-1 protein production by decidualized endometrial stromal cells. Human endometrial stromal cultures were decidualized *in vitro* for 11 days without IGF peptides, and then cultured for 6 additional days without IGFs (0 nM), or with various concentrations of IGF-II (●), Des(1-6) IGF-II (■), or with 6 nM Long R^3 IGF-I (◆). IGFBP-1 levels were measured in 2-day conditioned media collected after 6 days of IGF treatment. IGFBP-1 in the conditioned medium of each culture was assayed in duplicate by IRMA. Values represent the net IGFBP-1 protein production (nanograms) over the 2-day period (days 4–6), normalized against micrograms of total cellular RNA. Each point is the mean ± SEM (*error bars*) from duplicate cultures. *, Significantly different from no IGF ($P < 0.05$).

was detected in decidualized endometrial stromal cells, but not in nondecidualized cells. This observation is consistent with previous studies showing induction of TIMP-3 mRNA expression during the course of progesterone-induced decidualization of human endometrial stromal cell cultures (6). The unique expression of TIMP-3 in decidualized stromal cells in response to progesterone-induced decidualization compared with, *e.g.* TIMP-1 and TIMP-2, which are not menstrual-cycle (or gonadal steroid-hormone)-dependent (6, 8), suggests an important role for TIMP-3 in the periimplantation period. Regulation of this TIMP by a trophoblast-derived growth factor strongly supports paracrine interactions between the maternal and fetal compartments during the very early stages of implantation in humans.

In the current study, we found that IGF-II exerted a dose-dependent inhibition of TIMP-3 mRNA expression by *in vitro* decidualized endometrial stromal cells, by as much as 73%. The current study does not address mechanisms leading to different extents of inhibition of steady-state levels of TIMP-3 (and IGFBP-1) mRNA levels, and whether the inhibition occurs at the level of gene transcription or mRNA stability is planned in future studies in our laboratory. Compared with TIMP-3, IGFBP-1 mRNA expression and protein secretion by *in vitro* decidualized endometrial stromal cells were more effectively inhibited by IGF-II, which caused complete suppression at maximally effective doses. However, from a teleological perspective, if indeed TIMP-3 is important in inhibiting MMPs expressed by the invading trophoblast, persistent presence of some TIMP-3 may be important to prevent uncontrolled matrix degradation. Indeed, in most systems in which MMPs are expressed, TIMPs are coexpressed at various levels (6). In addition, if IGFBP-1 is also important in modulating trophoblast invasiveness, then efficient local control of IGFBP-1 expression may be important to this process. The data reported in this study suggest that the invading trophoblast has the capacity, via IGF-II, to inhibit maternal restraints on trophoblast invasiveness, by regulating decidual TIMP-3 and IGFBP-1. However, the current studies are *in vitro* observations, and whether TIMP-3 and IGFBP-1 and their regulation by IGF-II and other trophoblast-derived products are important in the process of implantation *in vivo*, remains to be determined.

References

1. **Cross JC, Werb Z, Fisher SJ.** 1994 Implantation and the placenta: key pieces of the development puzzle. Science. 266:1508–1517.
2. **Aplin J.** 1991 Implantation, trophoblast differentiation and haemochorial placentation: mechanistic evidence *in vivo* and *in vitro*. J Cell Sci. 99:68–692.
3. **Fisher SJ, Damsky CH.** 1993 Human cytotrophoblast invasion. Sem Cell Biol. 4:183–188.
4. **Kirby DRS.** 1965 The "invasiveness" of the trophoblast. In: Park WW, ed. The early conceptus, normal and abnormal. Edinburgh: University of St. Andrews Press; 68–74.
5. **Graham CH, Lala PK.** 1991 Mechanism of control of trophoblast invasion *in situ*. J Cell Physiol. 148:228–234.
6. **Hampton AL, Salamonsen LA.** 1994 Expression of messenger ribonucleic acid encoding matrix metalloproteinases and their tissue inhibitors is related to menstruation. J Endocrinol. 141:R1–R3.
7. **Higuchi T, Kanzaki H, Nakayama H, et al.** 1995 Induction of tissue inhibitor of metalloproteinase 3 gene during *in vitro* decidualization of human endometrial stromal cells. Endocrinology. 136:4973–4981.
8. **Rodgers WH, Matrisian LM, Giudice LC, et al.** 1994 Patterns of matrix metalloproteinase expression in cycling endometrium imply differential functions and regulation by steroid hormones. J Clin Invest. 94:946–953.
9. **Rutanen E-M, Menabawey M, Isaka K, Bohn H, Chard T, Grudzinskas JG.** 1986 Synthesis of placental protein 12 by decidua from early pregnancy. J Clin Endocrinol Metab. 63:675–679.
10. **Waites GT, James RFL, Bell SC.** 1989 Human pregnancy-associated endometrial α_1-globulin, an insulin-like growth factor-binding protein: immunohistological localization in the decidua and placenta during pregnancy employing monoclonal antibodies. J Endocrinol. 120:351–357.
11. **Irwin JC, Giudice LC.** 1998 Insulin-like growth factor binding protein-1 ninds to cytotropho-blast $\alpha_5\beta_1$ integrin and inhibits cytotrophoblast invasion into decidual multilayers. Growth Hormone IGF Res. 8:21–31.
12. **Irwin JC, Giudice LC.** 1998 Decidua. In: Knobil E, Neill JD, eds. Encyclopedia of reproduction. San Diego: Academic Press; 823–835.
13. **Librach CL, Feigenbaum SL, Bass KE, et al.** 1994 Interleukin-1β regulates human cytotrophoblast metalloproteinase activity and invasion *in vitro*. J Biol Chem. 269:17125–17131.
14. **Han VK, Bassett K, Walton J, Challis JRG.** 1996 The expression of insulin-like growth factor (IGF) and IGF binding protein genes in the human placenta and membranes: evidence for IGF:IGFBP interactions at the feto-maternal interface. J Clin Endocrinol Metab. 81:2680–2693.
15. **Ballard FJ, Walton PE, Bastian S, Thomas FM, Wallace JC, Francis GL.** 1993 Effects of interactions between IGFBPs and IGFs on the plasma clearance and *in vivo* biological activities of IGFs and IGF analogues. Growth Regul. 3:40–44.
16. **Francis GL, Aplin SE, Milner SJ, McNeil KA, Ballard FJ, Wallace JC.** 1993 Insulin-like growth factor (IGF)-II binding to IGF-binding proteins and IGF receptors is modified by deletion of the N-terminal hexapeptide or substitution of arginine for glutamate-6 in IGF-II. Biochem J. 293:713–719.
17. **Irwin JC, Kirk D, King RJB, Quigley MM, Gwatkin RBL.** 1989 Hormonal regulation of human endometrial stromal cells in culture: an *in vitro* model for decidualization. Fertil Steril. 52:761–768.
18. **Suwanickul A, Morris SL, Powell DR.** 1993 Identification of an insulin-response element in the promoter of the human gene for insulin-like growth factor binding protein-1. J Biol Chem. 268:17063–17068.